Ecological Risk Assessment of Multiple Chemicals: where are we?

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What an environmentally relevant mixture is?

The concept of mixture, from the point of view of Ecological Risk Assessment (ERA), is a complex issue.
At least three different levels may be taken into account:

1. Chemicals present in a commercial mixture (e. g.: PCBs in Arochlor)
2. Chemicals deriving from a given human activity (e.g.: the effluent from an industrial plant)
3. Chemicals present in an ecosystem due to the complex activities in the territory (e.g.: a river basin)
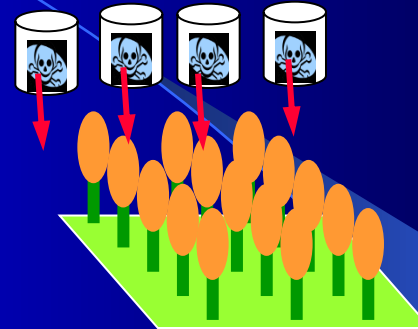
From a regulatory point of view, each level requires different control and mitigation approaches
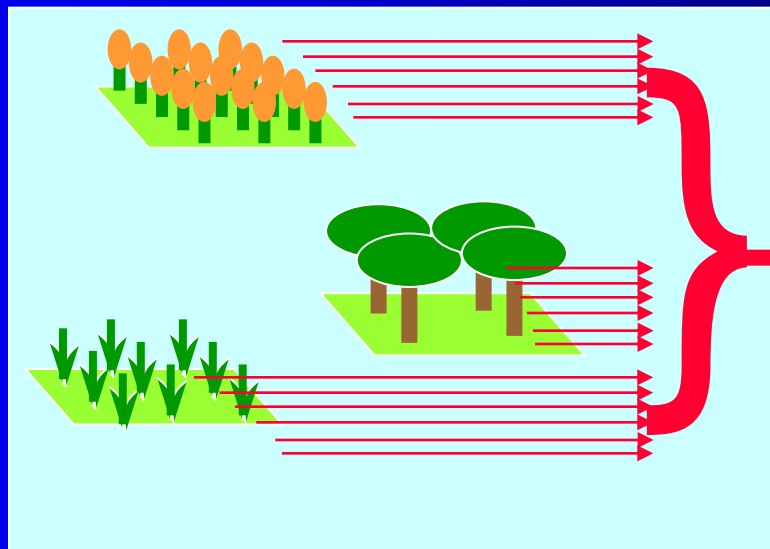
The case of pesticide mixtures

Many active ingredients may be present in the same formulation

Many formulations may be applied on the same crop

**Many crops may be present on the same agricultural area**

Final mixture

Mixture complexity in a river basin

An ecosystem is usually exposed to emissions deriving from several human activities.

For example, a river receives emissions from all the human activities present in the drainage basin (agricultural runoff, industrial discharges, urban sewage). In particularly complex situations hundreds of individual potentially dangerous chemicals may be present in a mixture.
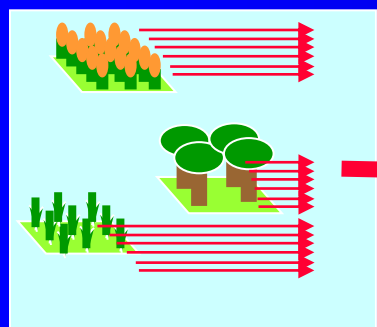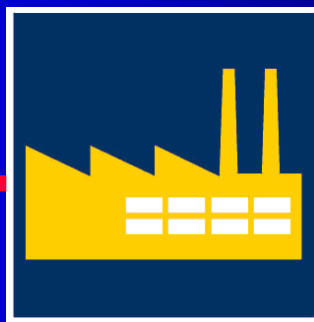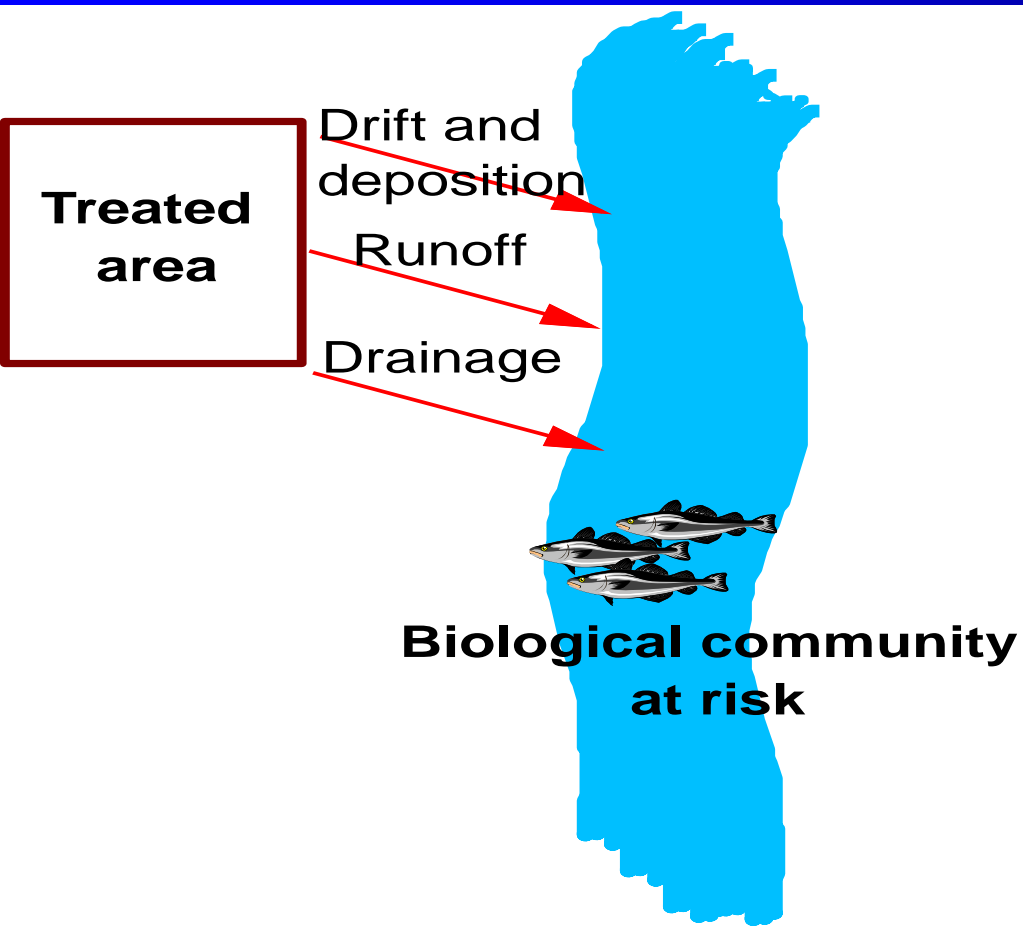
Agricultural mixture

Industrial mixture

Urban mixture

Assessing exposure to mixtures
Aquatic ecosystems

Treated area

Drift and deposition

Runoff

Drainage

Biological community at risk

Pesticides are moving toward the endangered community.

