UNIVERSITY OF PADUA

DEPARTMENT OF INDUSTRIAL ENGINEERING

Master Thesis in Environmental Engineering

COMPARISON OF THE DIFFERENT ECOLOGICAL RISK ASSESSMENT METHODOLOGIES APPLIED TO THE ITALIAN RIVER “PO”

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1 Introduction

Several indicators (EU-TGD,2003; M.G.D. Smit et al., From PEC_PNEC ratio to quantitative risk level using Species Sensitivity Distributions,2005) have been tested and discussed during previous years and currently, lots of methodologies are being developed for the evaluation of ecologic state of environmental ecosystem.

The aquatic environment risk assessment plays an important role as it is a procedure that permits to evaluate or predict the effects of different anthropogenic disruptions (spillage of chemicals compounds) on the ecosystem and, thus, to evaluate the actual or future ecological status.

The concept of ecological risk is the base of this work and of the examined methodologies. It is defined as the perturbation of an ecological status considered acceptable. The perturbation is meant as the concentration of the contaminants present in the environment, while the considered acceptable ecological status is defined with different parameters depending on the used methods (Guidelines for Ecological Risk Assessment,1998)

In order to measure the perturbation effects that impact an ecosystem, working methods are used to give risk indicators through several calculation approaches.

The object of this study is the comparison of three different methodologies for risk assessment, PEC/PNEC ratio(EU-TGD,2003), Species Sensitivity Distribution(J.R. Wheeler et al., Species sensitivity distributions: data and model choice,2002) and a modeling approach using Aquatox program(Laura Grechi,2003). Ecosystem examined is about a stretch of the river Po previously analyzed in Laura Grechi's thesis (2013) and the substances analyzed are LAS and Triclosan. For each substance have been calculated appropriate risk indicators which, compared, will permit to say if different methods give the same answers in the environmental risk evaluation.

The first two methodologies analyzed are united by the fact of being easily applicable and intuitive but at the same time they don't describe in a realistic way the analyzed ecosystem and the real consequences of the analyzed perturbation.

This happens because they are based on the analysis of eco toxicological data taken from the laboratory, under constant conditions that have little in common with the natural environment analyzed, for this reason the results obtained don't give any information on the relative impact of chemicals in a multi-stressed environment, exposed to several sources of natural and anthropogenic stress. So, the ecological risk measurement, cares exclusively about the direct ecological effects of the contaminant on the organisms and not of the indirect ecological effects due to the iteration among present organisms that can change the initial perturbation answer. The third analyzed method, the modeling one, permits the assessment of indirect effects of stressors, the analysis of the gap between individual effects and ecologically relevant endpoints, the interactions between combined stressors and environmental factors, and the potential to define standard scenarios to be used as the base for flexible adaptable models. To address different aspects of ecological realism, ecological models can be applied to individuals, populations, ecosystems, communities or landscapes.

2 Materials and Methods

2.1 Chemicals

2.1.1 LAS

LAS(Linear Alkylbenzene Sulfonate) is a non-volatile anionic surfactants with molecules characterized by a hydrophobic and a hydrophilic group. LAS does not undergo significant degradation by abiotic mechanisms under environmentally relevant conditions because photolyzable and hydrolysable groups are absent from the chemical structure (UNEP Chemicals, 2007).
Regarding LAS degradation operated by biotic factors, it is demonstrated the rapid and complete (ultimate) biodegradation of LAS in many of the available aerobic biodegradation tests, including soil and the aqueous environment (UNEP Chemicals, 2007). While LAS degrades rapidly under aerobic conditions, it does not degrade under anaerobic conditions, except under special conditions.

2.1.2 Triclosan

Triclosan is an antibacterial used in a range of consumer products including personal care products. It is very toxic for aquatic organisms and in particular for algal species, where it acts as a strong photosynthesis inhibitor.

Considering biotic degradation processes, as written by Lyndall et al. (2010), TCS can be biodegraded under aerobic conditions. Products of degradation are 2,8-dichlorodibenzo-p-dioxin and 2,4-dichlorophenol, they are not considered in this risk assessment model because it is demonstrated by several studies that they do not accumulate in fishes and are less toxic than TCS, moreover they occur at lower concentrations (see Lyndall et al., 2010; Laura Grechi, 2013).

2.2 Methodologies

In this comparison work, we used 3 approaches that are among the most known and conventional in the ones used in the evaluation of the environmental risk.

2.2.1 PEC/PNEC ratio

It is based on the concept that a perturbation due to a effective concentration of the contaminant (PEC) is compared to a limit value concentration that under it, the ecological status results acceptable (PNEC)

PEC (Predicted Environmental Concentration), can be obtained by actual field measurements (monitoring data) or by estimations using environmental random models.

PNEC is calculated by dividing the lowest LC50/EC50 or NOEC value for three tropic groups of aquatic organisms by an appropriate assessment factor that is applied to extrapolate from laboratory single-species toxicity data to multi-species ecosystem effects. Assessment factors are reduced with the increasing number of species.

So, the risk indicator will be given by the relationship R1= PEC/PNEC.

If it is less than or equal to 1,it is concluded that for the moment no measures are necessary to reduce the risk.

If the ratio is bigger than 1, there is a risk for the eco system e other factors must be evaluated such as:

i) indications of bioaccumulation potential;

ii) the shape of the toxicity / time curve in eco toxicity testing;

iii) indications to other adverse effects on the basis of toxicity studies

2.2.2 Species Sensitivity Distribution

A reference concentration is calculated using statistical extrapolation method from species sensitivity distribution by taking the prescribed percentage of this distribution. It has been decided that the concentration corresponding with the point in the SSD profile below which 5% of the species occur, should be derived as an intermediate value in the determination of a PNEC. This 5% point in the SSD is also identified as a hazardous concentration (HC) at which a certain percentage (in this case 5%) of all species is assumed to be affected. This methodology can only be applied in cases where sufficient NOECs of good quality for sufficient species are available. Reliability can be associated with a PNEC derived by statistical extrapolation if the database contains at least 10 NOECs (preferably more than 15) for different species covering at least 5 taxonomic groups (EC, 2003; Posthuma et al., 2002; Laura Grechi,2013).

For the graphic creation has been used the "SSD Generator" program.

Reference concentration = 5% SSD based on chronic NOECs

At the end let's calculate our risk indicator as the relationship R2 = PEC/ HC_5.

Also in this case if the relationship is less than 1 or 1 any risk is not expected, if t's bigger than 1, a risk is expected which has to be valuated more precisely with other measuring factors.

2.2.3 Aquatox

It is an evaluation model of the general ecological risk that describes the environmental destiny of an ecosystem and the effects of the conventional contaminants, such as nutrients and sediments, and toxic chemical substances. (Park & Clough, 2012).

