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Quantitative inter-specific chemical activity relationships of pesticides in the aquatic environment

Paolo Tremolada [a,*], Antonio Finizio [b], Sara Villa [b], Carlo Gaggi [c], Marco Vighi [b]

[a] *Department of Biology, University of Milano, via Celoria 26-20133 Milan, Italy*
[b] *Department of Environmental and Landscape Sciences (DISAT), University of Milano-Bicocca, P.zza della Scienza, 1-20126 Milan, Italy*
[c] *Department of Environmental Sciences "Giacomino Sarfatti", University of Siena, Via P.A. Mattioli, 4-53100 Siena, Italy*

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Abstract

Inter-species correlations could be a useful tool for predicting toxicity and for establishing sensitivity ratios among species. In this paper, quantitative inter-specific chemical activity relationships (QICAR) for aquatic organisms were developed to verify if such an approach could be utilised for estimating toxicological data when no other information is available. Inter-specific toxicity relationships on fish, *Daphnia* and algae were performed for pesticides considering a large data set (more than 600 compounds) and grouping the data either on a functional (herbicides, fungicides and insecticides) or chemical class base. Good correlations were found between several fish species and they were improved by excluding, from the data set, highly specific compounds such as organophosphorus insecticides. Relationship between fish (rainbow trout) and *Daphnia* was significant for the whole data set, but clearly improves if congeneric classes of pesticides are considered. The most significant results were found for azoles (fungicides) and for all data set of pesticides with the exclusion of organophosphorus and carbamate insecticides. As expected, toxicity on algae does not correlate either with fish or with *Daphnia* on the whole data set, but excluding the classes acting specifically toward one organism (insecticides and several classes of herbicides), good relationships were found. The analysis of the data permits the conclusion that the specificity in the mode action of pesticides is the key parameter for expecting or not inter-specific relationships. By the relative specificity of action of a group of compounds towards two species, the probability of obtaining a QICAR for this group can be derived. In general, compounds acting with the same level of specificity towards two different species, have a higher probability of showing inter-specific relationships and the lower the specificity of the mode of action of the compounds (e.g. narcotics or less inert chemicals), then the stronger are the relationships.

Keywords: Quantitative inter-specific chemical activity relationships; Acute toxicity; Pesticides; Aquatic organisms

1. Introduction

The massive use and the biocidal activity of pesticides enhance the probability of negative impacts on non-target organisms such as aquatic biota, plants, mammals and soil microorganisms. In order to prevent

* Corresponding author.
E-mail addresses: paolo.tremolada@unimi.it (P. Tremolada), antonio.finizio@unimib.it (A. Finizio), gaggi@unisi.it (C. Gaggi).

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negative impact on ecosystems, preventive risk assessment (ERA: environmental risk assessment) is necessary. The protection of a specific ecosystem, such as surface waters, require the preservation of its ecological functionality. Thus, generally, during ERA procedures, several representative species are selected, belonging to different ecological and taxonomic groups. For the aquatic environment (fresh-water) algae, *Daphnia* and fish are the most common organisms tested for the evaluation of toxic effects. Acute toxicity data on these three organisms represent the minimum requirement for ERA procedures for fresh-water ecosystems (EC, 1996). However, even this basic data set is incomplete for many substances. In addition, the comparability of available data is often impaired by differences in testing methods (static test or flow rate), water characteristics (pH, solutes, etc.), tested organism, (different fish species or ages of the organisms), experimental time (24, 48, 72, 96 h, etc.).

Thus, the development of predictive tools for the evaluation of toxic effects can be of great help in ERA procedures. In this frame, quantitative structure activity relationships (QSARs) are recognised to be a powerful tool in ecotoxicology to predict toxic effects on the basis of the physical and chemical properties of a compound (Hansch et al., 1995; Vighi et al., 1991).

The so-called inter-specific chemical activity relationships represents a different approach; through this analysis it could be possible to predict toxic effects on a particular organism, in case of absence of experimental values, starting from toxicity data obtained on a different species (Adema and Vink, 1981; Sloof et al., 1983; Janardan et al., 1984).

In mammalian toxicology, this approach is well established and allows the extrapolation of the biological activity of chemicals from test animals to man.

In ecotoxicology, inter-species relationships could also give an indication of the relative sensitivity of different species to toxicants or classes of them, and consequently could provide valuable information for selecting species best representing the wider range of biodiversity present in the environment.

Although promising, up to now, such an approach has been little investigated in ecotoxicology. Janardan et al. (1984) found correlation between fish and mammalian toxicity (rat) for organochlorine pesticides and for a list of priority pollutants. However, they found an appreciable scatter around the regression line limiting the predictive usefulness to an order of magnitude estimation. Cronin et al. (1991) found good correlations for a group of chemicals (alcohols, aldehydes and ketones) between the toxicity on fathead minnow versus Microtox (bacteria), *Daphnia magna* and *Tetrahymena pyriformis*. The correlations were improved on homogeneous chemical classes. Relationships between fathead minnow and *T. pyriformis* were found also by other authors for aromatic compounds (Schultz et al., 1986, 1989a,b).

Given the assumption that inter-species correlations may be the stronger the more similar the organisms are, and that improvement in correlations may be obtained on chemicals with a comparable mode of action, we believe that the usefulness of such an approach may be enhanced. However, there is need of a better understanding of the scientific basis for which inter-specific relationships can be expected and developed.

Within this framework, the aim of this paper was to analyse inter-specific relationships among aquatic organisms in order to supply a frame useful for interpreting the values and limitations of such an approach. Particularly, we examined: the existence of significant correlations among the toxicity levels on several aquatic organisms (algae, *Daphnia* and fish), the existence of functional groups (herbicides, fungicides and insecticides) or chemical classes (congeneric approach) which more than others outstand such correlations, the presence of constant sensitivity ratio among these organisms and the possibility of developing quantitative and predictive relationships.

Pesticides are an extremely heterogeneous group of toxicants developed for killing a specific target, so we considered such chemicals as a good starting point towards understanding inter-specific relationships. Several correlations were made, firstly by considering the whole data set and then by dividing pesticides into functional classes (herbicides, fungicides, etc.) or chemical classes (1,3,5-triazines, pyrethroids, etc.).

2. Material and methods

2.1. Pesticides

A total number of 679 pesticides were considered in this study, taking into account those listed in the 11th edition of the Pesticide Manual (Tomlin, 1997),

Table 1
Pesticides analysed grouped according to chemical class (major, minor and unspecified classes) and function (herbicide, fungicide, insecticide/acaricide and others)

	Herbicides	Fungicides	Insecticides/acaricides	Other functions	Total
Major classes					
1,3,5-Triazines	17	1	–	–	18
2-(4-Aryloxyphenoxy) propionic acids	14	–	–	–	14
2,6-Dinitroanilines	10	1	–	–	11
Aryloxyalkanoic acids	14	–	–	–	14
Azoles	–	30	–	–	30
Benzoylureas	–	–	9	1	10
Carbamates	6	1	18	1	26
Chloroacetanilides	10	–	–	–	10
Diphenyl ethers	9	–	1	–	10
Organophosphorus	6	4	68	6	84
Pyrethroids	–	–	36	–	36
Sulfonylureas	25	–	–	–	25
Thiocarbamates	14	–	–	–	14
Ureas	24	–	–	–	24
Minor classes	77	66	38	10	191
Unspecified classes	47	46	46	23	162
Total	273	149	216	41	679

Other functions account for several minor groups such as alghicides, bactericides, ixodicides, molluschicides, nematocides, rodenticides, soil sterilants, and wood protectors. When more then one function was reported, the first was considered.

together with several compounds included in the superseded entries section of the same manual, in order to increase the number of cases of congeneric chemical classes (Table 1). Major classes of Table 1 are those for which at least 10 chemicals are present; other compounds are listed in minor or unspecified classes.

