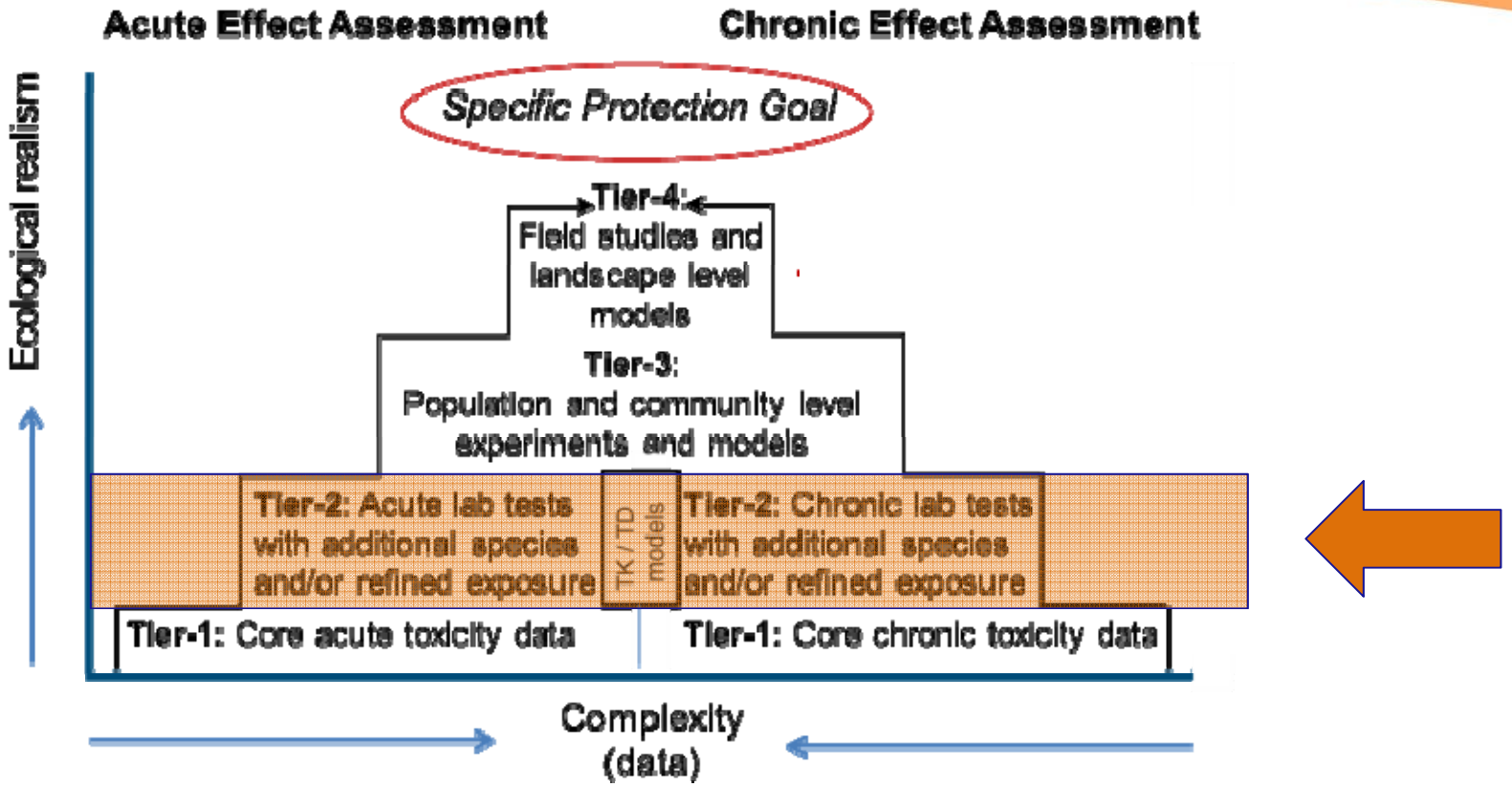
European Food Safety Authority

Tier 2 Effects and Risk Assessment - laboratory toxicity tests

Dan Pickford

Higher Tier Laboratory Tests



Reduce uncertainty in the risk assessment

- improved exposure scenarios
- more information on inter-species variation in sensitivity

Additional studies from Literature

Legal requirement in Data Requirements to submit data from peer-reviewed scientific literature

Data Quality important:

- statistical power
- verification of measurement methods
- control of experimental conditions
- universality of effects in validated test systems
- biological plausibility of results
- uniformity among substances with similar attributes

EFSA (European Food Safety Authority), 2011. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092, 49 pp. doi:10.2903/j.efsa.2011.2092.