It is a released model given by the United States Environmental Protection Agency (EPA) and it nowadays probably one of the most used and modern model to evaluate the environmental destiny and the ecological effects in the aquatic ecosystems.

It is an ecosystem model, it means that it doesn't concentrate on the variations of the individuals number of a single population (model population), but it considers processes like the aquatic organisms interdependence in the ecosystem, nutrient and scoria recycling and the combined effects of the toxic chemical substances.

Each ecosystem model is composed of different components that need input data: abiotic and biotic status variables, guide variables (temperature, light, nutrients, etc…), parameters and coefficient that permit to the user to specify the key characteristics of the process.

With AQUATOX it is possible to model a fluvial ecosystem dividing into more segments. Each segment to be modeled, requires the collect of specific data of the place for the construction and calibration of the model. Due to the data availability in literature and the required effort for the activity in terms of time of data research, for this project only a segment is considered.

The considered segment is modeled as a CSTR reactor in which the status simulated variables are: organisms, nutrients, scoria (batters and organic substance), sediments (inorganic matter) and chemicals. The status variables are expressed in terms of concentration or density of variation in time (in μg/L or g/m^2) and depend on forcing variables (water flow, temperature, light, nutrient charge, biotic/abiotic parameters values and inputs to the system) (Park R.A. and Clough J.S., 2012). Outputs model such as the biomass of each simulated organism and the concentration of chemicals in abiotic and biotic compartments can be plotted over time to compare the controlled ecosystem (that is the simulated ecosystem without polluting insertions) with the perturbed one. However, the large amount of outputs generated by AQUATOX is complex to analyze, and to assist their interpretation for using it in a prospective risk assessment and communicate results in a more straightforward way, an ecological parameter was so calculated:

the average biomass variation (εi) for a given organism i, that is calculated as the percentage difference between its average biomass during the one-year perturbed simulation (Bi,P, $g_{dry}\ m^{-2}$) and its average biomass during the one-year control simulation (Bi,C, $g_{dry}\ m^{-2}$). It can assume positive and negative values.

$$\varepsilon i = \frac{Bi,P - Bi,C}{Bi,C} \cdot 100$$

The absolute perturbation (AP) for an organism of the ecosystem is the absolute value of ε. (Lombardo,2013)

$APi = |\varepsilon i|$

At the end, we have chosen a risk indicator to be compared with the others obtained from the previous, as defined:

R3 = (absolute value of the average variation of all the species that are decreasing)/0.05).

This happens because we established that a maximum decrease of 5% for each species results is acceptable and it's not seen as a danger for the entire ecosystem balance.

2.3 Case of Study

The river Po is located in Northern Italy and flows 642 km from West to East along the whole Pianura Padana before entering in the Adriatic Sea with a delta of 380 km2. It is the longest Italian river, the one with the maximum annual average discharge (1450 m^3/s) and also the one with the largest catchment basin (approximately 74000 km^2). Along its course are 141 tributaries, in its watershed inhabit approximately 16 million people and is concentrated more than a third of industries and of the Italian agricultural production. Water withdrawals amounted on average to 20.5 billion cubic meters per year, of which 12 % are for drinking use, 81 % for irrigation use, 7 % for industrial uses (Autorità di bacino del fiume Po, 2006).
The system with the AQUATOX program is as a sort of continuous stirred-tank reactor, in which the variables change over time but not in space, it is necessary to consider the river ideally divided into segments (reactors), homogeneous in space and possibly linked one to another.
The modeled stretch is about 41 km long (L) from Pontelagoscuro to Serravalle, immediately before the branching section of the delta, the average latitude is 44.9° in North hemisphere, it has an average width (W) of 485 m and a mean depth (H) of 5,15 m (Autorità di bacino del fiume Po, 2006). The volume of the system was set constant at 1.02 * 108 m3. (H x L x W). Time series of water flow (yearly average 2700 m3 s−1) and temperature (yearly average 18.9 C °, range 6–29.0 °C) have been taken from Ufficio Idrografico e Mareografico di Parma - Bacino del Po (1988-1989) and Agenzia Regionale Prevenzione e Ambiente - Regione Emilia Romagna - Servizio Idrometeorologico - Area idrologia (1990). Water speed (yearly average 0.15 m s^{-1}) was calculated by AQUATOX as the ratio between the flow and the river cross section. Water evaporation (from AQUATOX available studies is about 15 in/year = 10^{-9} m/s) is negligible in comparison with the high flow modelled (on average 1540 m3/s), so in this study, it means a daily evaporation that is set to 0. The range of light intensity at the surface, used in AQUATOX to recreate the photoperiod, were determined through calibration to match the minimum and maximum values reported in Petrarca et al. (1999) for the range of years between 1994 and 1999. Chemical water quality parameters in the inflow water were assumed for pH (8.14), total soluble phosphorous (0.12 mg/L.), nitrate (3.02 mg/L.) and ammonium (0.29 mg/L). Dissolved oxygen (DO) and carbon dioxide (CO2) were converted from saturation ratios (Park & Clough, 2012) to concentrations (DO = 10 mg/L, CO2 = 0.25 mg/L). The TSS concentration was set equal to 36,5 mg L^{-1} based on measurements in ARPA Emilia Romagna in the section of Pontelagoscuro for the years 2010-2011.

2.4 Ecotoxicological data

The data used at the beginning for this comparison work include 13 species analyzed in the thesis of Laura Grechi (2013) in the Po River for which were researched eco toxicological values in the short term (EC50 and LC50) and in the long term (NOEC) available in the literature or websites for both the LAS and the Triclosan. It has been considered useful to undertake a further literature search to expand, with new ecotoxicological data of other species present in the PO, the data already used in the thesis of Laura Grechi (2013), this is because while the determination of the PEC is type “site- specific”, the determination of the PNEC is “substance-specific”, so more experimental data are collected, the PNEC approximation will be better.

Not all the analyzed species have eco-toxicological data available in the literature or on Web sites, so it was necessary for some of them to try the experimental data of species “similar” to those examined (Read Across procedure) that do not necessarily live in River PO. By “similar” is meant that they have diet, size, functional role in the ecosystem compatible with the species that we wish to study.

At the end we have obtained four PNEC values, 2 for the LAS and two for TCS, using assessment factor and extrapolation from SSD and will be included them as a input data in Aquatox to analyze their effect on the ecosystem in terms of variation of the present species biomass.

2.4.1 PEC/PNEC

In the Appendix tables 1 and 2 are reported the eco-toxicological data for 21 examined species, taken from the laboratory for LAS and Triclosan.

2.4.2 SSD

For the graphics creation where to take the HC5 value, it is suggested to use, for a better result, exclusively long-term eco-toxicological values (NOEC), for this reason values of acute toxicity have been taken (EC50, LC50) used in the first method and converted in NOEC through the following relationships:

$$\frac{EC50}{LC50} = 1.67 \text{ for LAS}$$

$$\frac{EC50}{LC50} = 3.86 \text{ for Triclosan}$$

$\frac{EC50}{NOEC} = 2$

These values were chosen by professional judgment (Marshal, 2013).
The 3th and 4th tables of the appendix show the eco-toxicological data modified for the two substances Las and Triclosan.

