

# **Valutazione del rischio chimico**

CdL Magistrale Interateneo in  
Analisi e Gestione dell'Ambiente  
Università di Udine e Università di Trieste

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# Tossicologia umana

## Dagli studi di laboratorio alla definizioni di massimo rischio accettabile (MPR) o dosi giornaliere accettabili (ADI/TDI/RfD) o esposizione

Table 5.11. Estimated conversion factors between an LD50, the NOEL of a subchronic or chronic study and the acceptable daily intake (ADI)

LD50	NOEL subchronic	NOEL chronic	ADI
100,000 - 5,000,000	300 - 1000	100	1

### Box 6.4. Maximum Permissible Risk level

RAoC, 2007

Van Leeuwen, Vermeire

Examples of oral MPRs for non-carcinogenic substances are the ADI (acceptable daily intake, for substances deliberately added to food items) and TDI (tolerable daily intake, for substances unintentionally present in food items), both are expressed in mg/kg bw/day and defined as the daily intake of a chemical which, during the entire lifetime, appears to be without appreciable risk on the basis of all known facts at the time. The RfD (Reference Dose) is similar to the ADI/TDI, but is more strictly defined. Inhalation MPRs are defined in a similar way and expressed as concentrations in air. An example of another health-based limit value is the AOEL (acceptable operator exposure level): the level that has no harmful effects on the health of operators (people working with the substance).

MPRs for carcinogenic substances are usually defined as the daily dose, taken during the entire lifetime that will cause 1:10<sup>4</sup>, 1:10<sup>5</sup> or 1:10<sup>6</sup> additional cancer cases during the entire lifetime.

+ tossicità di miscele («gestibile» per azione congiunta semplice)

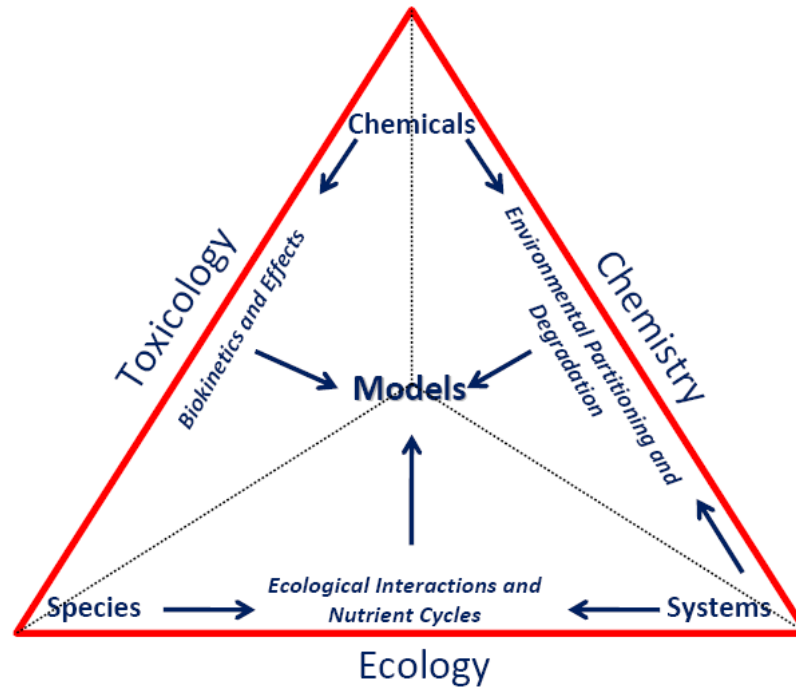
## **Tossicologia ambientale**

Identificazione e quantificazione dei *danni sui sistemi biologici (diversi dalla specie umana)* a diverso livello di organizzazione, prodotto dall'esposizione ai contaminanti ambientali

## **Ecotossicologia**

Studio del destino e degli effetti dei contaminanti nell'ambiente

# Ecotossicologia: scienza delle tre S



Redrawn from Figure 7.1 of van Leeuwen and Vermeire (2007)

Table 6.1. “Disciplines” of ecotoxicology and some of their research topics

Chemistry	Toxicology	Ecology	Mathematics
Exposure assessment	effects assessment	community structure	environmental fate models
Transport	modes of toxic action	community functions	pharmacokinetic models
Partitioning	bioaccumulation	population dynamics	LC50 and NOEC statistics
Transformation	biotransformation	nutrient/energy cycling	species-species extrapolation
SARs/QSARs	extrapolation	various interactions	population and ecosystem models

## **Differences between HRA and ERA**

- 1)• Taxonomic diversity
- 2)• Toxicological endpoints
- 3)• Spatial scales
- 4)• Temporal scales
- 5)• Complexity of exposure

Table 6.2. Numbers of classified species of some large taxonomic groups of the plant and animal kingdom [6]

Regnum vegetabile		Regnum animalia	
Algae	20,000	Protozoa	46,000
Lichens	20,000	Porifera	5,000
Fungi	100,000	Coelenterata	10,000
Bryophyta	23,000	Plathyhelminthes	12,000
Pterydophyta	11,000	Nematoda	10,000
Spermatophyta	250,000	Mollusca	120,000
		Annelida	8,000
		Arachnida	30,000
		Crustacea	35,000
		Insecta	750,000
		Diplopoda	7,200
		Echinodermata	5,000
		Chordata	45,000



Biodiversity (macrofauna) in soil

## 1) DIVERSITA' TASSONOMICA

## 2) Endpoints tossicologici

Sono le **risposte avverse che vengono misurate**, sono i criteri per valutare gli effetti.

Cambiano con il livello di organizzazione biologica considerato (***marker biochimici, attività enzimatiche - tassi di sopravvivenza, crescita, riproduzione - produzione primaria, cambiamenti nella struttura e nelle funzioni nella comunità biologica considerata***)

### Criteria for Selecting Ecological Endpoints

- Societal relevance
- Biological relevance
- Unambiguous operational definition
- Accessibility to prediction and measurement
- Susceptibility to the hazardous agent

*(Produzione primaria: produzione di composti organici dalla CO<sub>2</sub> presente nell'atmosfera o in acqua che avviene principalmente mediante processi fotosintetici o, in misura minore, chemosintetici)*

## ***Effetti inaccettabili***

**Riduzione nella sopravvivenza inaccettabile**

**Riduzione nella crescita inaccettabile**

**Riduzione nella riproduzione inaccettabile**

**Livello di *avoidance* inaccettabile**

**Percentuale di **deformità o tumori visibili** inaccettabile**

**Concentrazione inaccettabile di **residui tossici nei tessuti (edibili)****

**Odore/sapore inaccettabile nei tessuti (edibili)**



## Selecting Ecotoxicological Endpoints in Practice

“Which species and functions of ecosystems are to be protected, and at what levels?” are largely political questions.

### To what extent should ecosystems be protected?

- In ERA, clear choices for the protection of species, ecosystems, ecosystem functions or processes are normally not made.
- Many ecological effects assessments have an undefined or vaguely defined goal (Suter, 1993).



### Moria di api a Udine, il gip sequestra i terreni: "Non piantate mais conciato"

5 marzo 2019 Guido Minciotti Senza categoria



AGGIORNAMENTO DEL 25 APRILE 2019 - LA PROCURA DI UDINE RICORRE IN CASSAZIONE

*Meno di un arnia*



Dominella Trunfio **INFORMARSI ANIMALI** 03-05-2019

### Strage di api in Veneto: morte in 10mila per colpa dei pesticidi



Diecimila api sono morte a Musile, tra i fiumi veneti Piave e Sile. Gli insetti sono stati trovati sulla riva e per gli apicoltori, la colpa sarebbe dell'uso sconsiderato di diserbanti.

ilfattoquotidiano.it/2020/01/26/api-fitofarmaci-per-concia-del-mais-causano-danni-anche-allambiente-lir

f Flowers www.labsolutions.c... Posta in arrivo (19.7... 3 Empire Posta in arrivo (16.4...

SOSTIENICI

il Fatto Quotidiano.it

### Api, "fitofarmaci per concia del mais cau danni anche all'ambiente". L'indagine de procura di Udine si allarga

RSITA LESANO SI FERMA

te per i Online

BITO!



## 4) Scale temporali

### Generation Times for Some Taxa

- Bacteria ~0.1 day
- Green algae ~1 day
- Waterfleas ~10 days ← crostacei branchiopodi. Costituiscono una componente principale del plancton d'acqua dolce
- Snails ~100 days
- Rats ~1 year
- Politicians ~4 years
- Humans ~25 years

## **5) Complessità dell'esposizione nel ERA**

- Niche-partitioning
- Abiotic factors
- Surface Area/Volume
- Life-history
- Behaviour
- Exposure time
- Non-linearity
- Consumption patterns
- Feeding and growth rates
- Biotransformation

# Complessità dell'esposizione

Table 6.8. The relationship between surface area and volume of species.  
For the sake of simplicity, the shape of species is taken to be cubic

Edge (mm)	Surface area (mm <sup>2</sup> )	Volume (mm <sup>3</sup> )	Surface/Volume ratio	Examples
0.001	6x10 <sup>-6</sup>	10 <sup>-9</sup>	6000	cells/bacteria
0.01	6x10 <sup>-4</sup>	10 <sup>-6</sup>	600	algae ( <i>Chlorella</i> sp.) and fungi ( <i>Penicillium</i> sp.)
0.1	6x10 <sup>-2</sup>	10 <sup>-3</sup>	60	protozoans ( <i>Paramecium</i> sp.)
1	6	1	6	nematodes and crustaceans (e.g. <i>Ceriodaphnia dubia</i> )
10	6x10 <sup>2</sup>	10 <sup>3</sup>	0.6	earthworms/small fish (e.g. guppy)
100	6x10 <sup>4</sup>	10 <sup>6</sup>	0.06	rainbow trout/pigeon
1000	6x10 <sup>6</sup>	10 <sup>9</sup>	0.006	sharks/cows

# Complessità dell'esposizione

Fase del ciclo vitale in cui avviene l'esposizione

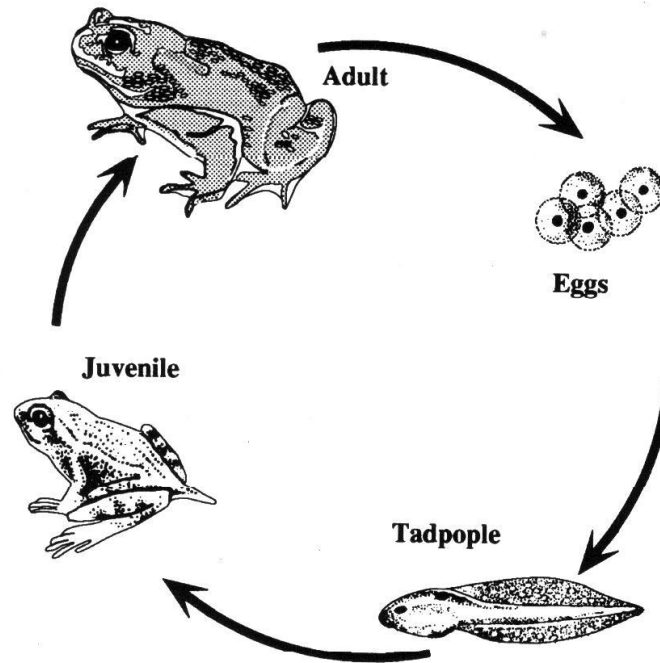


Figure 6.8. Life cycles of an insect and amphibian species with concomitant changes in exposure patterns.

