Multiple exposure of ecosystems to global contaminants and possibilities of mixture responses

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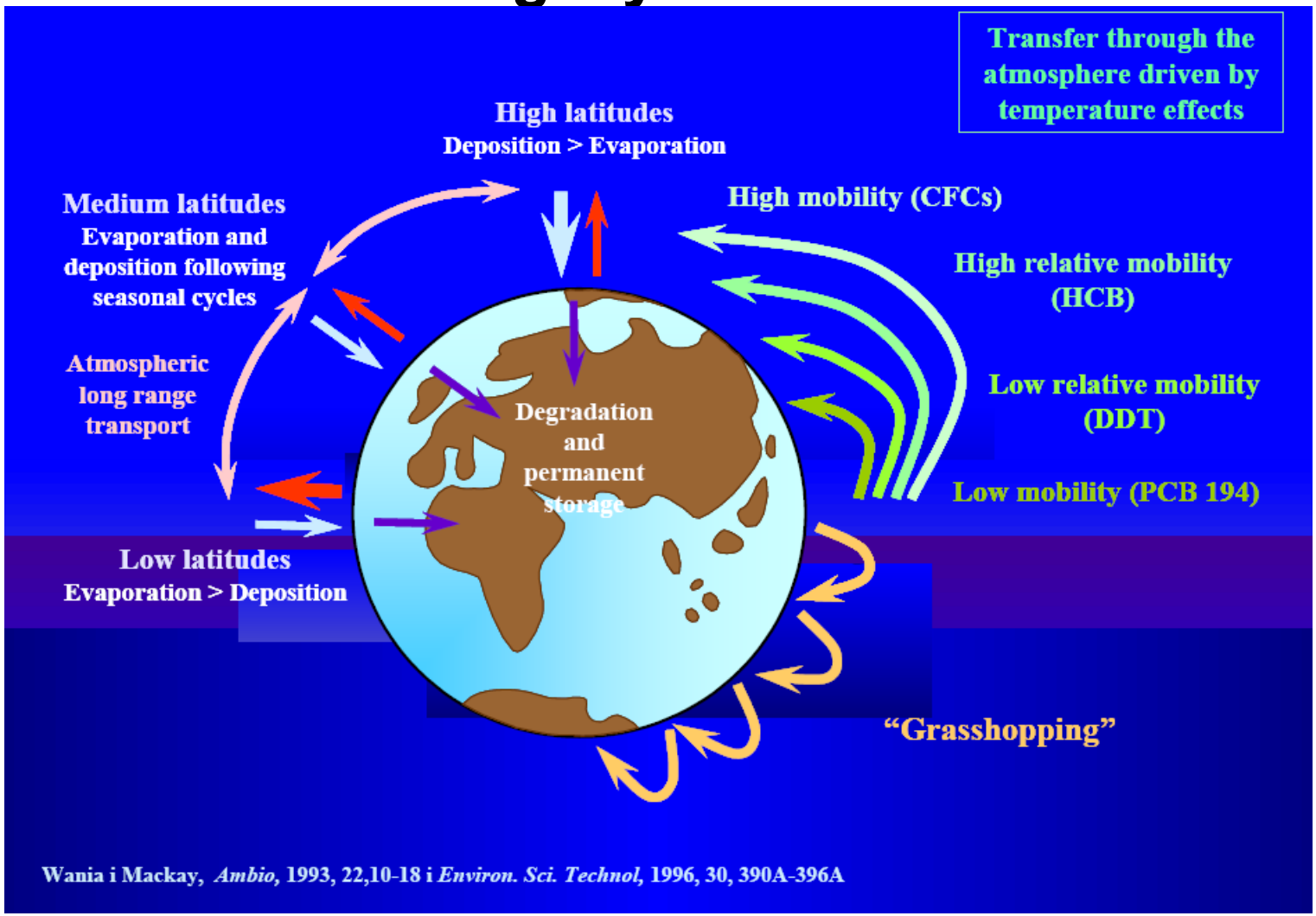
Global contaminants and POPs

The dirty dozen

- **Chlorinated pesticides**
  - **Aldrin**
  - **Chlordane**
  - **Dieldrin**
  - **DDT**
  - **Endrin**
  - **Heptachlor**
  - **Mirex**
  - **Toxaphene**
- **HCB**
- **Polychlorinated dibenzodioxins and furans PCDD/Fs (210 Chemicals)**
- **Polychlorinated byphenils PCBs (280 Chemicals)**

They are controlled by the Stockholm Convention. However, they are still a problem of global concern

The long range atmospheric transport (LRAT) of legacy POPs



Is LRAT applicable to emerging POPs?

