LABORATOIRE INTERDISCIPLINAIRE DES ENVIRONNEMENTS CONTINENTAUX

CNRS UMR 7360

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Ecotoxicologie des écosystèmes aquatiques : point sur les effets cocktails

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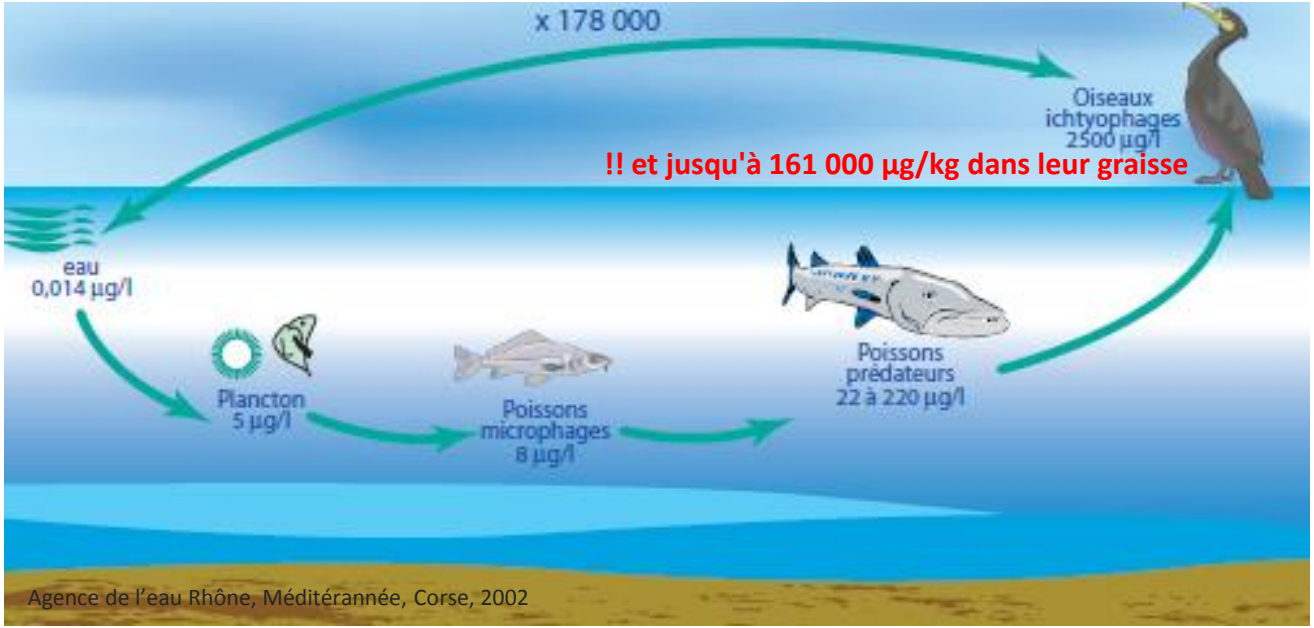
SEMINAIRE TECHNIQUE EVOLUER VERS UNE PRISE EN COMPTE DE LA TOXICITE DANS LES SUIVIS DE QUALITE DES EAUX

APRONA Strasbourg 19 mars 2019

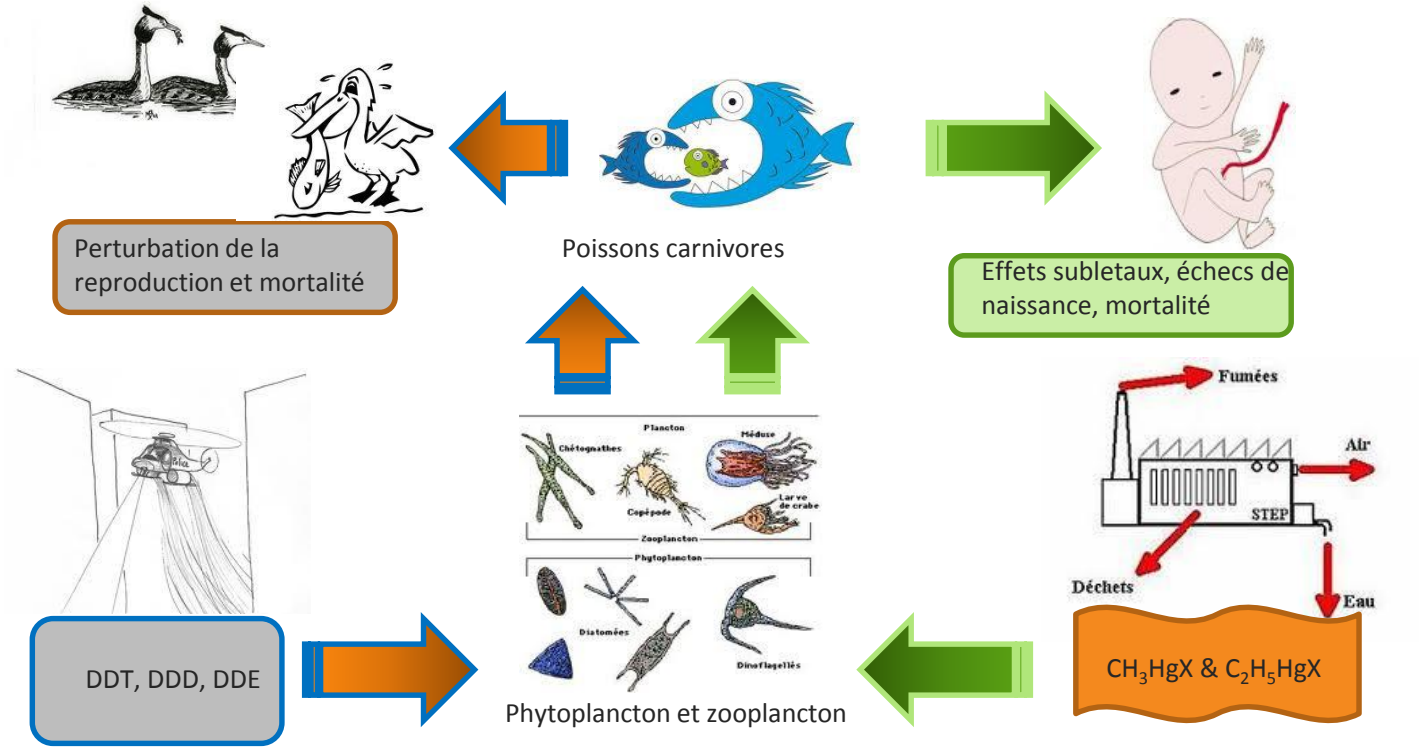
Clear Lake & DDT

Entre 1949 et 1957



Mesure des différentes concentrations en DDD dans la chaîne trophique du Clear Lake (USA). EAAP

En conséquence à la fin des années cinquante il ne restait environ que 30 couples de Grèbes, pour la plupart stériles alors que la population à l'origine comptait plus de 30000 individus

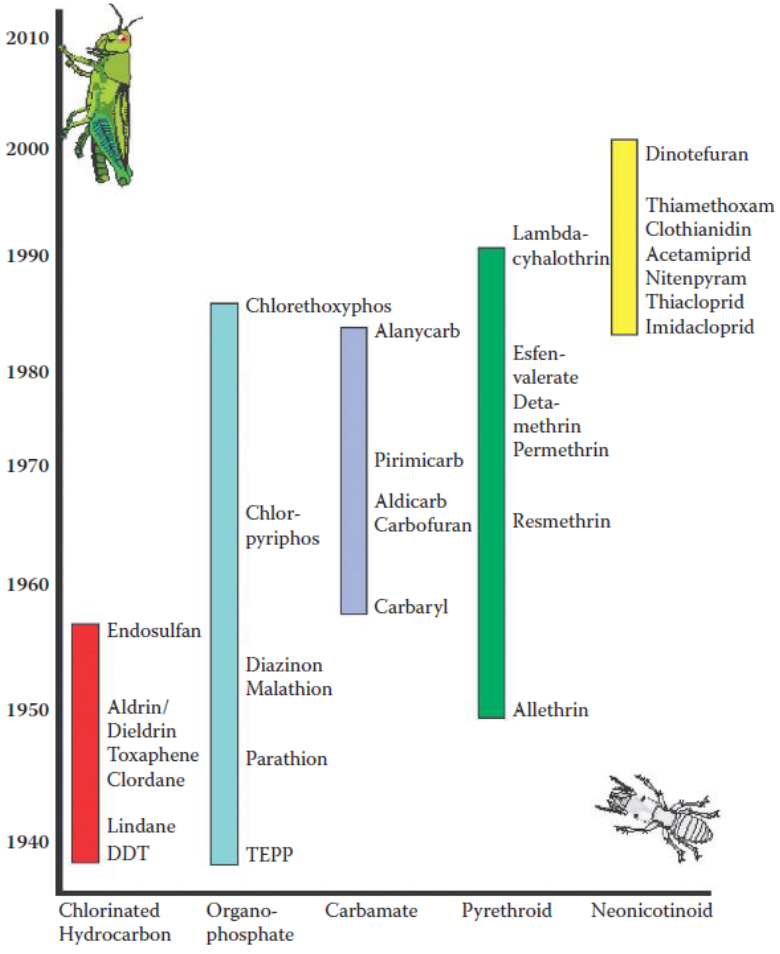


DDT et methylmercure, deux des premiers polluants qui ont attiré l'attention sur l'insuffisance du paradigme de dilution. Ils ont accéléré l'émergence du paradigme du boomerang.

(redessiné d'après Newman, 2010)

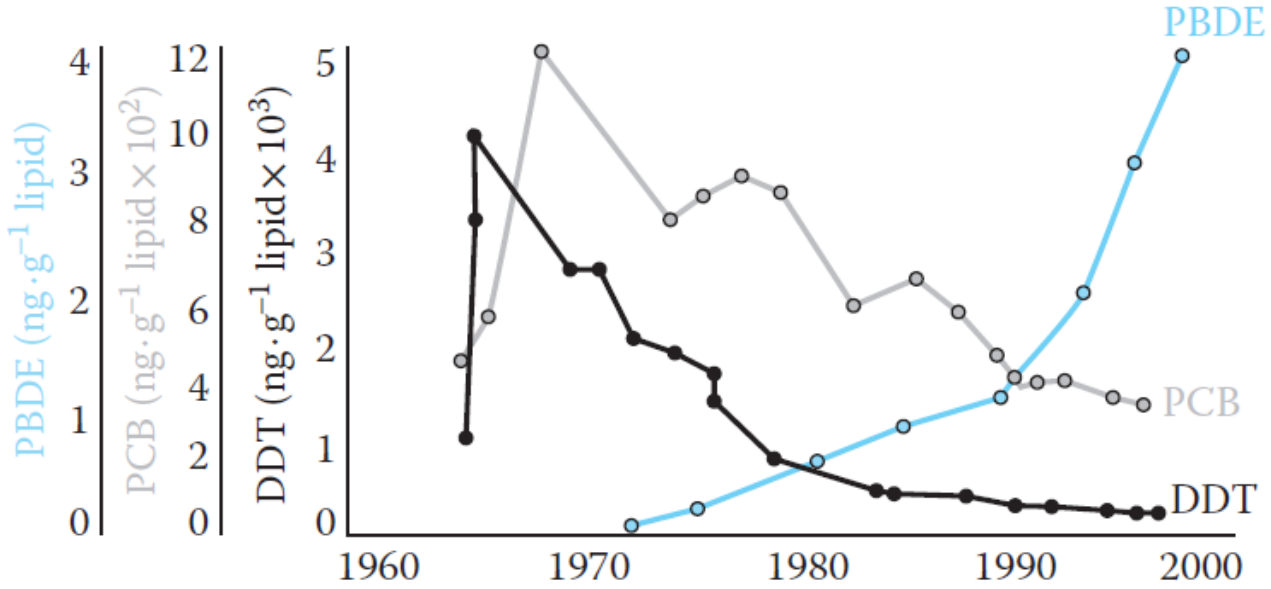
Images : edulcolnet.com; terreetvolcans.free.fr; noumea-boutique.nc; guadeloupensites.com ; dinosoria.com; grossesse.teteamodeler.com; vedura.fr ; illusion.over-blog.com

The general development chronology of the five major types of organic insecticides



In Newman, 2015

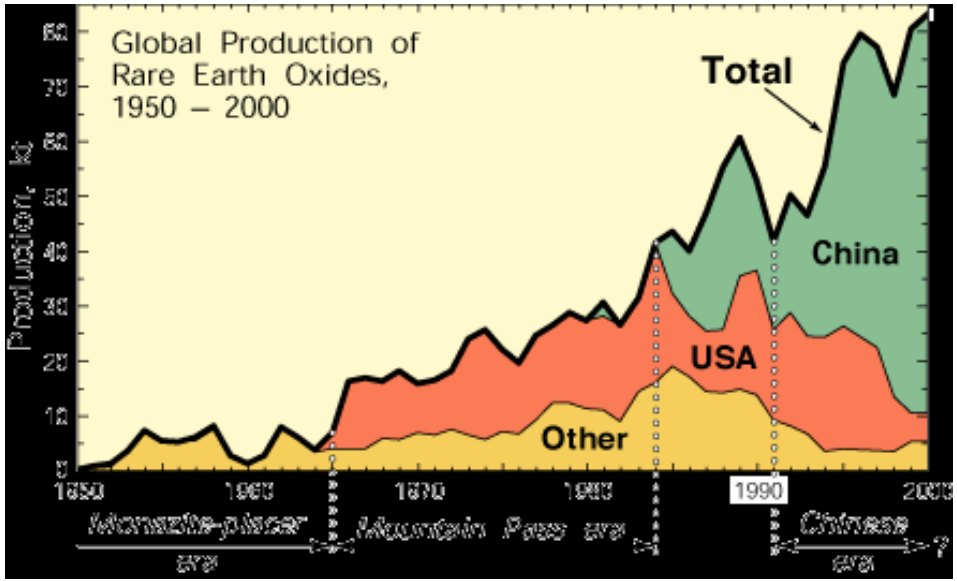
Tendances d'évolution de 3 POPs, DDT, PCBs et PBDE dans le lait maternel en Suède



Dispersion large et forte accumulation dans le biota loin de leur point d'émission

Convention Stockholm 1994 & Directive UE REACH : élimination graduelle des POPs dont PCB, dioxines, furanes et pesticides

Extraction des terres rares



Global rare earth element production (1 kt=10^6 kg) from 1950 through 2000, in four categories: United States, almost entirely from Mountain Pass, California; China, from several deposits; all other countries combined, largely from monazite-bearing placers; and global total.