Suitable models exist to predict PECs due to different transport patterns.

Mixture composition is a function of distribution and fate patterns of all chemicals applied in the hydrological basin of the water body.

Assessing exposure to mixtures
Terrestrial ecosystems: epigean communities (birds and beneficial insects)

Foraging area
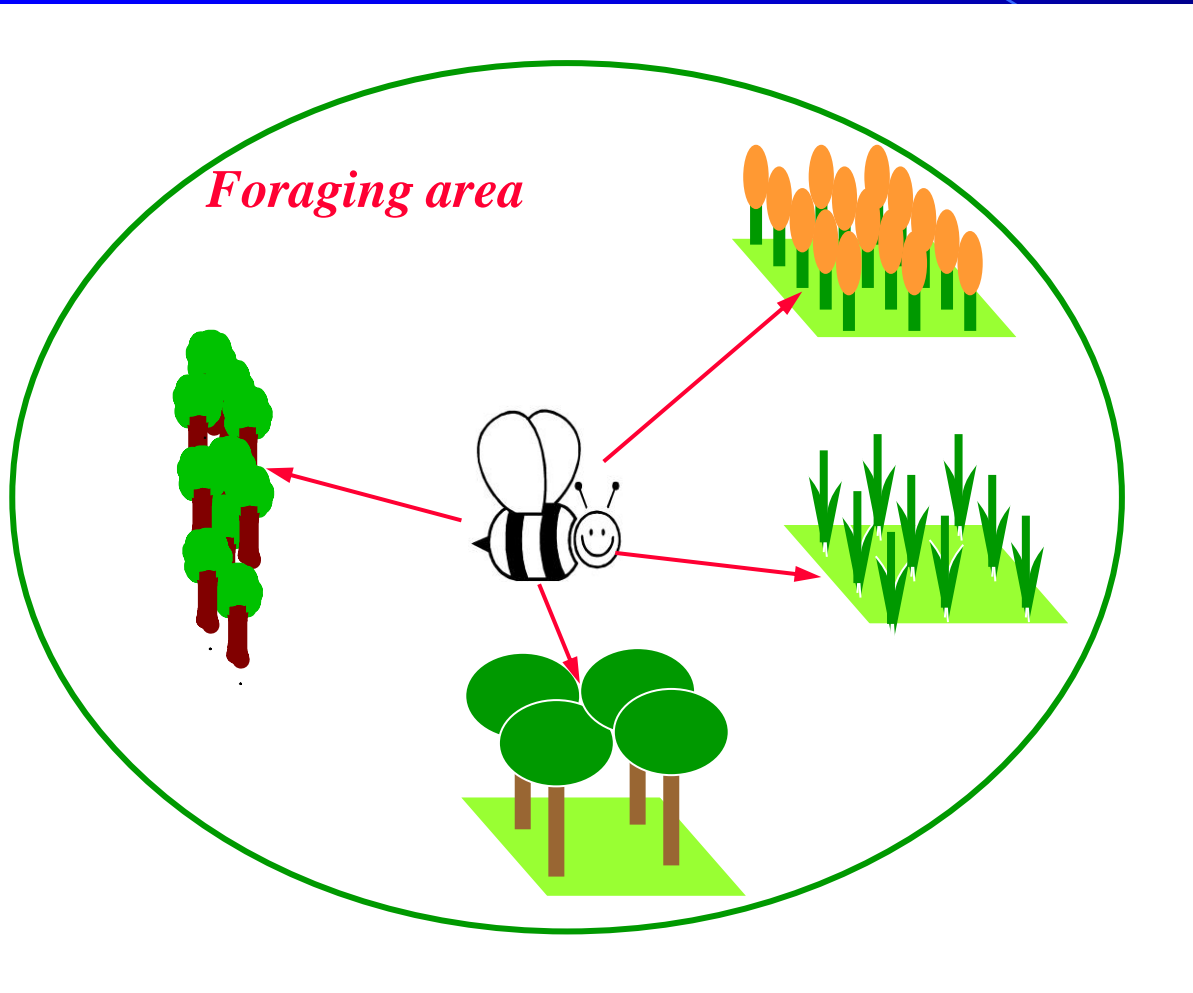
The community potentially exposed is moving within the area.

Exposure is a function of the variable concentrations on different systems in the area (e.g.: treated crops, non crop vegetation, etc) as well as of the behaviour and the ecological role and niche of the organism.

Assessing exposure to mixtures
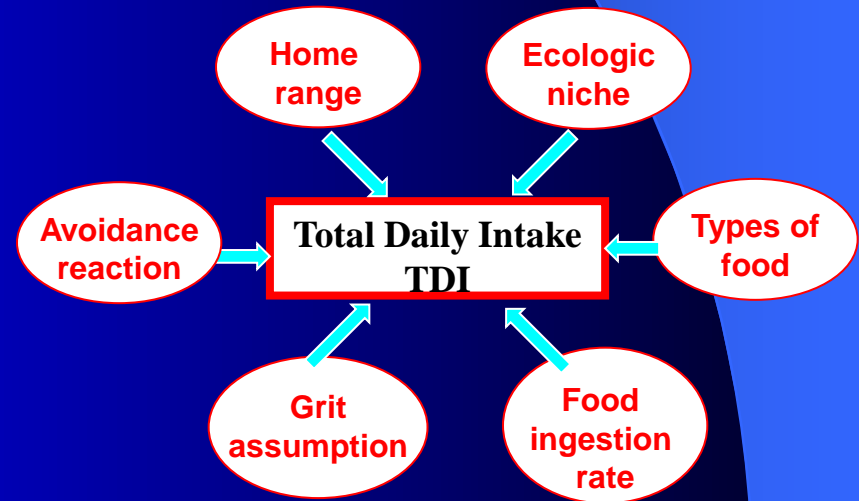# Terrestrial ecosystems (epigean): birds and beneficial insects

More or less complex models and procedures have been developed, considering behavioural and ecological traits, for assessing exposure (as Total Daily Intake)

for pollinators….