Freshwater vs Marine

Article 3

Definitions

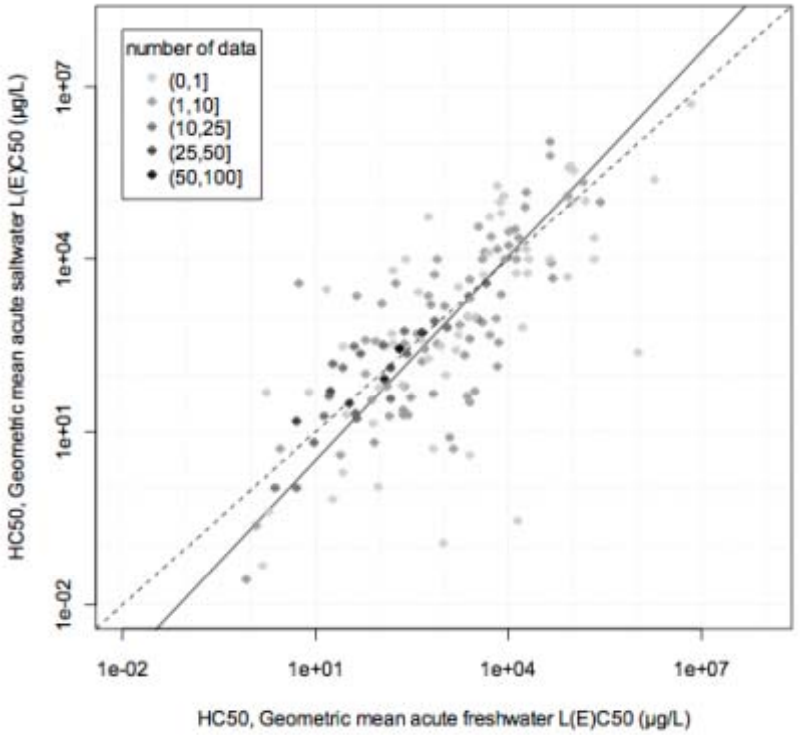
For the purposes of this Regulation, the following definitions shall apply:

13. 'environment' means waters (including ground, surface, transitional, coastal and marine), sediment, soil, air, land, wild species of fauna and flora, and any interrelationship between them, and any relationship with other living organisms;

No marine species in data requirements (except *A. bahia*)