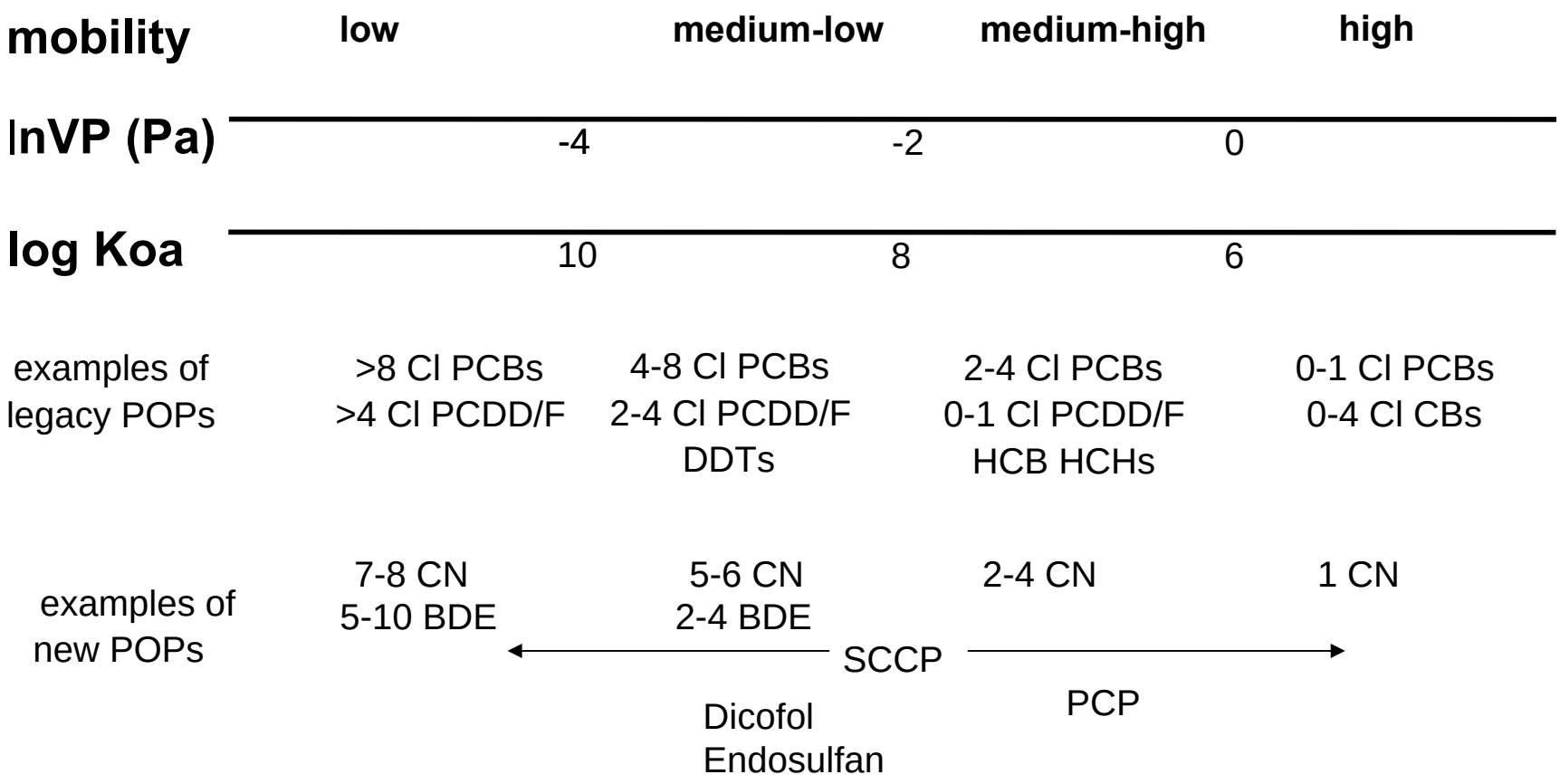
Emerging POPs

Many emerging POPs have physical-chemical properties comparable to those of traditional POPs:

- Polychloronaphtalenes (PCN)
- Polybrominated diphenyl ethers (PBDE)
- Short chained chlorinated paraffins (SCCP)
- Other chlorinated pesticides

They are semivolatile, persistent, hydrophobic.
Their global transport patterns can be reasonably described by the same scheme.

The mobility of old and emerging POPs



PFAs: perfluoro alkyl compounds

- **They have physical-chemical properties completely different in comparison to other POPs**
- **They are polar compounds, with low VP and low logKow**
- **However, there is experimental evidence for their role as global contaminants, in particular for their presence in the Arctic**
- **For some of them (particularly perfluoroctanesulfonate:PFOS) there is experimental evidence for biaccumulation and biomagnification**