# Complessità dell'esposizione - *consumption pattern*

Table 6.9. Fish consumption patterns and daily intakes of hexachlorobenzene (HCB) in The Netherlands (NL), Japan and in the cormorant (*Phalacrocorax carbo*)

	NL	Japan	Cormorant	
			male	female
Body weight (kg)	70	70	2	3
Fish consumption (kg <sub>wwt</sub> /d)	0.01	0.1	0.5	0.5
Fish consumption (70 kg <sub>bw</sub> ) <sup>a</sup>	0.01	0.1	17.5	11.6
Intake of HCB <sup>b</sup> (μg/kg <sub>bw</sub> ·d)	0.03	0.3	50	33.3

<sup>a</sup> Fish consumption expressed in terms of the body weight of man (70 kg).

<sup>b</sup> The Swedish product standard for HCB in fish (200 μg/kg fish) was used for the calculations

$$0,01 \cdot 200 / 70 = 0,028571 \text{ ug/kgbw d}$$

$$0,5 \cdot 200 / 2 = 50 \text{ ug/kgbw d}$$

Inadeguatezza dell'approccio  
"sanitario"

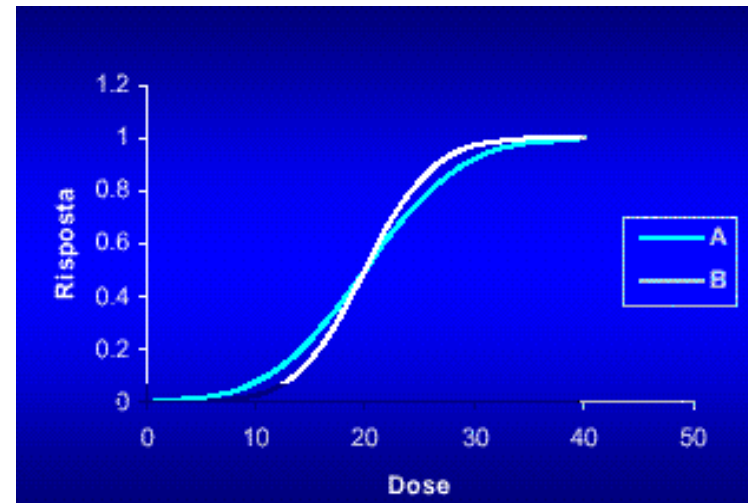
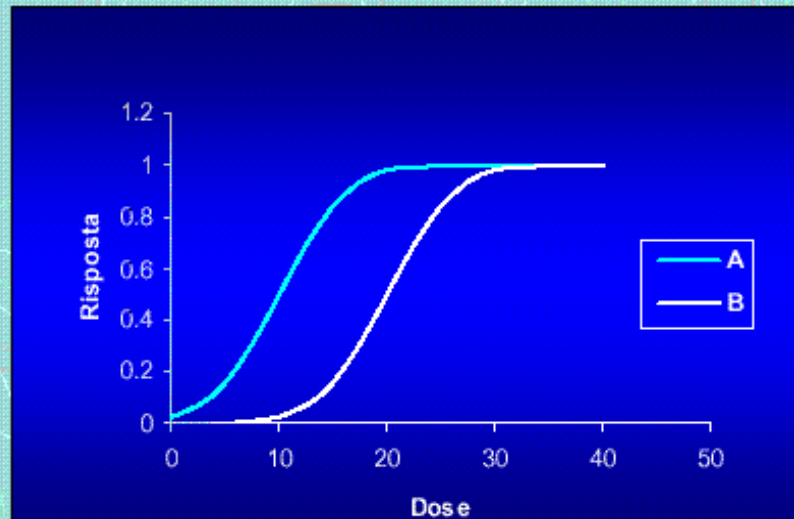


Figure 6.7. A food specialist: the cormorant (*Phalacrocorax carbo*). From A. Belfroid, Utrecht, The Netherlands. With permission.

# FOCALIZZIAMO L'ATTENZIONE SULLE RISPOSTE DI ORGANISMI DIVERSI ALLO STESSO INQUINANTE

A e B organismi (specie) testati diversi

## Confronto tra curve dose-risposta

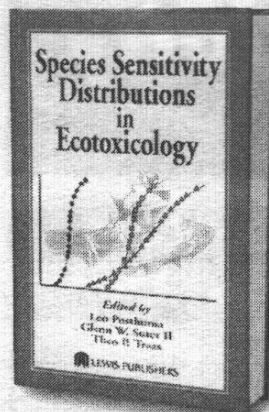




# TOSSICITÀ SELETTIVA

- Risposte multiple allo stesso composto tossico principalmente dovute a
  - Differenze nella superficie di esposizione
  - Differenze nell'accumulo
  - Tassi di trasformazione ("biotrasformazione")
  - Differenze nei percorsi biochimici

*Si possono definire **distribuzioni di sensibilità delle specie** (organismi su cui sono effettuati i test tossicologici)*



## **SPECIES SENSITIVITY DISTRIBUTIONS IN ECOTOXICOLOGY**

*Edited by*

**Leo Posthuma and Theo P. Traas**

*RIVM-Centre for Substances and Risks, The Netherlands*

**Glenn W. Suter II**

*U.S. Environmental Protection Agency, Cincinnati, Ohio, USA*

This book provides a critical review of the conceptual basis, strengths and weaknesses of using Species Sensitivity Distributions (SSDs) to model the effects of contaminants on ecosystems. The book covers when and how to use SSDs, and includes alternative assumptions, data treatments, computational methods, and available resources. It explains the basis for the SSD-based criteria standards, making it possible to correctly apply and defend them.

# Valutazione del Rischio

## Approccio Deterministico

Toxicity  

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Exposure  

---

TER  

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# Valutazioni sulla tossicità

- **A livello di Specie**
  - Laboratory toxicity experiments
  - Greenhouse studies
  - Field studies
- **A livello di Ecosistema**
  - Most sensitive species
  - Mesocosm studies
  - Species Sensitivity Distribution

# Species Level Assessment:

## NOEC (aka NOAEL) and EC<sub>x</sub>

- **LOEC** = lowest tested conc at which a statistically significant adverse effect is observed
- **NOEC** = highest tested conc < LOEC
  - LOEC, NOEC depend on experimental design & statistical test
- **EC<sub>x</sub>** = conc producing x% effect
  - EC<sub>x</sub> depends on experimental design and model and choice of x

# Ecosystem level assessment

## Current Method



- Determine the NOEC (or EC50) for each species representing an ecosystem
- Find the smallest NOEC (or EC50)
- Divide it by 10, 100, or 1000 (uncertainty factor)
- Regulate from this value
  - or argue against it

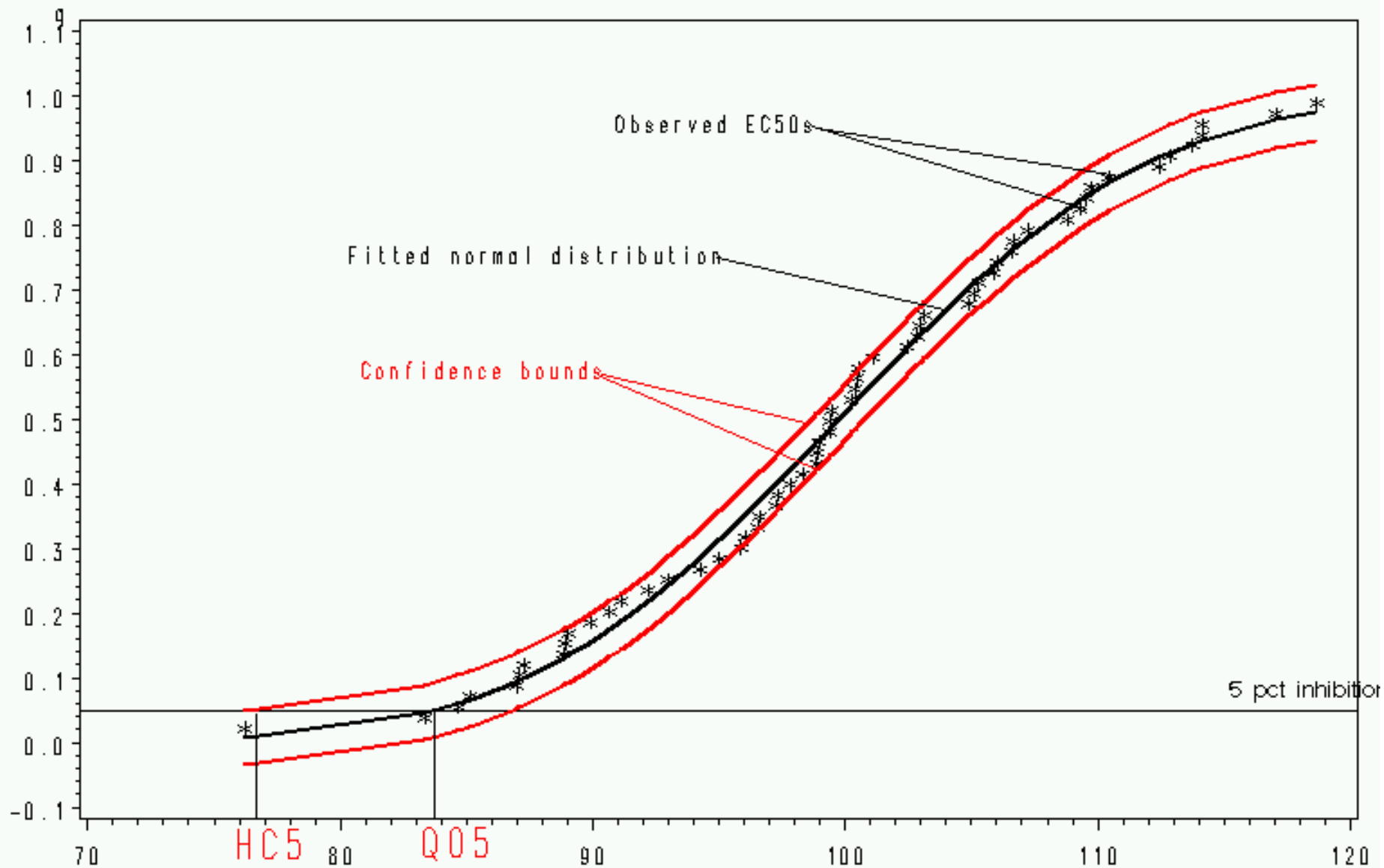
# Ecosystem level assessment

## Probabilistic Approach



- Collect a consistent measure of toxicity from **a representative set of species**
  - EC50s *or* NOECs (not both)
- Fit a distribution (**SSD**) to these numerical measures
- Estimate concentration, HC5, that protects 95% of species in ecosystem
- Advantages and problems with SSDs