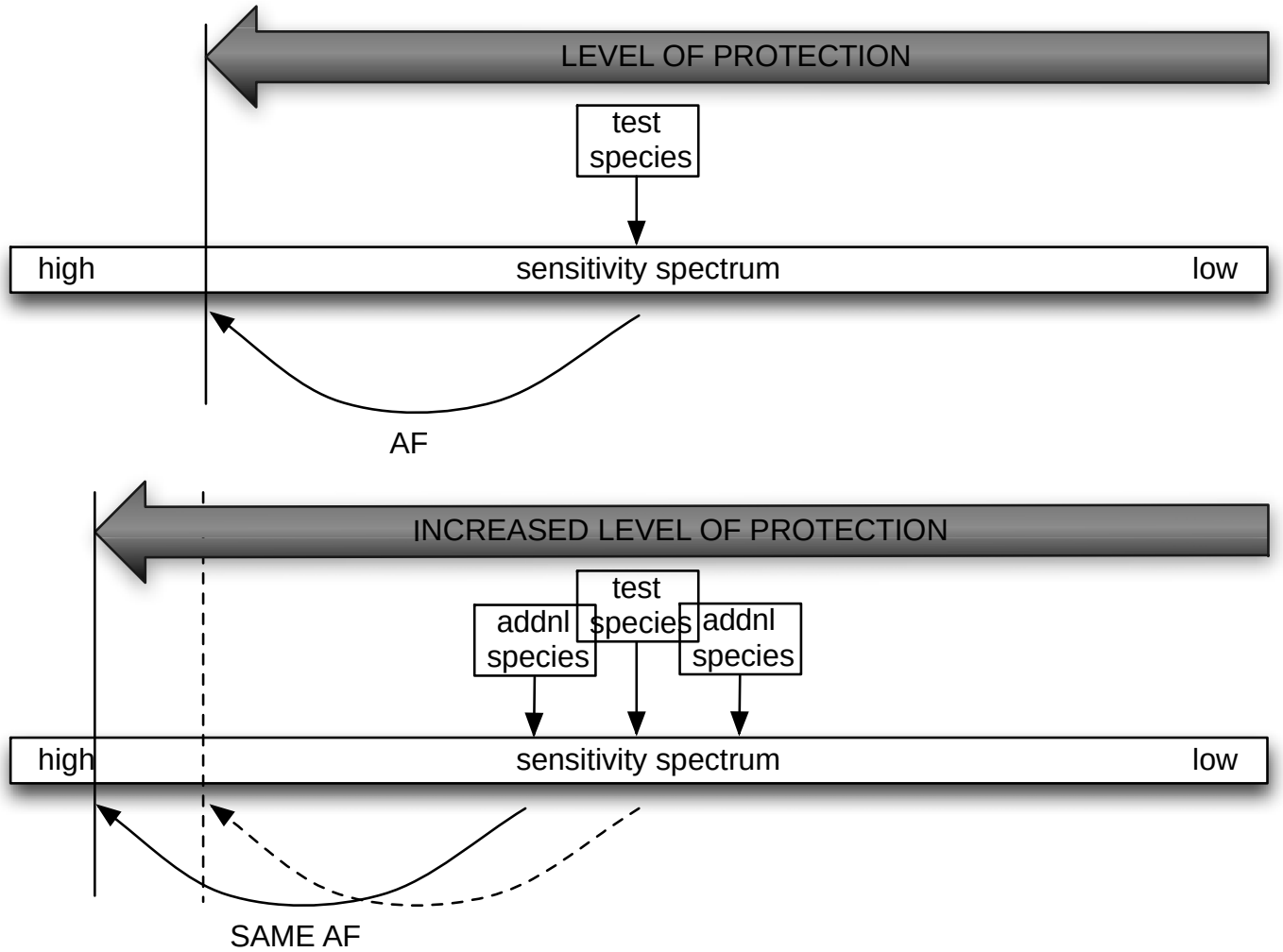
but potentially admitted through open literature requirement…

- relevance to edge of field surface waters?
- salinity and test conditions
- relative sensitivity



Klok C, de Vries P, Jongbloed R and Tamis J, 2012. Literature review on the sensitivity and exposure of marine and estuarine organisms to pesticides in comparison to corresponding fresh water species. Supporting Publications 2012:EN-357. [157 pp.].

Level of Protection



i.e. more than implied by the legislation (not specified)

Old guidance did not specify how much additional data required to allow decrease in AF

Geomean

Previous Approach

Taxa where only 1 species required:

- species sets level of protection
- with additional species: apply current AF to the geomean

(Option 1, EFSA 2006*)

Taxa where 2 species required:

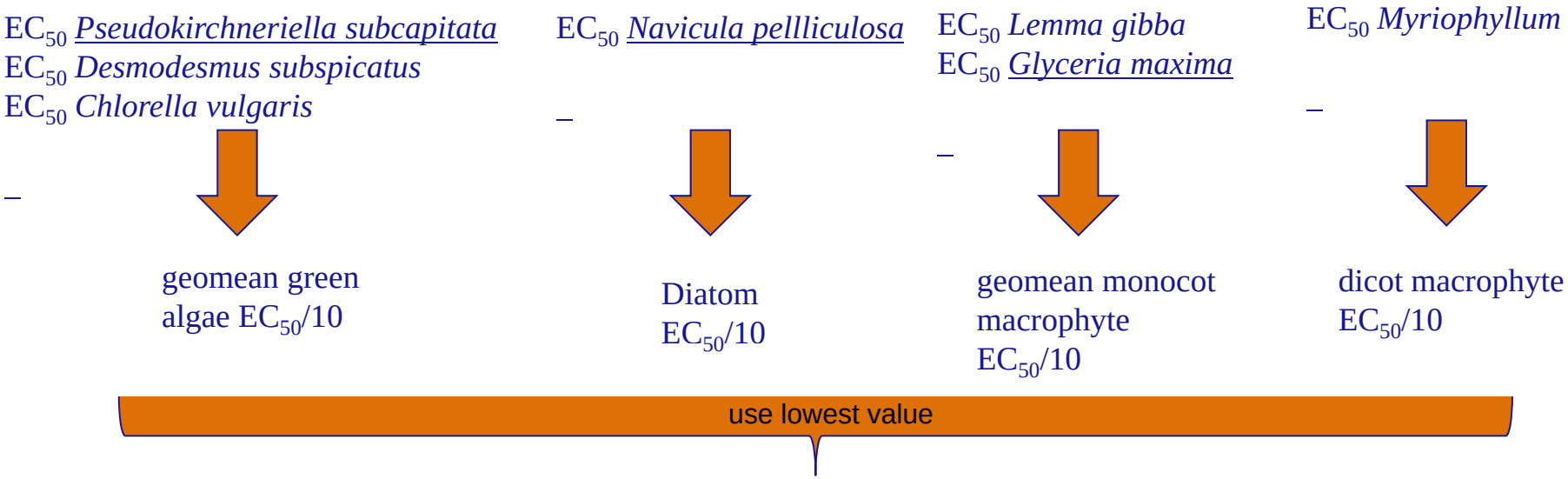
- higher level of protection implied
- with additional species: take 2nd/3rd most sensitive value