PFAs: perfluoro alkyl compounds

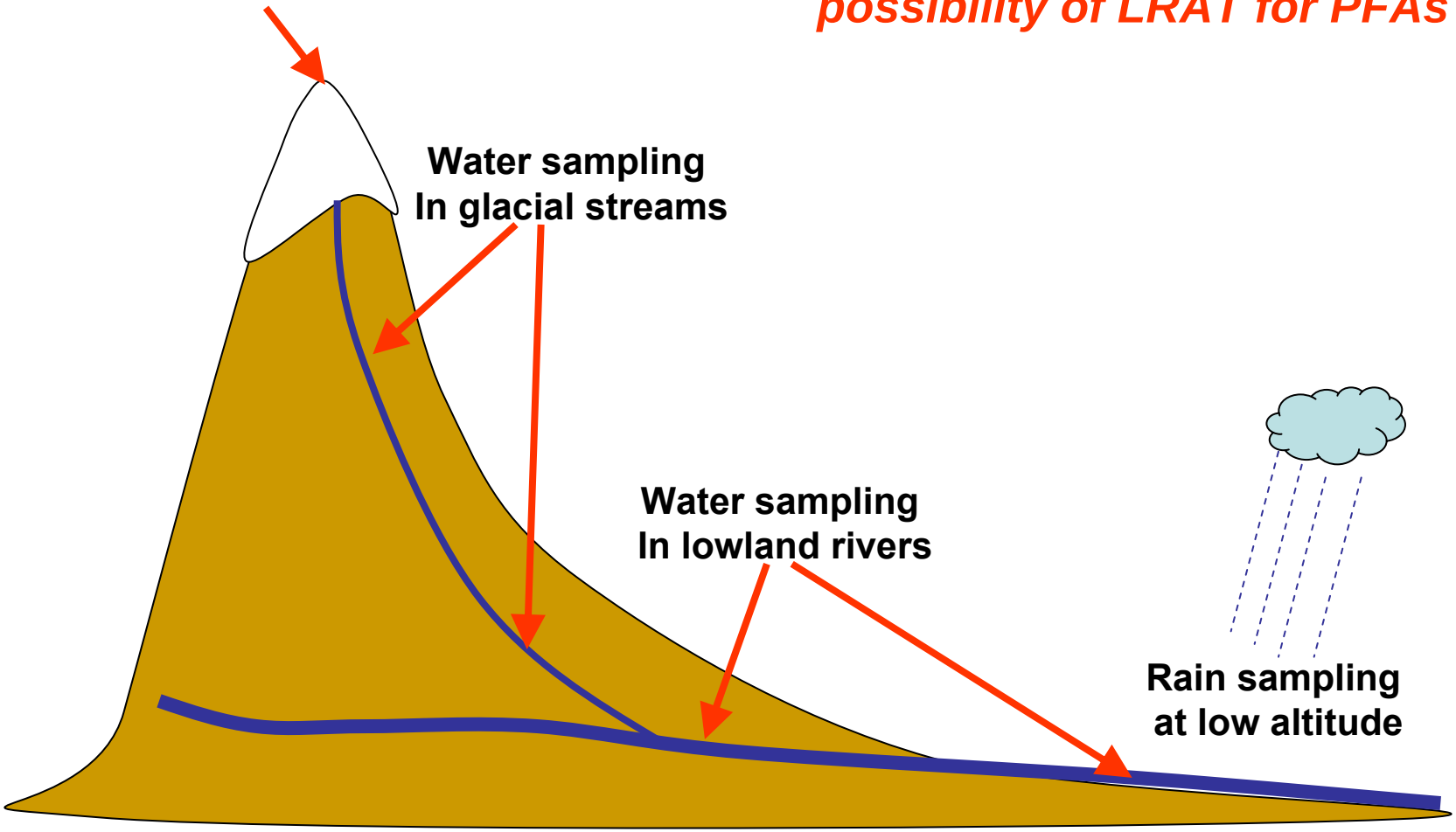
- **It is reasonable to suppose that transport patterns of PFAs are different in comparison to other POPs**

- **Several hypotheses have been developed (e.g. transport trough marine aerosols)**

- **However, at present, experimental evidence for supporting the diferent hypotheses is still lacking**

Confirming or rejecting LRAT for PFAs

A sampling campaign is ongoing to provide experimental evidence for supporting or rejecting the possibility of LRAT for PFAs



Legacy and emerging POPs in Arctic wildlife

Concentrations in polar bears

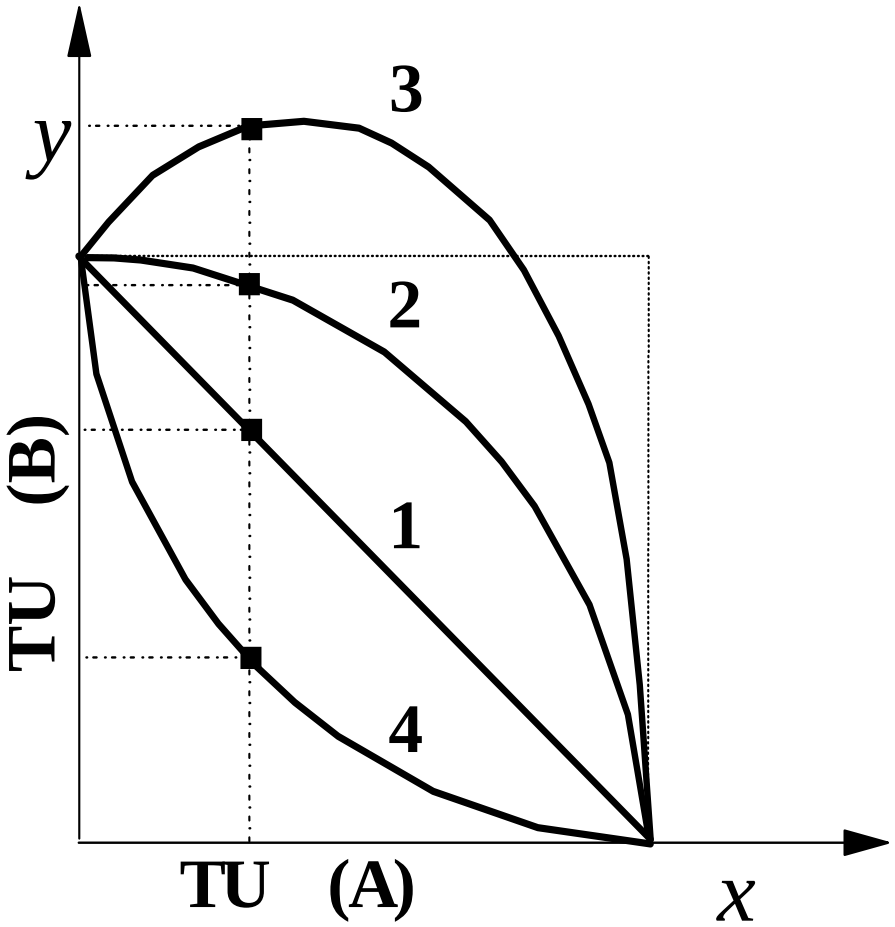
Location	Chemical	Tissue	Concentration	Units	Ref.
East Greenland	PFOS	liver	2140	ng/g ww	Smithwick *et al.*, 2005
Canadian Arctic	ΣPCBs	lipids	3240-8250	ng/g lipids	Norstrom et al., 1988
Canadian Arctic	DDTs	lipids	120-1190	ng/g lipids	Norstrom et al., 1988
Canadian Arctic	Chlordanes	lipids	1810-7090	ng/g lipids	Norstrom et al., 1988
Norvegian Arctic	ΣPBDEs	plasma	530	ng/g lipids	Verreault et al., 2005
Alaskan Arctic	PCDD/F+d.l.PCBs	liver	8 - 192	pg/g TEQ	Kumar et al. 2002
Alaskan Arctic	ΣPCNs	liver	370	ng/g ww	Corsolini et al. 2002