2.2. Acute toxicity tests and the n-octanol/water partition coefficient

The most common species used in the acute toxicity tests on algae are *Scenedesmus subspicatus*, *Selenastrum capricornutum* and *Chlorella* spp. Photosynthesis inhibition or growth reduction are the toxicological endpoints usually measured. Due to the lack of reliable data, all the three species were selected as representative of algal toxicity, if the testing procedure was comparable, even if inter-specific differences may exist.

Most of the tests on zooplancton are performed with *Daphnia* (both *D. magna* and *D. pulex*). Tests on both species were included as input data for zooplancton acute toxicity, when the end-point was 48 h EC_{50} on immobilisation.

A larger number of data is available on fish. Rainbow trout (*Oncorhynchus mykiss*), bluegill sunfish (*Lepomis macrochirus*), fathead minnow (*Pimephales promelas*), golden orfe (*Leuciscus idus*), catfish (*Ictalurus* sp.) and carp (*Ciprinus* sp.) were included as input data for fish acute toxicity, when the end-point was 96 h LC_{50}. The number of data available for each toxicological test is reported in Table 2.

n-Octanol/water partition coefficient (K_{ow}) was used to calculate predicted EC_{50} or LC_{50} for algae, *Daphnia* and fish, according to the standard QSAR equations for narcotics proposed by the European Commission (EC, 1996). K_{ow} values and toxicity data were taken from the literature as explained below.

It is evident that assessing the reliability of literature toxicity data is crucial in this kind of work. In the case of pesticides many compilations are available, e.g. the Pesticide Manual, but those data can not be taken without any critical revision, because, when data coming from different sources are compared, it is frequent to observe differences among the

Table 2
Numbers of data of several acute toxicity tests (EC_{50} = effective concentration on the 50% of the tested organisms and LC_{50} = lethal concentration on the 50% of the tested organisms) on different organisms for pesticides grouped per function (herbicide, fungicide, insecticide/acaricide and others)

Acute toxicity test	Herbicides	Fungicides	Insecticides/acaricides	Other functions	Total
EC_{50} (96 h) on algae	104 (8)	62 (4)	68 (16)	8 (1)	242 (29)
EC_{50} (48 h) on *Daphnia*	171 (43)	88 (9)	124 (6)	24 (2)	407 (60)
LC_{50} (96 h) on rainbow trout	200 (50)	94 (6)	130 (16)	23 (1)	447 (73)
LC_{50} (96 h) on bluegill sunfish	127 (43)	51 (5)	74 (8)	15 (0)	267 (56)
LC_{50} (96 h) on fathead minnow	10 (3)	1	6 (1)	1	18 (4)
LC_{50} (96 h) on catfish	24 (6)	6 (0)	11 (2)	2 (0)	43 (8)
LC_{50} (96 h) on carp	41 (12)	34 (2)	30 (7)	4 (1)	109 (22)
LC_{50} (96 h) on golden orfe	7 (3)	10 (0)	27 (1)	3 (0)	47 (4)
Total	714 (178)	352 (28)	486 (58)	81 (5)	1633 (269)

As Table 1, other functions account for several minor groups such as alghicides, bactericides, ixodicides, molluschicides, nematocides, rodenticides, soil sterilants, and wood protectors. First number accounts for the existence of data, either defined values or indicative values (> of), the second (in brackets) accounts for the existence of indicative values only.

toxicity values higher than one order of magnitude for the same pesticide and species. In this situation, it is necessary to select the most reliable one on the basis of the source and the coherence with other results (datum variability). This work was performed by A. Finizio for the Italian Environmental Agency (A.P.A.T.), in 1999, resulting in the FITOX data base (FITOX, 1999). All the acute toxicity data, for algae (96 h) *Daphnia* (48 h) and fish (96 h) were evaluated for most of the pesticides and, for each one, a reliable value was defined by an accurate selection or by calculating the geometric mean of the different experimental results, when the toxicity data were not very different in values. In this way, it was possible to include in the present study quite reliable values, but, in order to implement the pesticide survey on a higher number of molecules, several others compounds were added, using, as toxicity data, those reported in the Pesticide Manual 11th edition (Tomlin, 1997), taking into account that the level of reliability could be lower. The toxicity data considered in the present work, either coming from FITOX data base or the Pesticide Manual, were validated with those included in the EU risk assessment documents, when they were available, giving only minor or no differences.

Even if the reliability of the data used in this work can be considered sufficient, it must be kept in mind that a possible level of "noise" may be present, deriving from the experimental differences and the intrinsic variability of the toxicological tests. Therefore, the specific behaviour of individual chemicals should not be assumed as significant. Only general trends, referred to groups of chemicals are reasonably meaningful.

2.3. Statistical analysis

All the toxicity data (EC_{50} or LC_{50}) were expressed on a molar basis (mmol/l), and toxicity ratios among the species was calculated on the same basis. For the regression analysis toxicity data were log-transformed because it has been statistically assessed that they follow a log-normal distribution (Delistraty et al., 1998; Delistraty, 2000).

SPSS for Windows (version 5.02) program was utilised for descriptive statistics and for simple linear least-squares regression analysis. Analyses were performed on the whole data set or alternatively by grouping the data into different categories: functional classes (herbicides, fungicides, etc.) or chemical classes (1,3,5-triazines, pyrethroid, etc.), in order to verify whether specific functions (i.e. herbicides, insecticides and fungicides) or chemical classes show better correlations. These correlations were calculated when at least seven cases were available. If a relation exists, the median ratio between toxicity data on the species compared was calculated as an indicator of the sensitivity of the different species to the different groups of chemicals.

3. Results

3.1. Comparison among fish species

Toxicity data for pesticides were mainly found on rainbow trout, bluegill sunfish, carp, fathead minnow, golden orfe and catfish (Table 2).

Trout was taken as the reference species, because of the greater number of toxicity data available, thus, all correlations were calculated between trout and the other fish species. Fig. 1 shows the relationship between trout versus bluegill sunfish. In the figure, the line of the direct linear relationship with regression coefficient = 1 and intercept = 0 is reported. Dotted lines indicates an interval of one order of magnitude from the supposed direct linear relationship (±0.5 log unit). For the points inside the two dotted lines the extrapolation of the toxicity from one species to the other gives at most an error of a factor of about three that can be considered acceptable.

The regression equation on the whole data set is:

$$\log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ trout\ (mmol/l)} = 0.95 \times \log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ bluegill\ (mmol/l)} - 0.19$$

where $n = 199$; $R^2 = 0.92$; S.E. $= 0.44$; $F = 2168$; significance $P < 0.0001$.

The excellent correlation and the coefficient of the regression line close to 1 indicate that differences in sensitivity between the two species are negligible if the whole data set is considered, even if the median of the ratio trout/bluegill of 0.77 seems to indicate a slightly higher sensitivity in trout.

Comparable correlations and similar median ratios were found on functional classes (trout/bluegill median ratio of 0.77; 1.0; 0.77 for herbicides, fungicides and insecticides/acaricides, respectively indicating small or negligible sensitivity differences between the two species.

Some differences can be observed on a chemical class basis. Besides individual outliers or minor groups, with a number of cases too small for supporting significant evaluations, major differences have been observed for organophosphorus compounds for which the comparison is based on 25 cases. For these chemicals, a trend seems to exist, indicating higher sensitivity for bluegill, confirmed by the trout/bluegill median ratio of 1.5.