(Option 2, EFSA 2006*)

Current Guidance:
where more species are available than the Tier 1 requirements use the Geomean

* EFSA Journal 2005, 301, 45 pp. doi:10.2903/j.efsa.2006.301.

Deriving $RAC_{geomean}$

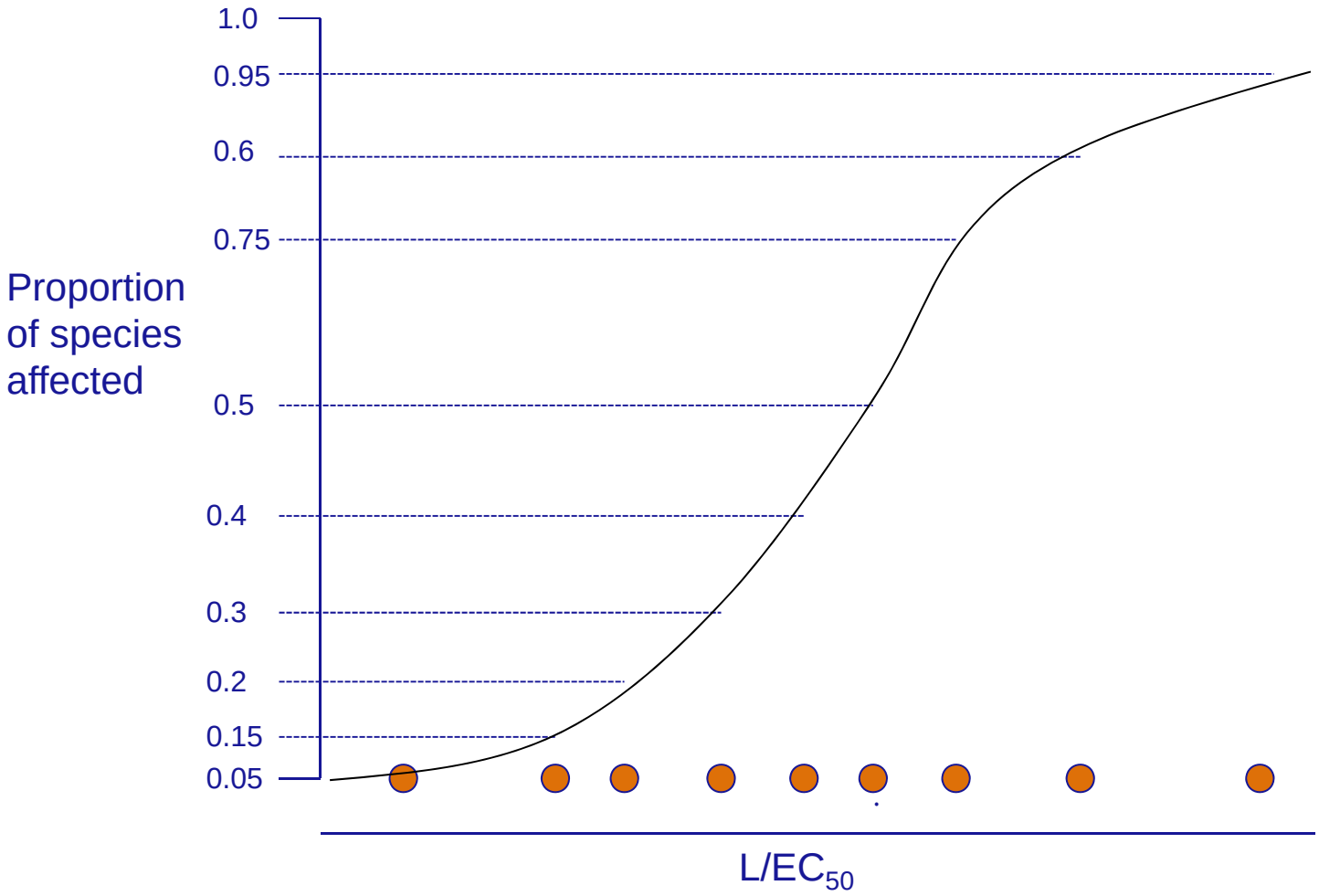


- taxonomic groupings must be scientifically justified i.e. consistent with data
- use Tier 1 taxonomic groupings as default
- use geomean value of most sensitive taxonomic group to generate $RAC_{geomean}$
- may be insensitive to a.s. with novel modes of action eg IGRs

How many values for $RAC_{geomeans}$?

Taxonomic group	No species values	RACgeomean Ecx/AF	Relevant environmental concentration
Acute Toxicity			
Aquatic vertebrates	< 5 acute LC_{50}s	geomean LC_{50}/100	$PEC_{sw;max}$