Isobologram describing possible toxicological responses to a binary mixture of toxic chemicals

$$TUi = Ci/ECi$$

$$xTU_A + yTU_B = 1TU_{(AB)}$$

1 additive response: x+y=1
2 less than additive: x +y>1 (x and y <1)
3 antagonism: x+y>1 (x or y >1)
4 synergism: x+y<1



"x" and "y" are the toxic units (TU) of the two chemicals (A and B). The four lines represent the loci where the response of the mixture corresponds to 1 TU

The Concentration Addition (CA) model

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

Where:

- **C_i is the actual concentration of the individual chemical "i" in the mixture;**
- **$EC_{x,i}$ is the ecotoxicological end-point (e,g, EC_{50}) of the individual chemical "i";**
- **TU_i are the toxic units of the individual chemical "i", i. e. the fraction of the ecotoxicological end-point produced by the individual chemical "i" ($TU_i=C_i/EC_{x,i}$);**
- **TU_m are the toxic units of the mixture.**

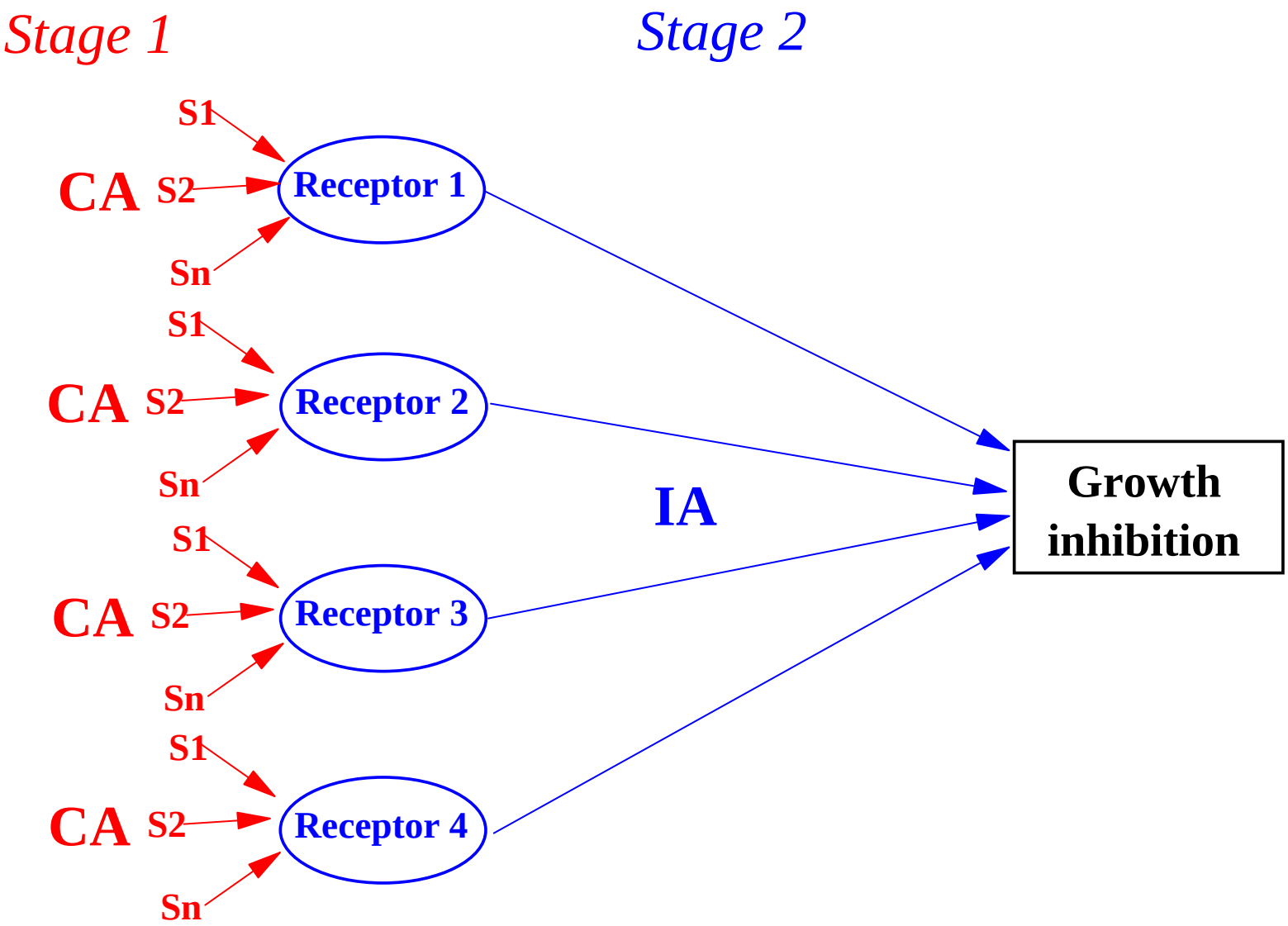
The Independent Action (IA) model

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

Where:

- **$E(c_{mix})$ is the effect caused by the mixture,**
- **$E(c_i)$ is the effect of the individual chemical "i".**