The A.1 and A.2 graphs in Appendix have been created and used for taking the HC_5 values.

2.4.3 Aquatox

The use of Aquatox program, provides the creation of several simulation sceneries.
The reference model has been used for the river Po stretch analyzed in Laura Grechi's thesis (2013), especially calibrated with the features of the river and the features of 13 species that have been reported in the table number 5 of the appendix. The simulation is one-year long.

2.5 Scenarios

The four PNEC values taken through the two previous methods for the two examined substances, are used for the creation of 4 sceneries, inserting them in the Aquatox input, simulating a concentration PEC.
To these, two values of LAS and Triclosan realistic concentration have been added in Pontelagoscuro section, calculated in Laura Grechi's thesis (pg105-109; pg.115-119) and other two values, which are referring to the limit concentration for which the Risk Indicator R3 is equal to 1.

Variables upstream	**Constant input concentration from**			
	Scenario 1[a]	Scenario 2[b]	Scenario 3[c]	Scenario 4[d]
LAS (µg/l)	3.22	23	139.26	262.5
Triclosan (µg/l)	0.926	0.16	1.738	0.325

Table 1. Chemical perturbation scenarios for LAS and TCS.

3 Result and Discussion

For each of the three methods a specific risk indicator has been calculated in order to compare the three approaches and make some considerations about their cautiousness.

3.1 Results

3.1.1 PEC/PNEC and Species Sensibility Distribution

Chart A.6 of the appendix shows the PEC values obtained choosing the most sensible eco-toxicological value among the 21 examined species for LAS and TRICLOSAN and dividing it in a proper assessment factor (TGD,2003).

Through statistic extrapolation of the graphics A.1 and A.2 in the appendix, the values of Hc5 for the two substances have been obtained. (David W. Pennington, Eco-toxicology and Environmental Safety, 25, 238-250, 2003).

The chart number 2 shows the obtained results.

For the first two methods, the risk indicators calculation is immediate:

	PEC	PNEC	HC5	R1(PEC/PNEC)	R2 (PEC/HC_5)
LAS (µg/l)	3.22	23	139.26	0.14	0.023
Triclosan (µg/l)	0.000926	0.16	1.738	0.057875	0.0005327

Table.2 Values of Risk Indicator for PEC/PNEC and SSD.

3.1.2 Aquatox

The calculation of the R3 Indicator Risk for the modelling method, required before the creation of scenarios in which the effect of the disturbance by substances LAS and Triclosan in the ecosystem, is evaluated by a parameter known as average biomass variation.

In the charts form A.9 to A.14 of the appendix, for each unsettled created scenario are brought back the biomass variation of each species.

Finally, the calculation of the risk indicator brought the following results:

LAS

Scenario (µg/l)	R3
3,22[a]	0.2734
23[b]	0.2374
139,26[c]	0.4027
262,5[d]	0.9913

TRICLOSAN

Scenario (µg/l)	R3
0,000926[a]	0.3340
0,16[b]	0.4045
1,738[c]	5.9692
0,325[d]	0.9971

Table.3 e 4 Values for Risk Indicator of Aquatox method for LAS and Triclosan

[a] Real Concentration of LAS measured in the section of Pontelagoscuro

[b] PNEC Value reached with the Assessment Factor method

[c] PNEC value obtained with the SSD method

[d] Concentration value where R3 is equal to 1

The procedure of comparison of the different methods has been done evaluating, with the same perturbation investing the ecosystem, the ecological risk prevision given by each indicator. (Guidelines for Ecological Risk Assessment,1998)

In the following charts number 5 and 6, the various obtained risk indicators R1, R2, R3 are resumed and compared for each examined scenario.

LAS

Scenario (µg/l)	R1	R2	R3
3.22	0.14	0.023	0.0122
23	1	0.165	0.0876
139.26	6	1	0.530
262.5	11.4	1.88	1

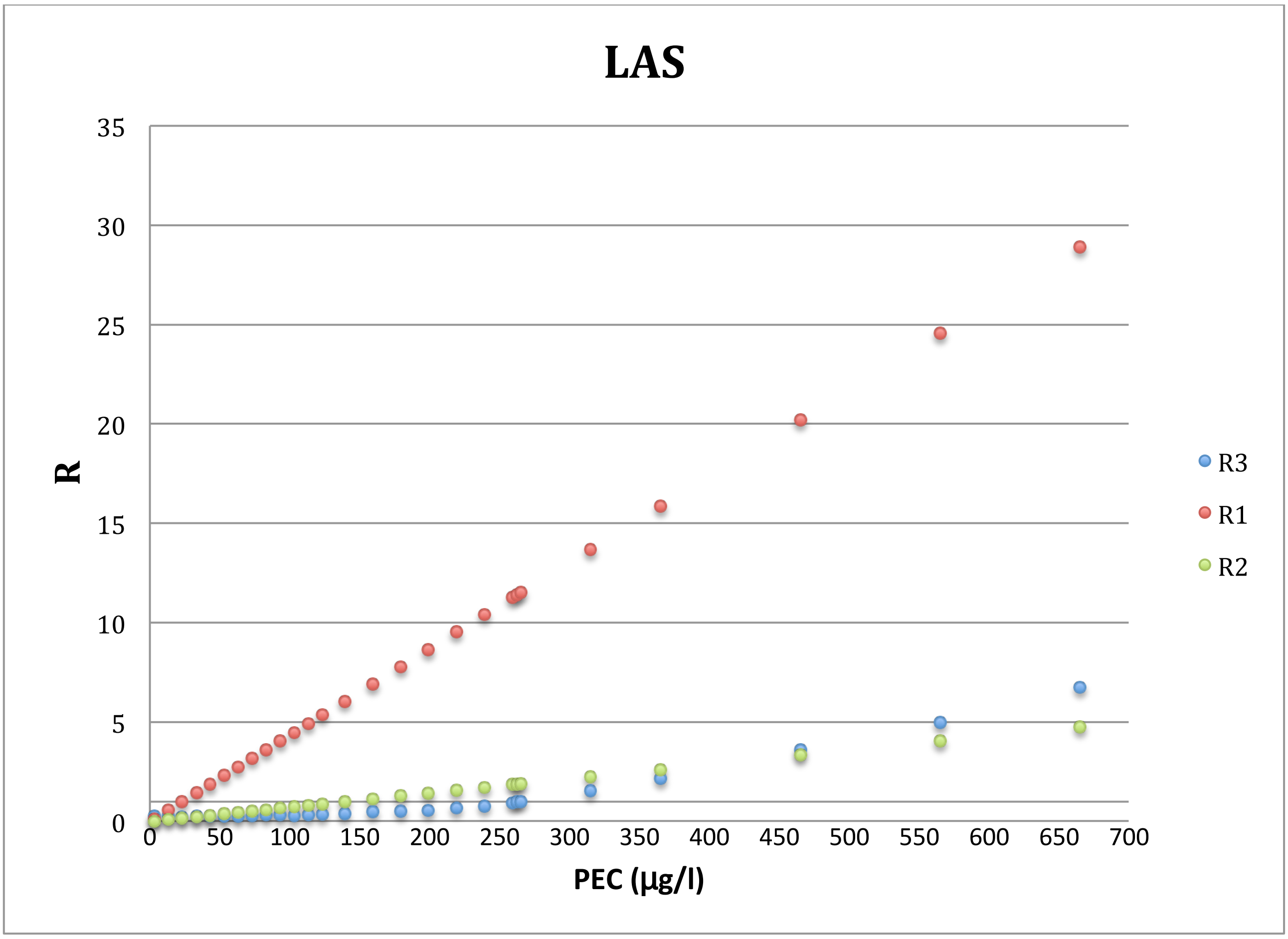
Table. 5 Results of comparison of the three methodologies for LAS