Four periods of production are evident: the monazite-placer era, starting in the late 1800s and ending abruptly in 1964; the Mountain Pass era, starting in 1965 and ending about 1984; a transitional period from about 1984 to 1991; and the Chinese era, beginning about 1991. ***U.S. Geological Survey Rare Earth Elements—Critical Resources for High Technology (2002)***

Les premières définitions

- Attribuée à Truhaut (1969) :

- La science décrivant les effets toxiques d'agents de nature diverse sur les organismes vivants, particulièrement sur les populations et communautés d'un écosystème. En 1977, elle devient un peu plus détaillée comme

- La branche de la toxicologie qui étudie les effets toxiques provoqués par les substances naturelles ou les polluants d'origine synthétique sur les constituants des écosystèmes animaux y compris l'homme, végétaux et micro-organismes, dans un contexte intégré

Les définitions les plus récentes de l'écotoxicologie

ont intégré 2 aspects qui dominent les approches actuelles :

- La première intègre la notion de danger des composés chimiques toxiques (risque) pour les organismes vivants à travers l'utilisation de données de suivis antérieurs, ce qui correspond à une **approche rétrospective** qui évalue le niveau des toxiques chimiques dans l'environnement et utilise cette information pour déterminer leurs potentiels impacts passés, présents et futurs.
- Cela ressemble si on peut dire à une « perspective environnementale historique » en analysant une situation passée et en reliant la cause (les pdts chimiques) aux effets (réponse de l'organisme).

Les définitions les plus récentes de l'écotoxicologie

- Au contraire, le second aspect tente de prédire l'impact des composés chimiques à l'aide d'études prospectives

- Cela nécessite l'utilisation de tests spécifiques qui permettent d'évaluer l'impact probable d'un composé unique ou bien d'un mélange complexe (effluents)

Objectifs

L'écotoxicologie a deux objectifs principaux :

- Etudier les processus de contamination des milieux
  (source, devenir, circulation et transformations biogéochimiques des polluants dans les écosystèmes)
- Evaluer les effets des polluants à l'égard de la structure et du fonctionnement des systèmes naturels

Contraintes reliées à l'écotoxicologie

L’écotoxicologie se heurte à une très grande complexité liée :

- à l’extrême diversité des constituants de l’écosphère,
- aux variations spatio-temporelles des facteurs écologiques,
- à la diversité qualitative et quantitative des contaminants et
- aux innombrables mécanismes d’adaptation mis en œuvre par les organismes

L'écotoxicologie: une science multidisciplinaire

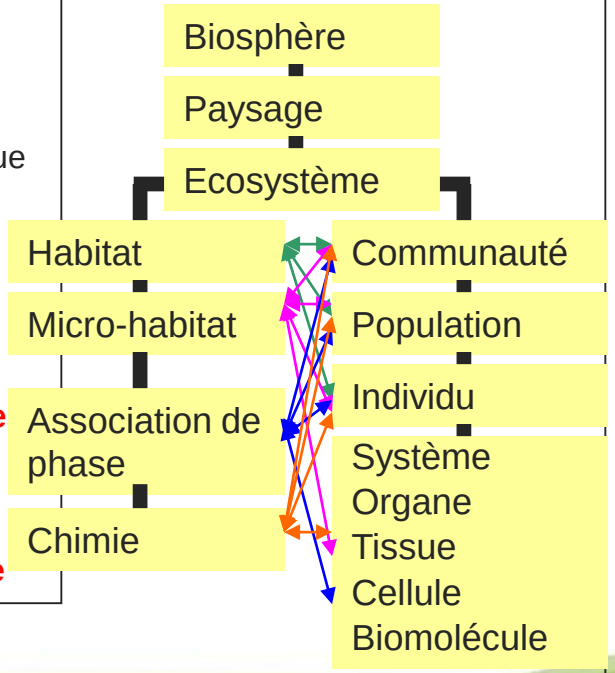
Influences majeures:

- **Chimie**
- **Toxicologie**
- **Ecologie**

SCIENCES PHYSIQUE
Géologie
Océanographie
Chimie atmosphérique
Climatologie

Géographie
Hydrologie
Géologie-Pédologie
Sédimentologie

Chimie des colloïde
Physico-chimie
Chimie analytique
Chimie organique
Chimie inorganique



SCIENCES DE LA VIE
Ecologie globale
Ecologie du paysage
Ecologie des systèmes
Microbiologie environnementale

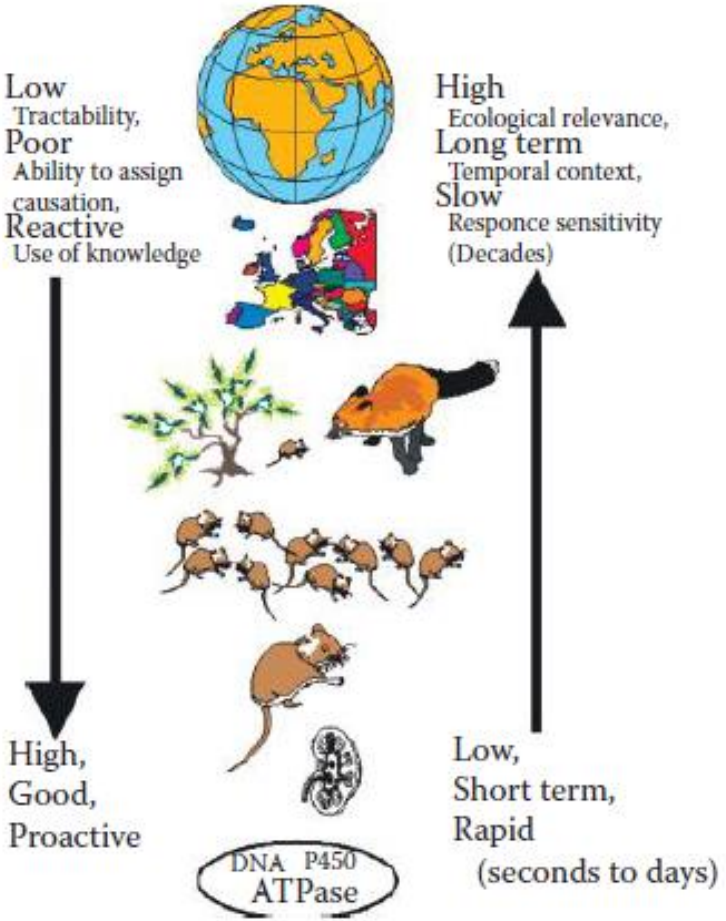
Ecologie des communautés

Métapopulation
Biologie
Biologie des populations
Génétique
Epidémiologie

Toxicologie
Physiologie/Anatomie
Comportement
Tératologie
Neurologie
Pharmacologie
Endocrinologie
Immunologie
Pathologie
Oncologie
Biochimie/Biophysique
Génétique moléculaire

DIVERS
Mathématiques
Statistiques-Modélisation
Informatique
Electronique

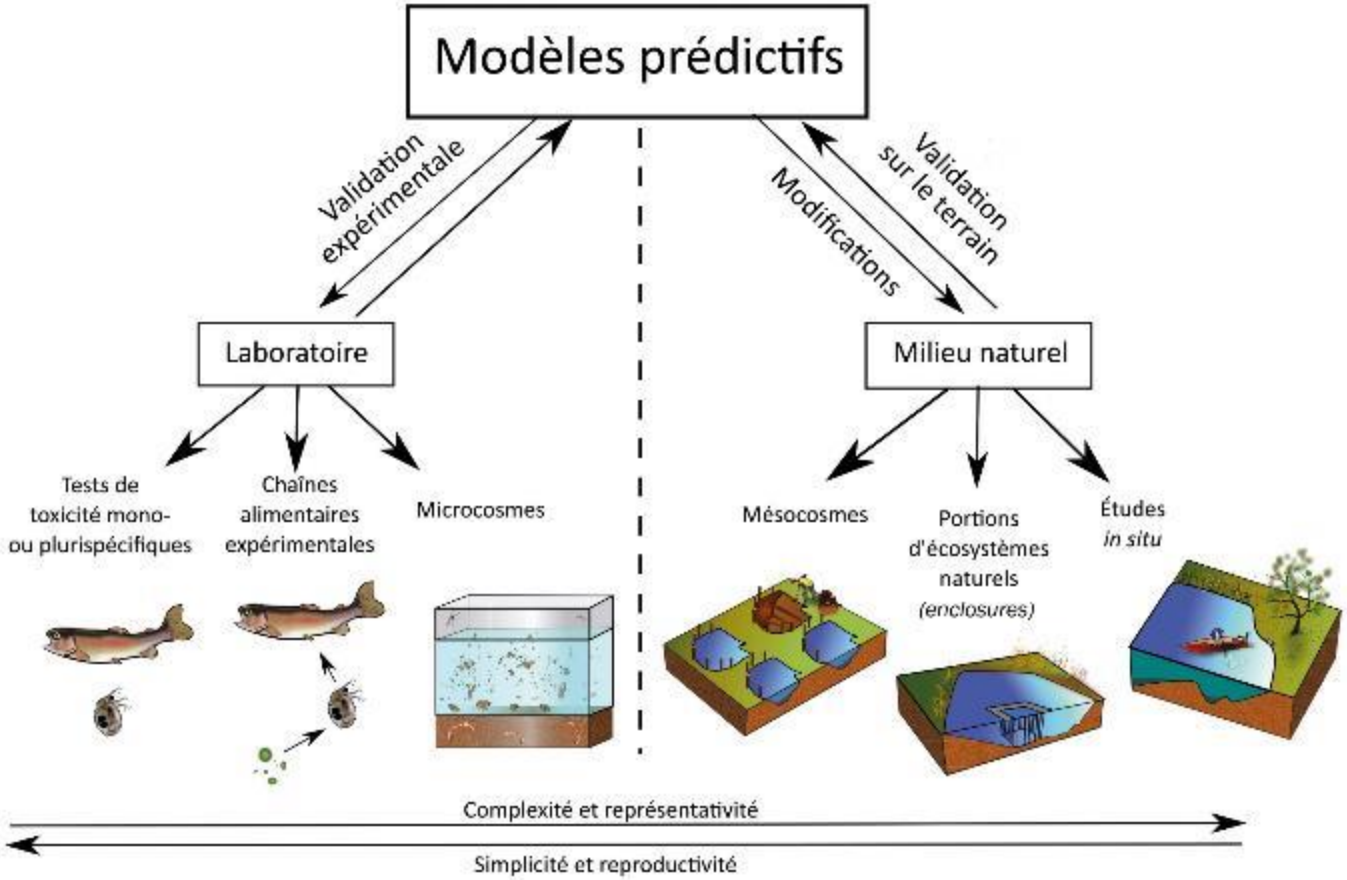
Source: Fundamentals of Ecotoxicology, 2nd Edition. Newman and Unger, 2003

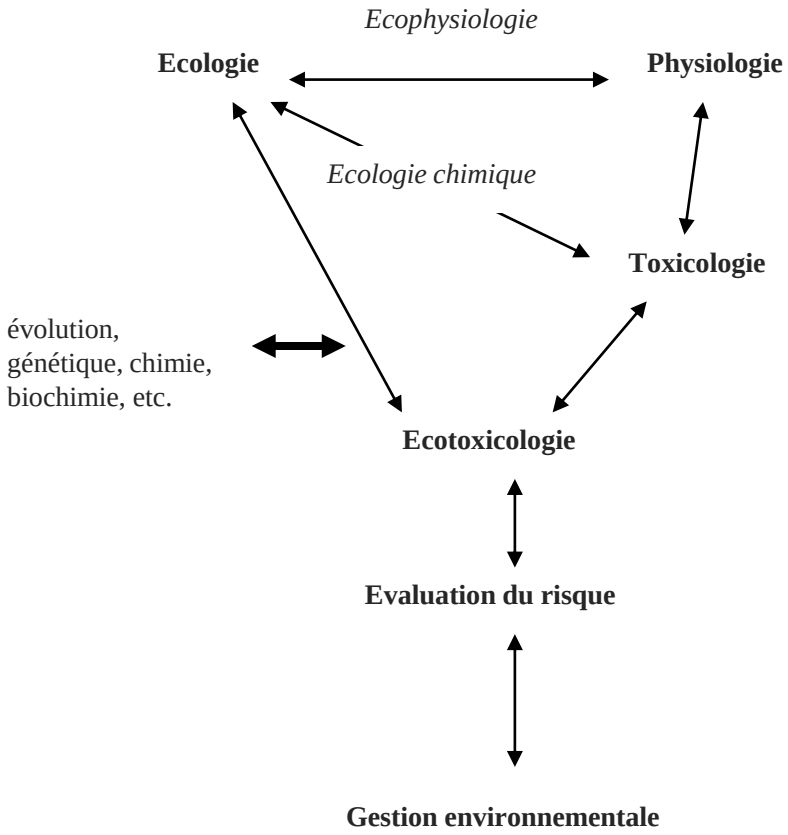


Hierarchical organization of topics in ecotoxicology relative to ecological relevance, general tractability, ability to assign causation, general use of knowledge, temporal context of consequence, and temporal sensitivity of response

L'information venant de tous les niveaux pertinents de l' organisation biologique doit être utlisée simultanément

Les outils de l'écotoxicologue



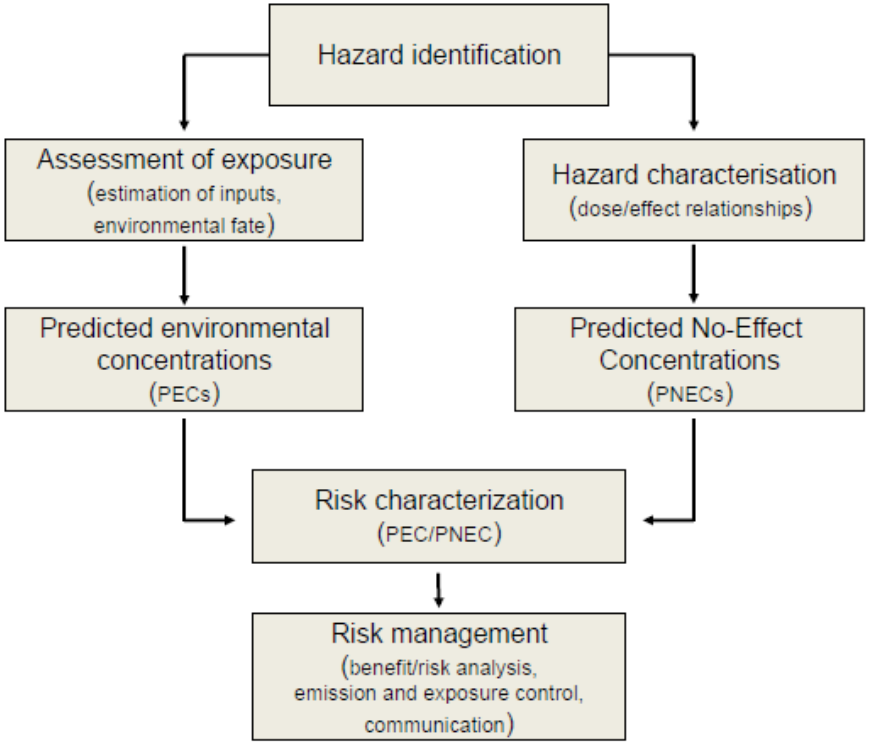


Relations de l'écotoxicologie avec les autres disciplines

Approche pluridisciplinaire, holistique

Evaluation du risque environnemental

Approche générale



Caractérisation de l’exposition

Evaluation PEC locale et régionale

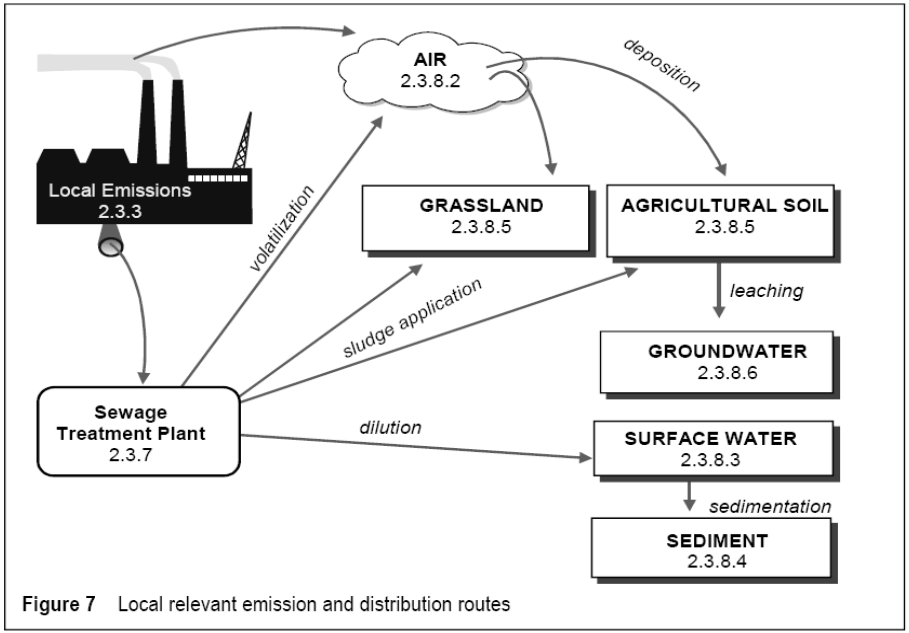


Figure 7 Local relevant emission and distribution routes

Figure 7 shows the relationship between the local emission routes and the subsequent distribution processes, which may be relevant for the different environmental compartments. For each compartment, specific fate and distribution models are applied.