# Normal Distribution

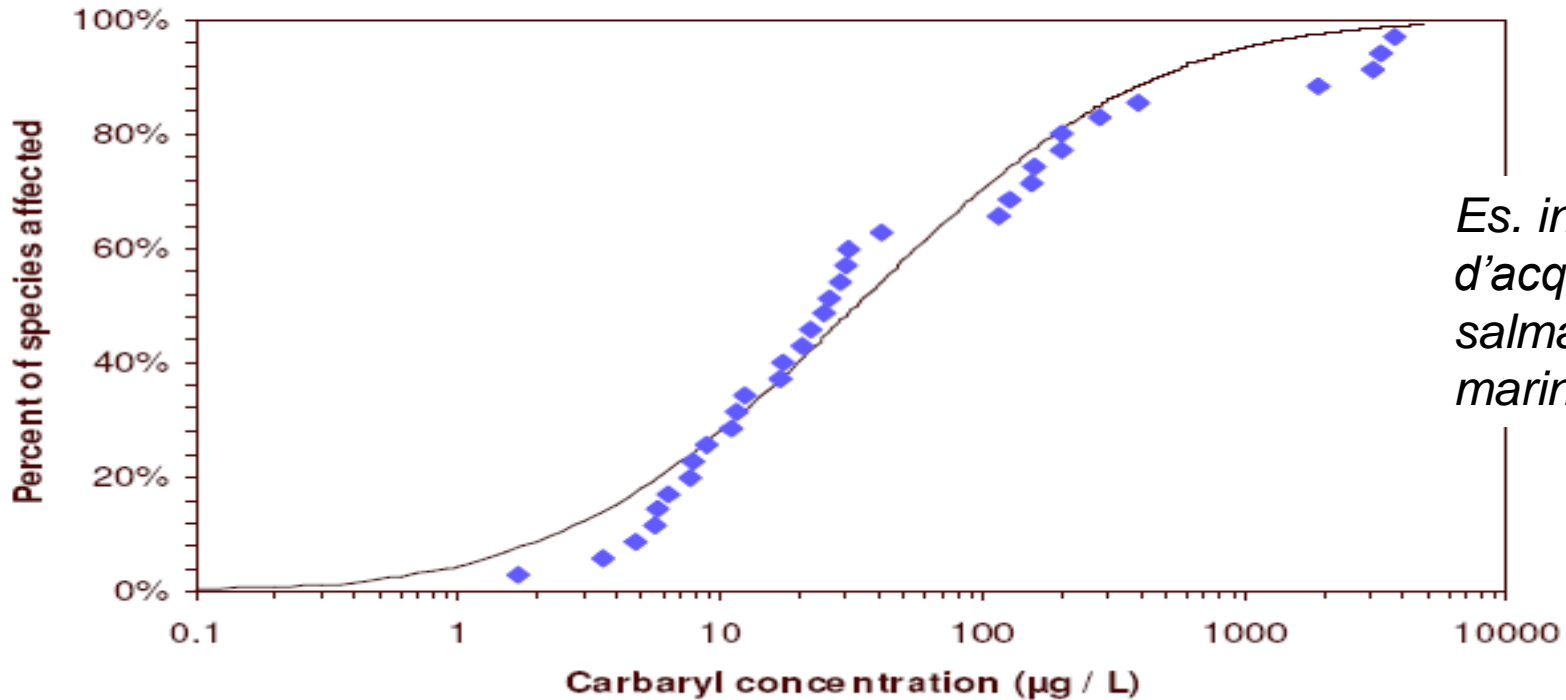


HC5 lower 5th percentile of the estimated hazardous concentration,

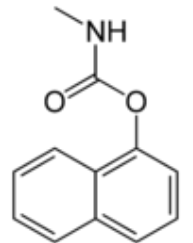


# Selection of Toxicity Data

Acute LC<sub>50</sub> values of Carbaryl for 34 aquatic invertebrate species. The fitted log-normal SSD has a mean of 3.497 and a standard deviation of 2.063.



*Es. invertebrati  
d'acqua dolce,  
salmastra e  
marina*



*insetticida*

## SSD by Habitat

Visual groupings are not taxonomic classes but defined by habitat, possibly related to mode of action

# How Many Species?

- **Newman's method: 40 to 60 species**
  - Snowball's chance...
  - Might reduce this by good choice of groups to model
- **Aldenberg-Jaworski: 1 species will do**
  - If you make enough assumptions,...
- **8 is usual target**
- **5 is common**
- **20-25 in some non-target plant studies**

# Which Distribution to Fit?

- **Normal, log-normal, log-logistic, Burr III...?**
  - **With 5-8 data points, selecting the “right” distribution is a challenge**
- **Does it matter?**
  - **Recent simulation study suggests **yes****
    - **Various distributions fit**
  - **Actual laboratory data suggests **yes****

# Which Laboratory Species?

One EUFRAM case study fits an SSD to the following

Species	Toxicity ( $\mu\text{g/l}$ )	Test
<i>Selenastrum capricornutum</i>	43	72h EC50
<i>Navicula pelliculosa</i>	60	120h EC50
<i>Skeletonema costatum</i>	69	48-120h EC50
<i>Myriophyllum heterophyllum</i>	132	14d EC50
<i>Lemna gibba</i>	180	7d EC50
<i>Anabaena flos-aquae</i>	342	72-120h EC50

Alga

Alga

Alga

Pianta acquatica

Pianta acquatica

Batterio/plankton

**Aquatic toxicologists can comment (and have) on whether these values belong to a meaningful population**

<http://www.eufram.com/outputsDraft.cfm>

# Variability and Uncertainty

**Uncertainty** reflects *lack of knowledge* of the system under study

Ex1: what distribution to fit for SSD

Ex2: what mathematical model to use to estimate EC<sub>x</sub>

Increased knowledge will reduce uncertainty

**Variability** reflects *lack of control*

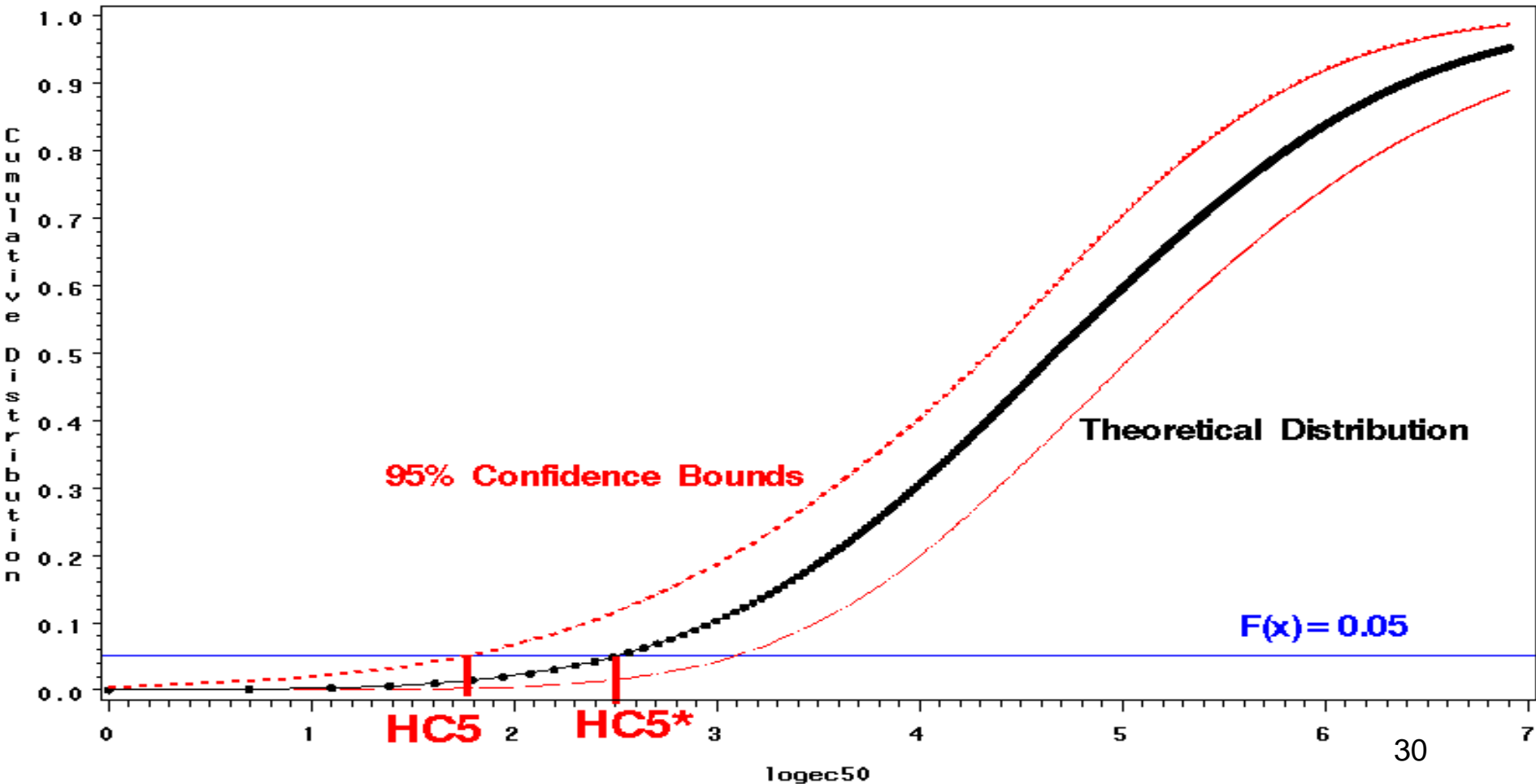
*inherent* variation or noise among individuals.