TRICLOSAN

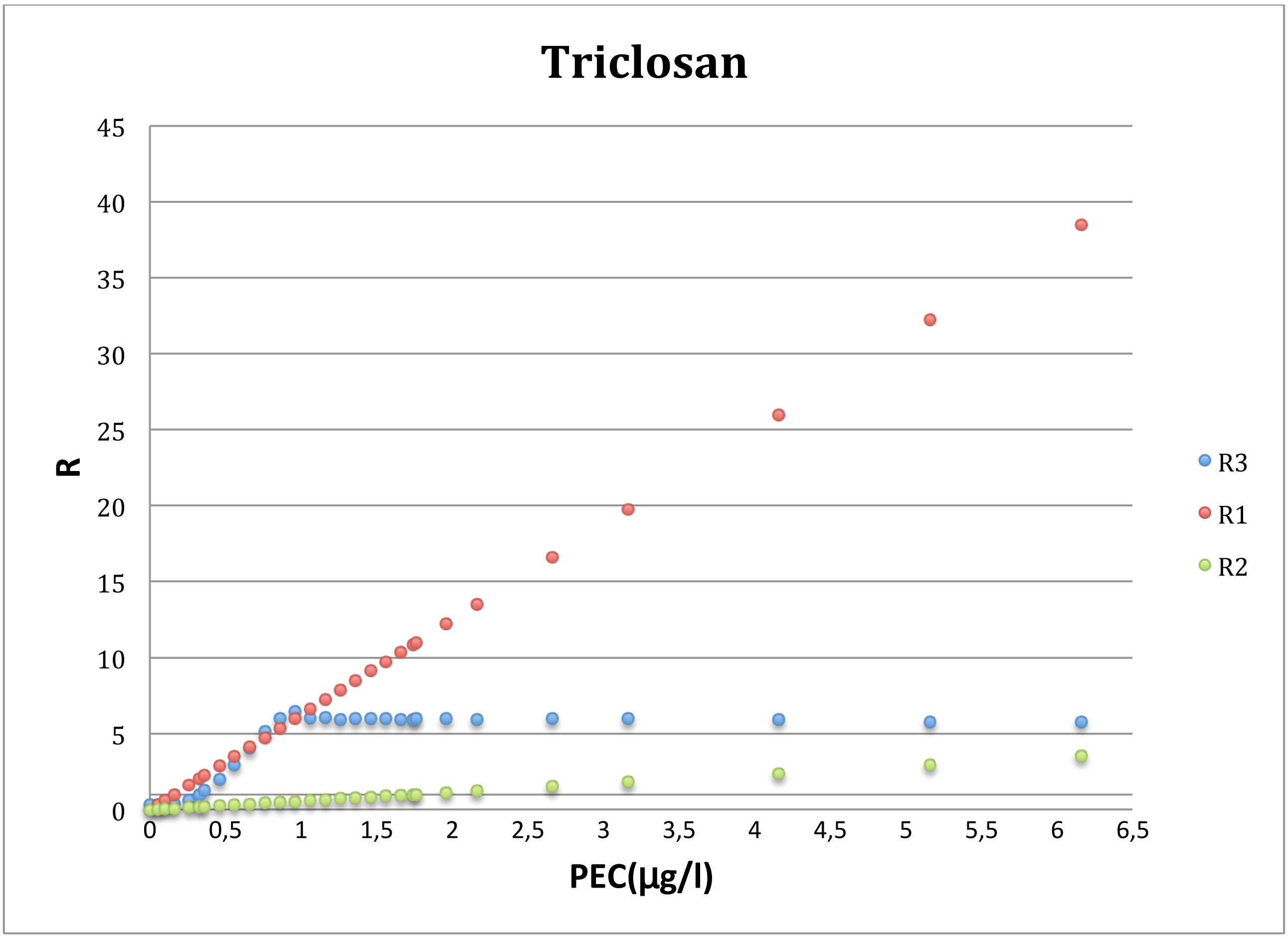
Scenario (µg/l)	R1	R2	R3
0.000926	0.0058	0.00053	$2.84*10^{-3}$
0.16	1	0.092	0.492
1.738	10.86	1	5.347
0.325	2.031	0.1869	1

Table. 6 Results of comparison of the three methodologies for Triclosan

To better understand how the different indicators behave, it has been proper to create some graphics that show the trend of them at the changing of the introduced polluting concentration in the ecosystem.



Graphic 1.
Trend of the risk indicators at the PEC concentration variation of the contaminant LAS



Graphic 2. Trend of the risk indicators at the PEC concentration variation of the contaminant TRICLOSAN

Analyzing the performance of three different indicators from tables 5 and 6 and on the graphics 1 and 2, it can be noticed that:

Taking into consideration the pollutant LAS, it can conclude that the indicator R1 is the most conservative of the three, this is given by the fact that the PEC / PNEC consider that the value ecotoxicological (LC50, EC50) is more sensitive in short term among those present (100% of protectiveness), but also because it is an indicator that doesn't consider of the biotic and abiotic iterations happening in the ecosystem.

Similarly, R2 grows linearly but slower than R1. This is because the SSD method is protective for 95% of the analyzed species and ecotoxicological values of Table 2 in the Appendix have been transformed into long-term values (NOECs), raising the accuracy of the order of magnitude of the PNEC.

More complicated is indicator R3: it describes a modelling approach that considers interactions of body-body and body-environment.