On the regional scale the region under consideration is viewed as a box, consisting of several,homogeneous compartments.

All flows of the substance between the different compartments (and with the outside world) are quantified.

Evaluation PEC milieu aquatique

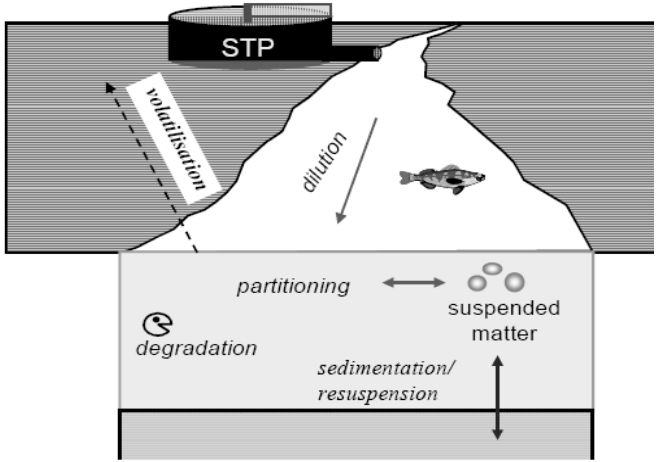


Figure 9 ***Fate processes in surface water***

$$Clocal_{water} = \frac{Clocal_{eff}}{(1 + Kp_{susp} \cdot SUSP_{water} \cdot 10^{-6}) \cdot DILUTION} \qquad (45)$$

Explanation of symbols

$Clocal_{eff}$	concentration of the substance in the STP effluent	$[mg \cdot l^{-1}]$	eq. (33)
Kp_{susp}	solids-water partitioning coefficient of suspended matter	$[l \cdot kg^{-1}]$	eq. (23)
$SUSP_{water}$	concentration of suspended matter in the river	$[mg \cdot l^{-1}]$	15
DILUTION	dilution factor	[-]	10
$Clocal_{water}$	local concentration in surface water during emission episode	$[mg \cdot l^{-1}]$	

$$DILUTION = \frac{EFFLUENT_{stp} + FLOW}{EFFLUENT_{stp}} \quad (46)$$

Explanation of symbols

$EFFLUENT_{stp}$	effluent discharge rate of stp	[l·d⁻¹]	eq. (34)
FLOW	flow rate of the river	[l·d⁻¹]	data set
DILUTION	dilution factor at the point of complete mixing	[-]	(max. = 1000)

For indirect human exposure and secondary poisoning, an annual average concentration in surface water is calculated:

$$Clocal_{water,ann} = Clocal_{water} \cdot \frac{Temission}{365} \quad (47)$$

Explanation of symbols

$Clocal_{water}$	local concentration in surface water during emission episode	[mg·l⁻¹]	eq. (45)
Temission	number of days per year that the emission takes place	[d·yr⁻¹]	App. IB
$Clocal_{water,ann}$	annual average local concentration in surface water	[mg·l⁻¹]	

The concentration at the regional scale ($PECregional_{water}$) is used as background concentration for the local scale. Therefore, these concentrations are summed:

$$PEClocal_{water} = Clocal_{water} + PECregional_{water} \quad (48)$$

$$PEClocal_{water,ann} = Clocal_{water,ann} + PECregional_{water} \quad (49)$$

Explanation of symbols

$Clocal_{water}$	local concentration in surface water during episode	[mg·l⁻¹]	eq. (45)
$Clocal_{water,ann}$	annual average concentration in surface water	[mg·l⁻¹]	eq. (47)
$PECregional_{water}$	regional concentration in surface water	[mg·l⁻¹]	2.3.8.7
$PEClocal_{water}$	predicted environmental concentration during episode	[mg·l⁻¹]	
$PEClocal_{water,ann}$	annual average predicted environmental concentration	[mg·l⁻¹]	

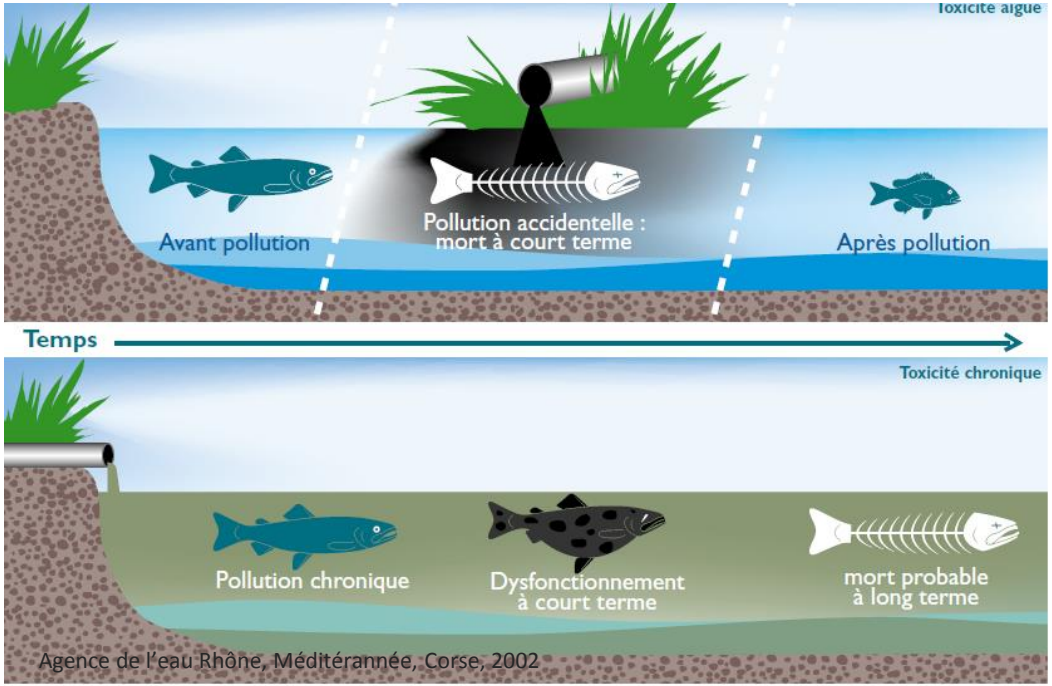
Utilisation des PEC calculées et /ou mesurées

Caractérisation du danger

Effets aigus et chroniques

Effets des polluants

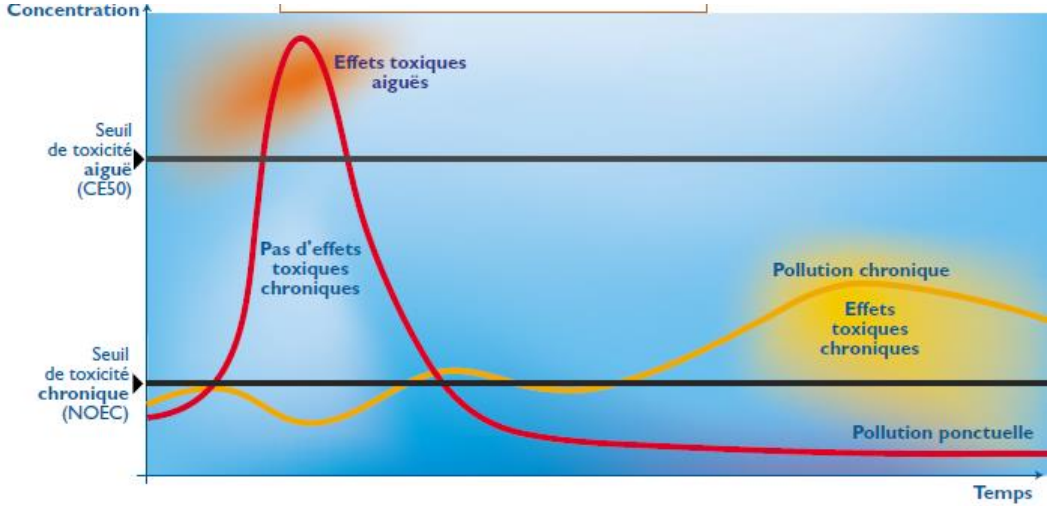
Pollution chronique ou aiguë : une toxicité différente



Agence de l'eau Rhône, Méditérannée, Corse, 2002

- Pour **évaluer la toxicité d'une substance, des tests de laboratoire standardisés sont utilisés**
- Le principe est de déterminer à quelle concentration une substance est toxique sur un organisme, afin d'appréhender les effets de cette substance sur les populations du milieu.
- Des organismes vivants sont mis en contact avec les substances à tester et les effets de cette exposition sont observés.
- Pour une évaluation correcte de la toxicité, il est nécessaire d'effectuer ces tests sur plusieurs organismes de la chaîne trophique (bactéries, algues, daphnies (microcrustacés), poissons…).

Principes des toxicités aiguës et chroniques



- Le danger d'une substance est **sa toxicité intrinsèque**
- Deux types de toxicité sont distinguées :
  - **La toxicité aiguë :** les effets sont rapides et généralement mortels.
  - **La toxicité chronique :** les effets apparaissent après une exposition prolongée à la substance, mais sont imperceptibles sur une courte échelle de temps.

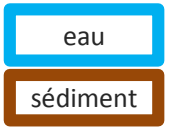
Agence de l'eau Rhône, Méditérannée, Corse, 2002

Essais (éco)toxiques

Organismes isolés

Réalisme écologique faible
Tests peu onéreux

Bactérie, Algue (ISO11348, OECD 201)



Daphnie (OECD 202, 211)

Ostracode (kit)

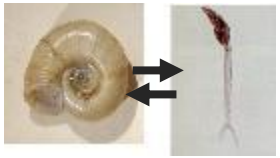
Poisson (OECD 203, 204, 212, 236)

Chironome (OECD 219)

Organismes en interaction

Réalisme écologique + important
Tests ± onéreux

Prédation

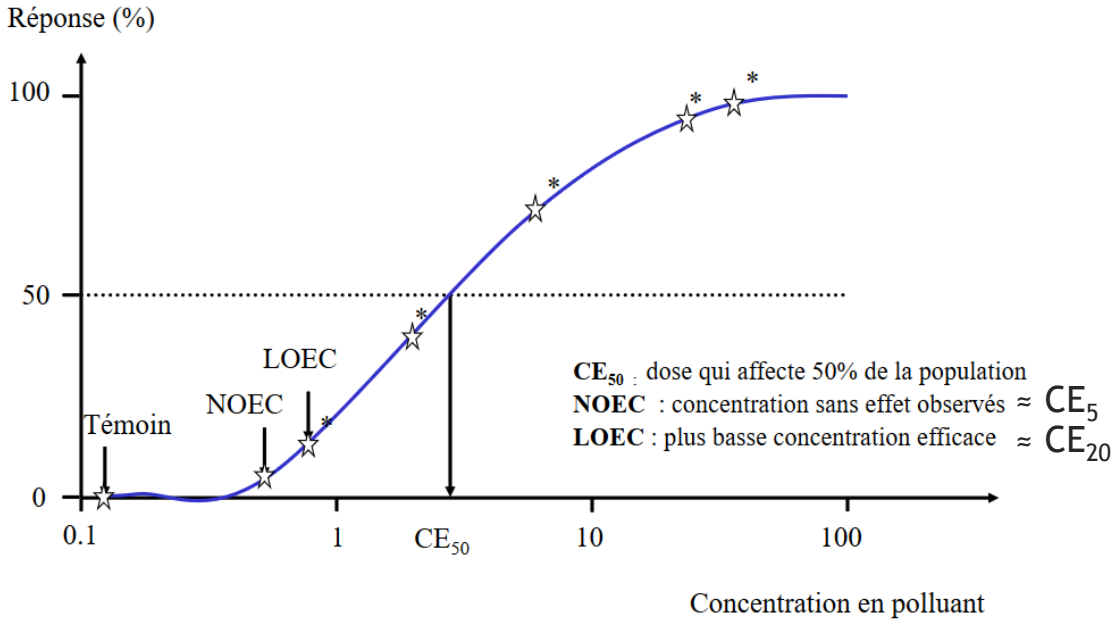


Parasitisme

Compétition

Evaluation de la toxicité

Courbe théorique dose réponse :



Calcul de la PNEC = HC5/AF (avec 1 < AF < 5)

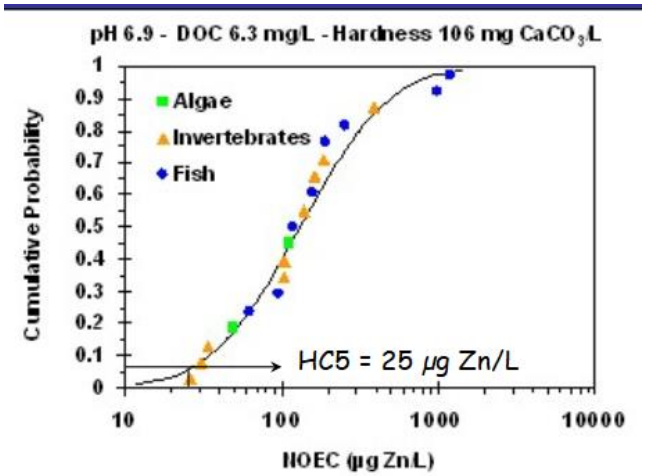
- **NOEC + Facteurs d'extrapolation (=sécurité)**