Increased knowledge of the animal or plant species will not reduce variability

# Summary Plot for SSD

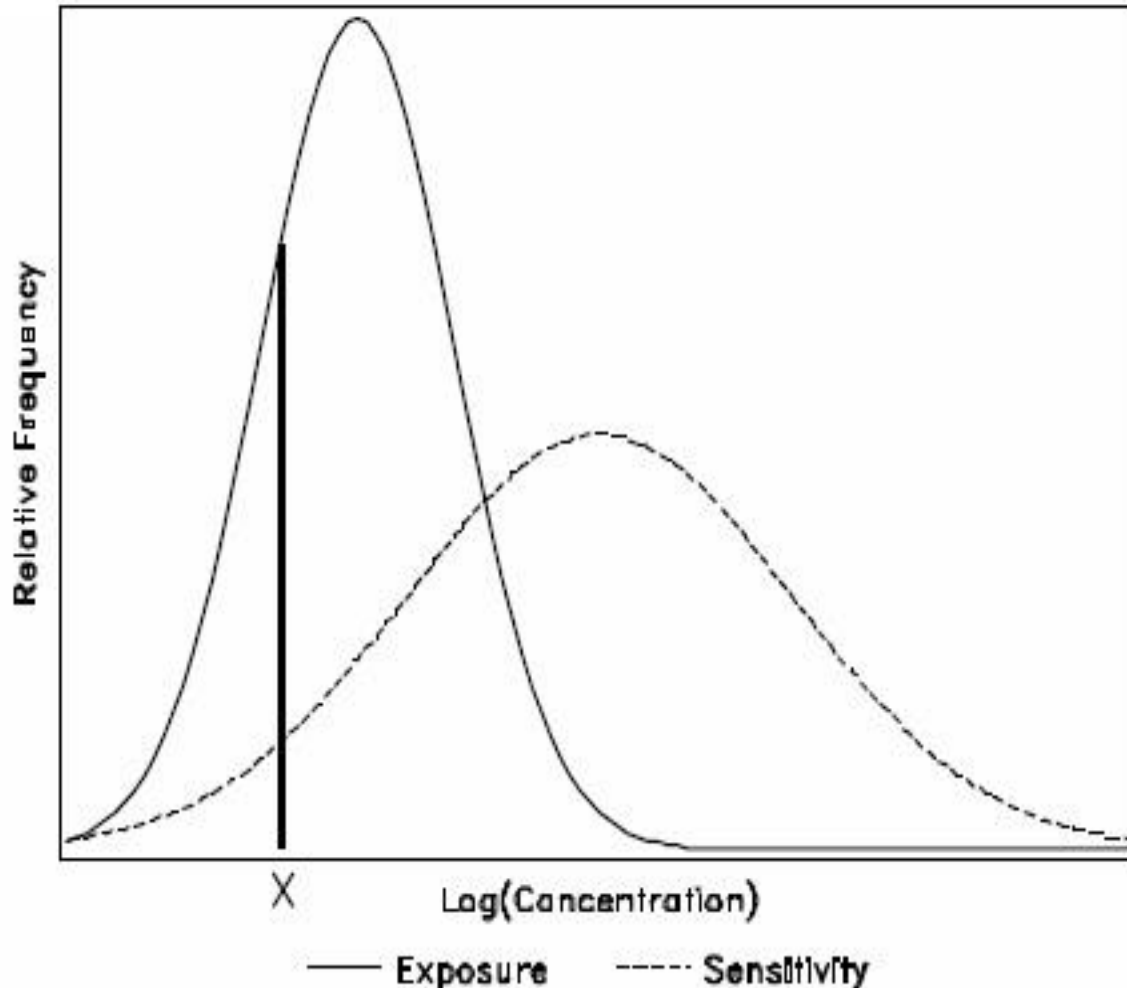
Distribution of Log(EC50) w/ 95% Confidence Bounds

Subset: Where Obs <=35,  
Subset: Where 0 < Obs,  
Mean=267.5159923, STD=533.77440553



# Putting it All Together

Probability Density Functions (PDF's)



## Joint Probability Curves

**Plot exposure and toxicity distributions together to understand the likelihood of the exposure concentration exceeding the toxic threshold of a given percent of the population**

# Calculating Risk

The risk is given by

$$\Pr[X_e > X_s]$$

where  $X_e$  = exposure,  $X_s$  = sensitivity or toxicity

**This is an “average” probability that exposure will exceed the sensitivity of species exposed**



# ICE and ACE Software Development

**ICE** (Interspecies Correlation Estimation)

Estimates ***acute toxicity*** for a species, genus or family ***from a surrogate species***

<https://www3.epa.gov/webice/> (2016)

**ACE** (Acute to Chronic Estimation)

Estimates ***chronic*** toxicity ***from raw acute toxicity data***

[https://cfpub.epa.gov/si/si\\_public\\_record\\_report.cfm?dirEntryId=62534](https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=62534)

Interspecies Correlation Estimation | Interspecies Correlation Estimation | US EPA - Windows Internet Explorer

US EPA http://www.epa.gov/ceampubl/fchain/webice/ Interspecies Correlation Estimation software

Interspecies Correlation Estimation | Interspecies Cor...

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## Interspecies Correlation Estimation

You are here: [EPA Home](#) » [Exposure Assessment](#) » [Food Chain](#) » [WebICE](#) » Interspecies Correlation Estimation

The data for this site is being updated, please postpone toxicity calculations until update is complete.

Exposure Assessment Models

- Web-ICE Home
- Aquatic Species
- Aquatic Genus
- Aquatic Family
- Wildlife Species
- Wildlife Family

Species Sensitivity Distributions

- Aquatic Wildlife

Endangered Species

- Aquatic Wildlife

Basic Information

- User Manual
- Download Model Data
- Bibliography

Web ICE Logo

The Web-based Interspecies Correlation Estimation (Web-ICE) application estimates acute toxicity to aquatic and terrestrial organisms for use in risk assessment. Please refer to the [User Manual](#) for detailed instructions on using Web-ICE.

### Web-ICE Modules

ICE Aquatic	ICE Wildlife
Aquatic vertebrates / invertebrates	Terrestrial Birds / Mammals
<a href="#">Species</a> <a href="#">Genus</a> <a href="#">Family</a>	<a href="#">Species</a> <a href="#">Family</a>

### Species Sensitivity Distribution Module

<a href="#">ICE Aquatic</a>	<a href="#">ICE Wildlife</a>
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### Endangered Species Module

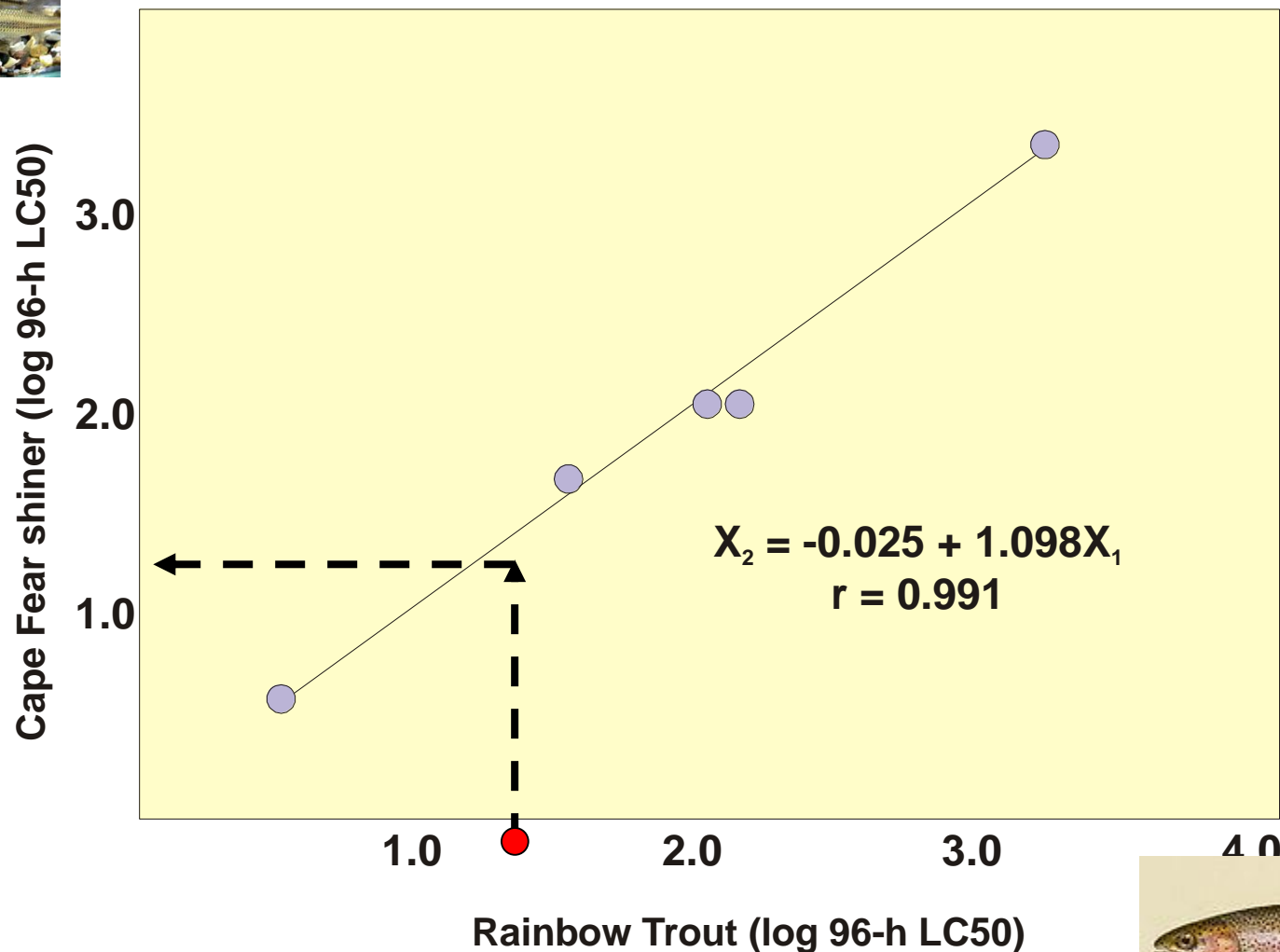
<a href="#">ICE Aquatic</a>	<a href="#">ICE Wildlife</a>
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# Acute toxicity estimates using interspecies correlations



- Exposure Assessment Modeling
- Modeling Products
  - Groundwater
  - Surface Water
  - Food Chain
  - Multimedia
  - Tools & Data
- Information Sources
  - About CEAM
  - Frequent Questions
  - CEAM Discussion
  - Related Links
  - Distribution & Support
  - Policy

# ACE

ICE and ACE were developed by the U.S. EPA in collaboration with other federal agencies, industry, and universities to address data gaps in species sensitivity and reduce reliance on uncertainty factors in ecological risk assessment.

The Acute-to-Chronic Estimation (ACE) with Time-Concentration-Effect Models software allows prediction of chronic toxicity from acute toxicity datasets. ACE uses linear regression and accelerated life testing to predict no-effect and low-effect concentrations for chronic mortality.

Specifications	
Current Version:	2.0
Release Date:	December 2003
Development Status:	General Release
Development Information:	<a href="#">Release Notes</a> - changes and known deficiencies
Operating System:	Win 9x, NT, 2000, XP
Development Language:	Visual Basic, Fortran
Intended Audience:	Scientist/Biologist
Key Words:	acute, chronic, toxicity, concentration, exposure
Related Web Sites:	<a href="#">Web-ICE Model Page</a> EPA National Health and Environmental Effects Research Laboratory (NHEERL)

### Text Files (ASCII Format)

File Name	File Description
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# ACE:

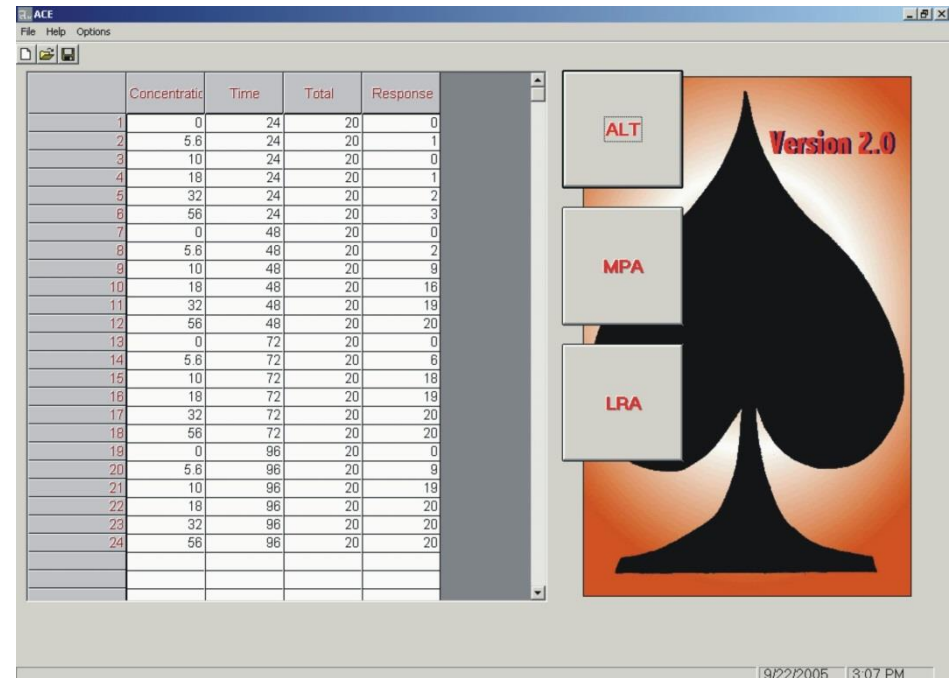
## Acute to Chronic Estimations

### Significance:

Provides estimated chronic toxicity for species with only acute data

Acute: ie. 96-hour LC50/ LD50

Chronic: long-term, sublethal



The screenshot shows the ACE software interface. On the left is a data table with columns for Concentration, Time, Total, and Response. On the right is a splash screen for Version 2.0 featuring a large black spade symbol and three buttons labeled ALT, MPA, and LRA. The status bar at the bottom indicates the date 9/22/2005 and time 3:07 PM.