The regression for the remaining chemicals is improved by excluding organophosphorus:

$$\log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ trout\ (mmol/l)} = 0.99 \times \log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ bluegill\ (mmol/l)} - 0.16$$

where $n = 174$; $R^2 = 0.94$; S.E. $= 0.39$; $F = 2557$; significance $P < 0.0001$.

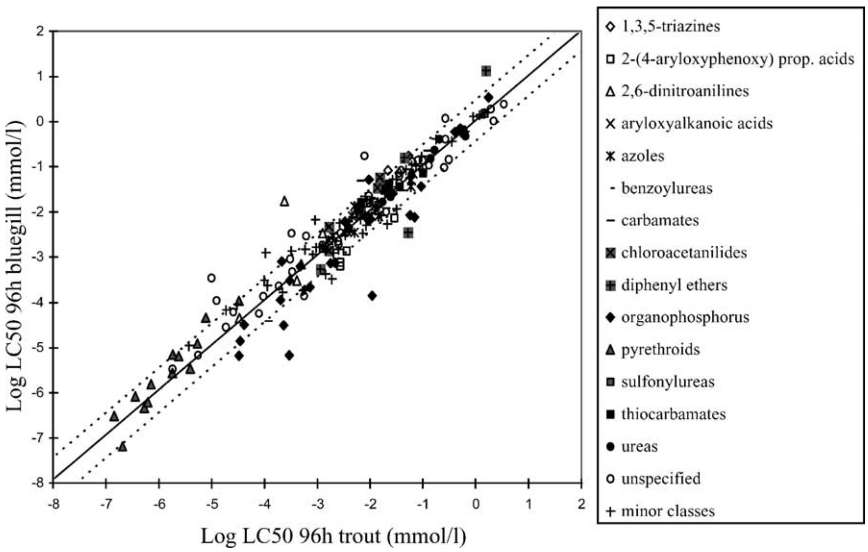


Fig. 1. Relationship between trout and bluegill sunfish toxicity of pesticides, grouped according to chemical class.

When considering the other chemical classes, good relationships can be found as well (e.g. $R^2 = 0.96$ for ureas) but in some cases the lower number of data and the smaller range of toxicity reduce their significance. The regression parameters of some of them seem to suggest different class-specific trends as for pyrethroid (higher trout sensitivity). In these cases, the median ratio between the toxicity on the two species can indicate the dimension of such differences (trout/bluegill median ratio for pyrethroids is 0.45).

Comparison with other fish species is less significant due to the minor number of cases available. Nevertheless, as a general rule, highly significant relationships were found. Regression equations between trout and golden orfe, catfish, and fathead minnow are the following:

$$\log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ trout\ (mmol/l)} = 0.97 \times \log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ golden\ orfe\ (mmol/l)} - 0.47$$

where $n = 39$; $R^2 = 0,92$; S.E. $= 0.48$; $F = 447$; significance $P < 0.0001$.

$$\log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ trout\ (mmol/l)} = 0.99 \times \log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ catfish\ (mmol/l)} - 0.14$$

where $n = 32$; $R^2 = 0.91$; S.E. $= 0.44$; $F = 298$; significance $P < 0.0001$.

$$\log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ trout\ (mmol/l)} = 1.0 \times \log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ fathead\ minnow\ (mmol/l)} - 0.22$$

where $n = 12$; $R^2 = 0,93$; S.E. $= 0,52$; $F = 125$; significance $P < 0.0001$.

Parameters of the regression lines indicate low or negligible differences of sensitivity between trout and other species. Only some organophosphorus compounds appear as outliers, showing in all cases higher sensitivity for trout.

In particular for carp (Fig. 2), organophosphorus compounds behave differently from the other chemicals and were not included in the calculation.

Regression equation, by excluding organophosphorus is the following:

$$\log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ trout\ (mmol/l)} = 0.98 \times \log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ carp\ (mmol/l)} - 0.36$$

where $n = 65$; $R^2 = 0.83$; S.E. $= 0.54$; $F = 314$; significance $P < 0.0001$.

The above reported equation shows again negligible differences between the two species. On the

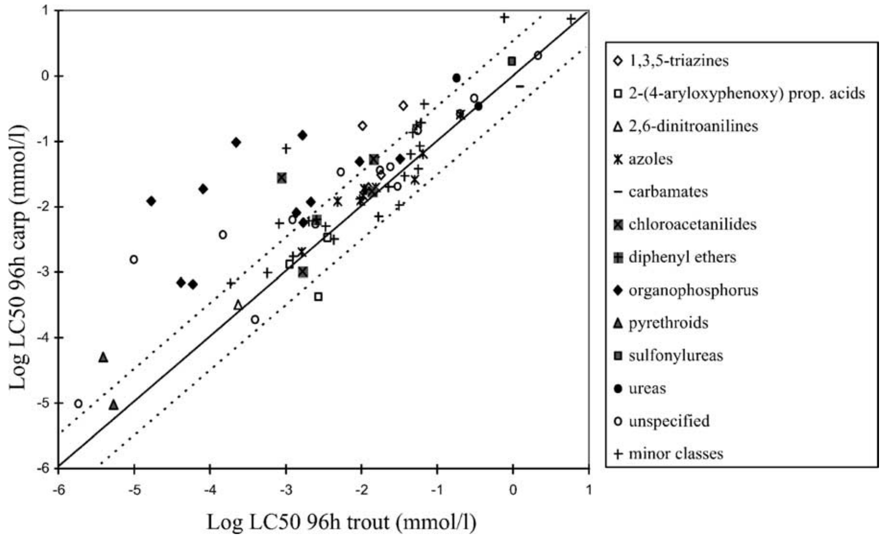


Fig. 2. Relationship between trout and carp toxicity of pesticides, grouped according to chemical class.

contrary, for organophosphorus, carp is substantially less sensitive than trout (median carp/trout ratio of 0.091).

It can be concluded that very good correlations exist between fish species and that sensitivity differences are small or unrelevant for most pesticide groups. The only exception are organophosphorus compounds for which decreasing sensitivity among species was observed as follows:

bluegill > trout > orfe > carp.

For the other two species (catfish and fathead minnow), the minor number of data available does not allow sound conclusions. In general for all species, it could be hypothesised that organophosphorus compounds may exert some specific toxicological mechanisms, capable of producing different effects on different fish species.

3.2. Comparison between Daphnia and trout

Daphnia and trout are significantly correlated on the whole data set (Fig. 3) even if a large number of outliers are present and the equations shows moderate predictive capability:

$$\log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ trout\ (mmol/l)} = 0.61 \times \log \mathrm{EC}_{50}\ 48\,\mathrm{h\ on}\ \mathit{Daphnia}\ \mathrm{(mmol/l)} - 0.65$$

where $n = 267$; $R^2 = 0.59$; S.E. $= 0.98$; $F = 379$; significance $P < 0.0001$.

In this case too organophosphorus compounds, together with carbamates, represent the most important outlier group, *Daphnia* being substantially more sensitive than trout. On the other hand, it is not surprising that arthropods show higher sensitivity for these insecticides with comparable mode of action (inhibitors of acethyilcholinesterase).

The regression equation for all other chemicals, without organophosphorus and carbamates is:

$$\log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ trout\ (mmol/l)} = 0.82 \times \log \mathrm{EC}_{50}\ 48\,\mathrm{h\ on}\ \mathit{Daphnia}\ \mathrm{(mmol/l)} - 0.41$$

where $n = 206$; $R^2 = 0, 77$; S.E. $= 0.75$; $F = 683$; significance $P < 0.0001$.