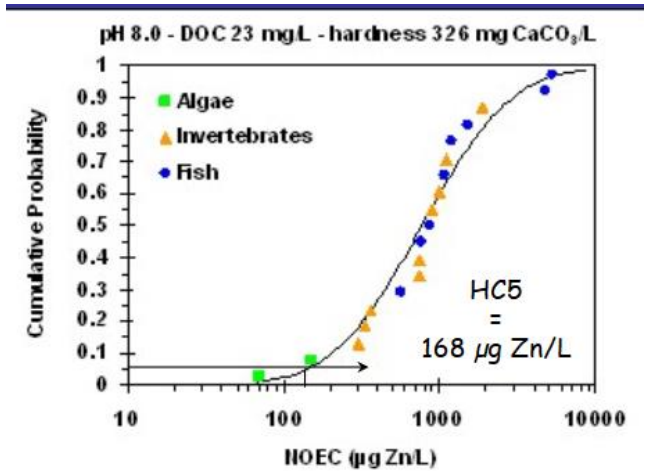
ou

- **Extrapolation statistique basée sur percentile SSD / facteur de sécurité (1 - 5)**

Distribution de la sensibilité de espèces

Hypothèse : la sensibilité de l'écosystème dépend de l'espèce la plus sensible





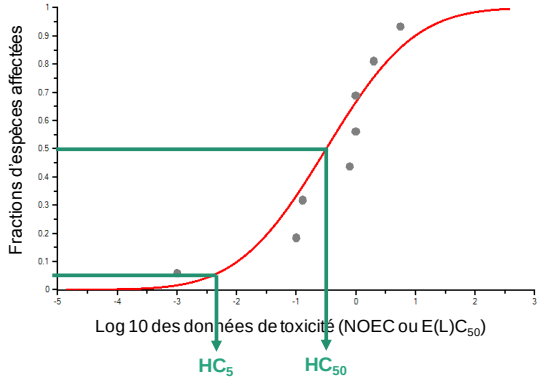
Utilisation des données de toxicité (e.g. NOEC)
Modèle de distribution statistique SSD
↳ Calcul HC_5 (conc. à laquelle 5% des espèces sont affectées)

Limite : valable pour un exposome donné → importance de la biodisponibilité

Distribution de la sensibilité de espèces

– Utilisation pour déterminer des classes d'évaluation de l'état écologique des masses d'eaux

Par exemple :



- Méthode statistique					
0	**1**	**2**	**3**	**4**	**5**
Indét.	**Très bon**	**Bon**	**Moyen**	**Médiocre**	**Mauvais**
<LQ	**< 0,1 HC5**	**0,1 HC5 - 0,2 HC5**	**0,2 HC5 - HC5**	**HC5 - HC50**	**>= HC50**

Table 16 Assessment factors to derive a $PNEC_{aquatic}$

Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels of the base-set (fish, Daphnia and algae)	1000 [a]
One long-term NOEC (either fish or Daphnia)	100 [b]
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50 [c]
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10 [d]
Species sensitivity distribution (SSD) method	5-1 (to be fully justified case by case) [e]
Field data or model ecosystems	Reviewed on a case by case basis [f]

Evaluation de l'exposition :

-Taux d'émission

-Distribution et

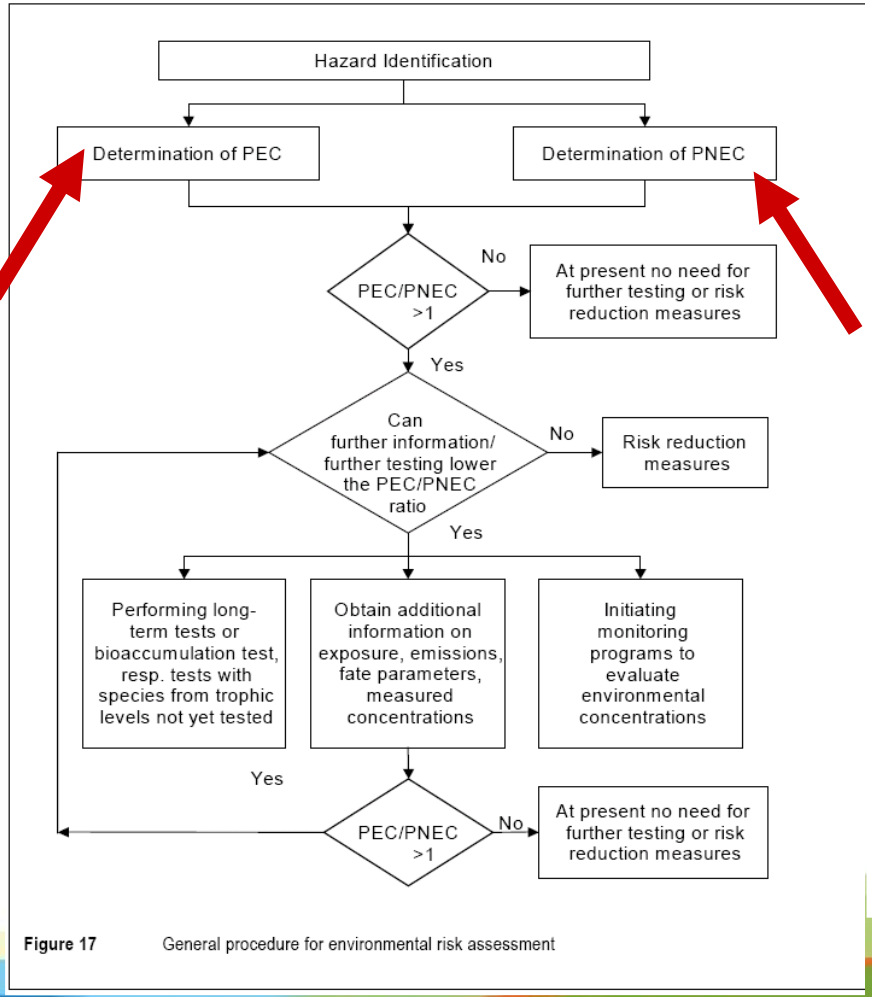


Figure 17 General procedure for environmental risk assessment

Evaluation des effets :

-Données toxicité une seule espèce

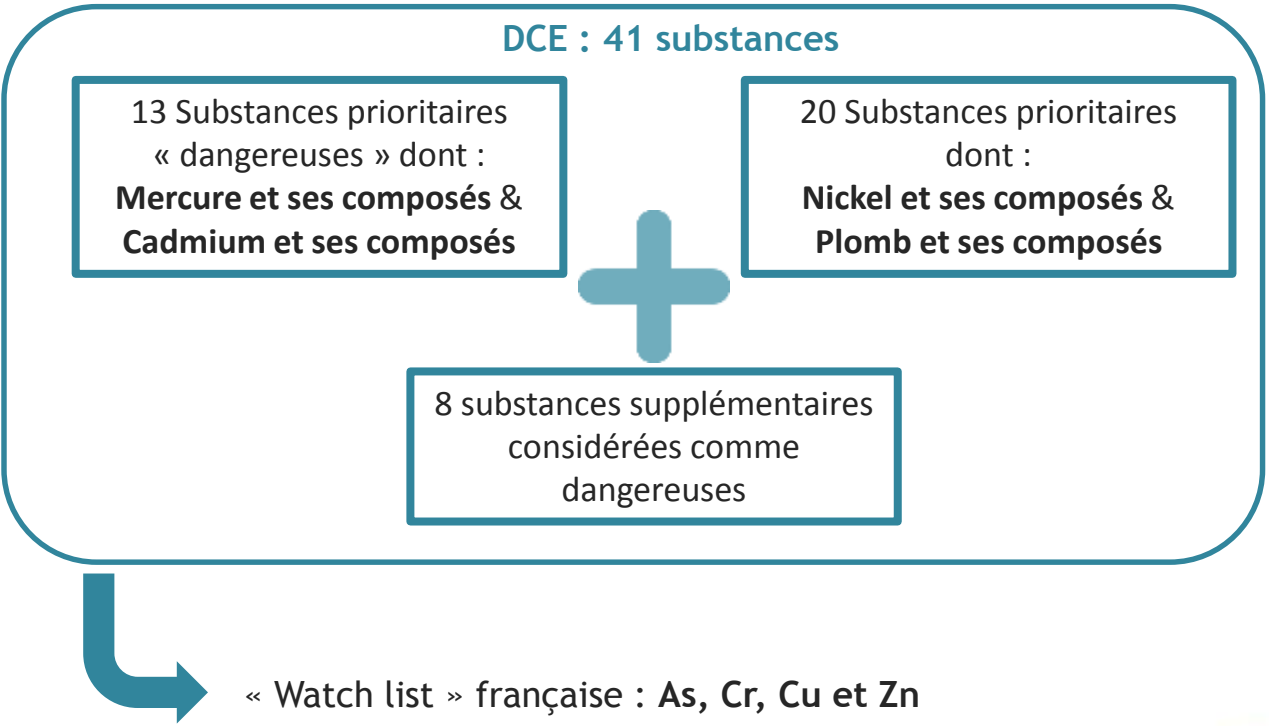
-Extrapolation et

Evaluation possible :

-Utilisation facteurs d'évaluation ou

-extrapolation statistique

Définition des NQE



La stratégie de lutte contre la pollution chimique définie dans la directive cadre sur l'eau (DCE) se concentre sur une liste restreinte de substances considérés comme prioritaires.

Une première liste a été définie lors de la rédaction de la DCE en 2000, révisable tous les quatre ans.

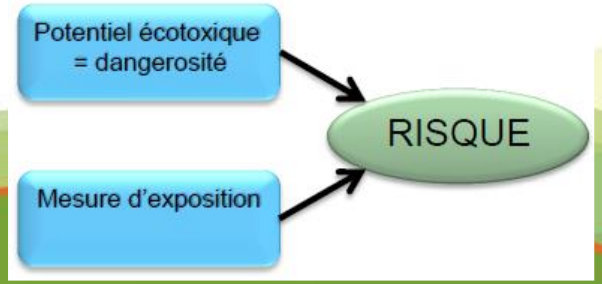
Des critères de qualité appelés normes de qualité environnementales (NQE) sont fixés.

Qu'est ce qu'une NQE?

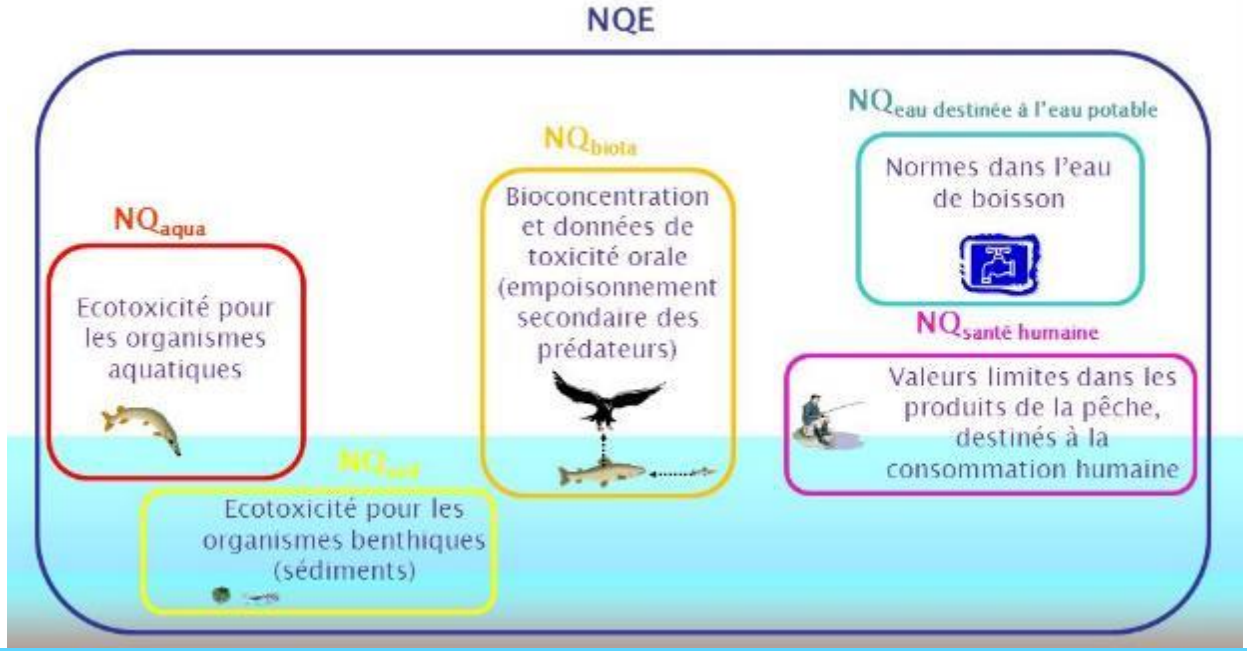
Normes de Qualité Environnementale (NQE) selon le texte de la DCE :
« *concentration d'un polluant ou d'un groupe de polluant dans l'eau, les sédiments ou le biote qui ne doit pas être dépassée, afin de protéger la santé humaine et l'environnement* »

La NQE a donc deux composantes :
- une composante environnementale qui permet de protéger les organismes du milieu aquatique d'un effet toxique direct ou par empoisonnement secondaire (ingestion de nourriture contaminée)
- une composante sanitaire qui permet de protéger la santé humaine de la toxicité des substances sur l'eau brute destinée à la consommation humaine ou d'un empoisonnement secondaire par consommation d'organismes éventuellement contaminés.