	Concentration	Time	Total	Response
1	0	24	20	0
2	5.6	24	20	1
3	10	24	20	0
4	18	24	20	1
5	32	24	20	2
6	56	24	20	3
7	0	48	20	0
8	5.6	48	20	2
9	10	48	20	9
10	18	48	20	16
11	32	48	20	19
12	56	48	20	20
13	0	72	20	0
14	5.6	72	20	6
15	10	72	20	18
16	18	72	20	19
17	32	72	20	20
18	56	72	20	20
19	0	96	20	0
20	5.6	96	20	9
21	10	96	20	19
22	18	96	20	20
23	32	96	20	20
24	56	96	20	20

# Tossicologia acquatica

Gli studi sugli organismi acquatici sono di prima generazione, i più diffusi, i più consolidati

Misure basate su effetti a breve termine, o Saggi acuti: da pochi minuti (batteri luminescenti) a 24 o 96 h (pesci, crostacei).

Valutazioni: effetti prodotti da immissioni, più o meno accidentali, di sostanze diverse, di pesticidi, di reflui industriali o domestici

Specie animali: pesci, invertebrati

Specie vegetali: microalghe

Scopo: rilevare la concentrazione o la dose di una sostanza o di una miscela, di un agente fisico (torbidità, livello termico, radiazioni ionizzanti) che hanno effetto avverso misurabile per gli organismi considerati

## **Motivazioni dell'uso di saggi di tossicità con organismi acquatici**

### **a) Sollecitazioni di carattere legale –**

controllo della qualità delle acque superficiali ai fini della *tutela della fauna ittica e della pesca*

### **b) Formulazione di criteri di qualità:**

saggi *preventivi all'immissione sul mercato di nuovi prodotti chimici*. Bersagli biologici – saggi con pesci e crostacei

### **c) Tutela ambientale:**

giudizi di *accettabilità di effluenti* di cui non è nota la composizione

## **ACQUA DOLCE**

Specie test

### **Pesci**

*Salmonidi* Oncorhynchus mykiss, Salvelinus fontinalis

*Ciprinidi* Pimephales promelas

*Ictaluridi* Ictalurus punctatus

*Centrarchidi* Lepomis macrochirus

### **Invertebrati**

*Cladoceri* Daphnia magna, D.pulicaria, D.pulex

*Anfipodi* Gammarus lacustris, G.fasciatus, G.pseudolimnaeus

*Decapodi* Orconects sp., Cambarus sp.

*Ditteri* Chironomus sp.

*Gasteropodi* Physa integra

## **ACQUA DI MARE**

### **Pesci**

*Ciprinodontidi* Cyprinodon variegatus, Fundulus heteroclitus, F.similis

*Aterinidi* Menidia sp.

### **Invertebrati**

*Copepodi* Acartia tonsa, A.clausii

*Decapodi* Peneus setiferus, P.duorarum, Palaemonetes pugio, P.vulgaris, Crangon septemspinosa,  
Mysidiopsis bahia, Callinectes sapidus, Uca sp.

*Lamellibranchi* Crassostrea virginica, C.gigas

*Policheti* Capitella capitata, Neanthes sp.



# Introduction: experimental

The toxicity towards **Fathead Minnow (*Pimephales promelas*)** – a freshwater fish from **north America** - has been tested [1] for

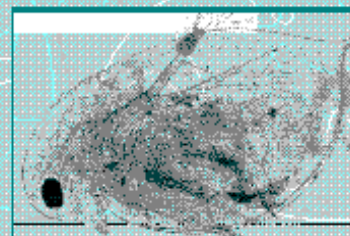
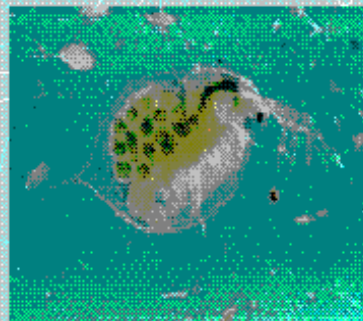
- 562 compounds representing a cross section of industrial organic chemicals [2], and
- Toxicity has been reported as median lethal concentrations LC50 (mmol/l) after 96 hours exposure

1. C.L. Russom, S.P. Brandbury, S.J. Broderius, D.E. Hammermeister, D.A. Drummond, *Environmental Toxicology and Chemistry*, 16 (1997) 948-967.

2. G.D. Veith, B. Greenwood, R.S. Hunter, G.I. Niemi, R. Regal, *Chemosphere*, 17 (1988) 1617-1630 .



## *Daphnia magna*, *D.pulex* e le altre



**Effetto rilevato:** morte, a volte si sceglie l'immobilizzazione (dafnie). Per gli organismi monocellulari si sceglie la diminuzione della crescita (alghe) o la compromissione della luminescenza (batteri)

**Somministrazione:** Nei saggi con organismi acquatici si contamina l'acqua (*effective concentration* – concentrazione efficace).

Tre sono i possibili tipi di approccio:

- **test statici.** Si allestiscono una serie di soluzioni con concentrazioni diverse senza ulteriori aggiunte di contaminante.

*Sono impiegati per i saggi di breve durata*

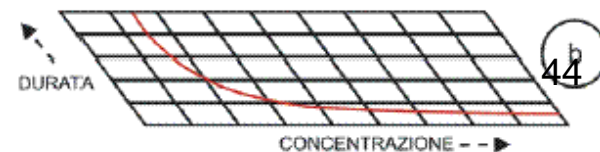
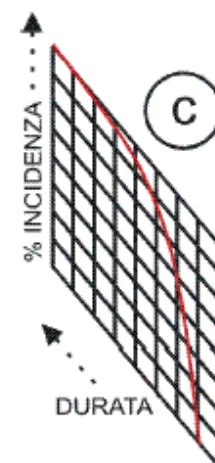
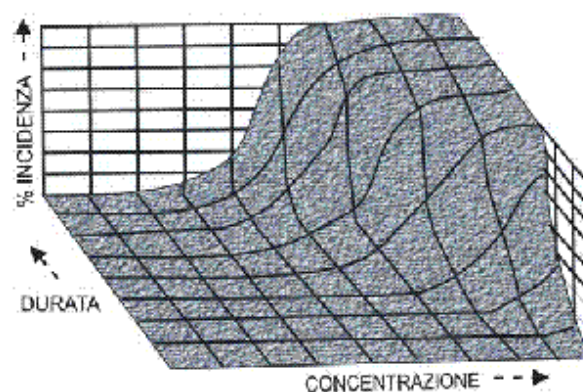
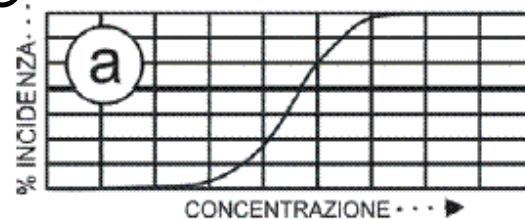
- **test con rinnovo periodico della soluzione:** si procede come con i test statici, ma dopo 24 h si procede ad una sostituzione dell'acqua a cui viene aggiunto di nuovo il tossico per ripristinare la concentrazione.

- **test a flusso continuo:** la soluzione test viene mantenuta in stato stazionario mediante un sistema di alimentazione automatico. *Sono i più utilizzati per gli esperimenti a lungo termine*

Si suppone che la sostanza impiegata nel test sia la causa dell'effetto osservato.

Nel corso del test deve essere verificato se a carico di tale sostanza si verificano trasformazioni chimiche **(b)**. Si suppone che la risposta osservata e la sua intensità siano in funzione della concentrazione della sostanza in esame nell'acqua in cui vengono posti gli organismi-test **(a)**. Livelli di esposizione non efficaci a breve termine possono produrre danni con tempi di trattamento più lunghi. ©

**Tuttavia, in genere, si tiene costante il tempo ma si varia la concentrazione.**



Produrre uno o più effetti sugli organismi tenuti in condizioni controllate

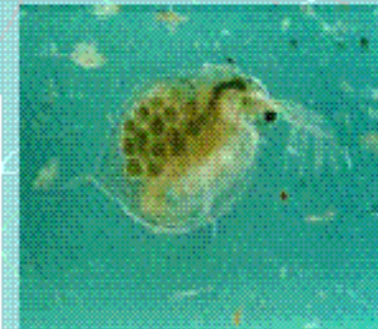
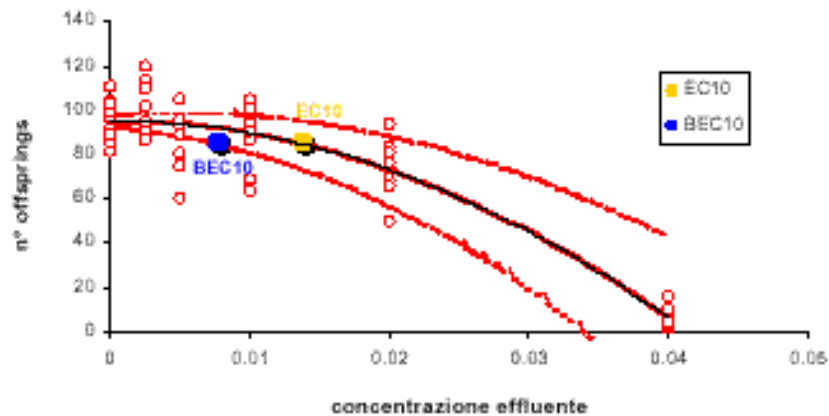
Esperimenti con più repliche:

- Variazione della concentrazione della sostanza in esame
- Scelta dei tempi
- Lettura dei danni prodotti allo scadere dei tempi prefissati
- Curva di tossicità
- EC 50 (*Median effective concentration – Concentrazione efficace mediana*)
- Quando l'effetto è la morte allora EC 50 = LC 50 (*Median lethal concentration – Concentrazione letale mediana*)
- Mediana indica che la risposta biologica è pari al 50%; alla LC 50 ci si aspetta la morte del 50% dei trattati.

