# Direct toxicity assessment

## methods, evaluation, interpretation

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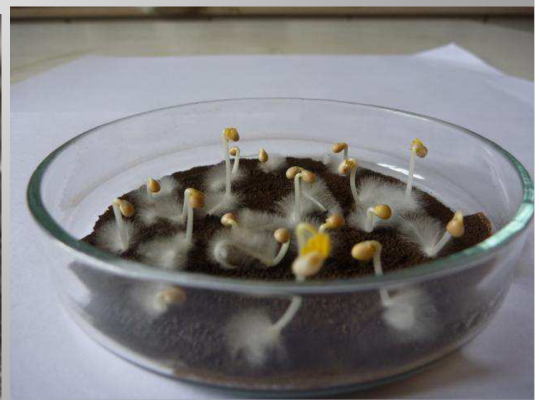
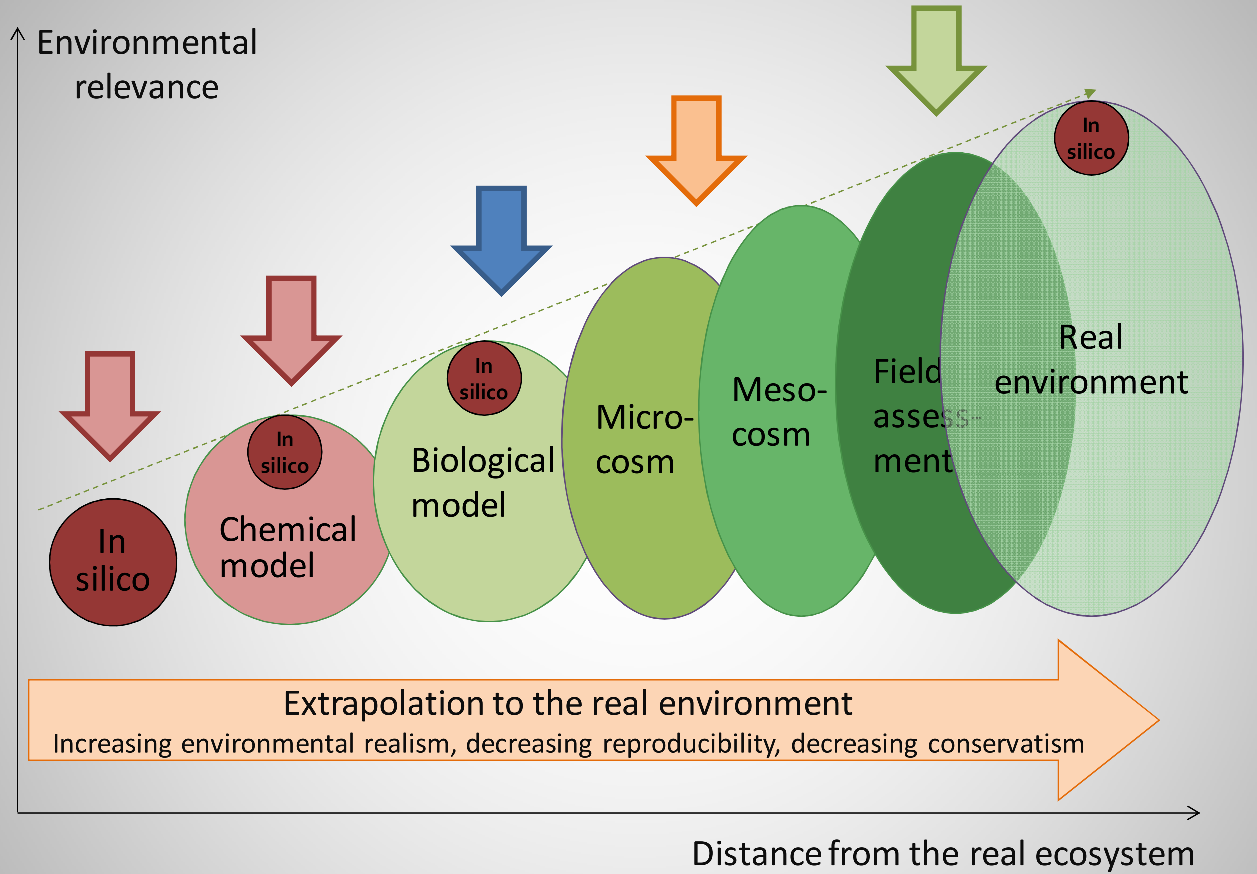
# DTA in contaminated land management