From graphic 1 it can be seen that the trend is not linear but tending to a superlinear curve.
For concentrations below 380-400 (μg/ l), the curve R3 is lower than R2, indicating that the interactions trophic is able to compensate the toxicity of the pollutant, over these, concentrations R3 exceeds R2 (toxicity is no longer compensated by iterations between species and the environment), but both remain under R1. From the mass variations reported in tables A.9,A.10,A.11 of appendix it can also notice how the increase or the decrease of the mass of the individual analyzed species is not proportional to the concentration of the pollutant, precisely because they are a lot of variables that influence as BCF (bio concentration factor), k1 (uptake rate constant) and k2 (desorption rate constant) (Laura Greeks, pg.92, 2013)
In the simulations carried out by analyzing the TCS, it will immediately notice that lower doses of LAS are required to obtain the same mass variations between species.
The indicator R1 remains the most conservative and R2 retains the same linear trend but with less precautionary values due to lower protectiveness of the SSD method, also considering only the direct effects (not considering indirect) of the pollutant species.
Greater attention is given to the performance of the indicator R3, the graphic has a pattern which grows up almost linearly up to a concentration of 0.96 μg / l and then decrease and stabilize at a value of R3 = 5.78 even for high concentrations (5-6 μg / l).
It can be seen that the curve R3 remains above R2 for all the performed simulations, a expected result given by the triclosan (compared to LAS) for its features, it has a high toxicity that leads to a very strong impact on organisms and hardly compensated by trophic interactions and the surrounding environment.
Analyzing in detail the results between 0.96 / 1.26 μg / l, it occurs that the Adult Catfish species at a concentration of 0.96 μg / l begins to decrease, this causes a rise in Bleak and Chub species that are part of its diet, and according to the average of the negative variations, it decreases and also R3 decreases.

3.2 Discussion

It's possible to conclude that for an accurate assessment of the ecological risk of an ecosystem, it is not recommended to base exclusively on one method, but you should make a comparison similar to that carried out in this thesis that allows to assess the direct of the analyzed pollutants species but also develops a simulation model that allows to understand how important can be the interconnections between the different species and the surrounding environment.

In this work, it has been obtained a result already found in the thesis of Laura Greeks (2013), analyzing the actual concentration of LAS and TCS (taken individually examined) in the tract in question, it can be concluded that such concentrations do not pose a risk to the ecosystem Po.

Finally, simulating extreme situations with high concentrations of LAS (500-600 μg / l) and TCS (5-6 μg /l) it can be concluded that the Po river ecosystem is more resistant to LAS Because even at high Concentrations (150 or 180 times the current concentration) no animal is extinct in the short term (1 years) while for TCS phytoplankton becomes extinct at high concentrations.

4 Conclusion

The work performed in this thesis, has permised to compare different methods of risk analysis of an aquatic ecosystem.

The basic concept of these methods is considered to compare a concentration of pollutant present in the environment which is the disruption of the ecosystem with a limit concentration threshold over which, the ecological state is no longer considered acceptable.

Depending on the considered method, it will have a different concentration limit and being compared with the actual disruption, it will provide an index that allows estimation of the ecological status of an ecosystem.

Three approaches were chosen that have given different results and in some ways it reproduced the predictions given by their definitions.

The indicator PEC / PNEC gave, for both the studied pollutants, the proof to be the most conservative, it is for definition 100% protective among the analyzed species.

The SSD method resulted the least precautionary that provides a threshold which is based on protecting 95% of the analyzed species.

Both of these methods have the advantage to be immediate and easy to use, but the disadvantage of analyzing only the direct effects that pollutants have on species, so they use eco toxicological data derived from laboratory experiments in constant conditions.

More complex and including Aquatox is the method that involves the use of a calibrated model for the ecosystem in question.

The estimated risk, based on the variation of biomass (positive or negative) of the species present in the ecosystem of a given disturbance, it is more difficult to understand, because this method considers the interactions that may occur between the species and the surrounding ecosystem. The obtained results allow to conclude that Aquatox although the greater complexity and lots of variables taken into consideration, is not "better" than the other two methods, essentially for two reasons:

- The answers that provides Aquatox are not proportional to the degree of disturbance which affects the ecosystem and this is given by the fact that the variations of biomass are influenced not only by the direct toxicity of the pollutant on the species but especially by such interconnections created between species with the other and with the environment.
- The model provided by Aquatox, although it is calibrated, it doesn’t represent the 100% of the reality both in space and in time.

It can be concluded that the eco toxicologial analysis, covering especially in recent times an increasingly important role for the preservation of our natural heritage terrestrial and marine environment, should be carried out using all the means at our disposal. Specifically, it is useful, as far as possible, to create a method of analysis that involves all three analyzed methods because each one has the possibility to compensate for the deficiencies of the other two, as far as possible, and provides a as precisely as possible estimate

5 Appendix

Organism	Value (μg/l)	Endpoint	Exposure time (h)	Reference/calculation
Elodea canadensis (Macrofite)	4000	NOEC	672	From test on *Elodea canadensis* (ECHA)
Lemna Minor (Macrofite)	3600	EC50	168	From test on *Lemna Minor* (CEPA)
Microcystis Aeruginosa (Cyanobacteria)	910	EC50	96	From test on *Microcystis Aeruginosa* (ECHA)
Desmodesmus subspicatus (Algae)	2400	NOEC	72	From test on *Desmodesmus*

				Subspicatus (ECHA)
Chlorella Kessleri (Algae)	3100	NOEC	360	From test on *Chlorella Kessleri* (ECHA)
Selenastrum capricornutum (Algae)	500	NOEC	96	From test on *Selenastrum capricornutum* (ECHA)
Naididae (aquatic worm)	286	NOEC	1344	From test on *Naididae* (C ECHA)
Mytilus Galloprovincialis (Mytilus)	7850	NOEC	168	From test on *Mytilus Galloprovincialis* (ECHA)
Chub (Fish)	250	NOEC	2160	From test on *Tilapia mossambica* (ECHA)
Bleak (Fish)	3200	NOEC	672	From test *Poecilia reticulata* (ECHA)
Pimephales promelas (Fish)	630	NOEC	4704	From test on *Pimephales promelas* (ECHA)
Salmo Gairdneri (Fish)	230	NOEC	1728	From test on *Salmo Gairdneri* (ECHA)
Hyalella Azteca (Crustacean)	7600	LC50	192	From test on *Hyalella Azteca* (ECHA)
Limnodrilus hoffmeisteri (Aquatic worm)	1800	LC50	96	From test on *Limnodrilus Hoffmeisteri* (ECHA)
Daphnia magna (Plancton)	2900	EC50	48	From test on *Daphnia Magna* (ECHA)
Paratanytarsus parthenogenica (Insect)	4000	LOEC	672	From test on *Paratanytarsus Parthenogenica* (ECHA)
Ceriodaphnia sp. (Plancton)	500	NOEC	168	From test on *Ceriodaphnia sp.* (ECHA)
Brachionus calyciflorus (Pelagic Invertebrate)	2000	EC50	48	From test on *Brachionus calyciflorus* (ECHA)
Wels catfish (young)	1670	LC50	672	From test on *Lepomis* (ECHA)
Wels catfish (adult)	1670	NOEC	1728	From test on *Lepomis* (ECHA)

Chironomus Riparius (Crustacean)	6500	LC50	96	From test on *Chironomus Riparius* (ECHA)

Table A.1. Ecotoxicological values for LAS used for organisms in Po river model.