- Generalmente si prendono come riferimento dei parametri fisiologici e/o riproduttivi (es. vel. di nuoto, tasso respiratorio, parametri indicativi del metabolismo) dell'organismo utilizzato nei test e se ne confrontano statisticamente i valori rispetto ad un gruppo di controllo.

## Definizione del NOEC

- Ad esempio può essere assunto come endpoint una diminuzione del 10% del tasso riproduttivo



## **PREGI**

Il saggio tossicologico diviene estremamente utile ai fini della valutazione delle interazioni tra le componenti tossiche e le caratteristiche naturali del corpo idrico ricevente.

## **LIMITAZIONI**

L'approccio tradizionale basato sull'utilizzazione di *una singola specie* può essere riduttivo rispetto alla complessità degli ecosistemi

L'utilizzazione di un *numero maggiore di specie lascia comunque irrisolti i problemi di incertezza* rispetto alla capacità di tolleranza delle innumerevoli specie (micro e macrospiche) di un ecosistema acquatico

I saggi a *breve termine*, prevalentemente utilizzati, non permettono di prevedere quali siano invece gli effetti derivanti da esposizione a lungo termine



*Soglia di tossicità.* Dose o concentrazione alla quale o al di sotto della quale non si manifesta un danno misurabile dopo un determinato tempo prestabilito. Tale concetto non si applica per quelle sostanze o agenti fisici (radiazioni ionizzanti) che agiscono sul DNA, per le sostanze mutagene, per quelle che producono un'inibizione enzimatica e dei meccanismi di trasporto, per quelle cancerogene.

*No-observed-effect level.* Max livello di esposizione ancora non efficace

*Lowest-observed-effect level.* Livello più basso tra quelli efficaci

Per definizione la soglia di tossicità si colloca tra NOEL e LOEL

*No-observed-adverse-effect level.* Concentrazioni che non producono effetti necessariamente dannosi (*adverse effect*) e pertanto anche se presenti non vengono considerati ai fini della valutazione della soglia di tossicità.

La conoscenza del NOEL **per gli organismi più sensibili di una comunità** consente di ricavare criteri di protezione accettabili. La difficoltà è rappresentata dalla possibilità di includere le specie più sensibili tra gli organismi con cui si effettuano i test di tossicità.

Individuazione *a priori* dei **percorsi critici** degli inquinanti (quelli in cui si prevedono le contaminazioni maggiori) e dei **gruppi critici** (specie o insiemi di individui più esposti alla contaminazione).

Si controllano gruppi critici e/o i percorsi critici, e si assume che se per essi sono verificate condizioni accettabili, allora anche altre specie, individui, siti si trovino in condizioni di sicurezza.

***Possibili forme di distorsione delle prove di tossicità:***

- l'impiego di un basso numero di animali può portare a valutazioni di tipo ottimistico.
- l'insorgenza di effetti a basse concentrazioni può confondersi con le risposte dei controlli
- il trasferimento dei risultati ricavati dal campione sperimentale all'intera popolazione (inferenza statistica).
- l'estrapolazione dei risultati conseguiti con una specie ad altre

**Fattori di sicurezza**

Ai NOEL sperimentali si applicano *fattori di incertezza o fattori di sicurezza* (1/5, 1/10, 1/100 del suo valore)

Spesso si effettuano *test tossicologici su organismi posti a diversi livelli della catena trofica.*

Ad esempio:

1 test su batteri (es. *Vibrio Fisheri* inibizione luminescenza)

1 test su alghe (es. *Dunaliella Tertiolecta* inibizione crescita)

1 test su invertebrati (es. *Daphnia Magna* inibizione mobilità)

1 test su pesci (es. *Pimephales promelas* LD<sub>50</sub>)

# SEDIMENTI

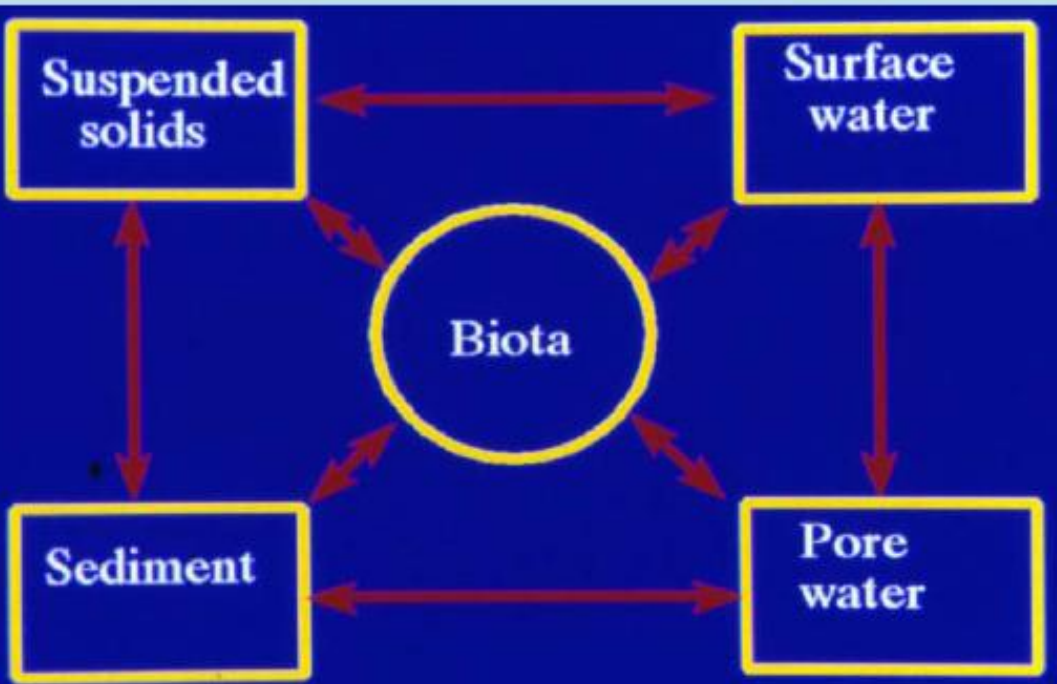


Fig. 7.26. Compartments and their interrelationships

## EQUILIBRIUM-PARTITIONING

(Van der Kooy et al., 1990)

CONCENTRATIONS IN WATER AND SOLIDS ARE RELATED THROUGH A PARTITION COEFFICIENT:

$$K_{sw} = \frac{C_s}{C_w}$$

WHERE:

$K_{sw}$  = SOLIDS-WATER PARTITION COEFFICIENT (L/KG)

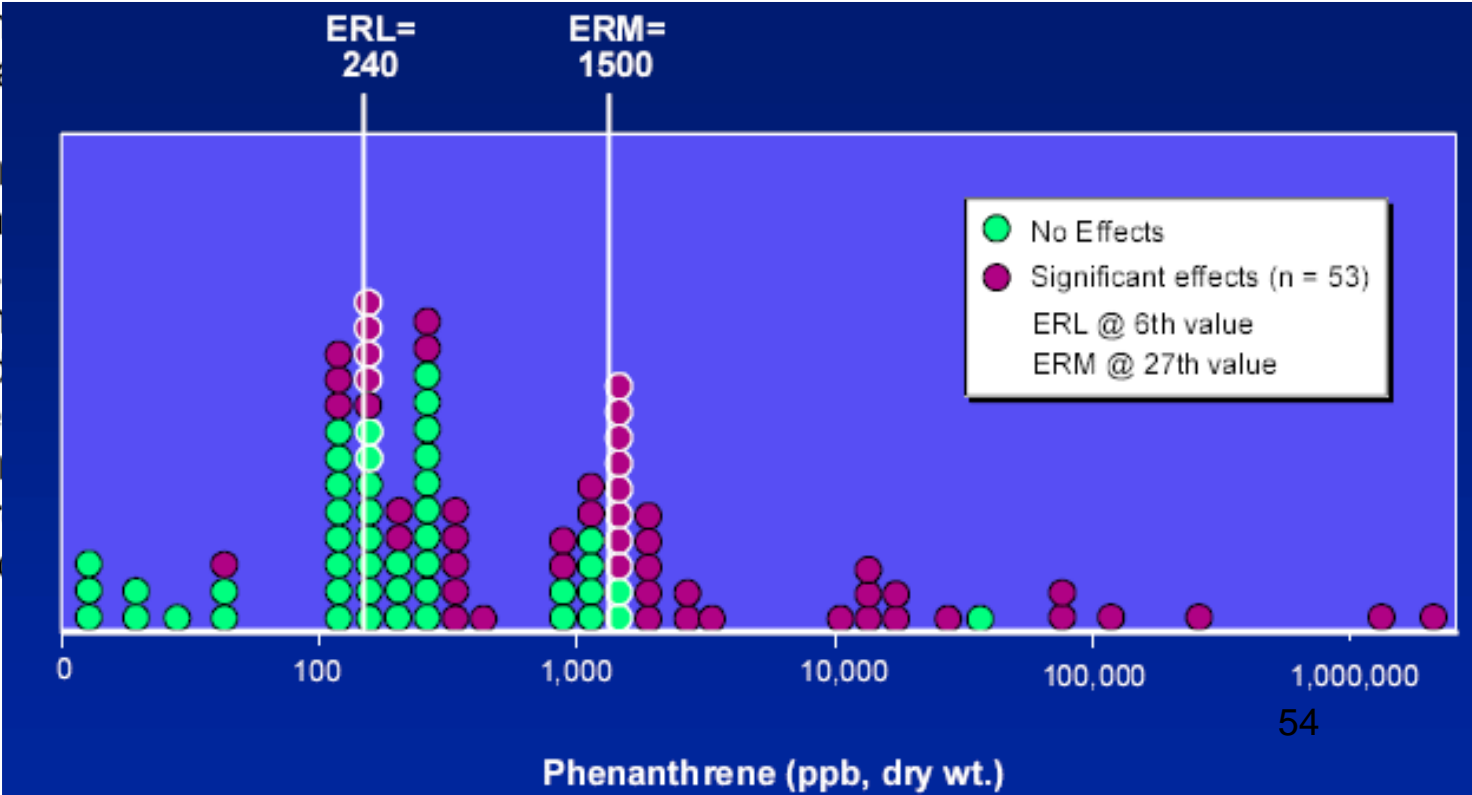
$C_s$  = CONCENTRATION IN THE SOLID PHASE (MG/KG)

$C_w$  = CONCENTRATION IN THE WATER PHASE (MG/L)

# Sediment Quality Guidelines developed for the National Status and Trends Program

Because guidelines were needed that were based on measures of biological effects associated with toxicants, data were compiled that included both chemical measures and biological effects.

SQGs were derived initially using a database compiled from studies performed in both saltwater and freshwater and published in NOAA Technical Memorandum NOS OMA 52 (Long and Morgan 1990). A larger database compiled from many studies performed since 1990 was used to revise and update the SQGs and/or of margin of safety in 1995, and a current database. Data on chemical concentrations and biological effects were identified. From this database, the 6th percentile (median) and the 27th percentile (median) were identified. The 10th percentile (median) was indicative of concentrations that were representative of background conditions.