Excluding organophosphorus and carbamates greatly increases the predictive capability of the equation (R^2 value from 0.59 to 0.77) and the slopes become nearer to 1, indicating that, without the most outstanding compounds, the differences in sensitivity are relatively low. The above equations are very

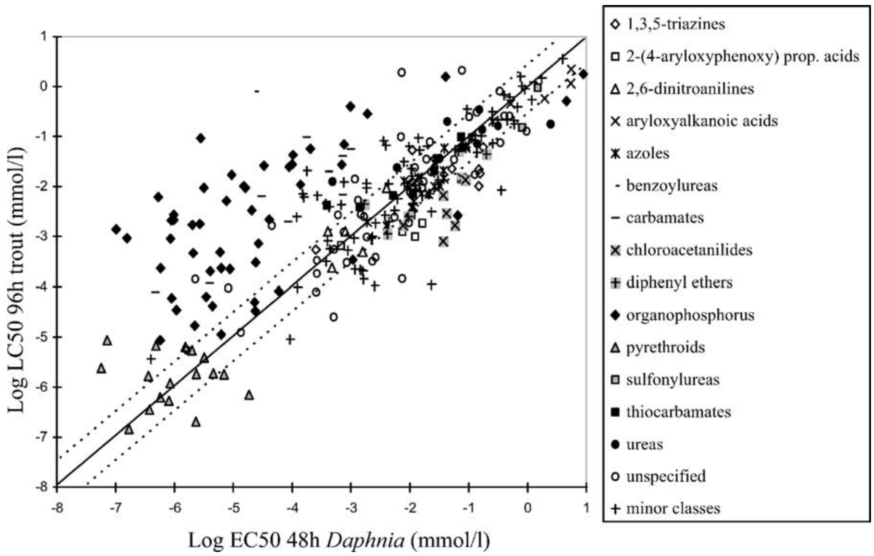


Fig. 3. Relationship between *Daphnia* and trout toxicity of pesticides, grouped according to chemical class.

similar to that found by Cronin et al. (1991) for fathead minnow and *D. magna*:

$$\log\left(\frac{1}{\mathrm{LC}_{50}}\right) 96\,\mathrm{h\ on\ mennow\ (mmol/l)} = 0.805 \times \log\left(\frac{1}{\mathrm{LC}_{50}}\right) 48\,\mathrm{h\ on}\ \mathit{Daphnia}\ \mathrm{(mmol/l)} + 0.061$$

where $n = 46$; $R^2 = 0.75$; S.E. $= 0.443$; $F = 307$.

As expected, the median *Daphnia*/trout toxicity ratio for insecticides (0.071) indicates higher sensitivity for *Daphnia*, while those of herbicides and fungicides are near to 1 (1.4 and 1.3, respectively). Most of these chemicals have probably a “narcotic type” mode of action on animals, thus sensitivity of the two species is comparable.

By grouping the data per chemical classes, it was possible to calculate seven relationships and different results were obtained. Good correlations were found for azoles and carbamate insecticides.

The regression equation for azoles is:

$$\log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ trout\ (mmol/l)} = 1.0 \times \log \mathrm{EC}_{50}\ 48\,\mathrm{h\ on}\ \mathit{Daphnia}\ \mathrm{(mmol/l)} - 0.21$$

where $n = 19$; $R^2 = 0.84$; S.E. $= 0.24$; $F = 86$; significance $P < 0.0001$.

For carbamate insecticides, a reliable equation can be calculated, where the slope near 1 indicates a constant ratio between trout and *Daphnia* toxicity, and the intercept accounts for the difference in sensitivity:

$$\log \mathrm{LC}_{50}\ 96\,\mathrm{h\ on\ trout\ (mmol/l)} = 0.87 \times \log \mathrm{EC}_{50}\ 48\,\mathrm{h\ on}\ \mathit{Daphnia}\ \mathrm{(mmol/l)} + 1.3$$

where $n = 8$; $R^2 = 0.80$; S.E. $= 0.57$; $F = 24$; significance $P = 0.0026$.

Lower correlations were obtained for ureas and 1,3,5-triazines ($R^2 = 0.65$ and 0.55, respectively), and even worse for chloroacetanilides and organophosphorus insecticides ($R^2 = 0.36$ and 0.21, respectively). No significant correlation was found for pyrethroids, due to several cases of completely divergent sensitivity of around two orders of magnitude. Pyrethroids have a specific toxicological mode of action on the gangliar nervous system of arthropods (Leahey, 1985). The high toxicity of pyrethroids on fish is well known but the mode of action is not completely clear. The high toxicity levels allow the hypothesis of a specific, even if different, mode of action on fish too.

3.3. Comparison between algae and Daphnia or trout

As expected, no correlation was found between algae and animal species on the whole data set. Statistically significant relationships were found within functional groups but with a very poor predictive capability (R^2 between 0.12 and 0.23). Comparable results were found by grouping data per chemical classes, in the few cases for which a sufficient number of data was available. The median ratio of the *Daphnia*/algae or trout/algae toxicities can give an idea of the relative specificity of each class. For example, *Daphnia*/algae median ratio of organophosphorus insecticides is 0.00056, meaning an high specificity on *Daphnia*, on the contrary, *Daphnia*/algae median ratio of 1,3,5-triazines is 1800, indicating an high specificity of these herbicides on algae. A relationships between algae and animals could only exist for compounds without any specificity of action nor on algae (as most of the herbicides have) nor on animals (as most of the insecticides have). When the median ratio of a congeneric class is near 1, an unspecificity toward the two organisms can be hypothesised. Indeed, good relationships with acceptable predictive capability were found for the non specific compounds (most of the fungicides and unspecifically acting classes of herbicides: thiocarbamates, 2,6-dinitroanilines, aryloxyalkanoic acids and 2-(4-aryloxyphenoxy) propionic acids. Between fungicides those belonging to 1,3,5-triazine, carbamate and organophosphorus chemical classes were excluded because highly specific toward one organism as well as *n*-trihalomethylthio-fungicides because highly specific especially toward fish. Algae versus *Daphnia* and algae versus trout relationships are shown in Figs. 4 and 5.

Algae versus *Daphnia* regression equation for unspecific compounds is:

$$\log \mathrm{EC}_{50}\ 48\,\mathrm{h\ on}\ \mathit{Daphnia}\ \mathrm{(mmol/l)} = 0.70 \times \log \mathrm{EC}_{50}\ 96\,\mathrm{h\ on\ algae\ (mmol/l)} - 0.40$$

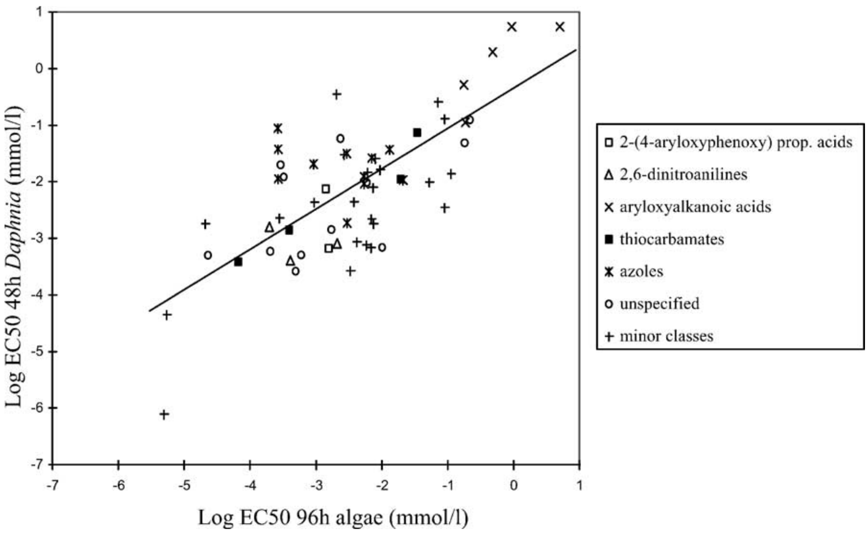


Fig. 4. Relationship between algae and *Daphnia* toxicity of unspecifically acting pesticides, grouped according to chemical class.

where $n = 60$; $R^2 = 0.52$; S.E. $= 0.83$; $F = 64$; significance $P < 0.0001$.