Organism	Value (µg/l)	Endpoint	Exposure time (h)	Reference/calculation
D. Tertiolecta(Plancton)	3,5	EC50	96	From test on *D. Tertiolecta* (CEPA)
A. Flos-aquae (Alga)	1,6	EC50	96	From test on A*. Flos-aquae* (CEPA)
Daphnia (Plancton)	200	NOEC	48	From test on *Daphnia* (CEPA)
Raimbow Trout (fish)	34,1	NOEC	48	From test on *Raimbow Trout* (CEPA)
Ceriodaphnia(Plancton)	240	EC50	48	From test on *Ceriodaphnia* (CEPA)
Grass Shrimp (Crustacean)	452	LC50	96	From test on Grass Shrimp (CEPA)
Trichoptera (Benthic insect)	860	LC50	48	From test on *Chironomus riparius* (CEPA)
Cyclotella (Phytoplankton)	1,61	EC50	72	From test on *Selenastrum* (CEPA)
Brachionus (Pelagic Invertebrate)	1544	EC50	48	From test on *Brachionus calyciflorus* (CEPA)
Amphipoda (Crustacean)	200	LC50	240	From test on *Hyalella azteca* (CEPA)
Chironomus (Benthic insect)	400	LC50	96	From test on *Chironomus riparius* (CEPA)
Oligochaeta (Aquatic worm)	1260	LC50	48	From test on *Corbicula* (CEPA)

Gastropoda (Benthic invertebrate)	135	EC50	48	From test on *Corbicula* (CEPA)
Odonata (Benthic insect)	400	LC50	240	From test on *Corbicula* (CEPA)
Bleak (Fish)	260	LC50	96	From test on *Oreochromis niloticus* (CEPA)
Chub (Fish)	260	LC50	96	From test on *Pimephales promelas* (CEPA)
Wels catfish (Young fish)	370	LC50	96	From test on *Lepomis macrochirus* (CEPA)
Wels catfish (Adult fish)	370	LC50	96	From test on *Oncorhynchus mykiss* (CEPA)
Selenastrum capricornutum(Microalga)	2,23	EC50	96	From test on *Selenastrum capricornutum* (CEPA)
L. gibba (Macrofite	10	NOEC	168	From test on *L. gibba* (CEPA)
Chromulina (Phytoplankton)	910	EC50	48	From test on *Chromulina* (CEPA)

Table A.2 Ecotoxicological values for TCS used for organisms in Po river model.

Organism	**NOEC (μg/l)**
D. Tertiolecta(Plancton)	1,75
A. Flos-aquae (Alga)	0,8
Daphnia (Plancton)	200
Raimbow Trout (fish)	34,1
Ceriodaphnia(Plancton)	120
Grass Shrimp (Crustacean)	58,5
Lepomis macrochirus(fish)	47,9
Trichoptera (Benthic insect)	60,8
Cyclotella (Phytoplankton)	0,8
Brachionus (Pelagic Invertebrate)	77,2
Amphipoda (Crustacean)	25,9
Chironomus (Benthic insect)	51,8
Oligochaeta (Aquatic worm)	163,2
Gastropoda (Benthic invertebrate)	67,5
Odonata (Benthic insect)	51,8

Bleak (Fish)	33,6
Chub (Fish)	33,6
Wels catfish (young)	47,9
Wels catfish (adult)	47,9
Selenastrum capricornutum(Microalga)	2,23
L. gibba (Macrofite)	10
Chromulina (Phytoplankton)	455

Table A.3. NOECs values for Triclosan

Organism	**NOEC (μg/l)**
Elodea Canadensis (Macrofite)	4000
Lemna Minor (Macrofite)	1800
Microcystis Aeruginosa (Cyanobacteria)	455
Desmodesmus Subspicatus (Algae)	2400
Chlorella Kessleri (Alga)	3100
Selenastrum Capricornutum (Algae)	500
Naididae (aquatic worm)	268
Mytilus galloprovincialis (Mytilus)	7850
Lepomis Macrochirus(Fish)	216
Tilapia Mossambica (Fish)	250
Poecilia Reticulata(Fish)	3200
Pimephales Promelas(Fish)	630
Salmo Gairdneri (Fish)	230
Hyalella Azteca (Crustacean)	984
Limnodrilus Hoffmeisteri (Aquatic worm)	233
Daphnia Magna (Plancton)	1450
Paratanytarsus Parthenogenica (Insect)	2000
Ceriodaphnia sp. (Plancton)	500
Brachionus Calyciflorus (Pelagic Invertebrate)	1000
Wels Catfish (young)	216
Wels Catfish (adult)	230
Chironomus Riparius (Crustacean)	841

Table A.4. NOECs values for LAS

Taxonomic Group	Organism
Phytoplankton	Cyclotella (Diatom) Chromulina(Chrysofyte)
Zooplankton	Rotifer Brachionus
Macroinvertebrates	Amphipoda Chironomids Oligochaeta Trichoptera Gastropoda Odonata
Fishes	Bleak Chub Young Wels Catfish Adult Wels Catfish

Table A.5. Species simulated in Aquatox model.

Chemical Compounds	PNEC (μ g/l)	Assessment Factor
LAS	23	10
Triclosan	0,16	10

Table A.6 PNEC values for Assessment Factor method

Chemical Compounds	HC_5 (μ g/l)
LAS	139.26
Triclosan	1.738

Table A.7. HC_5 values for Species Sensibility Distribution method

Variables from upstream	Constant input concentration			
	Scenario 1[a]	Scenario 2[b]	Scenario 3[c]	Scenario 4[d]
LAS (µg/l)	3.22	23	139.26	262,5
Triclosan (µg/l)	0.926	0.16	1.738	0,325

Table A.8. Chemical perturbation scenarios for LAS and TCS.

[a] Actual concentration in Pontelagoscuro section

[b] PNEC value obtained with Assessment Factor

[c] PNEC value obtained with Species Sensibility Distribution

[d] Limit value of concentration for R3 egual 1

1nd Scenario for LAS			
Organism	**Avarage biomass in control simulation (g/m2 dry)**	**Avarage biomass in Perturbed simulation (g/m2 dry)**	**Avarage Biomass variation (%)**
Chub	2,5211	2,5210	-0,0040
Siluro adult	5,5804	5,5741	-0,1129
Bleak	0,5600	0,5600	0,0000
Odonata	0,0660	0,0660	0,0000
Gastropoda	0,0015	0,0016	6,6667
Siluto young	5,7326	5,6971	-0,6193
Tricoptera	0,0470	0,0470	0,0000
Amphidona	0,0038	0,0039	2,6316
Chriso	0,0420	0,0420	0,0000
Chironomus	0,1500	0,1500	0,0000
Oligochaete	0,1532	0,1500	-2,0888
Rotifera	0,0470	0,0470	0,0000
Cyclotella	1,0418	1,0000	-4,0123

Table A.9. Results of 1st simulation of 1 year with Aquatox for LAS. The negative biomass variation are highlighted with red color.