**Table 1. ERL and ERM guideline values for trace metals (ppm, dry wt.) and percent incidence of biological effects in concentration ranges defined by the two values (from Long et al., 1995). ERL= Effects Range-Low; ERM= Effects Range-Median.**

Chemical	Guidelines		Percent incidence of effects*		
	ERL	ERM	<ERL	ERL - ERM	>ERM
Arsenic	8.2	70	5.0	11.1	63.0
Cadmium	1.2	9.6	6.6	36.6	65.7
Chromium	81	370	2.9	21.1	95.0
Copper	34	270	9.4	29.1	83.7
Lead	46.7	218	8.0	35.8	90.2
Mercury	0.15	0.71	8.3	23.5	42.3
Nickel	20.9	51.6	1.9	16.7	16.9
Silver	1.0	3.7	2.6	32.3	92.8
Zinc	150	410	6.1	47.0	69.8

\*Number of data entries within each concentration range in which biological effects were observed divided by the total number of entries within each range.

**Table 2. ERL and ERM guideline values for organic compounds (ppb, dry wt.) and percent incidence of biological effects in concentration ranges defined by the two values (from Long et al. 1995). ERL= Effects Range-Low; ERM= Effects Range-Median.**

Chemical	Guidelines		Percent incidence of effects*		
	ERL	ERM	<ERL	ERL--ERM	>ERM
Acenaphthene	16	500	20.0	32.4	84.2
Acenaphthylene	44	640	14.3	17.9	100
Anthracene	85.3	1100	25.0	44.2	85.2
Fluorene	19	540	27.3	36.5	86.7
2-methyl naphthalene	70	670	12.5	73.3	100
Naphthalene	160	2100	16.0	41.0	88.9
Phenanthrene	240	1500	18.5	46.2	90.3
Sum LPAH	552	3160	13.0	48.1	100
Benz(a)anthracene	261	1600	21.1	43.8	92.6
Benzo(a)pyrene	430	1600	10.3	63.0	80.0
Chrysene	384	2800	19.0	45.0	88.5
Dibenzo (a,h) anthracene	63.4	260	11.5	54.5	66.7
Fluoranthene	600	5100	20.6	63.6	92.3
Pyrene	665	2600	17.2	53.1	87.5
Sum HPAH	1700	9600	10.5	40.0	81.2
Sum of total PAH	4022	44792	14.3	36.1	85.0
p,p'-DDE	2.2	27	5.0	50.0	50.0
Sum total DDTs	1.58	46.1	20.0	75.0	53.6
Total PCBs	22.7	180	18.5	40.8	51.0

\*Number of data entries within each concentration range in which biological effects were observed divided by the total number of entries within each range.

# ***Preliminar Results From a Sediment Quality Triad Study in the Gulf of Trieste: the Choice of the Reference Site.***

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***Title anticipated in the Book of Abstracts:***

***“Sediment Quality Triads and the Integration of Information from Analytical Chemistry with Ecological Community Structure and Toxicological Data in Risk Assessment of Coastal Sites.”***

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## **Environmental quality criteria**

Aiming at the classification of ecosystems on the base of their environmental degradation, *environmental quality criteria* (numerical values) are required in order to determine if a zone is degraded or not.

Questions arise when the environmental quality criteria is based only on the assessment of chemical contamination of a certain environmental compartment since *chemical contamination does not necessarily imply effects on biological communities*. Moreover effects on biological communities are related to several factors, conditioning also the concentrations of contaminants, as – in aquatic systems – hydrodynamics, grain size of sediments, species being considered, etcetera.

## **Sediments**

Within aquatic ecosystems, sediments achieve importance in consideration of:

- Accumulation of contaminants (low solubility – affinity for particulate matter

- High residence time of c. (difficult biodegradation in reducing medium) → benthic organisms exposed to high levels of c.

- Sediment bound contaminants can be released to water if environmental conditions do vary.

Environmental agencies - as U.S.E.P.A. - thus consider *sediments as key environmental components within aquatic compartments*.

*Criteria classically determined* for environmental quality characterisation derive from approaches listed in Table 1, where examples and main limitations of each are reported.

Table1

<b>APPROACH</b>	<b>EXAMPLE OF MEASUREMENTS</b>	<b>LIMITATIONS</b>
Sediment chemical analyses	<ul style="list-style-type: none"> <li>- Individual contaminants</li> <li>- Complementary analyses (TOC, surface of grains etc.)</li> </ul>	<ul style="list-style-type: none"> <li>- Assumes that all chemical contaminants are measured</li> <li>- Contamination do not inform about biological effects</li> </ul>
Organism tissue chemical analyses	<ul style="list-style-type: none"> <li>- Individual contaminants</li> <li>- Complementary analyses (biometrical etc.)</li> </ul>	<ul style="list-style-type: none"> <li>- <i>Idem</i> as above</li> <li>- Organisms mobility</li> </ul>
Sediment toxicity tests	<ul style="list-style-type: none"> <li>- Survival</li> <li>- Sublethal effects (malformation, burial)</li> </ul>	<ul style="list-style-type: none"> <li>- Conditions different from reality;</li> <li>- Assumes that considered tests cover all responses</li> <li>- Toxicity is not linked causally to specific toxic agent</li> </ul>
Histopathological alterations	<ul style="list-style-type: none"> <li>- Individual pathological conditions</li> <li>- Complementary analyses (biometrical etc.)</li> </ul>	<ul style="list-style-type: none"> <li>- Organisms mobility</li> <li>- Disease is not linked causally to specific chemical agent</li> </ul>
Structure of the Benthic community	<ul style="list-style-type: none"> <li>- Taxa (Mollusca, Polichaeta etc.)</li> <li>- Biomass; indices of biodiversity</li> </ul>	<ul style="list-style-type: none"> <li>- Difficult to discriminate between natural and anthropogenic effects</li> </ul>

*Each single approach presents pros and cons; consequently two or more of the cited type of measurements can be applied on samples acquired simultaneously thus allowing an integrated assessment.*

## The case study

The growing degree of connection of urban and industrial sites of the Plain of the Isonzo River to the local sewage treatment plants and the high environmental pressure on the coast line of the Gulf of Trieste have brought to *plan the building of a new off-shore diffusor* that will be completed before the end of 2002.

Other diffusors within the same Gulf were demonstrated to bring metals to offshore sediments, thus extending the radius of impact of human activities, beside lowering the environmental strain on the coastline [].

An *integrated environmental assessment* has been performed before the building and exercise of the offshore dispersion device *at four sites located nearby* it -locations are ISO1, ISO2, ISO3 and ISO4 in Figure 1 - so to provide a reference for a future evaluation the possible impact of treated waters on benthic life. *Measurements describing chemical contamination of sediments, ecotoxicity tests with sediment elutriates, and quali-quantitative assessment of macrobenthic population have been produced.*

	Long.	Lat.
ISO1	13°35'.43	45°42'.08
ISO2	13°35'.17	45°41'.86
ISO3	13°35'.91	45°42'.13
ISO4	13°35'.33	45°42'.73

	Depth (m)	Sand %	Silt %	Clay %
ISO1	13.7	0.00	33.47	63.53
ISO2	13.7	0.00	33.16	66.84
ISO3	14.6	0.00	37.05	62.95
ISO4	11.5	0.00	42.82	57.18



## *Experimental methods:*

*Samples* for chemical and toxicological analyses have been collected by a Kc HAPS bottom corer with a sample area of 127 cm<sup>2</sup>; for the analysis of benthos three samples have been collected with a 0.1 m<sup>2</sup> van Veen grab.

*Chemical analyses:* metals (Cd, Ni, Pb, Ag, Cu, Cr, Fe, Zn, As and Hg) have been released from sediments and analysed according to I.R.S.A. methodologies []. The spectrometer was a PE-5100PC.

PAHs (Phenanthrene, Fluoranthene, Pyrene, Benzo(a)anthracene, Crysene, Benzo(b)fluoranthene, Benzo(a)pyrene), PCBs, 4,4'-DDE, 4,4'-DDD, 4,4'-DDT have been extracted again according to I.R.S.A. methods []; PCBs have been quantified as PCB1254 mixture. The separation were conducted by gas chromatography, with ECD for chlorinated compounds and MS for PAHs. PE-AutoSystem XL and HP-6980/5973 instruments were used.

*Toxicological analyses* on sediment elutriates considered here are the Microtox assay® [] and the assay on the alga *Dunaliella tertiolecta* [].

*In situ alteration of the benthic community* has been assessed by the *analysis of macrobenthos*. Macrobenthic organisms (Mollusca, Polychaeta, Crustacea, Echinodermata) have been determined to species level; furthermore abundance values of specimens were computed. From these data diversity indices (Shannon, Pielou) have been calculated.

Chemistry, Toxicity and Infauna Data from the four different sites can be combined into *the Sediment Quality Triad* (SQT) [] in order to determine the degree of degradation at each site. The normalization of data from the sampling sites towards those of one of them that is considered as an unpolluted reference makes the comparison relatively easy. For each site and for each parameter determined, the datum is converted into a *Ratio To Reference* (RTR) value:

$$(RTR_i)_k = (v_i)_k / (v_i)_0 \quad \text{where:}$$

$(RTR_i)_k$  is the RTR for parameter i-me at site k-me;

$(v_i)_k$  is the datum determined for parameter i-me at site k-me;

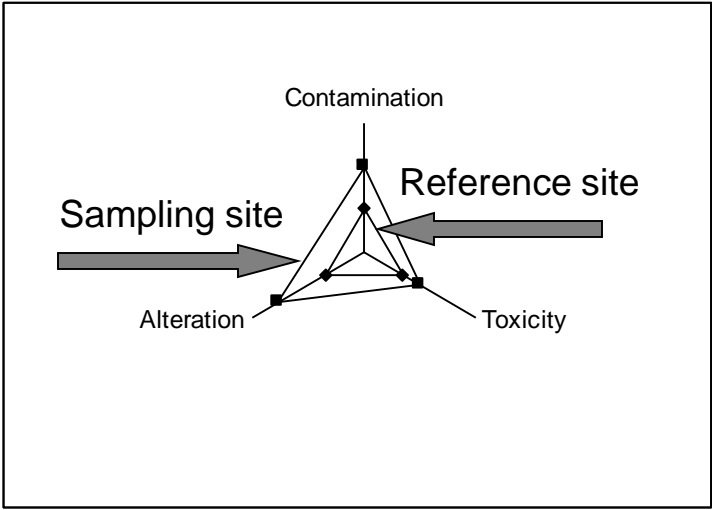
$(v_i)_0$  is the datum determined for parameter i-me at site chosen as reference.

This is straightforward for chemical parameters, while toxicological and infaunal parameters have been transformed so to show increase with biological damage. For instance, in a Microtox® test the result (endpoint) is expressed as EC20, the percentage of interstitial water sample causing a 20% inhibition of bioluminescence of the population of *Vibrio Fisheri*; this means that EC20 is low when sediment is highly polluted; the inverse (EC20<sup>-1</sup>) is thus considered.

$(RTR_i)_k$  for all i parameters describing chemical contamination are averaged, thus providing a single *Index of Contamination for each site, IC* ; the same is done for parameters describing sediment toxicity and *in situ* alteration; The result is a *Index of Toxicity (IT)* and a *Index of Alteration (IA)* for each site.

The three indices for each sampling site can be displayed in graphical form as three segments (for Contamination, Toxicity and Alteration ) departing from a central point, where the lengths of each segment equals the averaged values of the RTR for the three group of determined parameters.