Algae versus trout regression equation for unspecific compounds is:

$$\log LC_{50}\ 96\ \text{h on trout (mmol/l)} = 0.58 \times \log EC_{50}\ 96\ \text{h on algae (mmol/l)} - 0.71$$

where $n = 56$; $R^2 = 0.49$; S.E. $= 0.67$; $F = 53$; significance $P < 0.0001$.

These relationships cannot be considered sufficiently reliable for predicting unknown data because of the scatter around the regression line, but they suggest the crucial role of the specificity of action for establishing an inter-specific relationship.

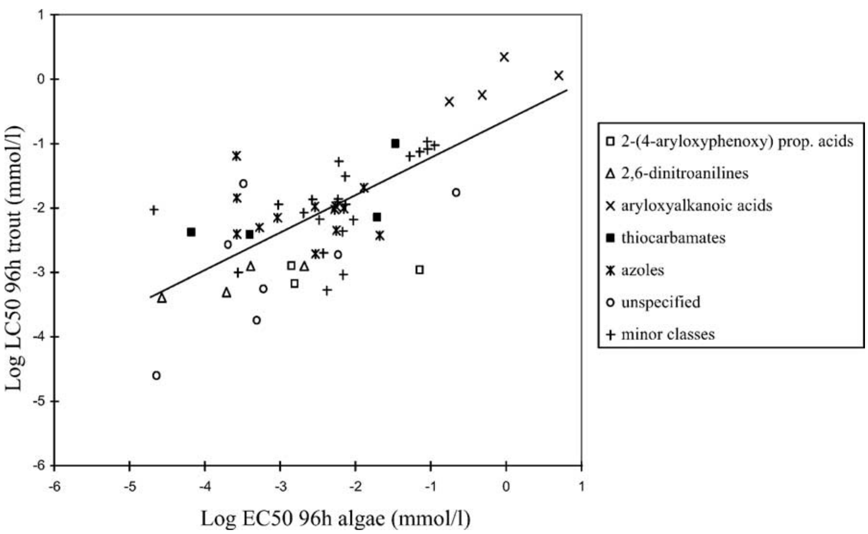


Fig. 5. Relationship between algae and trout toxicity of unspecifically acting pesticides, grouped according to chemical class.

4. Discussion

The results obtained confirm the existence of a correlation for the toxicity among different aquatic organisms as suggested by Cronin et al. (1991). These authors found good correlations between fathead minnow and a series of other organisms, very different from a phylogenetic point of view: an invertebrate (*D. magna*) a ciliate (*T. pyriformis*) and a bacterium (*Vibrio fisheri*) for a number of generic chemicals. Nevertheless, the results of the present paper suggest that limitations and exceptions must be kept in mind. Also in the case of a clear general inter-correlation (trout versus carp or *Daphnia* versus trout), several compounds behave differently, not simply as exceptions, but following a specific trend linked to their chemical class. This different behaviour mainly depends on the level of specificity of the toxicological mode of action toward a given organism.

A possible criterion for the definition of the specificity or unspecificity of action of a molecule could be the deviation from the "baseline" toxicity defined according to Verhaar et al. (1992). This criterion is based on the assumption that, in the absence of any specific mechanism of action, the biological activity of a chemical is "narcotic type" and is entirely dependent on its hydrophobicity measured by the $\log K_{ow}$. Therefore, narcosis type toxicity is also called "baseline" toxicity or minimum toxicity. Verhaar et al. (1992) proposed a scheme for classifying organic pollutants into four classes: narcotic, less inert chemicals, reactive chemicals and specifically acting chemicals. Each class is characterised by a specific mean toxicity ratio. The Toxicity Ratio (TR) is calculated for each compound by the ratio between estimated baseline toxicity and the corresponding experimental LC_{50} value ($TR = LC_{50baseline}/LC_{50experimental}$). For a given chemical class, mean toxicity ratio is calculated on logarithmic transformed TR values (log TR).

In the EU Technical Guidance Document (EC, 1996), several QSAR equations for narcotics were selected in order to predict aquatic toxicity on several organisms (algae, *Daphnia* and fish). These equations were utilised to plot a reference line in the three experimental toxicity versus K_{ow} relationships (Figs. 6–8) and to calculate TR values.

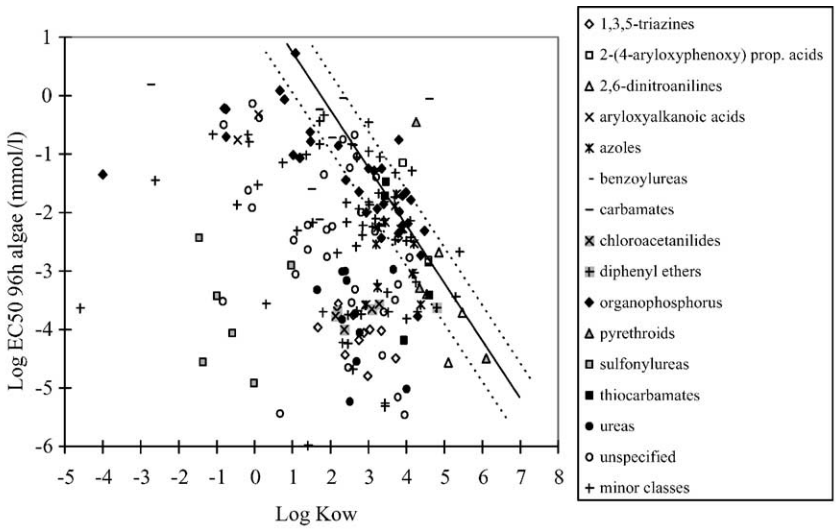
In Figs. 6–8 a few chemicals show a toxic concentration (EC_{50} or LC_{50}) far above the line, indicating a toxic effect substantially lower than the baseline. Considering that narcosis is the minimum toxicity, these values must be assumed as being of low reliability. Therefore, these chemicals were excluded from the elaboration. Moreover, compounds with $\log K_{ow}$ far below zero were also excluded, because narcosis type QSAR equations cannot be applied realistically to those compounds (Verhaar et al., 1992). Obviously, there are several uncertainties that could affect the results such as those related to the variability of literature data (both on toxicity data than on K_{ow} values) and to the uncertainties of QSAR model results. Taking into account these considerations, we assumed that one order of magnitude interval around the baseline toxicity reference line (±0.5 log unit) can be considered still acceptable for considering substances acting as narcotics.

Looking at Figs. 6–8, one can perceive the specificity in the mode of action of pesticides on the different aquatic organisms. The distance from the baseline can be assumed as a measure of the specificity of the mode of action. The measure of such distance can be calculated for each class of pesticides through log TR approach (Table 3).

The log TR-derived specificity categories reported in Table 3 are derived from the work of Verhaar et al. (1992) but they were adapted to a different log TR range found in this work (6 log unit). In the work of Verhaar et al. (1992) the highest log TR was 4.22 and it was considered as a maximum value of the class 4 distribution ($\mu = 1.79$ and $\sigma = 1.27$). In this work, many chemical classes show mean log TR much higher especially on algae. Therefore, a different specificity scale is proposed:

Narcotic	$-0.5 < \log TR < 0.5$
Less inert chemicals	$0.5 < \log TR < 1.5$
Reactive chemicals	$1.5 < \log TR < 2.5$
Specifically acting chemicals	$2.5 < \log TR < 4$
Highly specifically acting chemicals	$\log TR > 4$

Schemes of Figs. 6–8 represent the mean and the dispersion of the log TR distribution of each chemical class on algae, *Daphnia* and fish, to represent better the specificity and the homogeneity of action of the different classes of pesticides.