Two Stage Calculation



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)***

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

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

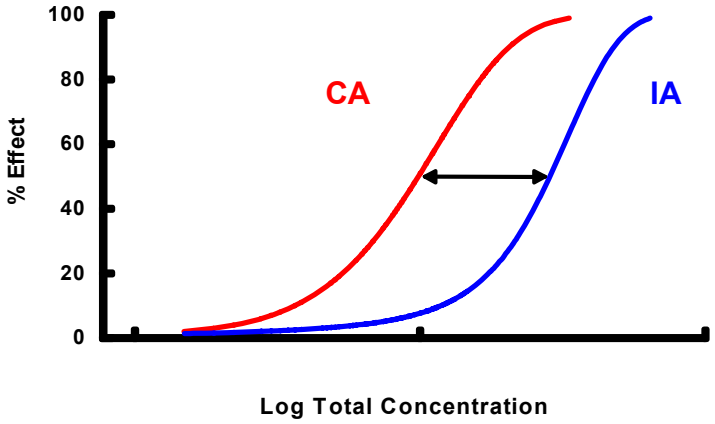
2:*IA by Default*

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

3:*CA by Default*

Problem: **Overestimations of mixture toxicity**

Differences between CA and IA models (BEAM European Project)



Theoretical approaches: Large differences may only occur with large numbers of components and steep concentration-response curves of individual chemicals

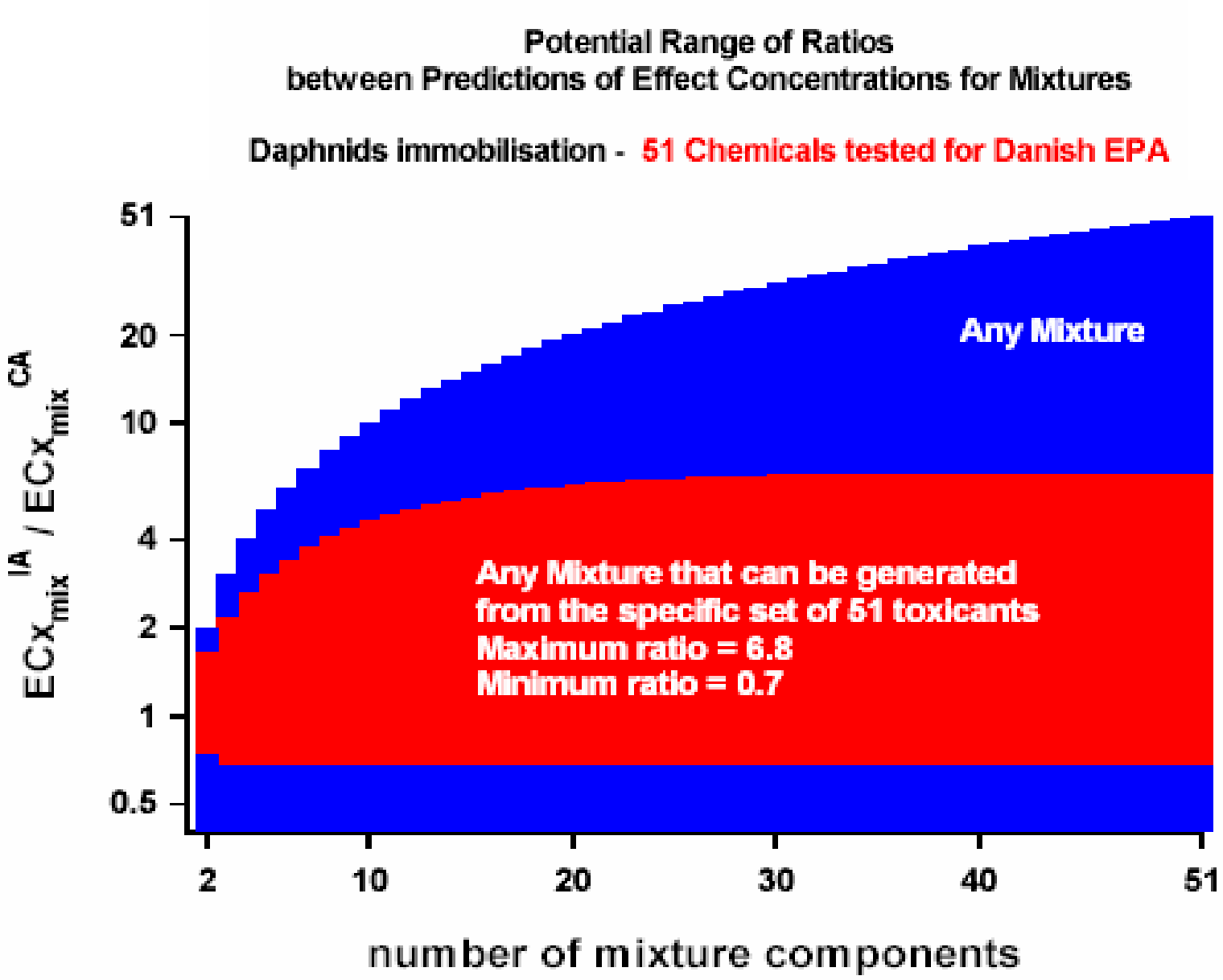
Experimental evidence: Reported differences do not exceed a factor 4 for mixtures with up to 40 components

Simulation studies: Concentration response data from aquatic toxicity testing

- algae, 232 toxicants
- daphnids, 176 toxicants
- fish, 69 toxicants

Results:Differences are relatively small: <of a factor 10 for mixtures with up to 100 components

An example of the experimental results



The rationale for the results

The main factors affecting the CA/IA ratio (the prediction window) are:

- The number of component of the mixture
- The role of each component in determining the total response
- The slope of the dose response curve

The number of component of the mixture

The number of components of mixtires naturally occurring in the environment is often very high.