Two triangles are identified; the inner one represents the reference site, the outer is one of the site for whom the environmental quality must be assessed. The difference between the areas of the outer and inner triangles can be retained as a synthetic ***index of degradation*** with respect of the reference site []. The difference between the sums of the three indices IC, IT and IA. for the site under investigation and the reference is a measure of degradation as well.



Del Valls et al. [] proposed a modified normalization procedure, where:

$$(RTM_i)_k = (RTR_i)_k / RTRmax_i$$

$(RTM_i)_k$  is the new normalized value for parameter i-me at site k-me;

$(RTR_i)_k$  is the RTR for parameter i-me at site k-me;

$RTRmax_i$  is the maximum value of RTR for parameter i-me;

The new indices of Contamination, Toxicity and Alteration for site k are computed as:

$$NIC_k = (\sum RTM_{ic})_k / (\sum RTM_{ic})_0;$$

ic = index running between chemical parameters;

$$NIT_k = (\sum RTM_{it})_k / (\sum RTM_{it})_0;$$

it = index running between toxicological parameters;

$$NIA_k = (\sum RTM_{ia})_k / (\sum RTM_{ia})_0;$$

ia = index running between alteration parameters

It is clear how results depend on the choice of the reference site, but no formal procedure has been proposed to select it, at the best of our knowledge.

The problem is not trivial, since in practical cases it is frequent to choose the reference site between stations which are not “completely unpolluted”; the quest for a “truly unpolluted” reference could lead to select a station being too heterogeneous from others.

A formal procedure for selecting the reference site is as follows:

1) for each possible reference site  $i$

compute IC, IT, IA (or NIC, NIT, NIA) and index of degradation  $P_{ij}$  (based on areas of triangle or on sums of indices) for each sampling site  $j$

2) the selected reference site  $i$  is the one for which

a)  $P_{ii} = \min P_{ij}$ ;

b)  $P_{ij} \geq 0$ .

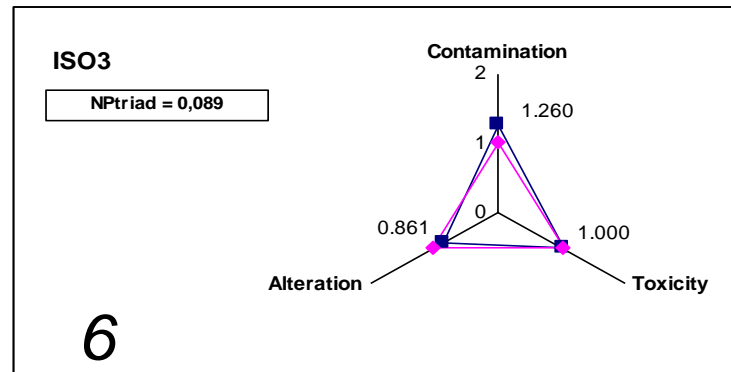
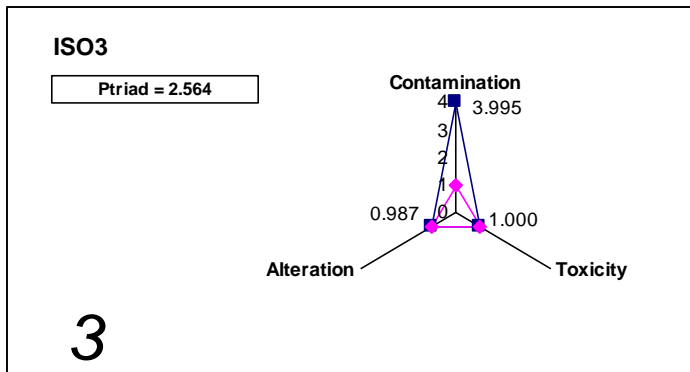
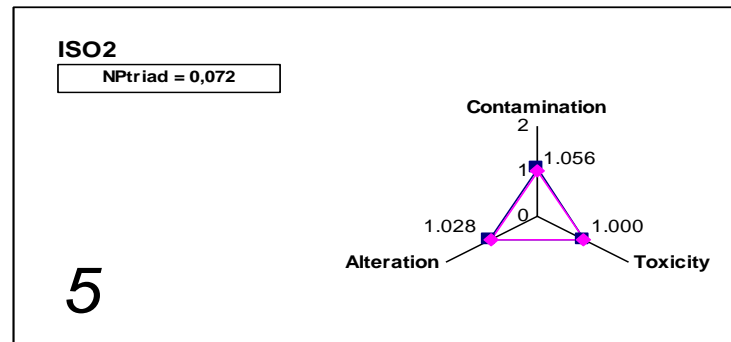
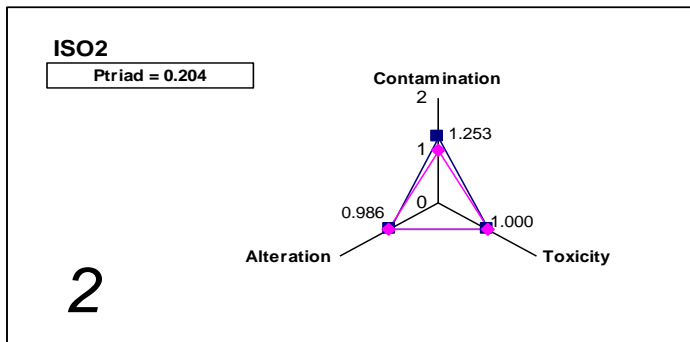
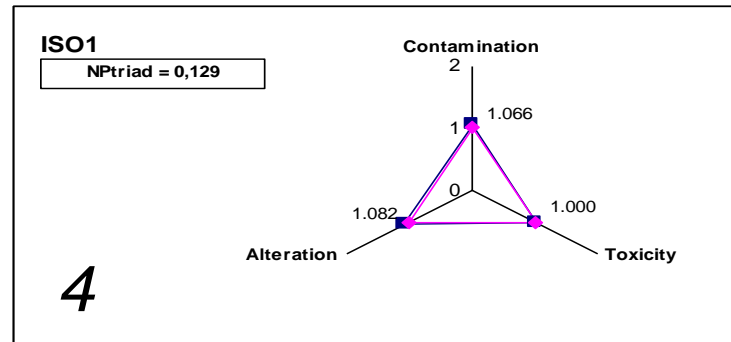
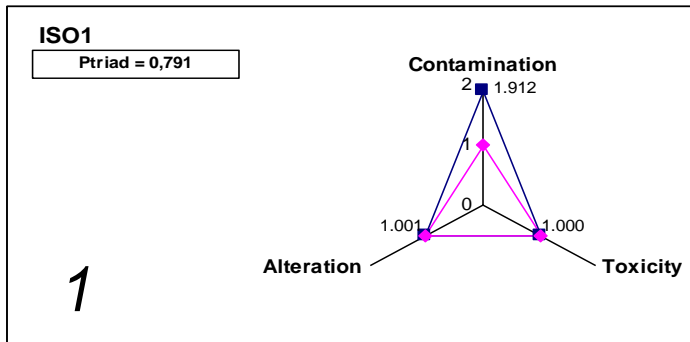
The results of the procedure described above for our data, using RTR, the areas for defining the index of degradation, are as follows:

Rif.	ISO1				ISO2				ISO3				ISO4			
	ISO1	ISO2	ISO3	ISO4	ISO1	ISO2	ISO3	ISO4	ISO1	ISO2	ISO3	ISO4	ISO1	ISO2	ISO3	ISO4
IC	1.000	0.888	1.760	0.908	1.193	1.000	2.423	0.903	1.193	1.000	2.423	0.908	1.012	1.253	3.995	1.000
IT	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IA	1.000	0.992	0.791	0.939	1.016	1.000	1.001	1.015	1.016	1.000	1.001	1.015	1.001	0.986	0.987	1.000
<b>P<sub>triad</sub></b>	<b>0.000</b>	<b>-0.017</b>	<b>0.407</b>	<b>-0.131</b>	<b>0.181</b>	<b>0.000</b>	<b>1.233</b>	<b>-0.073</b>	<b>0.181</b>	<b>0.000</b>	<b>1.233</b>	<b>-0.073</b>	<b>0.791</b>	<b>0.204</b>	<b>2.564</b>	<b>0.000</b>

Underlined numbers stand for condition (a), Italics stand for condition (b), from the table above, ISO4 is selected as reference site.



# Sediment Quality Triad Plots



Plots 1, 2, 3 report results derived after RTR normalization; plots 4, 5, 6 report results derived after RTM normalization; Degradation Indices (P or NP) are differences between areas of triangles defined for the sampling sites ISO1, ISO2, ISO3, and the reference site ISO4.

## Conclusions

Examining the plots it can be seen how the three sites are very similar to the reference station; some differences can be appreciated with respect to the chemical contamination, but they seem not to be severe enough to alter in a significant way population of macrobenthos, and neither to determine a significant toxicity of sediments. This scenario will be compared with SQT analysis obtained when the wastewater diffuser will be operative.

From a methodological point of view, the SQT approach presents an interesting way of synthesising complementary information, providing a rich -informative- comparison between sites of a certain area.

In order to gain more widespread acceptance of the methodology, detailed guidelines are needed so to apply SQT “on objective bases”.

Clear indications (“how to”) on the selection of contaminants to be considered, on ecotoxicological tests to be applied, and on measures of the *in situ* alteration should be set. Moreover an exhaustive study on benefits of the different normalization procedures and a general criterium for the selection of the reference site are required.

In this work we have proposed a procedure for the choice of the reference station.

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- **Relative Taxa Sensitivity (RTS) of aquatic invertebrates with respect to organic and metal compounds. (39)**
- **Von der Ohe, P. & Liess, M. 2004. Environmental Toxicology and Chemistry. 23, 150-156.**
- In the field, a multitude of species can be exposed to numerous toxicants; thus, the sensitivity of individual species to particular toxicants must be known to predict effects and to analyze changes in species composition. For most species, no information about their toxicant sensitivity is available. To address this limitation, we have grouped the available information to assign sensitivities to aquatic invertebrate taxa relative to *Daphnia magna*. With respect to organic compounds, most taxa of the orders Anisoptera, Basommatophora, Coleoptera, Decapoda, Diptera, Ephemeroptera, Eulamellibranchiata, Heteroptera, Hirudinea, Isopoda, Oligochaeta, Prosobranchia, Trichoptera, Tricladida, and Zygoptera are less sensitive than *D. magna*. Some taxa of the Amphipoda, Plecoptera, and Cladocera (other than *D. magna*) are significantly more sensitive. For organic compounds, approximately 22% of the investigated taxa were more sensitive than *D. magna*. Most taxa of the orders Amphipoda, Basommatophora, Diptera, Ephemeroptera, Eulamellibranchiata, Heteroptera, Isopoda, Oligochaeta, and Tricladida are significantly less sensitive than *D. magna* to metal compounds. The taxa belonging to the Crustacea, with the exception of the order Isopoda, are much more sensitive. For metal compounds, approximately 30% of the investigated taxa were more sensitive than *D. magna*. Hence, *D. magna* is among the most sensitive taxa regarding both groups of toxicants. The sensitivities for several taxa are listed, and use of the relative sensitivity distribution to link toxicant effects in mesocosm studies and field investigations is discussed.