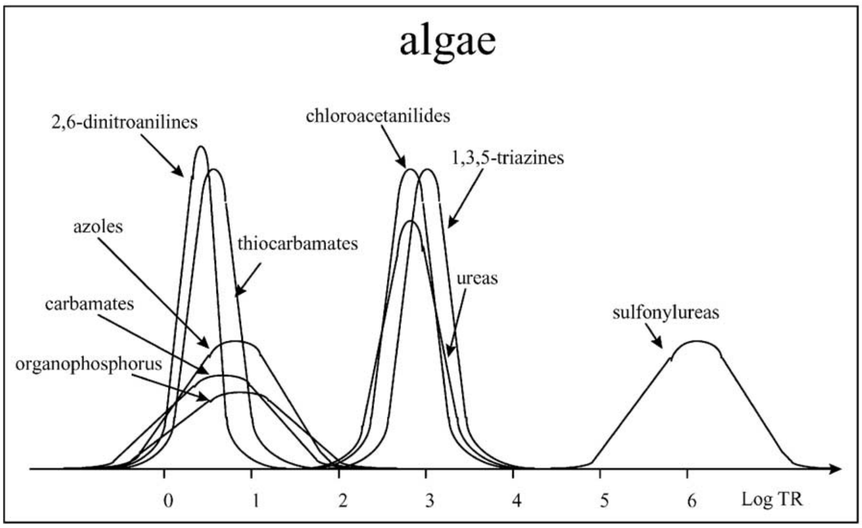


Fig. 6. Relationship between $\log K_{ow}$ and algae toxicity of pesticides, grouped according to chemical class. The line correspond to the calculated baseline toxicity, according to the equation: $\log EC_{50}$ *S. capricornutum* 72–96 h (mmol/l) $= -1 \times \log K_{ow} + 1.77$ (EC, 1996). The scheme below shows the normal distribution approximations of the logarithm of the toxicity ratios (log TR) of the different classes of pesticides, as reported in Table 3.

On algae, the most specific compounds are sulfonylureas (highly specifically active compounds) and chloroacetanilides, ureas and 1,3,5-triazines (specifically active compounds). Aryloxyalkanoic acids seem to act in between specifically active or reactive compound classes, but only two cases were available. Other classes of herbicides (2,6-dinitroanilines, thiocarbamates, and 2-(4-aryloxyphenoxy) propionic acids) as well as main classes of insecticides and fungicides seem not to act specifically (less inert compounds or narcotic). The specificity of the above mentioned classes is in agreement with the main mechanism of action. Compounds most toxic on algae are those acting on specific processes of

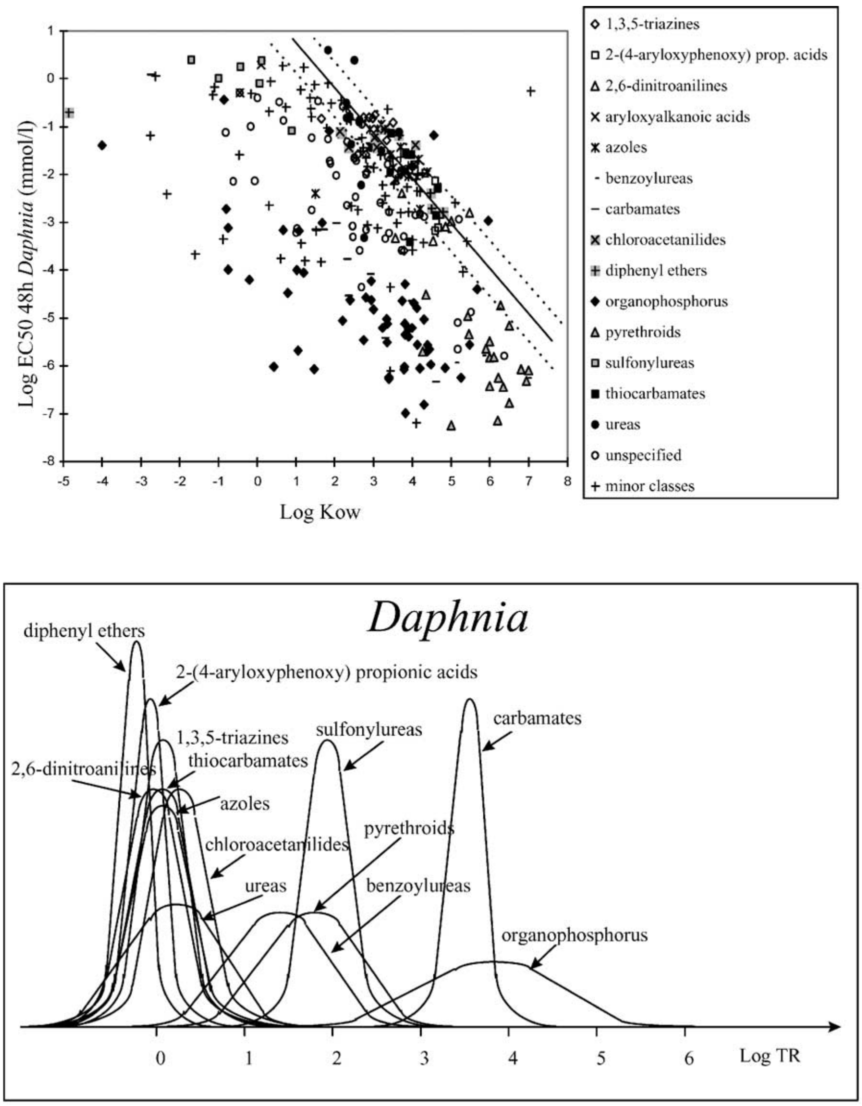


Fig. 7. Relationship between $\log K_{ow}$ and *Daphnia* toxicity of pesticides, grouped according to chemical class. The line correspond to the calculated baseline toxicity, according to the equation: $\log \mathrm{EC}_{50}$ *D. magna* 48 h (mmol/l) $= -0.95 \times \log K_{ow} + 1.68$ (EC, 1996). The scheme below shows the normal distribution approximations of the logarithm of the toxicity ratios (log TR) of the different classes of pesticides, as reported in Table 3.

the vegetal cell (sulfonylureas inhibit branched chain amino acid synthesis and 1,3,5-triazines and ureas inhibit photosynthetic electron transport). Others classes such as 2,6-dinitroanilines, thiocarbamates and 2-(4-aryloxyphenoxy) propionic acids are expected to be less specific on algae because they act on less specific processes such as cell division or lipid metabolism. Sulfonylureas in particular, act so specifically on vegetal cells that their effective doses for weed control are at g/ha level (1000 times less than the average dose of the other herbicides). Its log TR is 3 log unit higher than that of the other reactive classes (chloroacetanilides, ureas and 1,3,5-triazines).