Aquatic inverts	< 8 acute EC_{50}s	geomean EC_{50}/100	$PEC_{sw;max}$
Chronic toxicity			
Aquatic vertebrates	< 5 chronic EC_{10}s or NOECs	geomean EC_{10}/10	$PEC_{sw;max}$ or $PEC_{sw;twa}$
Aquatic inverts	< 8 chronic EC_{10}s or NOECs	geomean EC_{10}/10	$PEC_{sw;max}$ or $PEC_{sw;twa}$
Primary producers	< 8 chronic EC_{50}s or NOECs	geomean EC_{50}/10	$PEC_{sw;max}$

Species Sensitivity Distributions SSDs



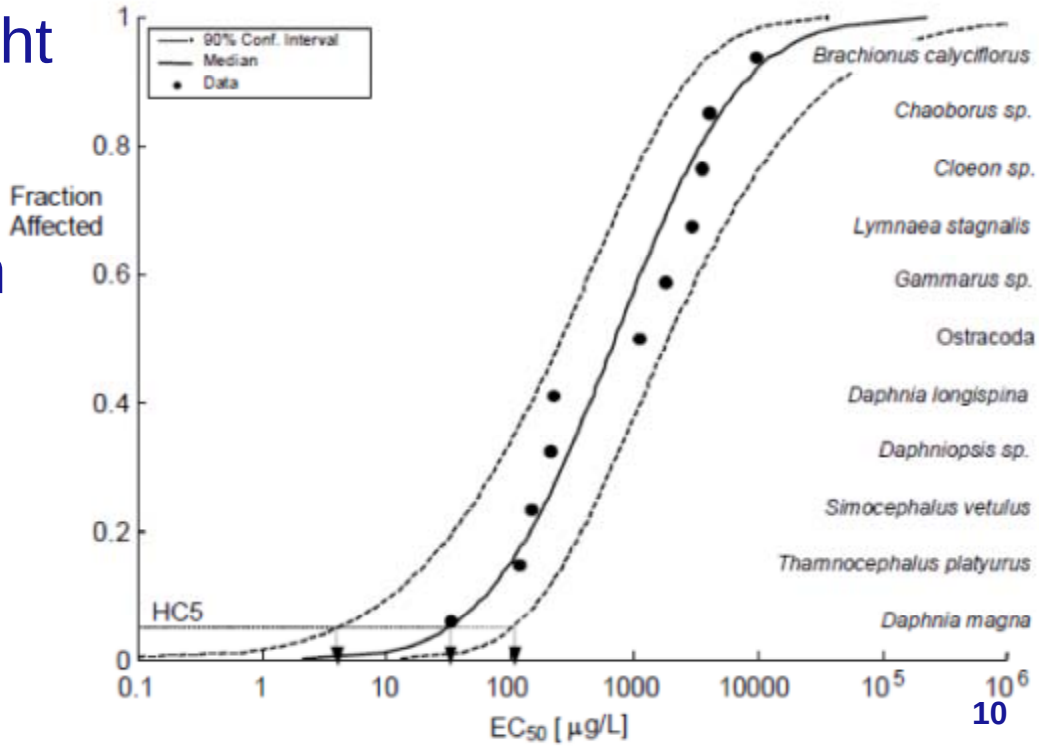
SSDs – HC_5

Probabilistic approach to better estimate toxicity to a wide range of organisms

Statistical distribution estimated from lab tox data

All species have equal weight

Estimate a concentration at which a specified proportion of species expected to experience toxicity
- HCx