2nd Scenario for LAS			
Organism	**Avarage biomass in control simulation (g/m2 dry)**	**Avarage biomass in Perturbed simulation (g/m2 dry)**	**Avarage Biomass variation (%)**
Chub	2,5211	2,5210	-0,0035
Siluro adult	5,5804	5,5740	-0,1147
Bleak	0,5600	0,5600	0,0000
Odonata	0,0660	0,0660	0,0000
Gastropoda	0,0015	0,0016	6,6667
Siluto young	5,7326	5,6972	-0,6175
Tricoptera	0,0470	0,0470	0,0000
Amphidona	0,0038	0,0039	2,6316
Chriso	0,0420	0,0420	0,0000
Chironomus	0,1500	0,1500	0,0000
Oligochaete	0,1532	0,1544	0,7833
Rotifera	0,0470	0,0470	0,0000
Cyclotella	1,0418	1,0000	-4,1230

Table A.10. Results of 2nd simulation of 1 year with Aquatox. The negative biomass variation are highlighted with red color.

3nd Scenario for LAS			
Organism	**Avarage biomass in control simulation (g/m2 dry)**	**Avarage biomass in Perturbed simulation (g/m2 dry)**	**Avarage Biomass variation (%)**
Chub	2,5211	2,5197	-0,0555
Siluro adult	5,5804	5,5507	-0,5322
Bleak	0,5600	0,5600	0,0000
Odonata	0,0660	0,0660	0,0000
Gastropoda	0,0015	0,0016	6,6667
Siluto young	5,7326	5,6787	-0,9402
Tricoptera	0,0470	0,0470	0,0000
Amphidona	0,0038	0,0039	2,6316
Chriso	0,0420	0,0420	0,0000
Chironomus	0,1500	0,1500	0,0000
Oligochaete	0,1532	0,1500	-2,0888
Rotifera	0,0470	0,0470	0,0000
Cyclotella	1,0418	0,9746	-6,4502

Table A.11. Results of 3th simulation of 1 year with Aquatox for LAS. The negative biomass variation are highlighted with red color.

1nd Scenario for Triclosan			
Organism	**Avarage biomass in control simulation (g/m2 dry)**	**Avarage biomass in Perturbed simulation (g/m2 dry)**	**Avarage Biomass variation (%)**
Chub	2,5211	2,5211	-0,0000
Siluro adult	5,5804	5,5749	-0,0986
Bleak	0,5600	0,5600	0,0000
Odonata	0,0660	0,0660	0,0000
Gastropoda	0,0015	0,0016	6,6667
Siluto young	5,7326	5,7000	-0,5687
Tricoptera	0,0470	0,0470	0,0000
Amphidona	0,0038	0,0039	2,6316
Chriso	0,0420	0,0420	0,0000
Chironomus	0,1500	0,1500	0,0000
Oligochaete	0,1532	0,1500	-2,0888
Rotifera	0,0470	0,0470	0,0000
Cyclotella	1,0418	1,0022	-3,9259

Table A.12. Results of 1st simulation of 1 year with Aquatox for Triclosan. The negative biomass variation are highlighted with red color.

2nd Scenario for Triclosan			
Organism	**Avarage biomass in control simulation (g/m2 dry)**	**Avarage biomass in Perturbed simulation (g/m2 dry)**	**Avarage Biomass variation (%)**
Chub	2,5211	2,5210	-0,0040
Siluro adult	5,5804	5,5740	-0,1147
Bleak	0,5600	0,5600	0,0000
Odonata	0,0660	0,0660	0,0000
Gastropoda	0,0015	0,0016	6,6667
Siluto young	5,7326	5,6949	-0,6576
Tricoptera	0,0470	0,0470	0,0000
Amphidona	0,0038	0,0039	2,6316
Chriso	0,0420	0,0410	-2,3810
Chironomus	0,1500	0,1500	0,0000
Oligochaete	0,1532	0,1500	-2,0888
Rotifera	0,0470	0,0470	0,0000
Cyclotella	1,0418	0,9700	-6,8919

Table A.13. Results of 2nd simulation of 1 year with Aquatox for Triclosan. The negative biomass variation are highlighted with red color.