NQE = exprimée sous forme de moyenne annuelle,
ou de concentration maximale admissible
Approche basée sur l'appréciation d'un risque



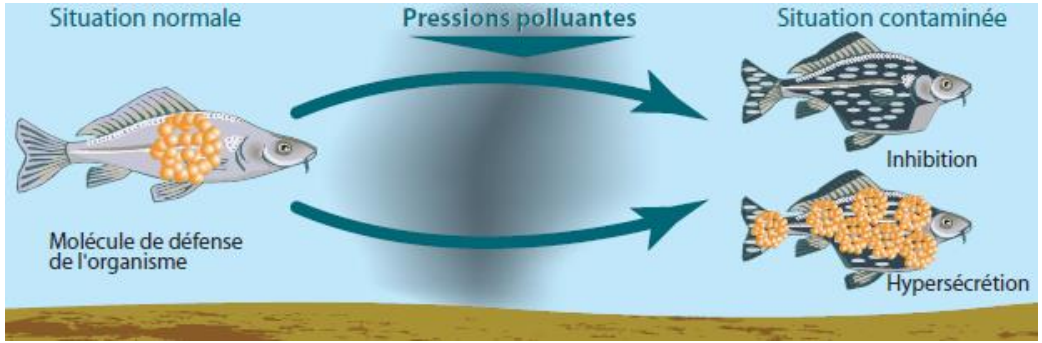
Norme de Qualité Environnementale (NQE)



NQE = min (QS water_eco, QS sediment, QS biota_sec pois, QS biota_hh, QS dw-_hh)

Evaluation de la toxicité - Biomarqueurs

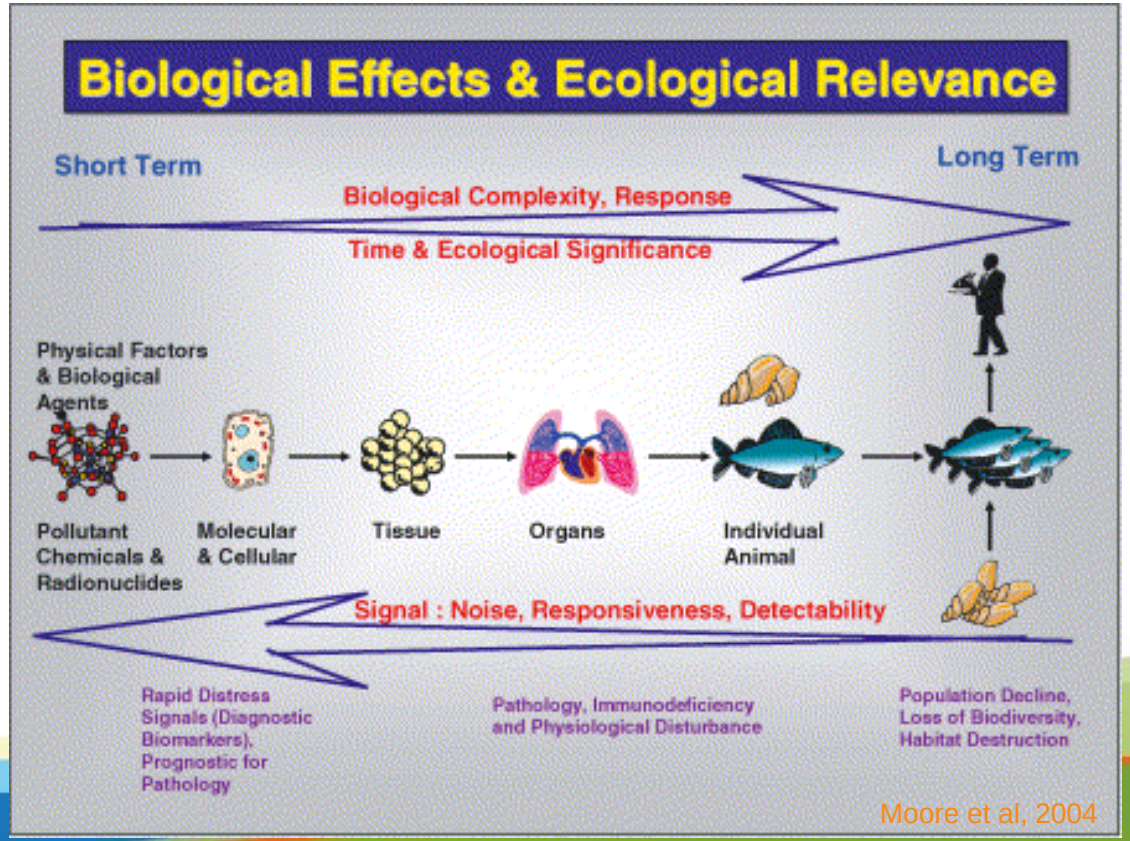
Concept de **biomarqueurs** : « réponses biochimiques, cellulaires, physiologiques ou comportementales observables et mesurables » (Depledge, 1994)



- Principe d'un biomarqueur : sa concentration dans l'organisme reflète que celui-ci est exposé à un polluant
- On distingue deux types de biomarqueurs :
  - **les biomarqueurs d'exposition**, qui signent l'activation de mécanismes de régulation, d'adaptation et de défense
  - **les biomarqueurs d'effet**, qui diagnostiquent un dépassement, éventuellement transitoire des capacités de régulation de l'organisme et constituent un signal d'alerte d'apparition de perturbations du fonctionnement de l'organisme, voire de sa population

Agence de l'eau Rhône, Méditérannée, Corse, 2002

A theoretical visualization of the relationships between ecological relevance and time-scales of pollutant-induced biomarker responses



Exemples de biomarqueurs
(poissons, invertébrés et périphyton)

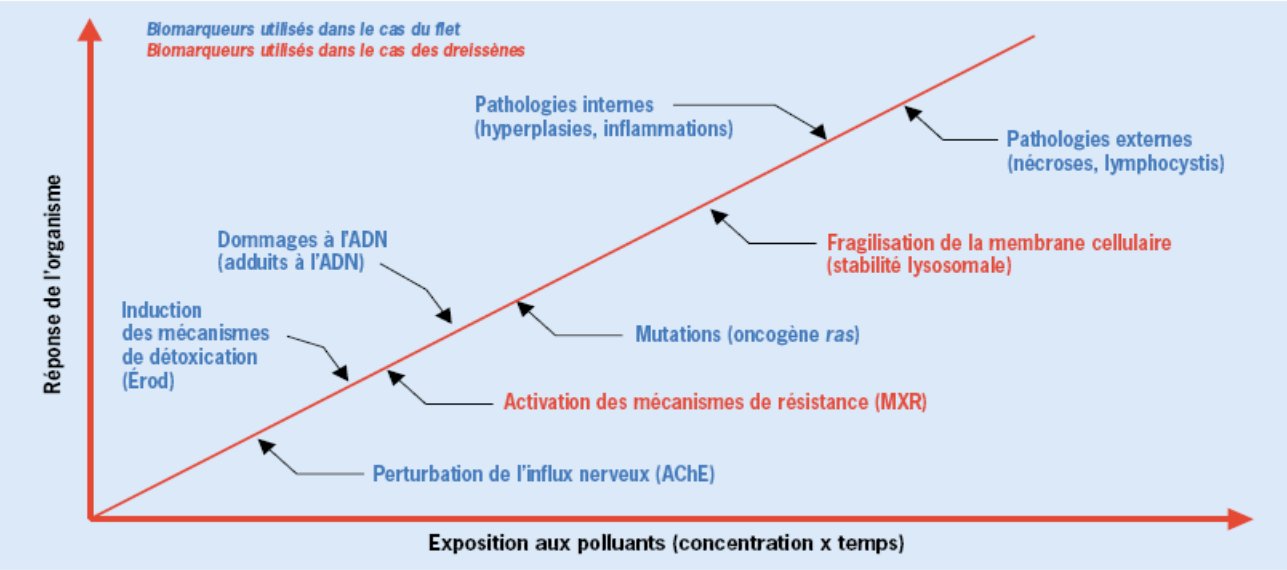
	Biomarqueurs	Réponse	Substances chimiques
Biomarqueurs d'exposition	CYP 1A1, expression et activité EROD	Induction	Pesticides organochlorés PCBs, dioxines, HAPs
	BaP hydroxylase	Induction	BaP
	Glutathion-S-transferase	Induction	Non spécifique
	Acetyl cholinesterase*	Inhibition	Pesticides (organophosphorés, carbamates)
	Métallothionéines / Phytochélatines	Induction	Métaux
	Protéines de stress	Induction	Métaux , autres xénobiotiques ?
	Dommage ADN, adduits ADN*	Occurrence	mutagènes, génotoxiques, ?
	Fluorescence spécifique	Inhibition	Non spécifique
Biomarqueurs d'effet	Dommage ADN	Occurrence	mutagènes, génotoxiques ?
	Acetyl cholinesterase	Génotoxiques Inhibition	Pesticides (organophosphorés, carbamates)
	Peroxydation lipidique	Induction	Non spécifiques
	Enzyme antioxydant	Induction/Inhibition	Non spécifiques
	Intégrité lysosomiale	diminution	Non spécifiques

Biomarqueurs biochimiques et cellulaires utilisés couramment en écotoxicologie pour une recherche d'exposition et /ou d'effet. D'après Vasseur et Cossu-Leguille 2003, In Garric 2009

- exemples de biomarqueurs mis en œuvre actuellement dans le cadre d'étude ponctuelle de la qualité des milieux aquatiques d'eau douce ou marines, avec leurs caractéristiques en terme de pistes d'interprétation quant à la nature chimique du stresseur

A ce jour, la mise en œuvre et l'interprétation de ces outils nécessitent une compétence appropriée, et ils ne disposent pas encore, sauf pour la mesure de la mesure de l'activité EROD sur poisson, de protocoles opératoires en eau douce.
Pour le milieu marin, au contraire des procédures standards de mise en œuvre ont été élaborées dans le cadre de la convention OSPAR 2007.

Exemple d'étude intégrant des biomarqueurs : programme Seine -Aval



Biomarqueurs suivis dans les flets et dans les dreissènes.

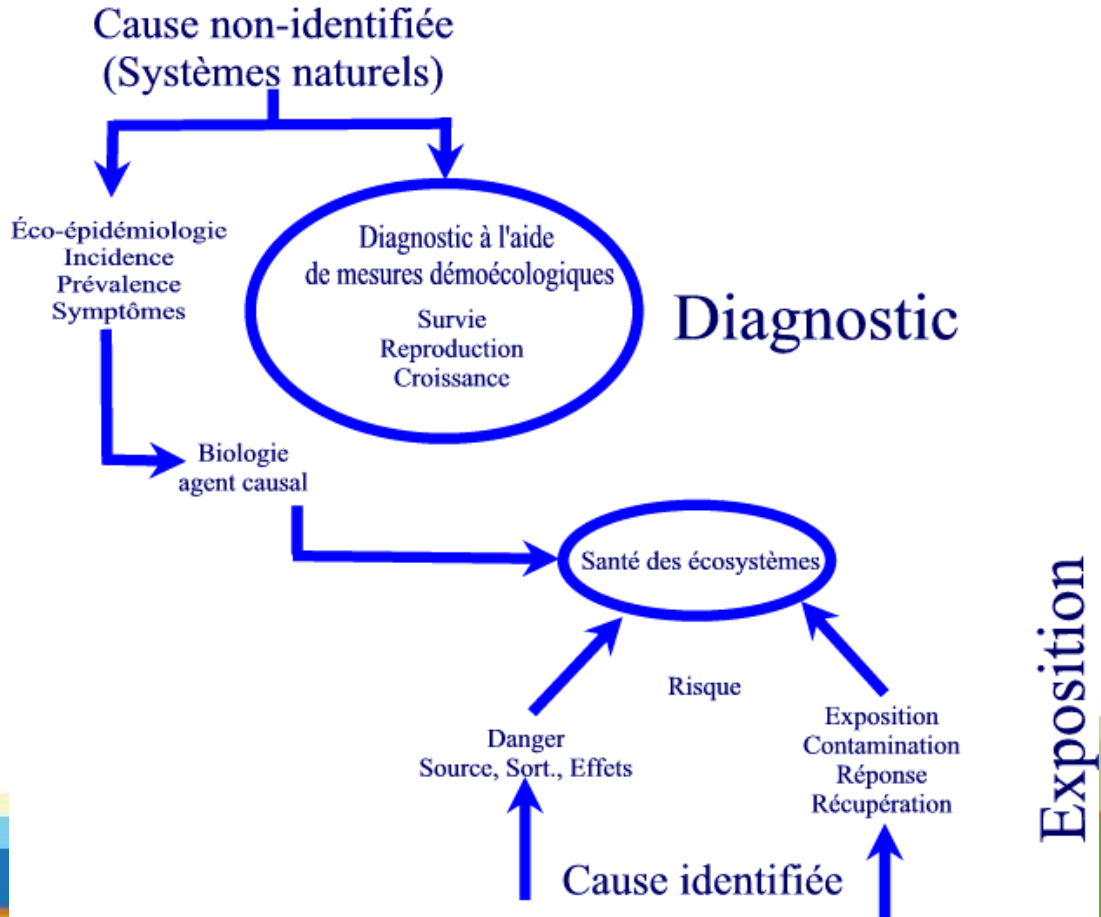
Ifremer 2000, N°12

Evaluation de la toxicité de métaux - Biomarqueurs

- évaluation de l'état de santé des bioindicateurs
- image dynamique des variations des quantités de polluants bio disponibles (molécules mères et produits de dégradation)
- Evaluation intégrée dans le temps et l'espace des polluants biodisponibles, en termes de présence mais aussi d'effets

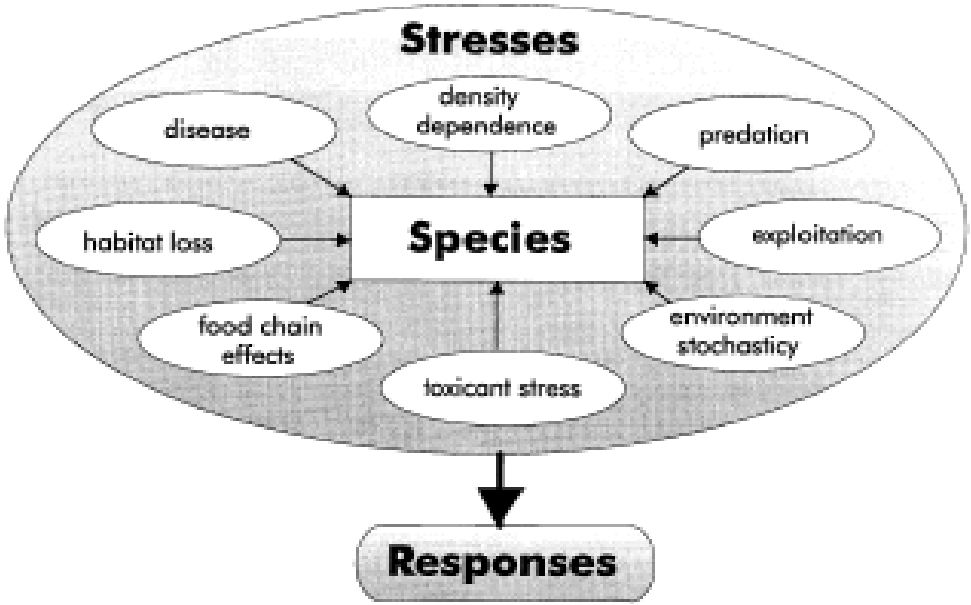
- Interférence avec d'autres facteurs de l'environnement :
  - -caractéristiques physico-chimiques du milieu
  - -relations interindividuelles et/ou interspécifiques
  - -particularités génétiques des espèces
  - -interactions entre polluants
- Problème de choix sites de référence, mais solution références relatives (sites de niveaux de contamination différents, organismes en conditions contrôlées)

Observation et interprétation des effets : deux approches opposées et complémentaires pour évaluer la santé des écosystèmes



(d'après Bernes, 1999).

Point sur les Effets cocktails



2. Contaminants with similar or different Mode of Action (MoA) can influence each other's toxicity;

resulting in an almost unlimited number of possible additive, synergistic or antagonistic combinations.