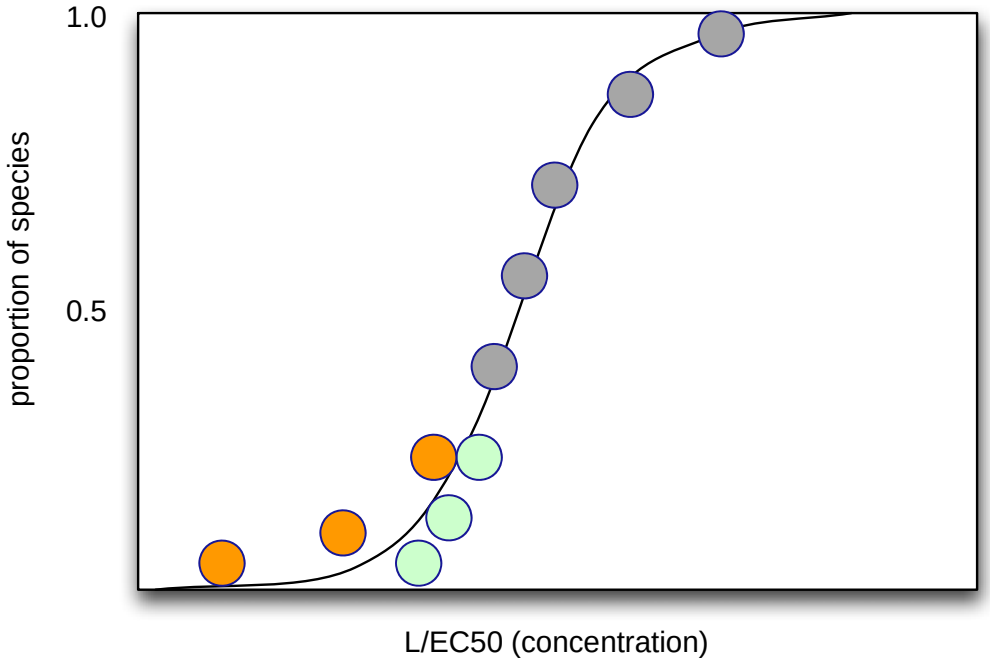


Statistical considerations

Expect log-normal/log-logistic distributions

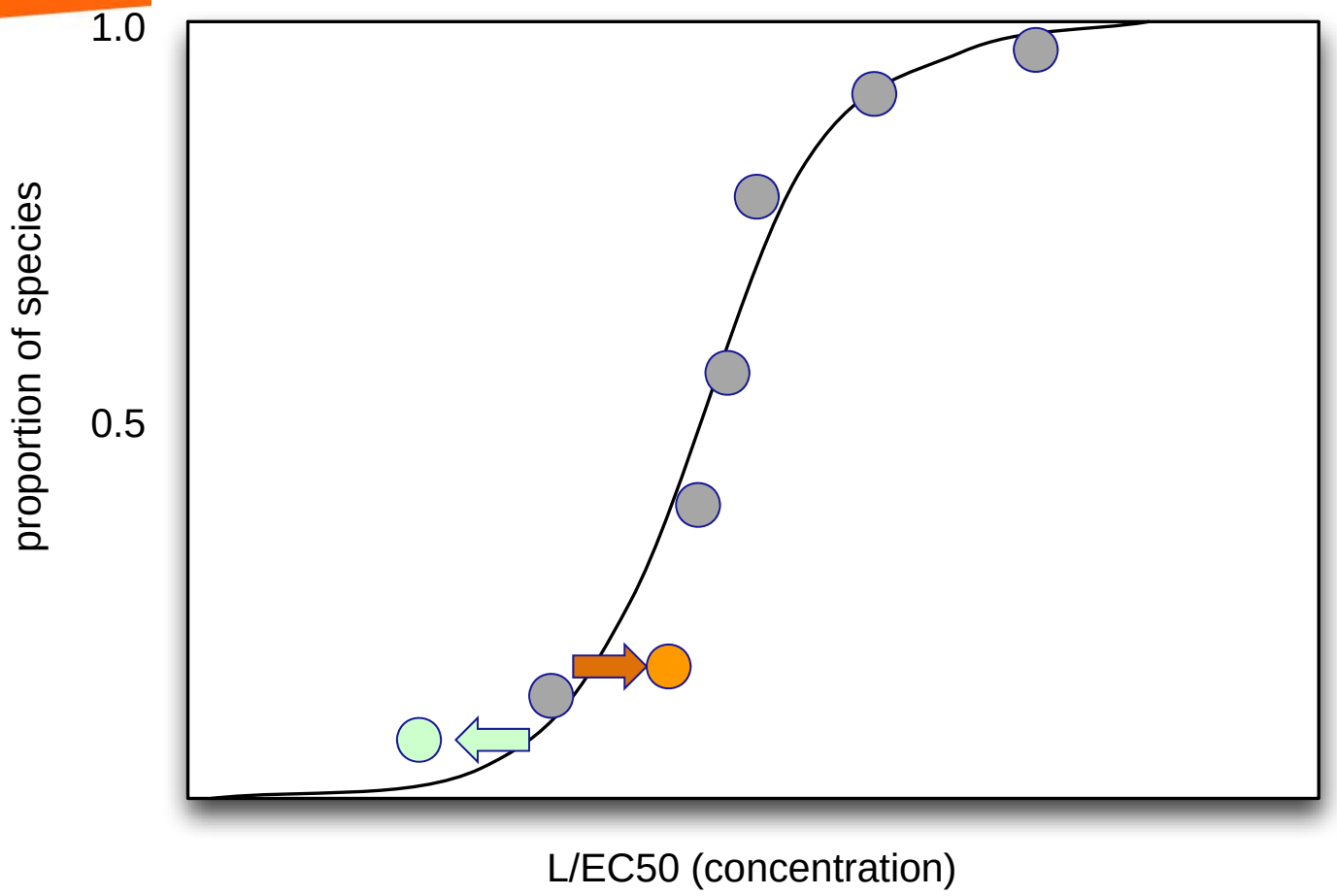
Tests for normality sensitive to amount of data

Judgement at lower end



Follow HARAP recommendations:
- separate SSDs for acute and chronic
- minimum of 5 species for fish
- otherwise minimum of 8 species

Unbounded values



Only use unbounded value
if it lies outside the range of other values

Selecting taxonomic groups for SSDs

Guided by Tier 1 data –