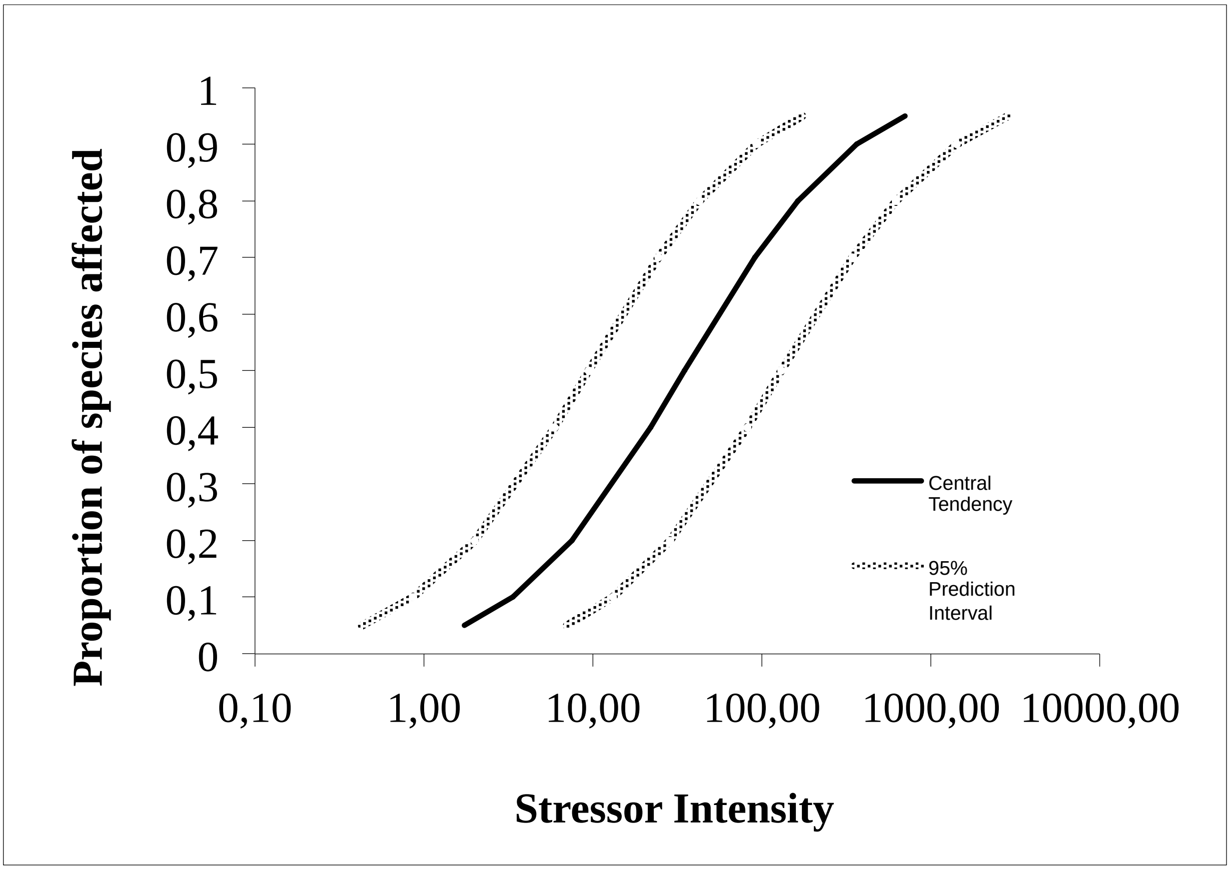
3nd Scenario for Tricosan			
Organism	**Avarage biomass in control simulation (g/m2 dry)**	**Avarage biomass in Perturbed simulation (g/m2 dry)**	**Avarage Biomass variation (%)**
Chub	2,5211	2,5204	-0,0278
Siluro adult	5,5804	5,4814	-1,7741
Bleak	0,5600	0,4198	-25,0357
Odonata	0,0660	0,0680	3,0303
Gastropoda	0,0015	0,0013	-13,3333
Siluto young	5,7326	5,1163	-10,5263
Tricoptera	0,0470	0,0370	-21,2766
Amphidona	0,0038	0,00340	-10,5263
Chriso	0,0420	0,00510	-87,8571
Chironomus	0,1500	0,14980	0,0000
Oligochaete	0,1532	0,16000	4,4386
Rotifera	0,0470	0,03300	-29,7872
Cyclotella	1,0418	0,02000	-98,0802

Table A.14. Results of 3th simulation of 1 year with Aquatox for Triclosan. The negative biomass variation are highlighted with red color.

	PEC	**PNEC**	**HC_5**	**R1(PEC/PNEC)**	**R2 (PEC/HC_5)**
LAS (µg/l)	3.22	23	139.26	0.14	0.023
Triclosan (µg/l)	0.000926	0.16	1.738	5.7875	0.5252

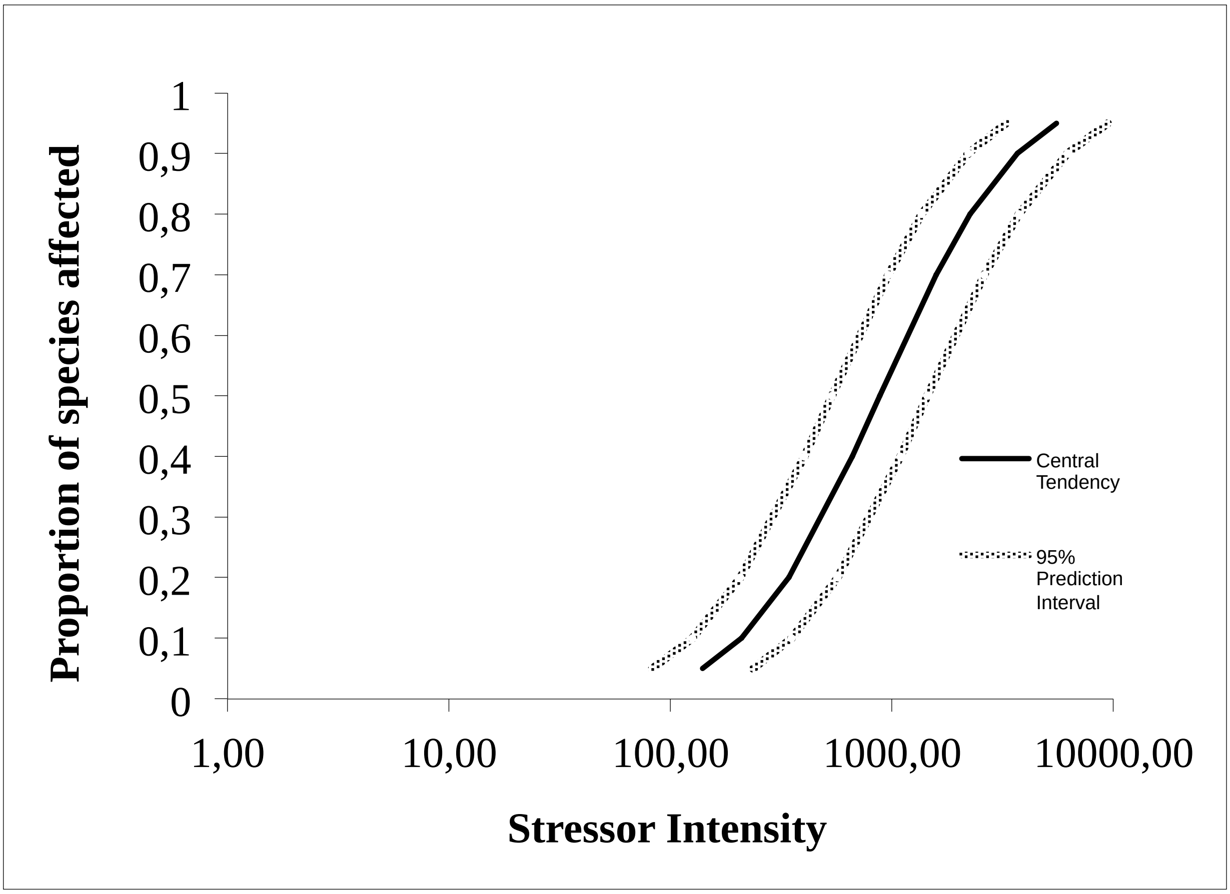
Table A.15. Values of Risk Indicator for PEC/PNEC and SSD method

Proportion	Central Tendency
0,05	1,738
0,1	3,373
0,2	7,527
0,4	22,020
0,5	34,961
0,7	91,021
0,8	162,376
0,9	362,362
0,95	703,137



Graph A.1. Results of SSD Generator program for LAS

Proportion	Central Tendency
0,05	139,262
0,1	209,159
0,2	342,277
0,4	661,313
0,5	878,190
0,7	1579,650
0,8	2253,196
0,9	3687,234
0,95	5537,891



Graph A.2. Results of SSD Generator program for Triclosan

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Programs

Aquatox (US-EPA)

SSD Generator Program