….and for birds

Foraging area

Pollen collection

Total Daily Intake TDI (oral and contact)

Nectar feeding

Foliage surface contact

Home range

Ecologic niche

Avoidance reaction

Total Daily Intake TDI

Types of food

Grit assumption

Food ingestion rate
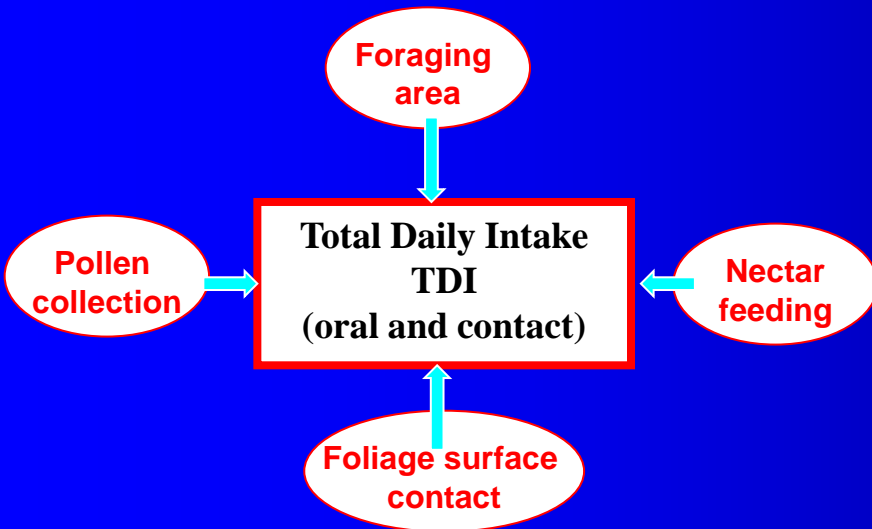
Mixture composition as a function of time

Exposure to pesticide mixture is the result of the combination of exposure indicators (e.g.: PEC, TDI) for individual chemicals.
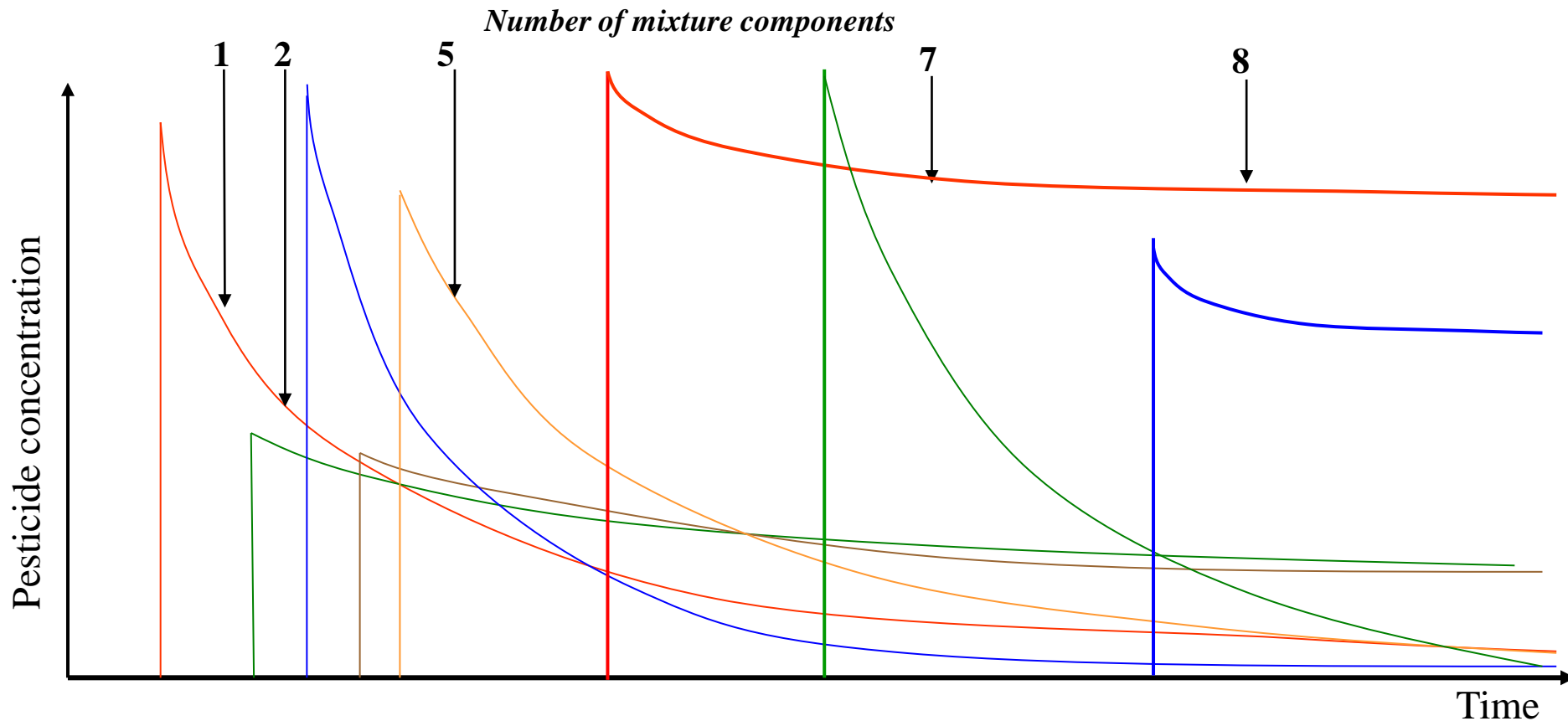
Mixture composition is highly variable with time and depends upon pesticide application patterns (rate and time) and persistence of individual chemicals.

But, how many components are really relevant at any time?