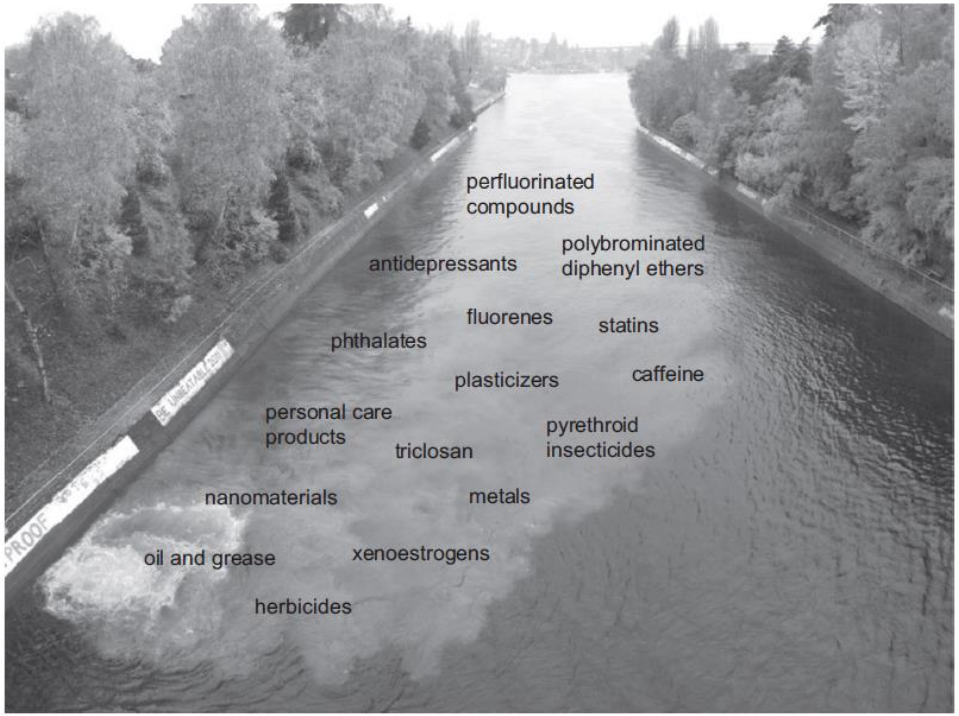
The aspects of combined effects have not yet been implemented in ERA in a standardised manner, nor has the combined effect issue become an integrated part of chemical regulation edicts

1. The complexity of stress/response relationships.

The dose/ response paradigm, although necessarily simple for experimental practice, does not adequately account for the multiple, simultaneous stressors to which all species are subjected in natural environments.

Adapted from Power and McCarty (1997), in van der Oost et al (2003)

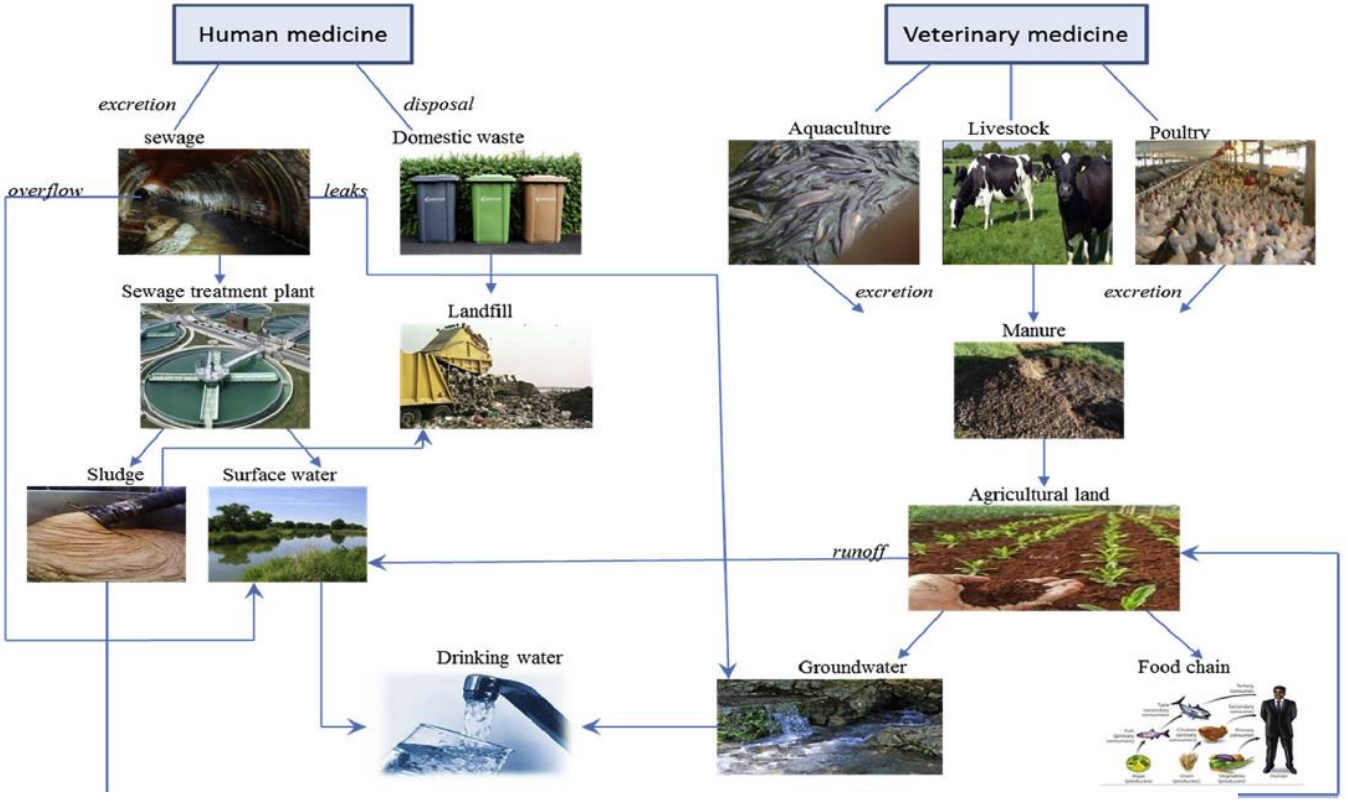
Contaminant exposure scenario : Mixture



Combined inputs of stormwater and untreated sewage to an urban waterway in Seattle, Washington, during a period of high rainfall. Subsurface discharge from a combined sewer overflow (CSO) is evident in the lower left.

Photo by Blake Feist, National Oceanic and Atmospheric Administration (NOAA). In Amiard-Triquet et al 2015

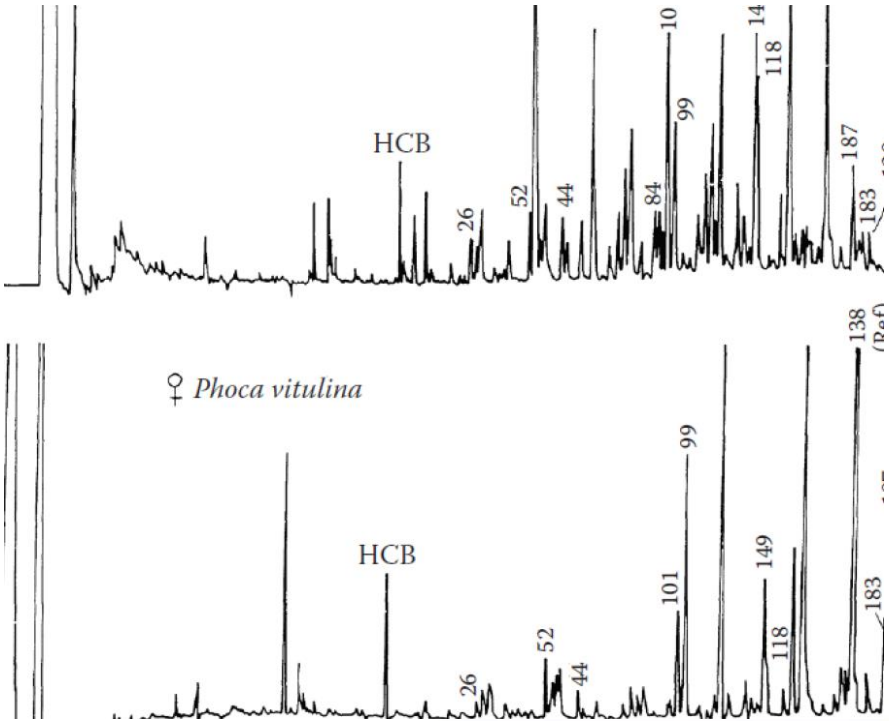
Illustration des sources de contamination environnementale pour les PPCPs



PPCPs : Pharmaceutiques et produits de soin personnels

Ebele et al, 2017

Determination of Toxicities of Mixtures : context

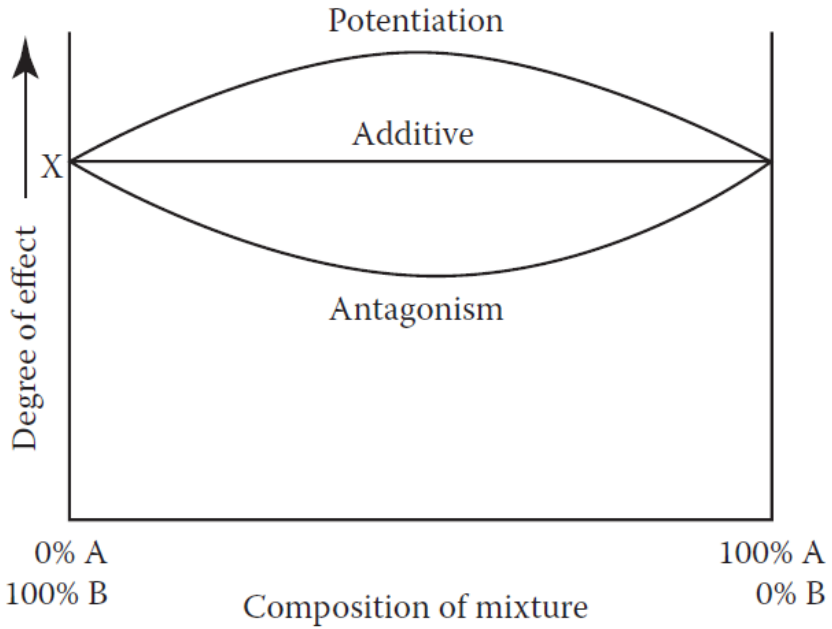


Example of complexity of the situation : residues of PCBs in tissues of organisms from a polluted area

- A number of different PCB congeners are found in both species, with a wider selection in mollusks than in harbor seals
- When effluents or contaminated environmental samples are subjected to testing, toxicity is often caused by more than one chemical component of a mixture, and questions arise concerning possible potentiation

PCB congeners in tissues of marine organisms [mussels (Macoma baltica) and harbor seals (Phoca vitulina)] from the Dutch Wadden Sea.
The compounds were separated, identified, and quantified by capillary gas chromatography. Each of the numbered peaks represents a PCB congener. HCB (hexachlorobenzene) served as an internal standard. Walker 2012 Principles of Ecotoxicology, Fourth Edition

Interactive Effects of Pollutants



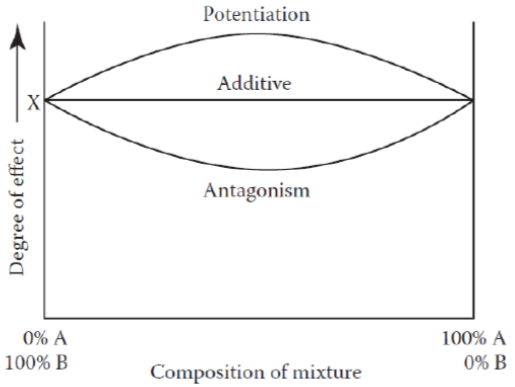
- **Additive Effects:** The toxicity of a mixture is often roughly equal to the total toxicity values of its individual components.
- **Antagonism**: the combined effect is less than the sum of the individual effects
- **Potentiation (Synergism):** the combined effect is more than additive

Potentiation of toxicity. The vertical axis indicates the degree of toxic effect of the compound, and the horizontal axis represents the composition of the mixture. The maximum doses of compounds A and B both yielded the same degree of toxic response *X*. Potentiation occurs when the toxicity of a mixture of two compounds exceeds the total toxicities of the individual components. (*Source:* Moriarty, F. (1999). *Ecotoxicology*, 2nd ed. Academic Press. With permission.)

Additive effects : theory

- The toxicity of a mixture is often roughly equal to the total toxicity values of its individual components
- In other words, each chemical expresses roughly the same toxicity in a mixture as it would when tested alone
- Where no evidence indicates potentiation or antagonism, estimates of the toxicity of a mixture can be made by adding together the expected contributions from each component
- The toxicity of each component in a mixture depends on its concentration and can be estimated from a dose–response curve, e.g., a percentage of mortality from an LD50 plot
- Thus a mixture containing three components at concentrations that would, if tested individually, cause 5, 10, and 15% mortality would be expected to cause 30% mortality overall if toxicity were simply additive
- Clearly, where potentiation or antagonism exist, the estimated toxicity will differ markedly from the measured toxicity

Interactive effects of pollutants



The maximum doses of compounds A and B both yielded the same degree of toxic response *X*.
Potentiation occurs when the toxicity of a mixture of two compounds exceeds the total toxicities of the individual components.

Potentiation (Synergism) : the toxic effect should be greater than expected

Sometimes the term synergism is used to describe this phenomenon.

However, many scientists restrict the use of this term to situations in which only one of two components is present at a level that can cause a toxic effect and the other compound (synergist) would have no effect if applied alone.

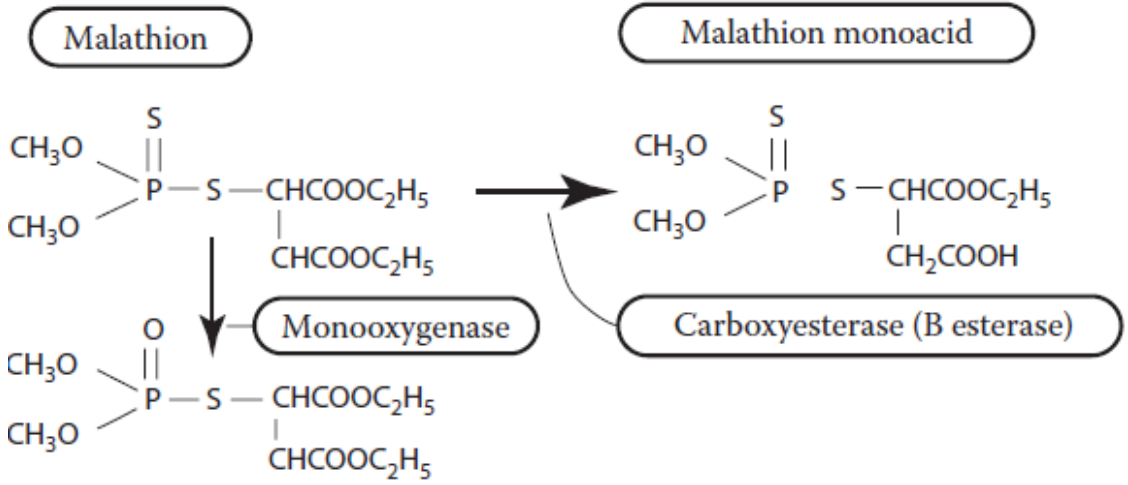
Potentiation of toxicity

- Care is needed when deciding whether toxic effects of combinations of chemicals are truly greater than additive.
- In the first place, because of errors in measurement, interest is confined to situations where toxicity is *substantially* greater than additive (e.g., where SRs exceed 2).
- Smaller differences usually reflect no more than the compounding of errors
- The question of interactive effects is also complicated by the determination of end point used for measuring toxicity.
- For example, in tests with the springtail *Folsomia candida* (Collembola), Van Gestel and Hensbergen (1997) found that mixtures of cadmium and zinc were antagonistic for growth but additive with respect to reproduction when comparison was made with the effects produced by the metals administered singly

Problems of potentiation

- The identification of combinations of pollutants that give rise to problems of potentiation might seem an impossible task
- However, there are guidelines that aid the recognition of such combinations
- In particular, recent rapid advances in biochemical toxicology have given more insight into the potentiation of toxicity due to interactions at the toxicokinetic level
- When one compound (A) causes a change in the metabolism of another (B), two types of interaction are recognized which lead to potentiation of toxicity:
  - 1. Compound A inhibits an enzyme system that detoxifies compound B. Thus the rate of detoxication of B is slowed down because of the action of A.
  - 2. Compound A induces an enzyme system that activates compound B. Thus the rate of activation of B is speeded up because of the action of A.