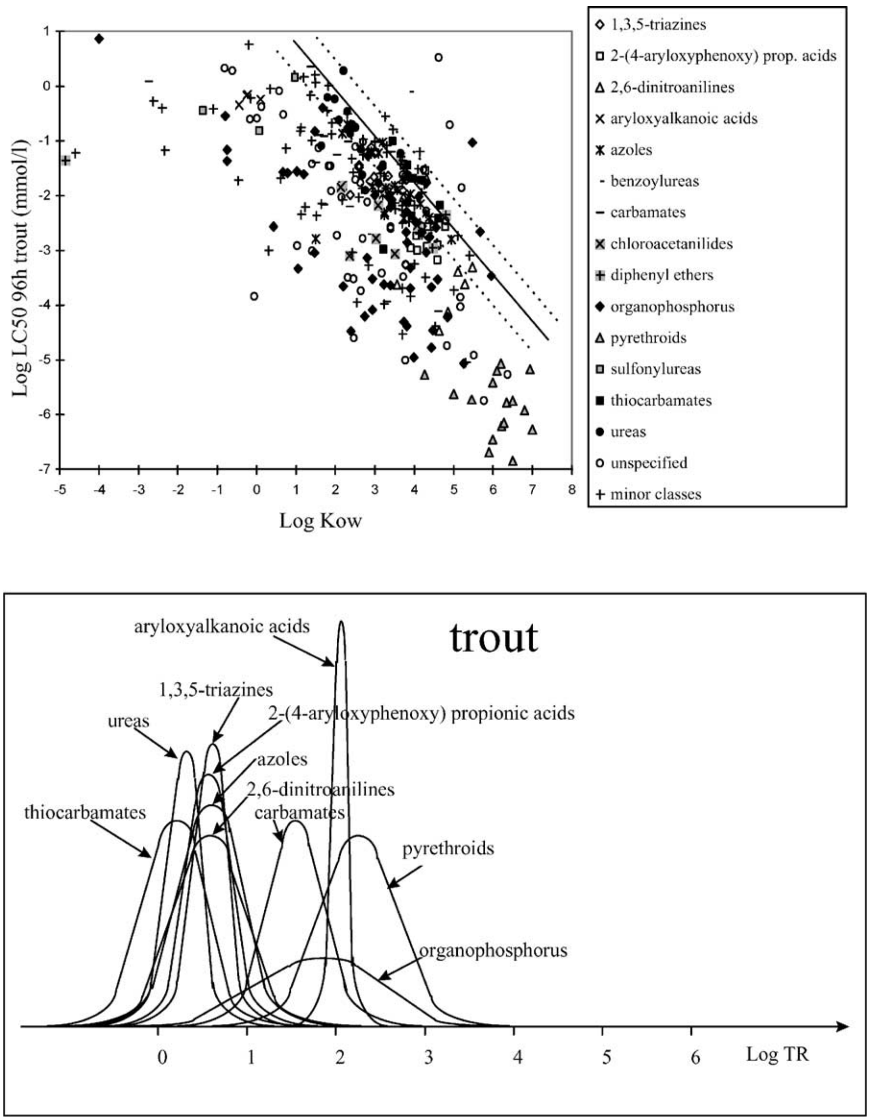


Fig. 8. Relationship between $\log K_{ow}$ and trout toxicity of pesticides, grouped according to chemical class. The line correspond to the calculated baseline toxicity, according to the equation: $\log LC_{50}$ *Pimephales promelas* 96 h (mmol/l) $= -0.85 \times \log K_{ow} + 1.61$ (EC, 1996). The scheme below shows the normal distribution approximations of the logarithm of the toxicity ratios (log TR) of the different classes of pesticides, as reported in Table 3.

On *Daphnia* and fish, almost all insecticides show a high specificity of action, especially organophosphorus and carbamates on *Daphnia*. Carbamates and organophosphorus are much more specific on *Daphnia* than on trout (two log TR units), probably because the target action site (nervous system) is more easily reached. On the contrary, pyrethroid are slightly more specific on fish than on *Daphnia* (0.5 log TR unit). It is interesting to note that pyrethroids are less specific than organophosphorus or carbamate considering their K_{ow}, although these compounds are much more toxic if compared with the latter. This indicates that $\log K_{ow}$ plays a key role in explicating the toxicity of pyrethroids, while for organophosphorus or

Table 3
Mechanism of action, distribution parameters of the logarithm of the calculated/experimental toxicity ratio (log TR) and specificity of action (specif.) of the different classes of herbicides, fungicides and insecticides on algae, *Daphnia* and fish

Pesticides	Mechanism of action	Algae		*Daphnia*		Fish	
		Log TR	Specif.	Log TR	Specif.	Log TR	Specif.
Herbicides							
1,3,5-Triazines	Photosynthetic electron transport inhibitors	3.1 ($\sigma = 0.54$; $n = 10$)	Specif. acting	0.13 ($\sigma = 0.57$; $n = 12$)	Narcotic	0.6 ($\sigma = 0.43$; $n = 11$)	Narcotic: less inert
2-(4-Aryloxyphenoxy) propionic acids	Lipid biosynthesis inhibitors	−0.3 ($\sigma = 0.58$; $n = 3$)	Narcotic	−0.13 ($\sigma = 0.45$; $n = 6$)	Narcotic	0.6 ($\sigma = 0.51$; $n = 11$)	Narcotic: less inert
2.6-Dinitroanilines	Cell division inhibitors	0.4 ($\sigma = 0.4$; $n = 4$)	Narcotic: less inert	−0.1 ($\sigma = 0.65$; $n = 4$)	Narcotic	0.6 ($\sigma = 0.71$; $n = 8$)	Narcotic: less inert
Aryloxyalkanoic acids	Growth inhibitors	2.5 ($\sigma = 0.7$; $n = 2$)	Reactive: specif. acting	1.8 ($\sigma = 0.8$; $n = 2$)	Reactive	2.0 ($\sigma = 0.24$; $n = 4$)	Reactive
Chloroacetanilides	Protein synthesis inhibitors	2.9 ($\sigma = 0,54$; $n = 5$)	Specif. acting	0.2 ($\sigma = 0.64$; $n = 7$)	Narcotic	1.5 ($\sigma = 0.66$; $n = 3$)	Less inert: reactive
Diphenyl ethers	Protoporphyrinogen oxidase inhibitors			−0.2 ($\sigma = 0.37$; $n = 4$)	Narcotic	0.3 ($\sigma = -0.2$–0.76)	Narcotic
Sulfonylureas	Branched chain amino acid synthesis inhibitors	6.1 ($\sigma = 0.92$; $n = 6$)	Highly specif. acting	2.0 ($\sigma = 0.62$; $n = 6$)	Reactive	2.1 ($\sigma = 1.32$; $n = 3$)	Reactive
Thiocarbamates	Lipid matabolism inhibitors	0.6 ($\sigma = 0.55$; $n = 4$)	Narcotic: less inert	−0.001 ($\sigma = 0.69$; $n = 7$)	Narcotic	0.2 ($\sigma = 0.68$; $n = 17$)	Narcotic
Ureas	Photosynthetic electron transport inhibitors	2.9 ($\sigma = 0.65$; $n = 11$)	Specif. acting	0.2 ($\sigma = 0.95$; $n = 12$)	Narcotic	0.4 ($\sigma = 0.46$; $n = 17$)	Narcotic: less inert
Insecticides/acaricides							
Carbamates	Cholinesterase inhibitors	0.7 ($\sigma = 1.1$; $n = 6$)	Less inert	3.5 ($\sigma = 0.43$; $n = 7$)	Specif. acting	1.6 ($\sigma = 0.66$; $n = 9$)	Less inert: reactive
Organophosphorus	Cholinesterase inhibitors	0.8 ($\sigma = 1.2$; $n = 28$)	Less inert	3.8 ($\sigma = 1.49$; $n = 45$)	Specif. acting	1.9 ($\sigma = 1.37$; $n = 41$)	Reactive
Pyrethroids	Nervous system acting	0.4 ($\sigma = 0,39$; $n = 2$)		1.8 ($\sigma = 0.97$; $n = 20$)	Reactive	2.3 ($\sigma = 0.71$; $n = 16$)	Reactive
Benzoylureas	Chitin synthesis inhibitors			1.3 ($\sigma = 0.98$; $n = 5$)	Less inert		
Fungicides							
Azoles	Steroid demethylation inhibitors	0,8 ($\sigma = 0.9$; $n = 13$)	Less inert	0.1 ($\sigma = 0.7$; $n = 18$)	Narcotic	0.6 ($\sigma = 0.65$; $n = 25$)	Narcotic: less inert