Content of the presentation

- Basics of DTA in contaminated land management
- Methods and tools
- Evaluation, endpoints
- Integrated assessment
- Interpretation
- Uncertainties, statistics



# Models applied in environmental assessment

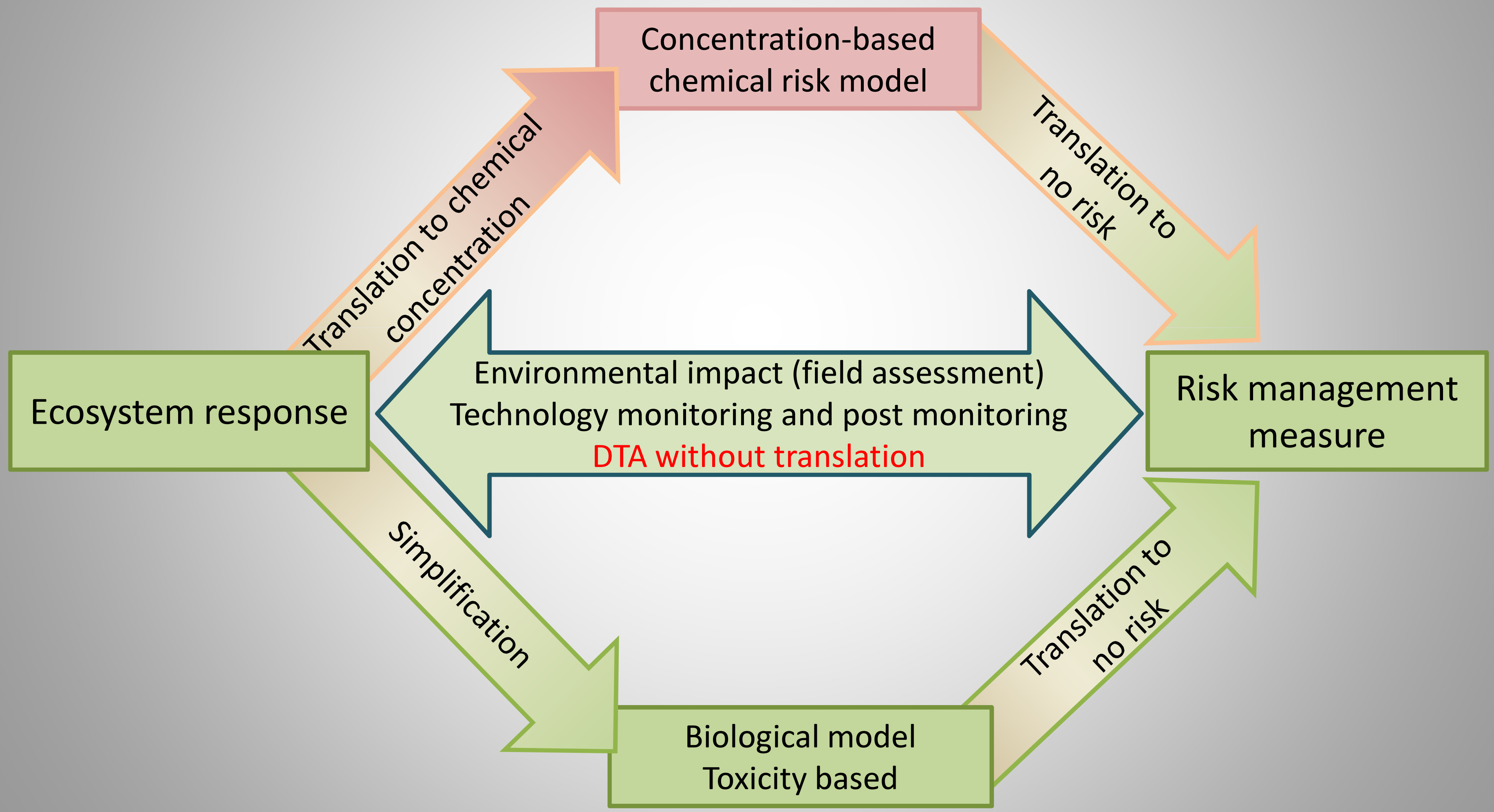



Biodegradability: QSAR → standardized tests → simulated degradation → real degradation
Toxicity: QSAR → standardized toxicity tests → simulation → controlled real environment