EXAMPLE OF POTENTIATION FROM INHIBITION OF DETOXIFICATION



Metabolism of malathion.
Malathion is detoxified by the action of a carboxyesterase but activated by monooxygenase. Toxicity depends on the relative importance of these competing enzymes. Chemicals that induce the monooxygenase system can make malathion more toxic.

Top-down evaluation of chemical mixture effects

- Strategies have been developed that use biological responses to direct the identification of causal agents in chemical mixtures
- The most relevant of these are Effect-Directed Analysis (EDA) and Toxicity Identification & Evaluation (TIE).
- The EDA procedure includes a combined use of chemical fractionation, sequential bioassay and subsequent chemical analyses and builds on the assumption that toxicity can be assessed for separated classes of chemicals or for matrices deprived of specific classes of chemicals

Top-down evaluation of chemical mixture effects

- The TIE procedures were developed by US EPA, as one of the first standardised EDA procedures, and mainly used for identification and evaluation of contaminants in aqueous samples
- Basically, the concept in TIE is to remove groups of compounds with certain properties (e.g. organics, metals, ionic and non-ionic compounds) from a test matrix until the toxicity of the sample disappears.
- Then, suspected chemicals are identified by analytical chemistry, and lastly their toxicity is confirmed by means of the same bioassay as used in the initial toxicity characterization phase

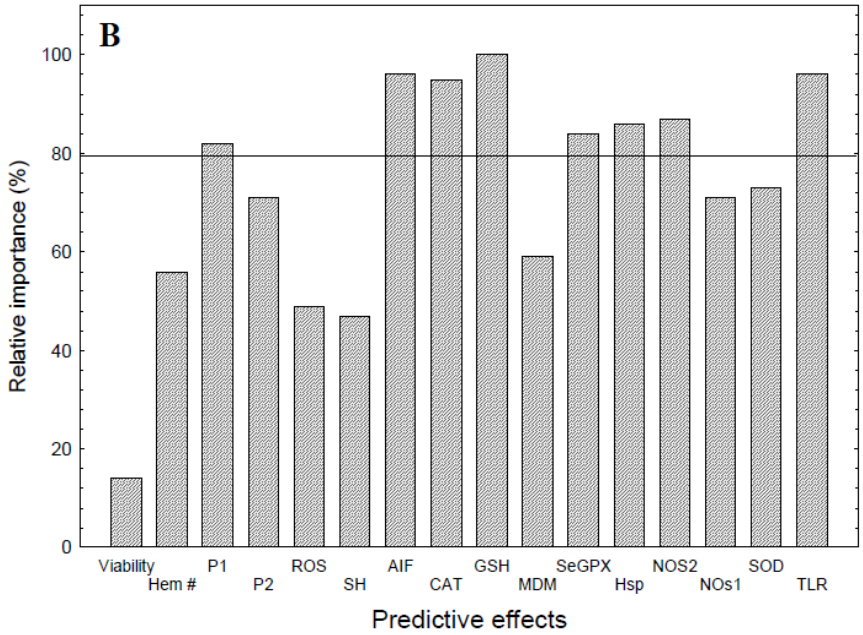
Limitations

- However, both EDA and TIE approaches have limitations with regard to assessing the nature and magnitude of combined toxicity, such as additivity of chemical mixtures, synergism and antagonism.
- In connection with a top-down study approach, the involvement of ecotoxicity tests and biomarkers might represent a means for identifying the major targets for toxicity, for quantifying the adverse effect of concern and for defining “key events” along the sequence of biological responses leading to certain toxicological endpoints.

Effects of short-term exposure to environmentally relevant concentrations of different pharmaceutical mixtures on the immune response of the pond snail Lymnaea stagnalis

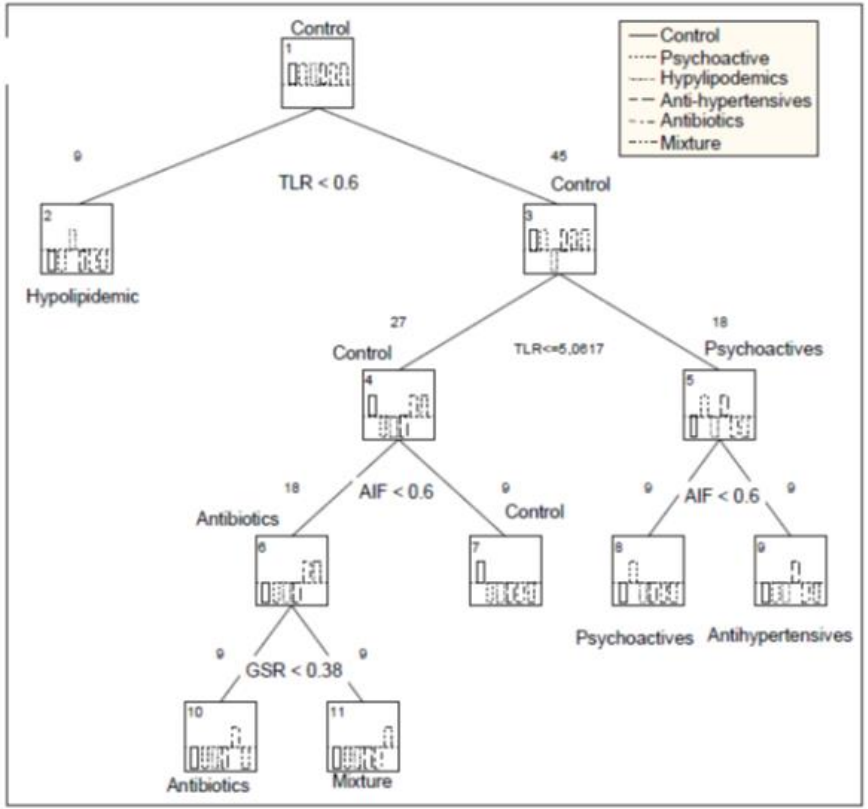
- Four drug mixtures were tested, and regrouped pharmaceuticals by main therapeutic use:
  - psychiatric (venlafaxine, carbamazepine, diazepam),
  - antibiotic (ciprofloxacine, erythromycin, novobiocin, oxytetracycline, sulfamethoxazole, trimethoprim),
  - hypolipemic (atorvastatin, gemfibrozil, benzafibrate) and
  - antihypertensive (atenolol, furosemide, hydrochlorothiazide, lisinopril).
- Their effects were then compared with a treated municipal effluent known for its contamination, and its effects on the immune response of Lymnaea stagnalis.

Gust et al 2012. Science of The Total Environment 445–446, 1



Adult L. stagnalis were exposed for 3 days to an environmentally relevant concentration of the four mixtures individually and as a global mixture.

Effects on immunocompetence (hemocyte viability and count, ROS and thiol levels, phagocytosis) and gene expression were related to the immune response and oxidative stress:
catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), Selenium-dependent glutathione peroxidase (SeGPx), two isoforms of the nitric oxide synthetase gene (NOS1 and NOS2), molluscan defensive molecule (MDM), Toll-like receptor 4 (TLR4), allograft inflammatory factor-1 (AIF) and heat-shock protein 70 (HSP70).



Analyse en arbre décisionnel des réponses immunes de la limnée L'arbre décisionnel montre la discrimination des classes thérapeutiques par la réponse immunitaire,

Les effets observés, suite à l'exposition au mélange global, sont du même type que ce qui est obtenu avec l'effluent, ce qui suggère que les médicaments sont responsables en partie des effets de l'effluent.

Ainsi, en faisant une analyse en arbre décisionnel, les effets du mélange complexe sont le plus proches de celui de l'effluent.

De plus, le mélange d'antibiotiques est celui qui provoque des réponses les plus proches de celles du mélange global.

Bottom-up evaluation of chemical mixture effects

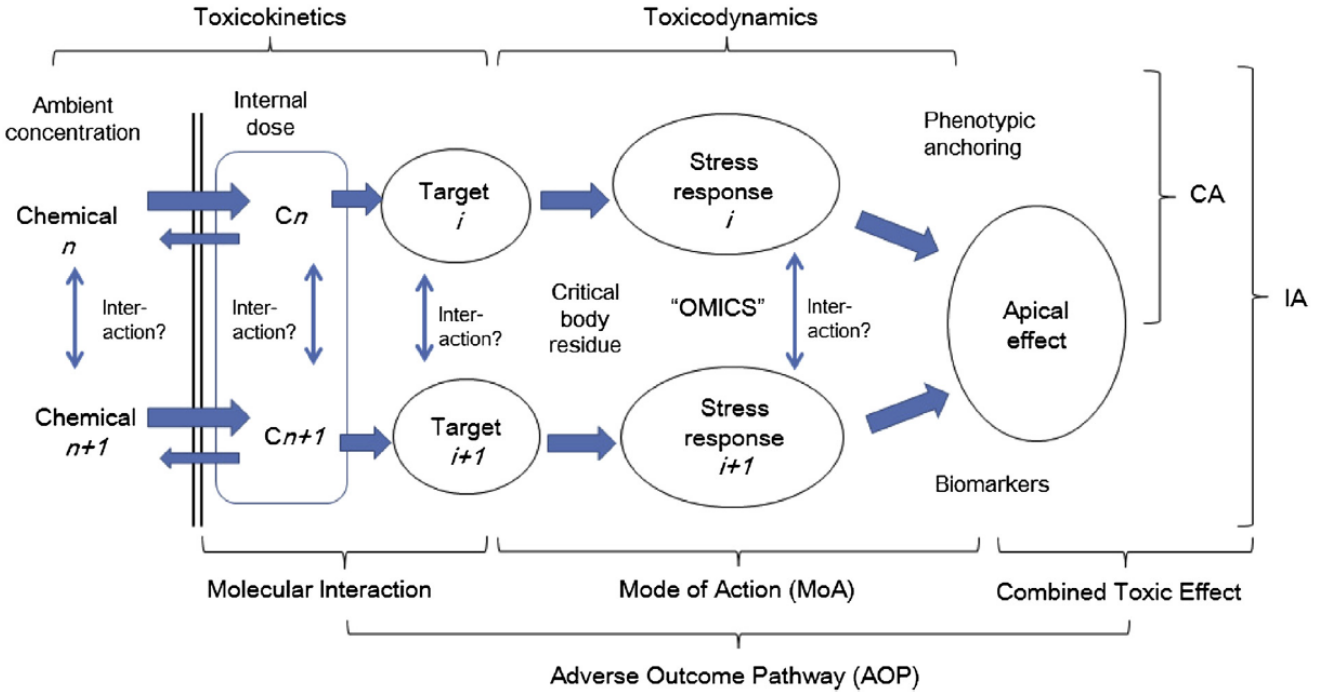
- Toxicants contributing to combined effect are thought to exert their effect along two major avenues, namely by
  - **concentration addition** (CA, also called dose addition or Loewe additivity), or by
  - **independent action** (IA, also called response additivity or Bliss independence)
- CA occurs when two or more chemicals **with similar MoA** affect the same target of toxic action (endpoint),
- whereas IA occurs when two or more chemicals affect the same endpoint but **through dissimilar MoAs**

Reference Models Used in Mixture Toxicology

		target/mechanism	
		same	different
mode of action	noninteractive	$\sum_{i=1}^{n}(c_{Si}/EC_x(Si)) = 1$ concentration addition	$X = 1 - \prod_{i=1}^{n}(1 - F_i(p_{Si}\cdot(EC_{xmix})))$ independent action
	interactive	no quantitative prediction model	no quantitative prediction model

[a]Abbreviations used: c_{Si}, concentration of substance i (Si) in the mixture; EC_x, effect concentration at the response level x; F, function describing the relation between concentration and response for the individual component; p_{Si}, fraction of substance i (Si) in the mixture; X, expected combined response; mix, mixture.

A conceptual framework of studying adverse outcome pathway (AOP) in a mixture design using toxicogenomic ("OMICS") approaches



(modified from Altenburger et al. (2012)). CA: Concentration addition; IA: Independent action In Beyer et al 2014.

Calculation of effect concentrations and prediction of mixture toxicity

- Different theoretical indices of mixture toxicity can be found in the literature and apply to quantitative assessment of the deviation of data from the CA model (Altenburger et al., 2003).
- Ex: The sum of toxic units (STU).
- A toxic unit (TU) = concentration of a compound present in the mixture with a total effect of x% divided by its individual concentration that would alone cause the same x% effect.
- All individual TU values are then summed to obtain the STU parameter.
- To avoid false positives, the STU limits distinguishing a synergistic effect from a simple addition was set at 0.8.
- Thus, $0.8 \leq STU \leq 1.2$ indicate perfect fit to the applied model and $STU < 0.8$ indicate underestimation of mixture toxicity, and $STU > 1.2$ indicate antagonism.

Joint effects of nine antidepressants on Raphidocelis subcapitata and Skeletonema marinoi: A matter of amine functional groups

Minguez et al Aquatic Toxicology 196 (2018) 117–123

Characteristics of tested antidepressants.