The log TR distribution parameters are, in order, mean, standard deviation and number of cases. The specificity of action accords to the same classification reported in the text: narcotic (narcotic), less inert chemicals (less inert), reactive chemicals (reactive), specifically acting chemicals (specif. acting), highly specifically acting chemicals (highly specif. acting). Calculated baseline toxicity, to obtain log TRs were derived by the following equations (EC, 1996): $\log EC_{50}$ *S. capricornutum* 72–96 h (mmol/l) $= -1 \times \log K_{ow} + 1.77$; $\log EC_{50}$ *D. magna* 48 h (mmol/l) $= -0.95 \times \log K_{ow} + 1.68$; $\log LC_{50}$ *Pimephales promelas* 96 *h* (mmol/l) $= -0.85 \times \log K_{ow} + 1.61$.

carbamate, the toxicity is more linked to the specificity of the mechanism of action. Most of the herbicides classes as well as fungicides act unspecifically (narcotic of less inert compounds) on these two animal species, only sulfonylureas, aryloxyalkanoic acids and chloroacetanilides (for fish only) show a specific mode of action (reactive compounds).

Given this definition of specificity of action, it consequently derives that when the specificity of action toward two organisms is different, the correlation between the two species become impossible; on the other hand, when the specificity is similar, even with very different organisms, the correlation is possible. In algae versus *Daphnia* or algae versus trout relationships, the specificity of action of all the insecticides and several classes of herbicides excludes any correlation on the whole data set, but, by excluding these classes, the relationships became evident. The same consideration can be made for the *Daphnia* versus trout relationship, where many organophosphorus and carbamate compounds outstand the main trend. Between the two organisms, these classes show a difference in the specificity of action of 2 log TR units.

5. Conclusion

According to the current environmental policy, the protection of the aquatic environment requires toxicity tests on several organisms, at least on algae, *Daphnia* and fish. In many cases not enough information is available for a complete risk assessment, so that it is necessary to derive missing values from other data (predictive relationships). With this framework, inter-specific relationships were analysed on a variety of aquatic organisms (algae, *Daphnia* and fish) for a wide set of pesticides. These compounds are rarely considered in this type of study essentially for two reasons: they are an extremely heterogeneous set of data and they are highly active compounds for which toxicity is specifically looked for. The correlations found confirm the validity of such approach and can help towards drawing up useful general guidelines for risk assessment studies.

Experimental values are always to be preferred to calculated ones for risk assessment. Nevertheless predictive models, such as QICAR or QSAR studies, may be of a great help both in giving general indications of the behaviour of a group of compounds, and in furnish a “calculated toxicity value” chemical-by-chemical. These data can be kept, in the case of absence of experimental ones, as an indication of the compound behaviour or, in the case of contrasting results, as a confirmation of one of them. Predictive models are affected by the variability and unreliability of the input data, but, if the data set is large enough, it is difficult that single unreliable results, eventually present, may affect the general trend observed. The intrinsic variability of the experimental results can be estimated at least in a factor 2 interval or higher in spot situations, considering that sometimes it is possible to found in the literature differences higher than one order of magnitude. This variability is intrinsically present also in the model, but, if a sufficiently high number of compounds are considered, as those of this study, the correlations, eventually observed, overcome the “noise” of the data. On the contrary, if the set of data is not large enough, any quantitative conclusion can be derived. An other uncertainly element of the predictive models derives from the compounds that do not fit the correlation (outstanding compounds). Their experimental toxicity can differ from the predicted one also by several order of magnitude. There are two possible reasons for explaining outlier behaviour:

(a) a specific toxicological effect can be hypothesised for one of the organisms involved; this may occur in the comparison between organisms with different taxonomic and physiological characteristics (e.g. fish and *Daphnia*); moreover, this behaviour is generally referred to class of chemicals (e.g. organophosphorus, carbamates, etc.) and not to individual substances;
(b) the deviation from the correlation is due to the bad quality of the datum.

As previously mentioned, this last possibility cannot be excluded within the data set used, but it does not affect the general validity of the model. Obviously, if a toxicity datum has to be predicted from another, the reliability of this last must be carefully checked.

Good correlations were found among fish considering acute toxicity (LC_{50} at 96 h) on six species (rainbow trout, bluegill sunfish, carp, catfish, golden orfe and fathead minnow). Only organophosphorus compounds resulted as outstanding class in several relations (rainbow trout versus carp and rainbow trout versus catfish) suggesting the opportunity of proposing predictive equations for all the pesticides excluding organophosphorus. For all species, regression equations do not greatly differ from the direct linear relation (slope $\approx$ 1 and intercept $\approx$ 0), suggesting that, in general, the datum on one fish species is highly representative of the whole ecological group and that this datum is inter-changeable from one species to another within a factor 3 tolerance interval. Only a few exceptions were observed, mainly belonging to the organophosphorus class, for which the highest toxicity discrepancies were observed. For this class, in the carp versus trout relationship, only the qualitative indication of a median trout/carp toxicity ratio of 0.091 is given, because the variability of the data do not allow any reliable quantitative relationship.

In general, trout has been demonstrated as being the most sensitive species, on which toxicity tests should focus, to be protective. For organophosphorus compounds alone, bluegill sunfish may be more sensitive than trout for a mean factor of 2.

Daphnia and trout show an unexpectedly high correlation even on the whole data set. The most reliable equation was found by excluding organophosphorus and carbamate compounds because they behave as outlier classes in the main relationships. For these compounds different class trends were found suggesting a high and different specificity on the two organisms. Both classes act as cholinesterase inhibitors on the nervous system, showing a high specificity: these classes are both, on average, 100 times more toxic on *Daphnia* than on trout. Organophosphorus and carbamate should require specific predictive models, but only for carbamate a reliable equation was found. Several other class-specific relationships were found as well, but the regression parameters were not much different from the general equation.

Between algae and *Daphnia* or algae and fish many more differences exist, deriving from diversity in the species metabolism and thus in compound specificity. Algae do not correlate either with *Daphnia* or with trout considering the whole set of data. On a functional or chemical class base, on the contrary, many correlation were significant and by considering only compounds acting unspecifically on both organisms a good relationship was found.

The difference in compound specificity seems to be the key parameter for determining the existence or not of inter-species relationships. This general assumption has been verified in this work, using a measure of the specificity of action derived from K_{ow} (log TR). By this parameter five classes of specificity were defined for pesticides on non-target species: narcotics, less inert chemicals, reactive chemicals, specifically acting chemicals and highly specifically acting chemicals. Even dealing with very different organisms (algae versus *Daphnia* or trout) it seem that compounds acting within the same specificity class toward two organisms have a high probability of showing inter-species relationships.

A second general observation, verified in this work, is that inter-species relationship have a greater probability of being the stronger the lower is the specificity class which the compounds belong to. Compounds acting as narcotics or less inert chemicals toward two organisms have much more probability of giving highly reliable quantitative relationships, consequently compounds acting more specifically have more probability of giving only qualitative relationships, For the latter, only the median inter-species ratio can be proposed as a possible toxicity indication in the case of absence of experimental values, taking into account that big discrepancy may exists upon compounds.

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