Most appropriate model: regulatory RA/RM, contaminated sites RA/RM, global RA/RM

# Concepts for ecosystem management

## Application of DTA



# Benefits of DTA

The adverse effects of environmental samples differ from the aggregated adverse effects extrapolated from chemically determined/analyzed contaminants.

Why?

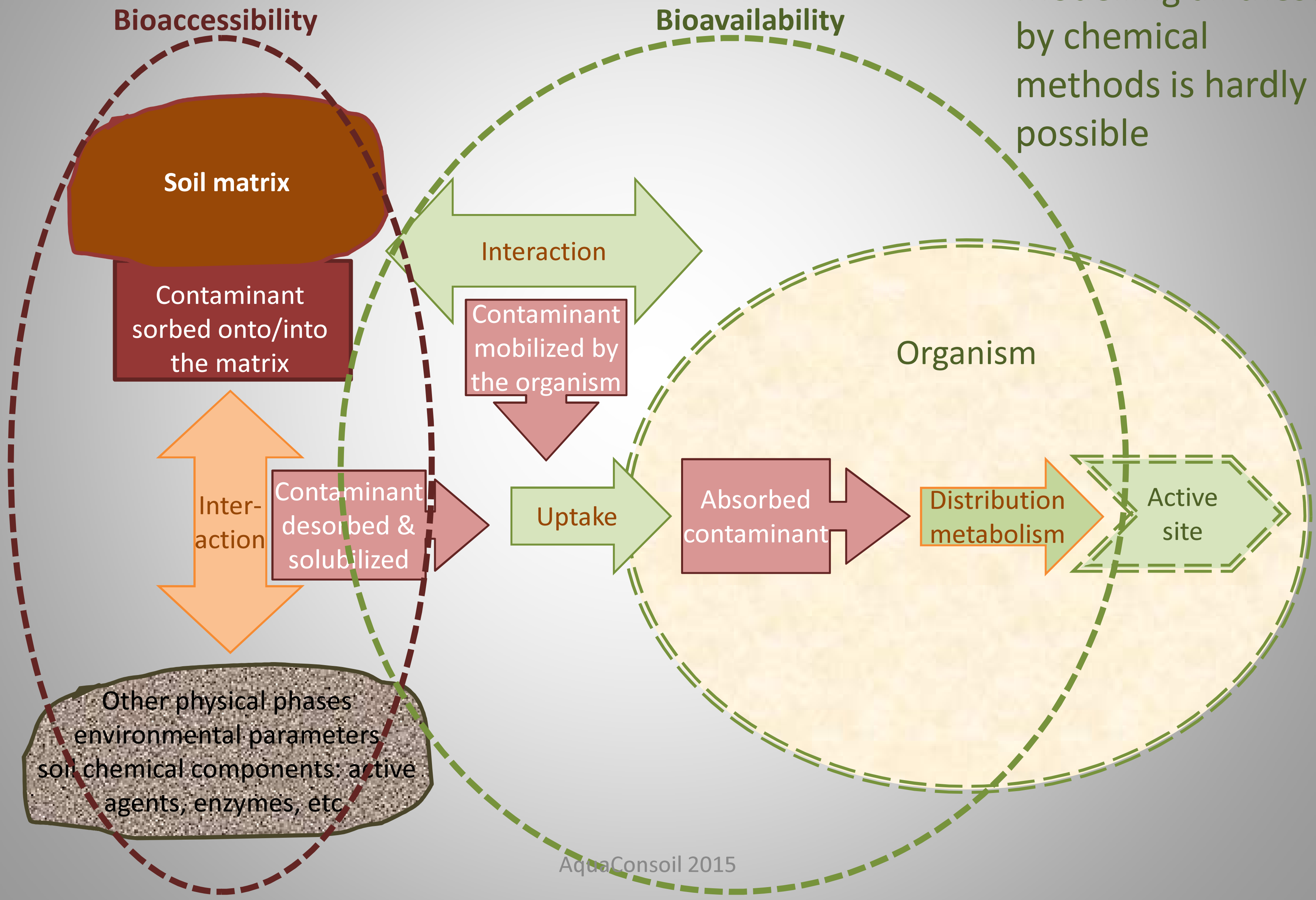
1. The analytical programme does not include all possible chemicals having adverse effects;
2. Analytical methods cannot measure some contaminants in their effective concentration range
3. Chemical availablility (extraction by solvents) differs from bioavailability;