This is also true for POP mixtures.

HOWEVER……

The role of each component in determining the total response

Even in mixtures with a high number of components, the difference between CA and IA is small if one or a few components are responsible for a large percentage of the total mixture potency.

There is experimental evidence that in most naturally occurring mixtures a few components (usually no more than two-three) are responsible for more than 80% of the mixture potency.

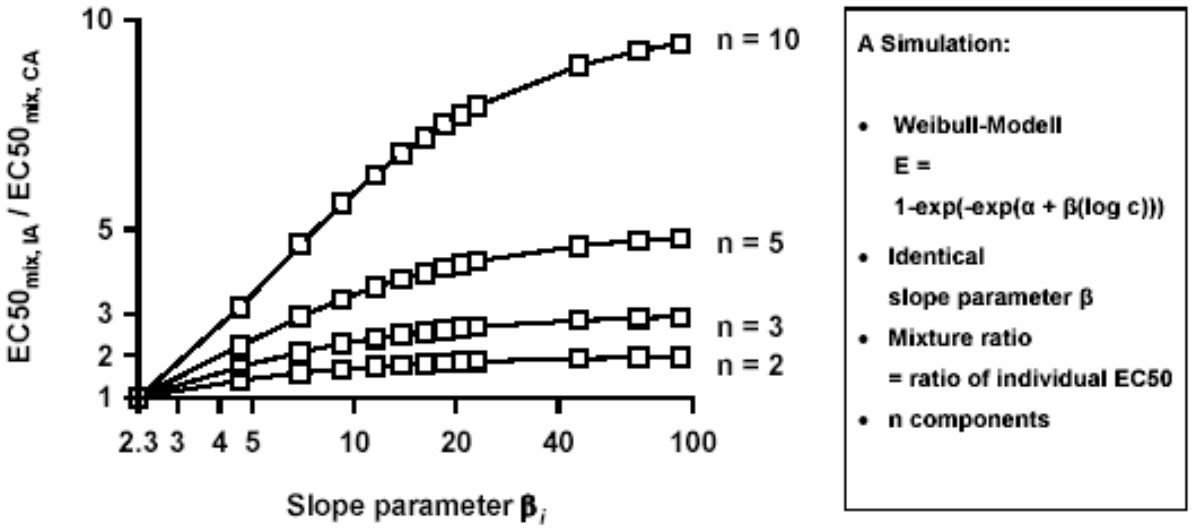
Is this the case of POP mixtures?

The slope of the dose response curve

It has been demostrated that the difference between CA and IA is small if the slope of the concentration-response curves is low.

$$\frac{ECx_{mix,\,IA}}{ECx_{mix,\,CA}}$$

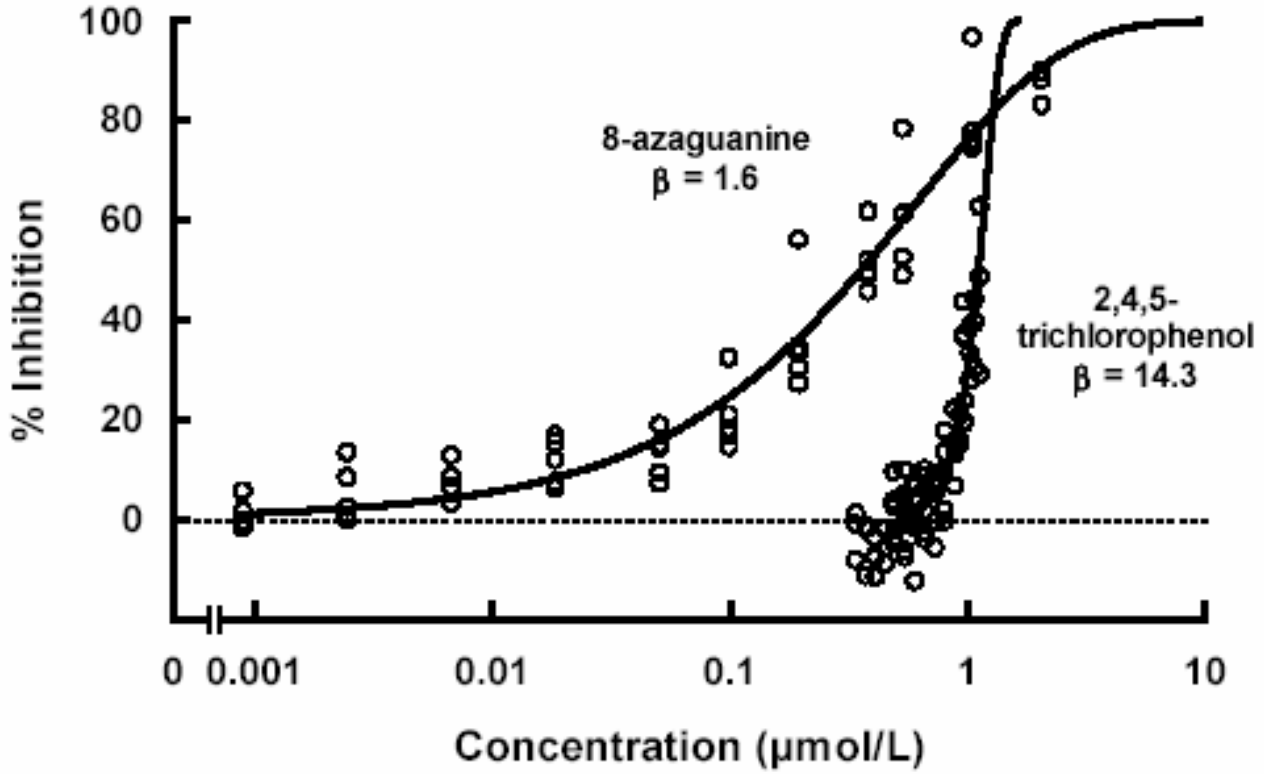
Slope of concentration response curves: a crucial parameter