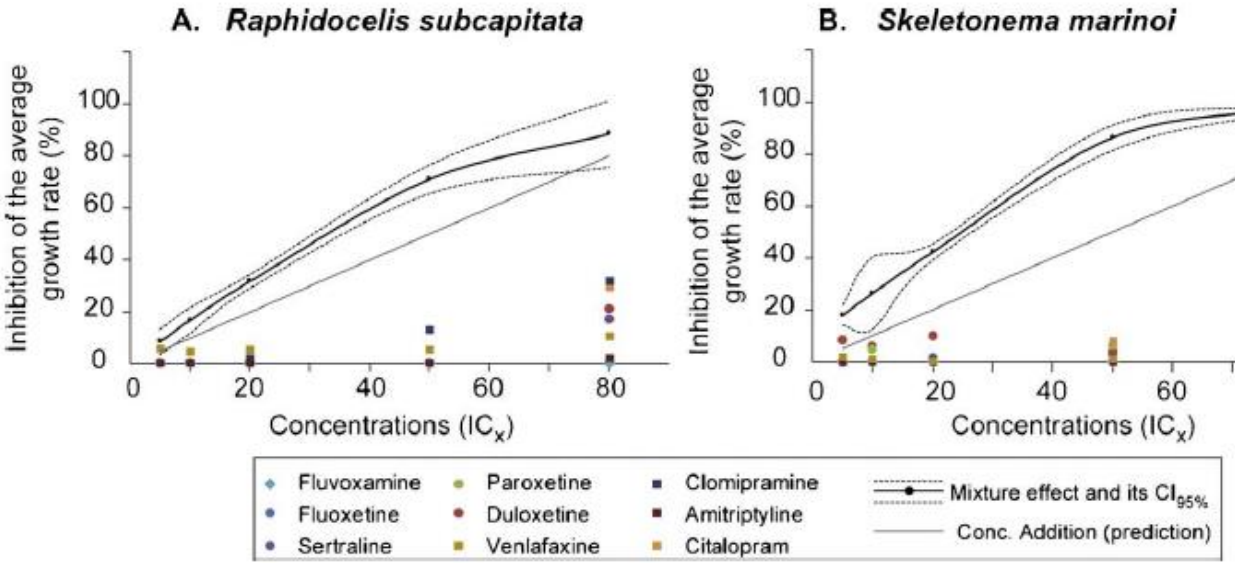
Compounds	CAS number	Description[a]	Subclass of amine	$LogK_{OW}$[b]
Fluvoxamine	61718-82-9	SSRI	Primary	2.8
Fluoxetine	56296-78-7	SSRI	Secondary	4.1
Sertraline	79559-97-0	SSRI	Secondary	5.1
Paroxetine	78246-49-8	SSRI	Secondary	3.6
Duloxetine	136434-34-9	SNRI	Secondary	4.2
Venlafaxine	99300-78-4	SNRI	Tertiary	2.8
Clomipramine	17321-77-6	TCA	Tertiary	5.2
Amitriptyline	549-18-8	TCA	Tertiary	4.9
Citalopram	59729-32-7	SSRI	Tertiary	3.5

[a] SSRI: Selective serotonin reuptake inhibitor, SNRI: selective serotonin-norepinephrine reuptake inhibitor, TCA: Tricyclic antidepressant.

[b] Drugbank database (august 2017): https://www.drugbank.ca/.

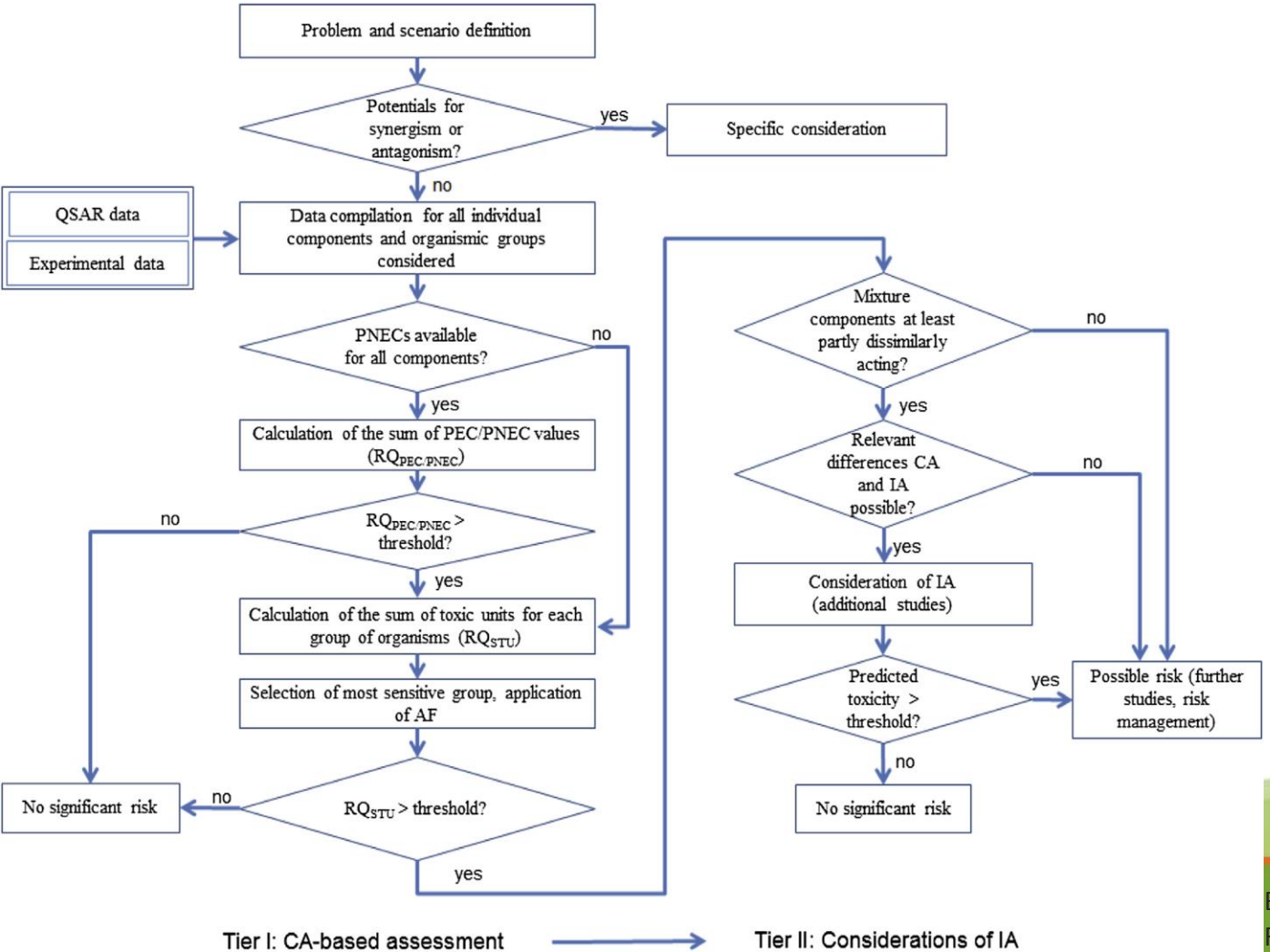
The octanol-water partition coefficients LogKOW were obtained from Drugbank database (experimental data).

Joint effects of nine antidepressants on Raphidocelis subcapitata and Skeletonema marinoi: A matter of amine functional groups



defined by a synergistic effect to R. subcapitata and S. marinoi (i.e. STU < 0.8)

The mixture toxicity predicted by the concentration addition model is indicated by a grey line.



Suggested two-tiered outline for predictive ecotoxicological risk assessment of chemical mixtures

Beyer et al. / Marine Environmental Research 96 (2014) 81e91

Merci de votre attention

Interactive effects of pollutants

- When regulatory authorities consider the toxicities of mixtures, it is usually assumed (without definite evidence to the contrary) that the toxicity of a combination of chemicals will be approximately additive
- In other words, the toxicity of a mixture of compounds will approximate to the total toxicities of its individual components. This is usually a correct assumption
- However, in a relatively small yet very important number of cases, toxicity may be substantially more than additive—i.e., when organisms are exposed to a combination of two or more chemicals, potentiation (synergism) of toxicity may occur
- The effectiveness of a synergist is usually measured as a synergistic ratio (SR):
- If synergism is present, the SR will be greater than 1; in effect, the synergist will lower the median lethal dose (MLD) or concentration of the chemical

$$\frac{\text{MLD (or conc.) for chemical alone}}{\text{MLD (or conc.) for chemiocal + synergist}} = \text{SR}$$

Interactive effects of pollutants

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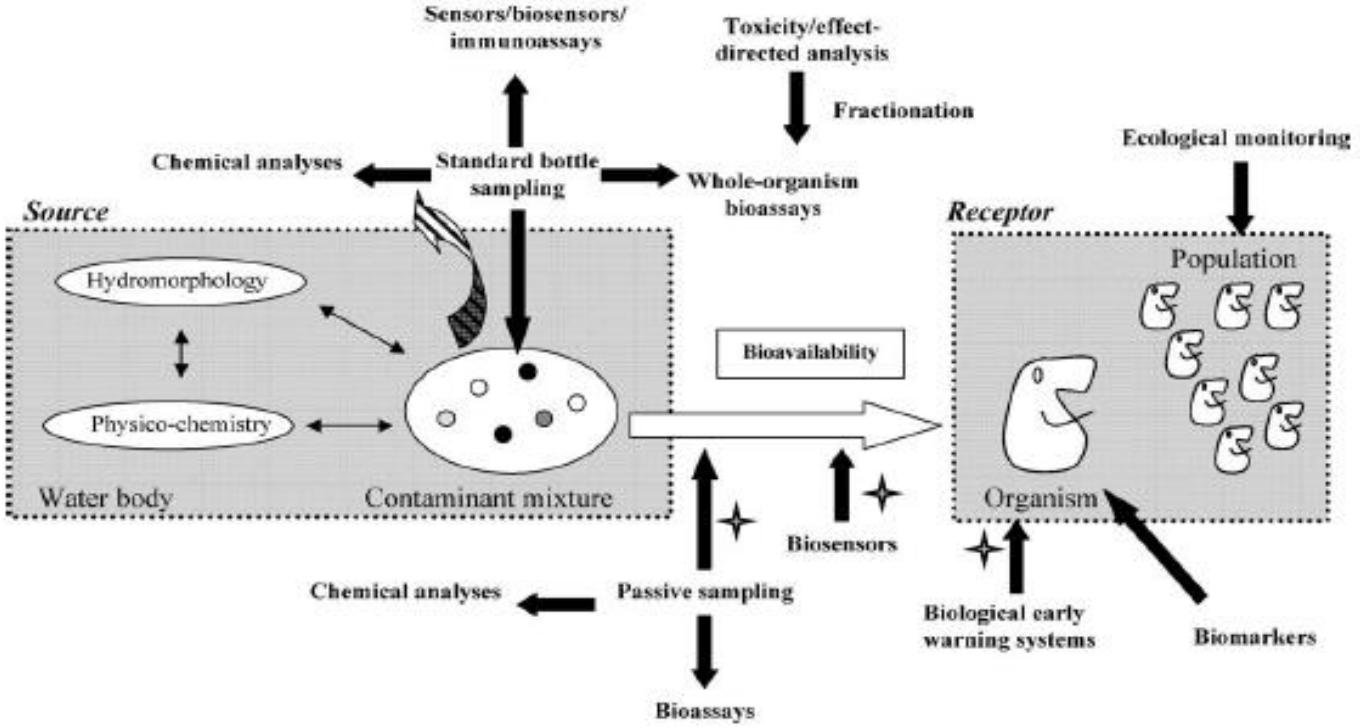
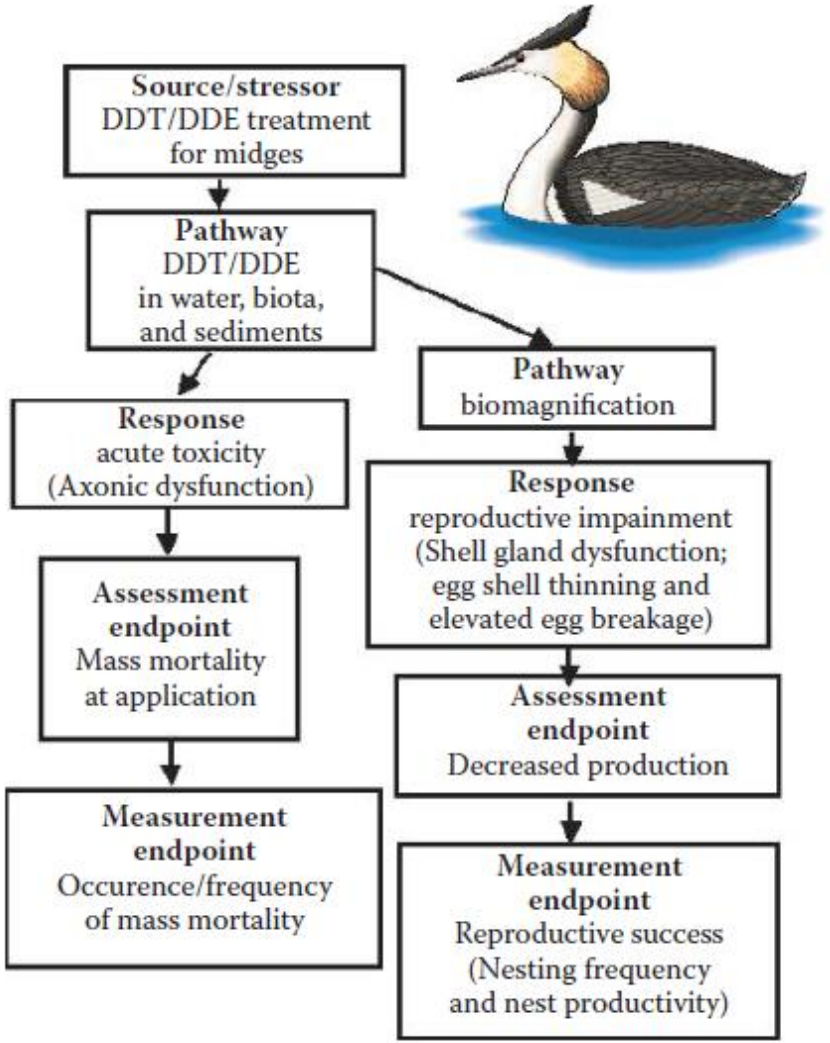
Additive effects : theory

- Where several compounds share a common mechanism of action and interact with the same site of action, it is probable that they will show additive toxicity when present as mixtures unless the picture is complicated by toxicokinetic factors
- Differences in affinities for the site of action (receptor) and corresponding differences in the relationship between concentration (or dose) and toxic effect are bound to arise within such groups of compounds.
- However, toxicity is frequently closely related to the percentage of receptor sites to which the toxic molecules bind
- In this case, the concentrations of individual compounds are corrected by affinity factors, and all toxicity data are fitted to a single dose–response curve.

Suitability of existing and emerging techniques and methods for water quality monitoring under Water Framework Directive. (Allan et al, 2005)



Thin arrows represent the interaction of the hydromorphology, physico-chemical properties of a water body with contaminants present in the water. Thick arrows represent possible monitoring strategies that may be employed to assess ecosystem health and water quality, while the four-point stars and the curved arrow represent sampling methods that may incorporate an additional temporal dimension and standard spot sampling, respectively.



conceptual model diagram for the pesticide spraying of nonbiting midges as described in Chapter 1 and depicted in Figure 1.2. Here, the assessment and measurement endpoints are separated for clarity. They could have been combined as the assessment endpoint. The valued
ecological entity is the Western Grebe population.