Number of mixture components

The relevant number of chemicals in a mixture
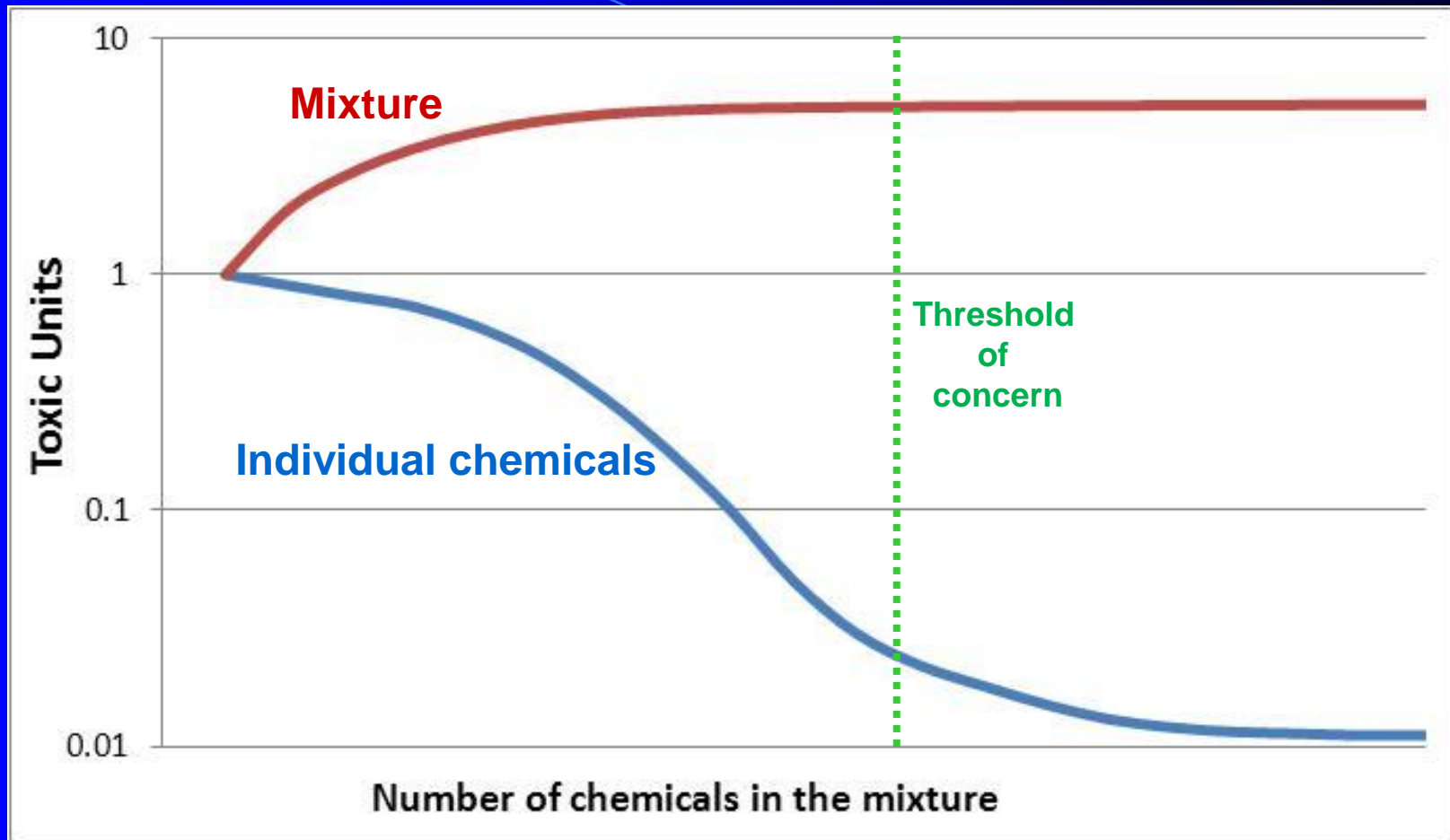
Even if chemicals present at very low TU levels may affect mixture response in the CA model, for practical reasons, a threshold of concern must be established to exclude from the assessment chemicals with a negligible effect on the cumulative response.

Assessing effects of mixtures
CA or IA?

Two reference models are generally used for predicting the effects of mixtures in ecotoxicology:

The Concentration Addition (CA) model, applicable to chemicals with the same mode of action

$$TUm = \sum_{i=1}^{n} TUi = \sum_{i=1}^{n} \frac{C_i}{EC_{x,i}}$$

The Independent Action (IA) model, applicable to chemicals with different mode of action

$$E(C_{mix}) = 1 - \prod_{i=1}^{n} (1 - E(C_i))$$

Options for a Predictive Hazard Assessment of Chemical Mixtures

1: *Case by case selection of the most appropriate concept:* *Independent Action (IA) OR Concentration Addition (CA) OR Two Stage Calculation (TSC) that is a combination of both*

      Prerequisite: **Sound criteria for classifying chemicals into groups of similar or dissimilar action**

      Problem: **Need for knowledge on the mechanisms of action of chemicals**

2: *IA by Default*

      Problems: **Underestimations of mixture toxicity**
                           Extensive data requirements

3: *CA by Default*

      Problem: **Overestimations of mixture toxicity**

Ecologically relevant enpoints and toxicological modes of action

The objectives of protection in ecotoxicology are not individuals but structure and functions of biological communities and ecosystems.

Therefore, ecologically relevant endpoints (survival, growth, reproduction, population dynamics) are much broader than in human toxicology where highly specific (physiology oriented, organ oriented) modes of action are considered.
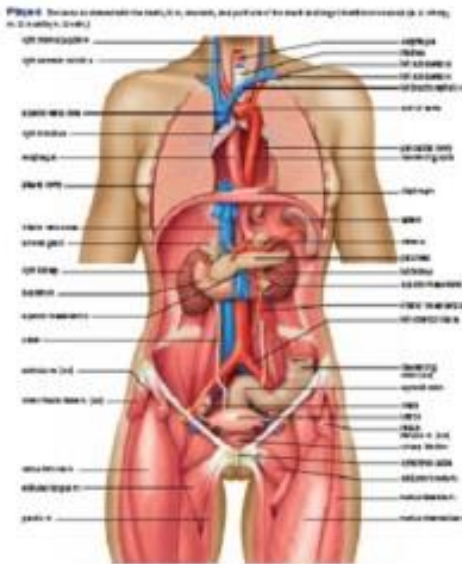
The problem of modes of action in ecotoxicology

In ecotoxicology, the knowledge on the toxicological modes of action on the different species that may be present in an ecosystem is largely incomplete.