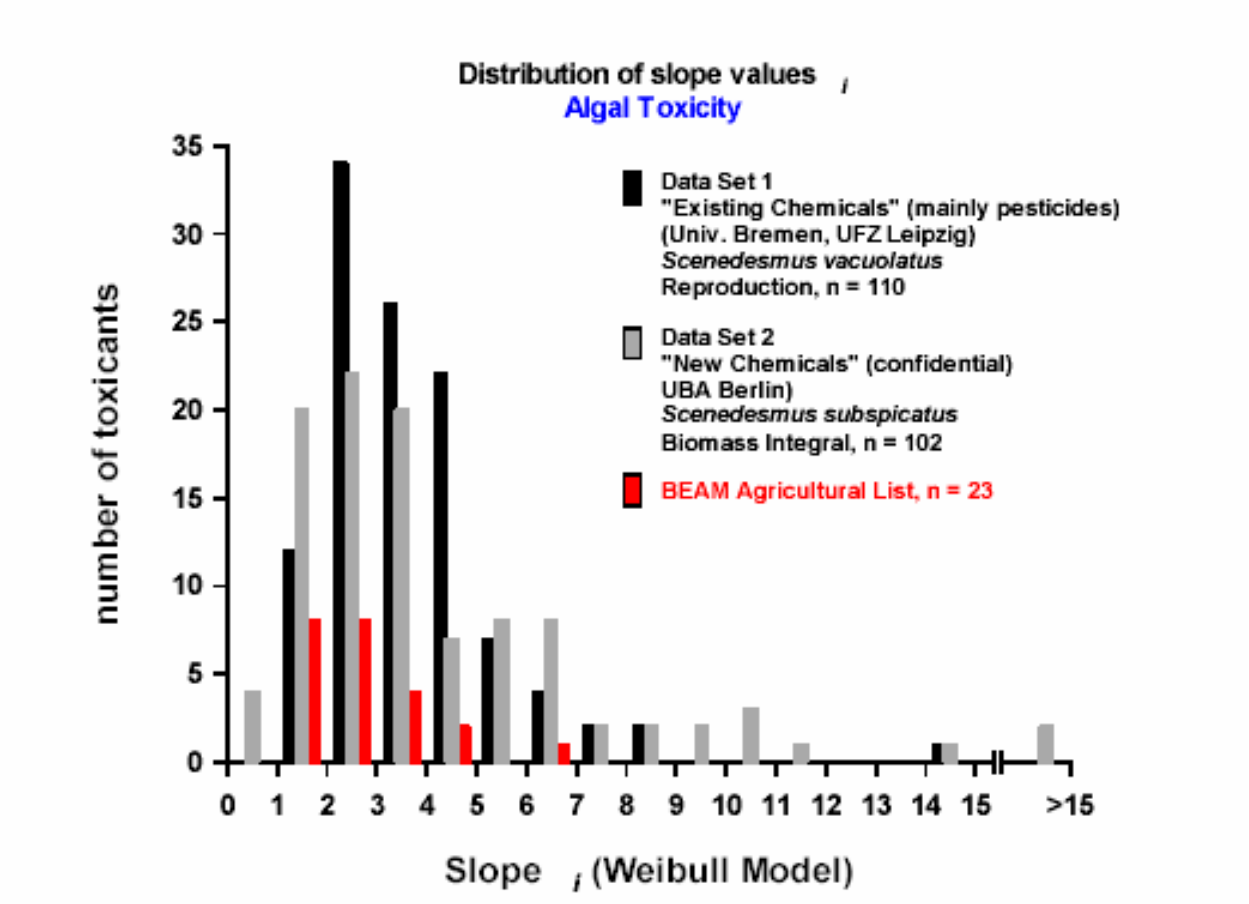
A Simulation:

- **Weibull-Modell**
  E =
  1-exp(-exp(α + β(log c)))
- **Identical**
  slope parameter β
- **Mixture ratio**
  = ratio of individual EC50
- **n components**

Extreme values observed



It has been observed that among many classes of potentially toxic chemicals the majority of slopes is lower than 5



Can this be demonstrated for the various toxicological end points of POPs?

CA as an acceptable worst case

$$\frac{ECxmix,\ IA}{ECxmix,\ CA}$$

1.All available evidence indicates that quantitative differences between predictions of effect concentrations for multi-component mixture derived from the competing concepts of *Independent Action* and *Concentration Addition* usually are relatively small (< or ≈ 1 order of magnitude).

2.Thus,our current status of knowledge may justify the general use of *Concentration Addition* as a pragmatic default approach to the predictive hazard assessment of chemical mixtures.

Advantages of the CA model

- **It is a conservative approach**
- **If applied by default, it does not require knowledge on mode of action**
- **It requires only knowledge on simple toxicological end-point (e.g. EC50)**
- **It is a powerfool tool for screening-level mixture assessment**

The problem of antagonism and synergism

The three approaches described (CA, IA, TSP) don’t take into account the possibility of antagonistic and synergistic effects of the components of a mixture.

Indeed, all approaches assumes that no interactions would occur among the chemicals, while antagonism or synergism are determined by chemical interactions that may produce effects lower or higher than predicted respectively.

The possibilities of interactions are extremely complex and different.