SSD for sensitive taxonomic groups (>10 x)

Guided by mode of action

example 1. Auxin inhibitor herbicide may require macrophyte-only SSD

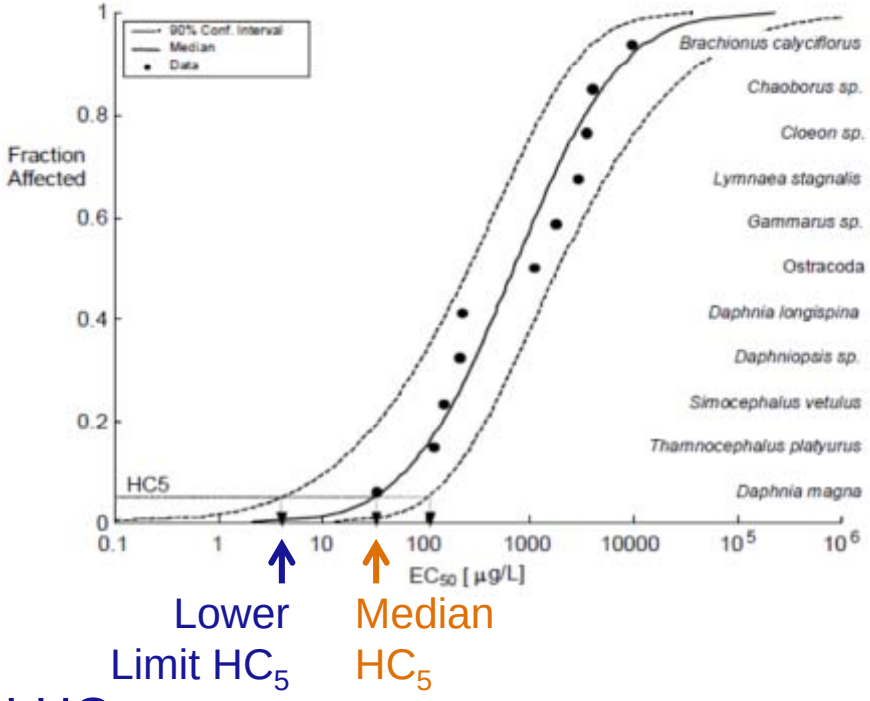
Use largest taxonomic grouping whose distribution gives an acceptable goodness of fit

example 2. for an insecticide, if an arthropod SSD gives a good fit, don't use a smaller grouping (insects or crustaceans)

example 3. for a general biocidal fungicide, use all aquatic organisms (ideally from ≥6 orders/families)