Even for chemicals with specific activity (e.g. pesticides) the toxicological mode of action is well known for target organisms but not for the non target ones. Highly specific toxic chemicals exert their effect on a particular physiological or metabolic function that, usually, is not common to all living organisms present in a biologic community. Therefore, for non-target organisms, taxonomically far from the target ones, the effect of the chemical is likely to be narcotic-type (baseline toxicity).
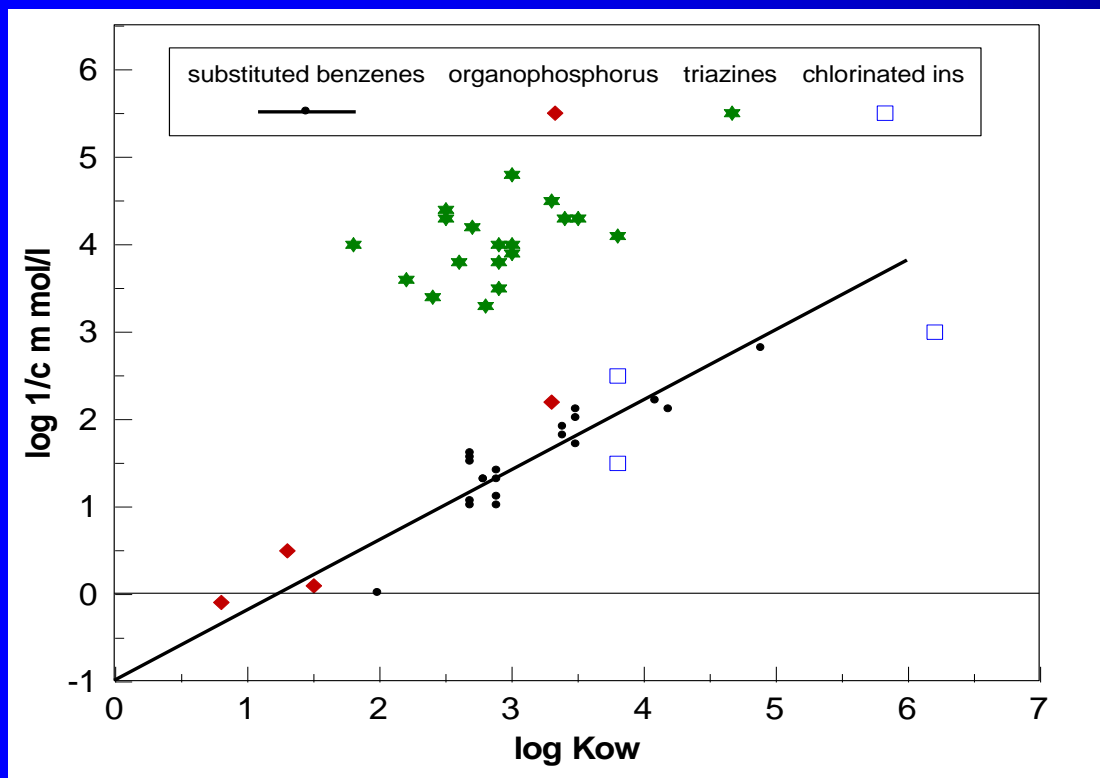
Relationship between toxicity on algae (Log 1/EC50) and Log Kow for chemicals with specific (triazines) and non-specific (all others) toxicological mode of action on algae.

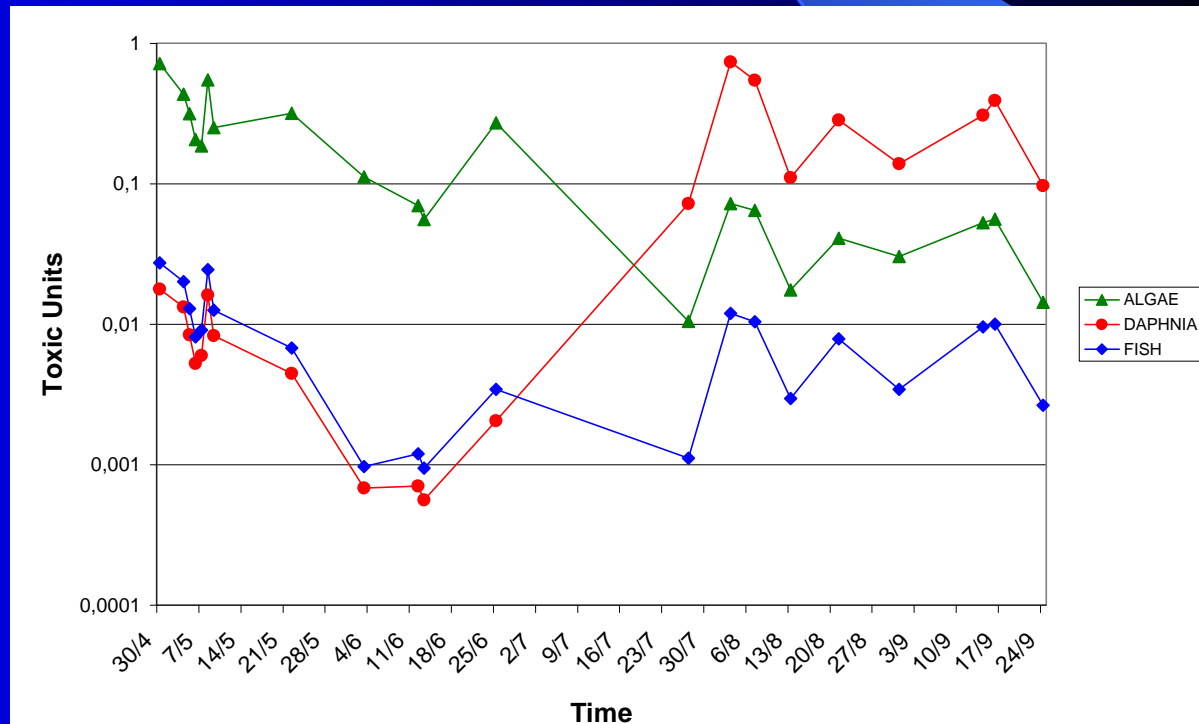# Examples of mixture composition in experimental areas Surface water in the River Meolo basin