At present, no models are available for predictive approaches and there is the need for a case by case evaluation.

The concept of Toxic Equivalency Factor (TEF)

The concept of TEF is a pragmatic approach for comparing and combining the effects of the PCDD/F and the so-called “dioxin-like” PCBs.

It is based on the hypothesis of concentration (or dose) additivity, referred to the effects of 2,3,7,4-TCDD (TEF=1).

The common mechanism for these compounds involves binding to the aryl hydrocarbon receptor (AhR) as an initial step.

However, it should be understood that the TEF concept is based on a number of assumptions and has many limitations.

It must be highligted that the toxic response of these chemicals is extremely complex, involving dermal toxicity, immunotoxicity, carcinogenicity, adverse effects on reproduction, development and endocrine functions.

The table of TEFs

TEFs for Mammals

Dioxins	**TEF**	**Furans**	**TEF**	**Dioxin-like PCBs**	**TEF**
2,3,7,8-TCDD	1	2,3,7,8-TCDF	0.1	*Non-orto*	
1,2,3,7,8-PnCDD	1	1,2,3,7,8-PnCDF	0.05	3,3’,4,4’-TCB	0.0001
1,2,3,4,7,8-HxCDD	0.1	2,3,4,7,8-PnCDF	0.5	3,4,4’,5-TCB	0.0001
1,2,3,6,7,8-HxCDD	0.1	1,2,3,4,7,8-HxCDF	0.1	3,3’,4,4’,5-PnCB	0.1
1,2,3,7,8,9-HxCDD	0.1	1,2,3,6,7,8-HxCDF	0.1	3,3’,4,4’,5,5’-HxCB	0.01
1,2,3,4,6,7,8-HpCDD	0.01	1,2,3,7,8,9-HxCDF	0.1	*Mono-orto*	
OCDD	0.0001	2,3,4,6,7,8-HxCDF	0.1	2,3,3’,4,4’-PnCB	0.0001
		1,2,3,4,6,7,8-HpCDF	0.01	2,3,4,4’,5-PnCB	0.0005
		1,2,3,4,7,8,9-HpCDF	0.01	2,3’,4,4’,5-PnCB	0.0001
		OCDF	0.0001	2,3,3’,4,4’,5-HxCB	0.0005
				2,3,3’,4,4’,5’-HxCB	0.0005
				2,3’,4,4’,5,5’-HxCB	0.00001
				2,3,3’,4,4’,5,5’-HpCB	0.00001

A comparable table has been developed for birds fish

Can the TEF approach be generalised to POPs?

TEF represent a low-confidence interim approach to describe the highly variable toxicities of dioxins and dioxin-like compounds.

They are set using single compound studies.

Therefore, they have a comparative value, but the scientific bases for using them for predicting combined effectes are weak.

The comparison is mainly based on the Ah receptor response and does not account for the extreme complexity of toxicological response to POPs.

Caution must be used in including new compounds into TEF assessment.

Toxicological effects of some POPs

DDT

Some effects on higher vertebrates (mammals and birds)

- Neurotoxicity
- Enzimatic inhibition
- Effects on cardiovasculary system
- Effects on reproductive system
- Effects on endocrine system
- Carcinogenicity
- Effects on bird egg tickness

Toxicological effects of some POPs

PCBs

Some effects on higher vertebrates

- Neurotoxicity
- Immunosuppression
- Effects on reproduction
- Effects on liver enzimes
- Carcinogenicity

Toxicological effects of some POPs

PCDD/Fs

Some effects on higher vertebrates (mammals and birds)

- Hepatotoxicity
- Porphyria
- Dermal toxicity (chloracne)
- Immunotoxicity
- Effects on bone haematopoiesis
- Endocrine effects
- Effects on reproduction
- Carcinogenicity
- Mutagenicity

Endocrine disruption

Most POPs are classified as endocrine disruptors.

However, endocrine disruption can hardly be considered as a common mode of action.

It is a general term indicating extremely different modes of action on different receptors.

The unique common factor of these mechanisms is that they produce adverse effects on reproduction, development, growth or other functions regulated by hormonal activities.

The complexity of toxicological modes of action

The two models available for predicting mixture responses (CA and IA) require to be referred to a given toxicological end-point.

For example, we know that the major effect of organophosphorus insecticides is inhibition of acetylcholinesterase and that the effect of triazines is inhibition of photosystem-2 in photosinthesys.
So they can be considered as concentration additive for animals and plants respectively.
The hypothesis is supported by experimental evidence.

The complexity of toxicological effects of most POPs make the problem extremely difficult.

Which toxicological end-point should we consider?

Possible synergism

Even if available information is largely insufficient, there is some experimental evidence for synergism among POPs and between POPs and other contaminants.

For example, the effect of acethycolinesterase inhibitors (e.g. organophosphorous insecticides) is increased in presence of DDT.

Other potential synergistic effects need to be investigated more in depth.

Conclusions

The Concentration Addition (CA) concept is a very valuable approach for a screening-level assessment of mixture response.

It can be applied even if information on modes of action of chemicals is lacking amd if a complete dose-effect ciurve is not available

The TEF approach is a pragmatic tool, with some sound conceptual bases and some limitations, useful for PCDD/F and dioxin-like PCBs

BUT…

Legacy and emerging POPs are chemicals of extremely high concern for human and environmental health.

A precise and detailed knowledge of their toxicological behaviour must be considered as an absolute priority.

In particular, considering the experimental evidence of combined exposure at levels of concern, screening-levels or approximated approaches are not satisfying for assessing combined effects.

Moreover, the complexity of modes of action of POPs requires more knowledge on the possible combined effects, including synergism, based on detailed experimental information.