Calibration wrt Mesocosms

calibration



Lower Limit HC_5

Median HC_5

Insecticides: acute Median $HC_5/3$ or $LLHC_5$ (Van Wijngaarden et al., 2013)

Herbicides: $LLHC_5$ (acute) or median HC5 (chronic) (Van den Brink et al., 2006)

Fungicides: acute Median $HC_5/3$, $LLHC_5$, median HC_1 (Maltby et al., 2006)

Median $HC_5/3$ or $LLHC_5$ protective of longer term exposure due to single or repeated pulse exposure (Maltby *et al.*, 2009)

Calibration wrt real world

?

calibration

Variability in mesocosm data
- low replicate number
- effects thresholds
- community composition

Representativeness of real world

Additional AF – 2 for effects class 1
2-3 for effects class 2

$SSD\text{-}RAC_{sw;ac}$

Derivation RACs protective for Field exposure characterised by single and repeated pulses

- Vertebrates: Median HC_5/3 (based on NOEC/LC_{10}) or
  Median HC_5/9 (based on LC_{50})

- Invertebrates: Median HC_5/3-6 (i.e. 3 x [1 or 2]) (based on LC/EC50 from standard or prolonged acute tests)

Factors to consider in selecting AF for invertebrate SSD-RAC

- Representativeness of the acute toxicity data (including EPT taxa?)
- Confidence limits of the distribution (higher if $LLHC_5$ < 1/3 median)
- Comparison to RACs from other tiers
- Steepness of the SSD curve (steeper – higher AF)
- Read across from compounds with similar MOA
- Consideration of chronic effects (higher ACR- higher AF)

Chronic SSDs

Limited experience, less available data

More variability in endpoints

- comparability across taxonomic groups

Relevance of exposure duration

– what constitutes chronic?

Uncertainties arising from use of NOECs

Combine EC10 (preferred) and NOEC

SSD-RAC$_{sw;ch}$ = median HC$_5$/3

Taxon. Group and Type of effect	Relevant PEC	Hazardous concentration	AF to derive RAC
Invertebrates acute and chronic effects after single / repeated pulse exposure	$PEC_{sw;max}$	• Latency of effects NOT expected: Median HC_5 from L/EC_{50} data from acute tests • Latency of effects EXPECTED: Median HC_5 from *prolonged* acute tests • or use chronic.	3-6 3-6
Invertebrates chronic effects after long-term exposure	$PEC_{sw;max}$ or $PEC_{sw;twa}$	Median HC_5 from chronic NOEC and/or EC_{10}	3
Primary producers	$PEC_{sw;max}$	Median HC_5 from EC_{50} data	3
Vertebrates acute effects/risk assessment	$PEC_{sw;max}$	• Latency of effects NOT expected: Median HC_5 from 96h NOEC and/or acute LC_{10} data • Or Median HC_5 based on 96h LC_{50} or EC_{50} data • Or if latency of effects is expected go to chronic effects assessment	3 9
Vertebrates chronic effects and long-term exposure	$PEC_{sw;max}$ or $PEC_{sw;twa}$	Median HC_5 from chronic NOEC and/or EC_{10}	3

Tier 2C: refined exposure lab tests

- Option if predicted exposure profiles differ substantially from exposure profile in standard tox tests
- Should use however realistic worst-case exposure profile
- Tests need to be long enough to allow expression of maximum effects, usually prolonged tests needed
- Usually performed with standard tier 1 species
- RAC should always be expressed as peak concentration in the test and compared to $PEC_{sw;max}$

Summary

- Tier 2A (Geomean) and 2B (SSD) refine effects assessment by using more species data
- Geomean method where insufficient data is available for SSD – will still reduce uncertainty relative to single species test in Tier 1
- SSD allow probabilistic methods – confidence intervals on hazardous concentrations
- Considerations on inclusion/exclusion of taxonomic groups important
- Selection of AF to reflect confidence in the data
- Tier 2C allows addressing the exposure side in the lab tests