Legend
- Corn
- Vineyards
- Soybean
- Spring sampling station
- Rovarè sampling station
- Monastier sampling station-Autosampler
- Castelletto sampling station
- Meolo river
- Meolo boundary catchment area
- Urban area

0 1000 2000 3000 Meters
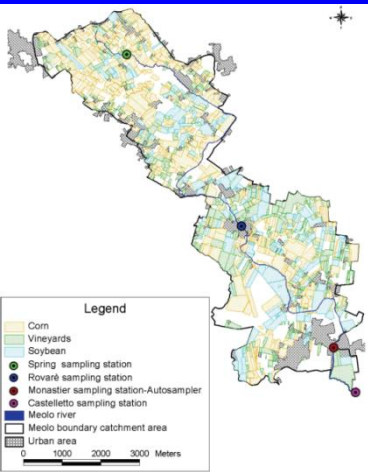
54 active ingredients are applied on the main crops of the basin. They reach the river by direct drift or by runoff after rain events.

PECs in river were calculated for all active ingredients and for all drift and runoff events during the productive season. The aquatic community is exposed to peaks of mixtures of different composition.

The time course of the potency of the total mixtures (as acute TUs) present in Meolo River has been calculated for algae, Daphnia and fish.

Different components of the biological community are affected as a function of the mode of action of active ingredients (herbicides, insecticides, fungicides)

Verro *et al.*, 2009.
Environ. Sci. Technol. 43 (2), 522-529.

- ALGAE
- DAPHNIA
- FISH

y-axis: **Toxic Units** (1, 0,1, 0,01, 0,001, 0,0001)

x-axis: **Time** (30/4, 7/5, 14/5, 21/5, 28/5, 4/6, 11/6, 18/6, 25/6, 2/7, 9/7, 16/7, 23/7, 30/7, 6/8, 13/8, 20/8, 27/8, 3/9, 10/9, 17/9, 24/9)

CA and IA models: how large is the prediction window?

All available evidence (theoretical and experimental) indicates that differences between predictions of effect concentrations for multi-component mixture based on CA or IA usually are relatively small (< or ≈ 1 order of magnitude).
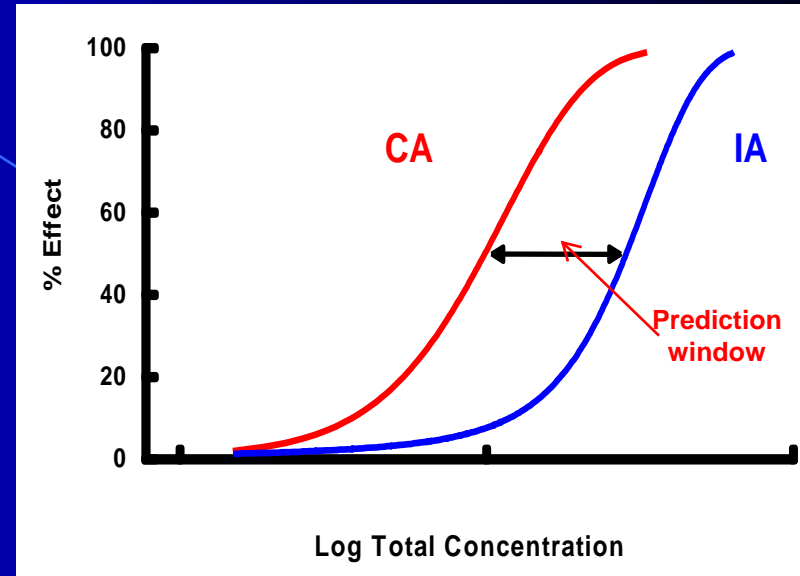
Main factors affecting the prediction window are:

- The number of (really relevant) component of the mixture

- The slope of the concentration/response curve of individual components

- The effect level

How many compondents are really relevant ?

In realistically occuring mixtures, however large the number of component is, the total potency of the mixture is usually determined by a few chemicals.
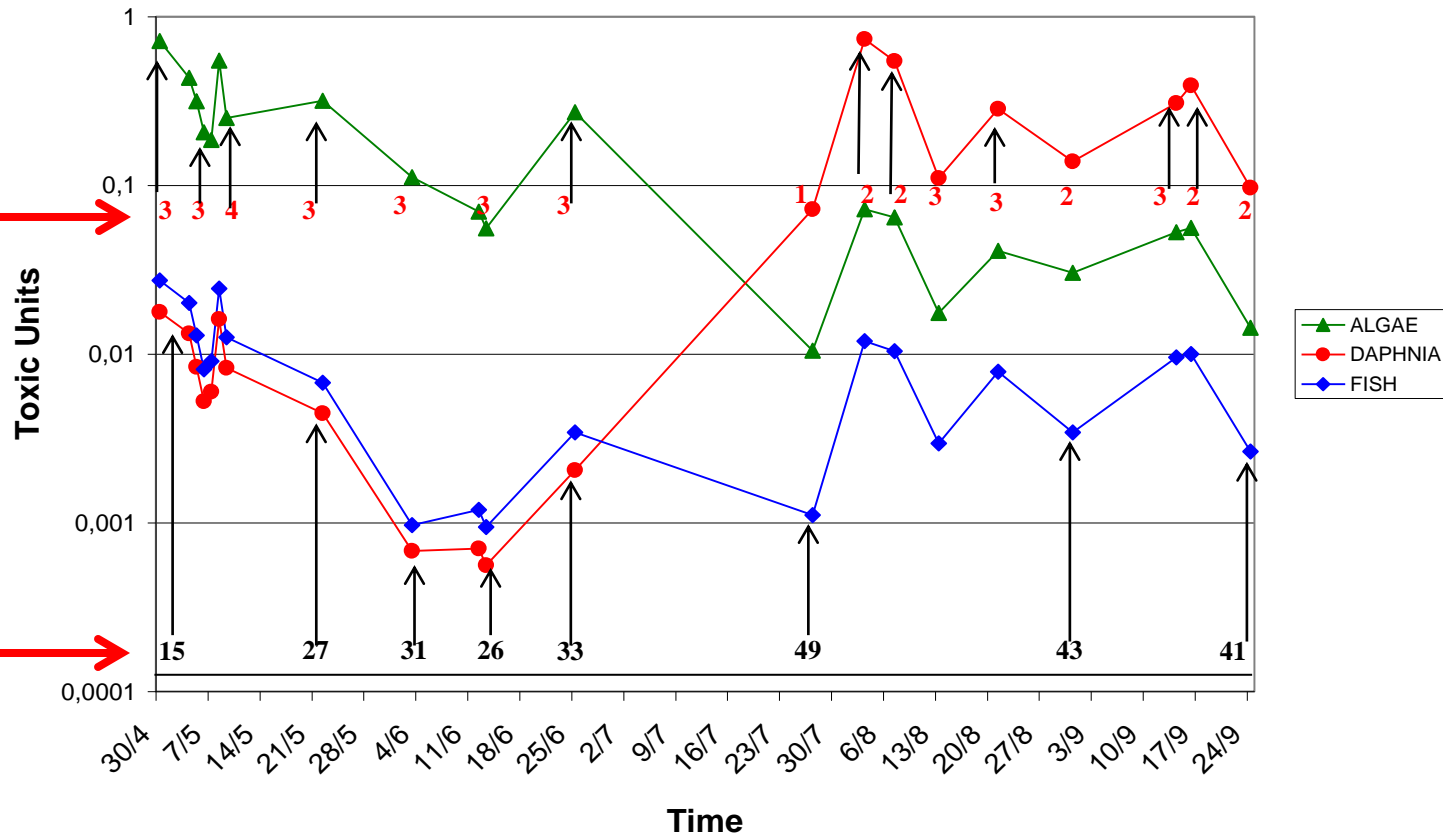
River Meolo case study (again)