**DTA measures:**

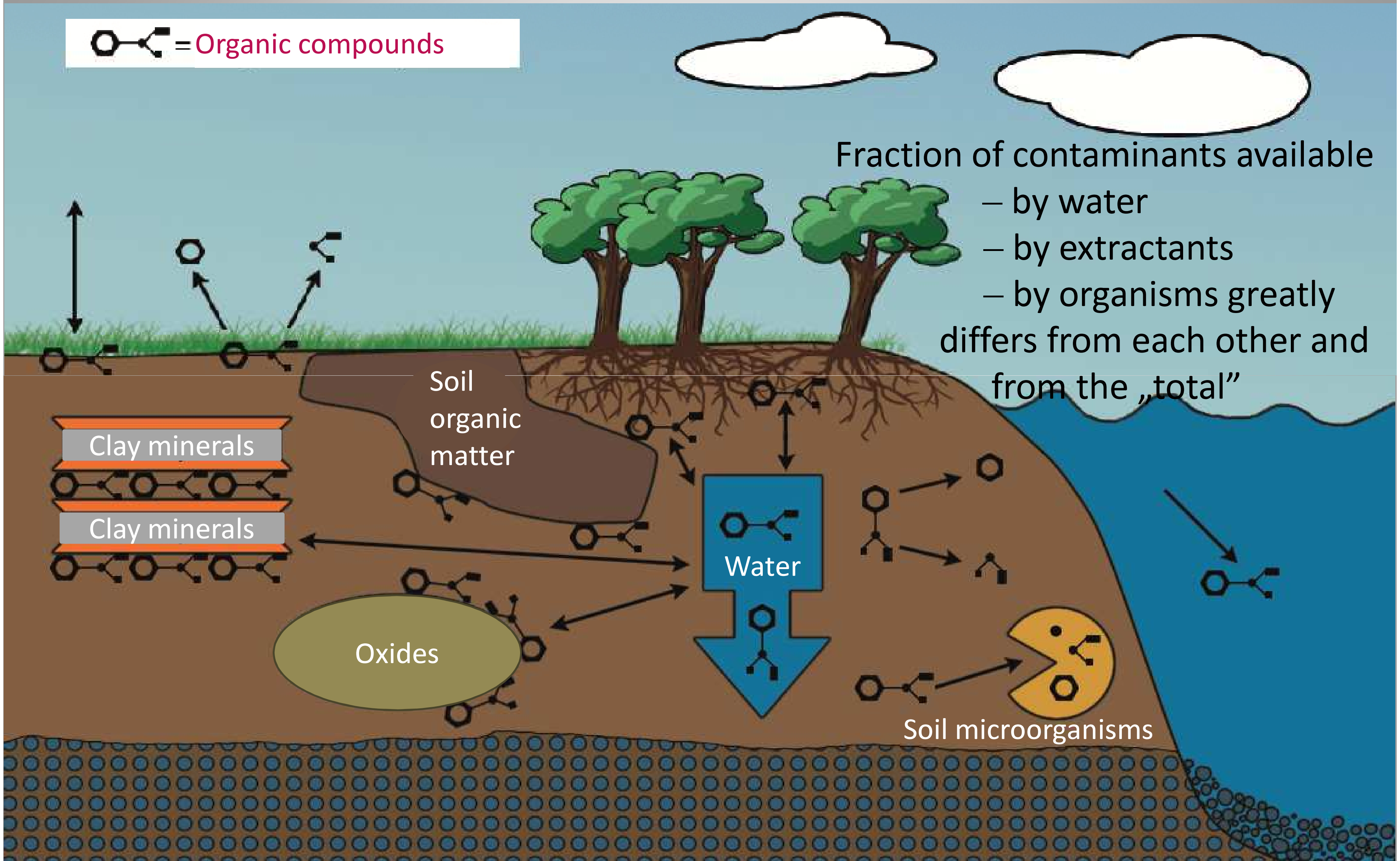
- the toxicity of entire effluents (wwtp, leachates, runoffs);
- characterizes sediment and soil toxicity with high environmental realism;
- accounts for the aggregated effect of chemical mixtures;
- may provide results including different exposure routes and effects;
- measures a response proportional to the bioavailable fraction;
- accounts for the effects of not analyzed and chemicals of not known effects,
- ensures safety by identifying toxicity of samples despite complying with chemical-based limits.