The total number of AI applied in the basin is 54.
The maximum number of AI detected above the DL is 49.
The number of AI producing more than 90% of the mixture potency ranges between 1 and 4

Number of individual components explaining more than 90% of the mixture potency

Total number of individual components (above analytical detection limit)

# The concept of Maximum Cumulative Ratio (MRC)

It is defined as:

$$MRC = \frac{\textit{Cumulative toxicity of the mixture}}{\textit{Maximum toxicity from one component}}$$

There is experimental evidence of the decrease of MRC with the increase of the toxic potency of the mixture.

This support the hypothesis of a reduced number of relevant component in realistic environmentally occurring mixtures.

However, MRC cannot be considered as a measure of the need for mixture risk assessment.

Controlling one individual chemical is practically never sufficient to eliminate the risk.

Price and Han, 2012

MCR vs Risk (Hazard Index)
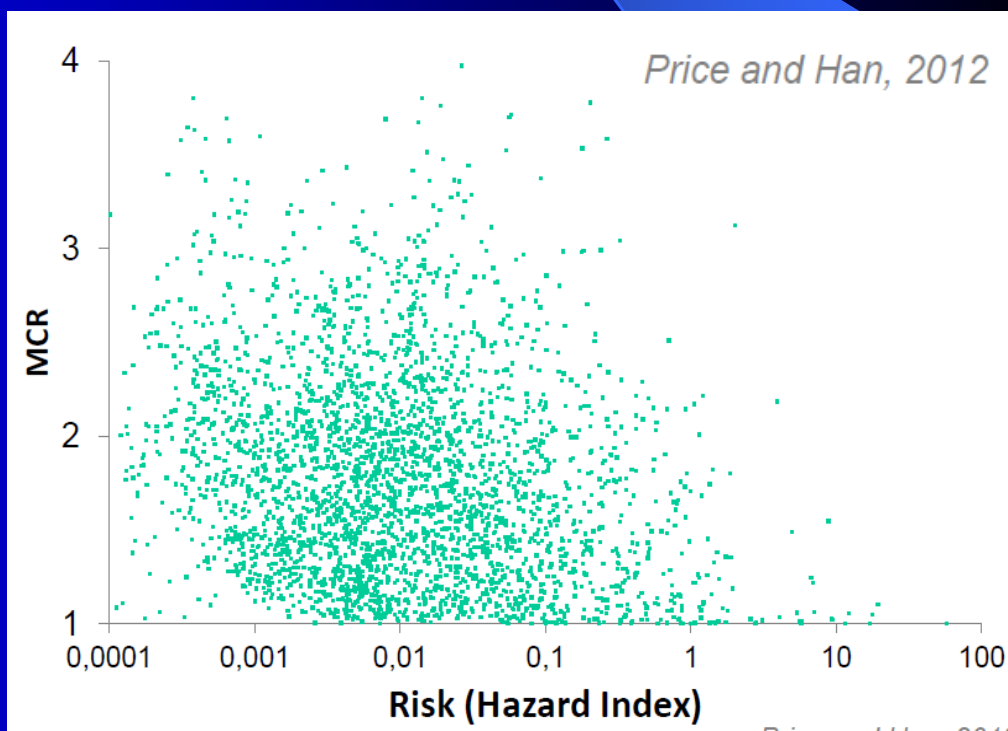
Advantages of the CA model

➢ **It is a conservative approach**

➢ **If applied by default, it does not require knowledge on mode of action**

➢ **It is a powerfool tool for screening-level mixture assessment**
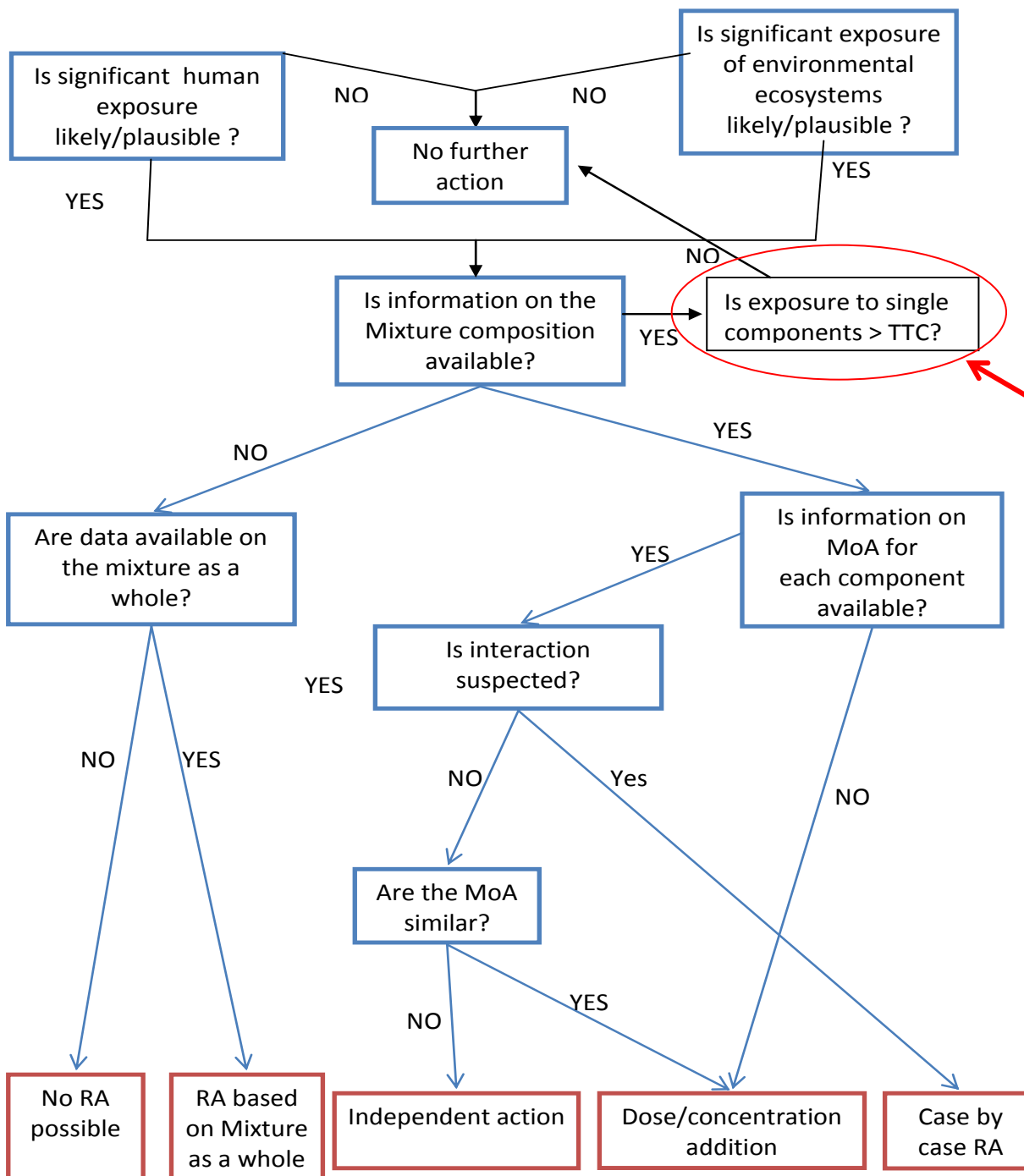
Drawbacks of the CA model

➢ **It does not allow predicting synergistic effects.**

At the present level of our knowledge, predictive approaches for synergistic and antagonistic effects are not available.

However, experimental evidence seems to indicate that, at environmental realistic exposure levels, synergism is unlikely to occur.

Decision Tree for the Risk Assessment of Mixtures

(EC - SCHER, SCCS, SCENIHR, 2012)

Is significant human exposure likely/plausible ?

Is significant exposure of environmental ecosystems likely/plausible ?

NO

NO

YES

YES

No further action

NO

Is information on the Mixture composition available?

YES

Is exposure to single components > TTC?

NO

YES

Are data available on the mixture as a whole?

Is information on MoA for each component available?

YES

Is interaction suspected?

NO

YES

NO

YES

NO

Yes

NO

Are the MoA similar?

NO

YES

No RA possible

RA based on Mixture as a whole

Independent action

Dose/concentration addition

Case by case RA

This box has been included as relevant for Human Health Risk Assessment.
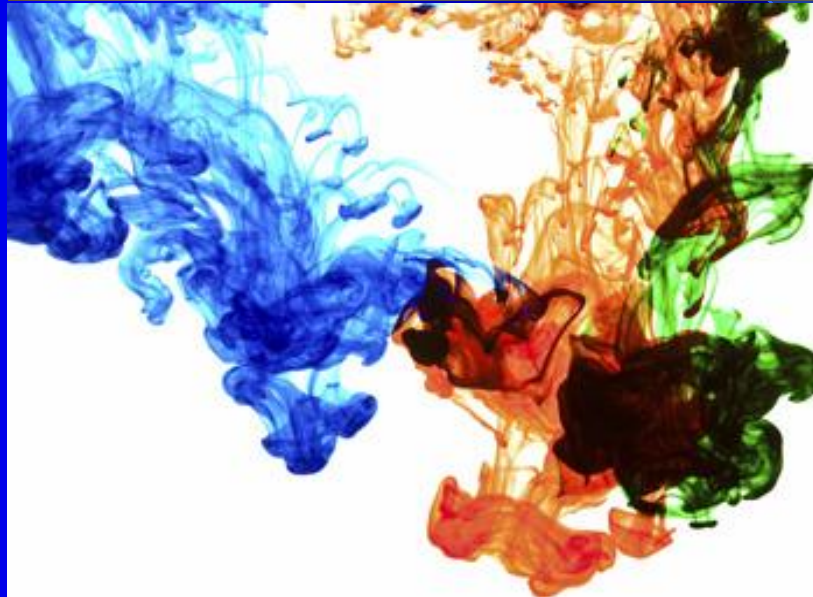
HHRA is a hazard driven procedure. So, a TTC is a commonly used concept.

ERA is an exposure driven procedure. So, the TTC concept is usually not considered.

Conclusions
ERA for mixtures: where we are?

➢ In recent years the knowledge on exposure and effect assessment of mixture needed for the development of regulatory ERA procedures have been substantially grown.

➢ Nevertheless, information is still lacking on data required for a proper prediction of mixture effect (e.g. modes of action).

➢ Therefore, the CA approach is a practically applicable, conservative compromise for predicting mixture response if information for a more precise assessment is lacking.

➢ It can be empirically demostrated that, in mixtures likely to occur in the real environment, a large percentage of the total potency of the mixture is covered by a reduced number of chemicals.

➢ This is an additional reason for supporting the general use of CA as a pragmatic default approach and a reasonable "worst case" for predicting effects of chemical mixtures.

Thanks for your attention