# DTA in soil testing

Modelling all these by chemical methods is hardly possible



# Complex and dynamic mobility/binding in soil



# DTA usability

- Direct toxicity assessment (DTA) ensures high environmental relevance, representing all possible interactions between contaminants, ecosystem members and soil . The result aggregates the effects of all contaminants present in the sample.
- DTA can simulate different soil uses and real, multiple exposures.
- Difficulty: directly measured toxicity of environmental samples cannot be expressed in concentration, thus it does not fit the chemistry based risk assessment model and the concentration-based screening values cannot be applied.

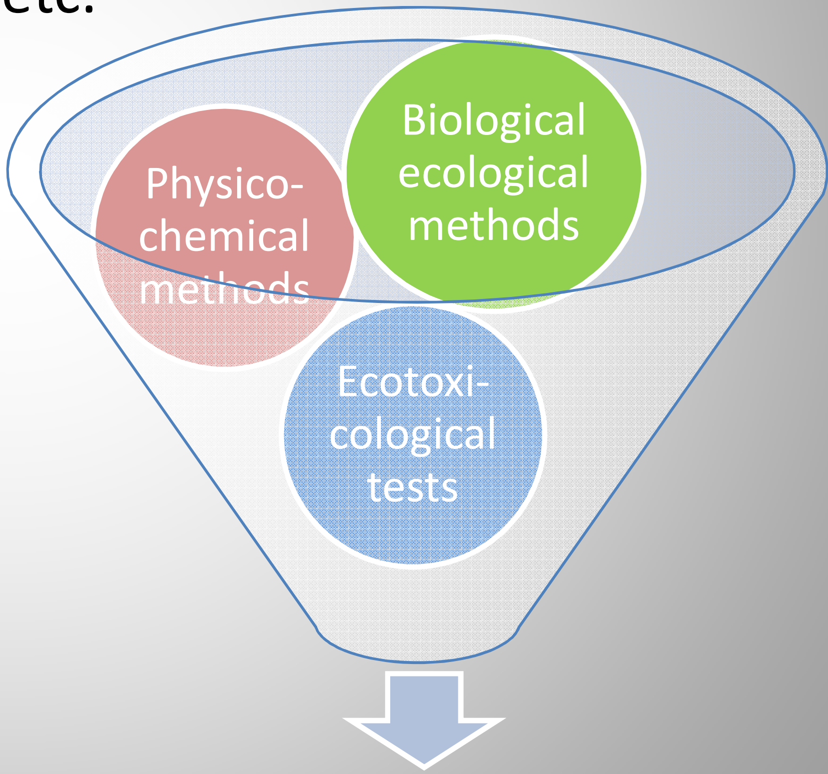



# Direct toxicity assessment applications

1. Non-targeted screening: not known or uncertain contaminants and site history (general toxicity on soil ecosystem);

2. Targeted screening: known contaminants / specific effects / contaminant-specific biosensors, etc.

3. Integrated evaluation by paralel toxicity testing, chemical analysis and biological/ecological and toxicological characterization




Integrated evaluation

# Can chemistry truely model the biological response?

Example of a zinc-lead mine tailing dump

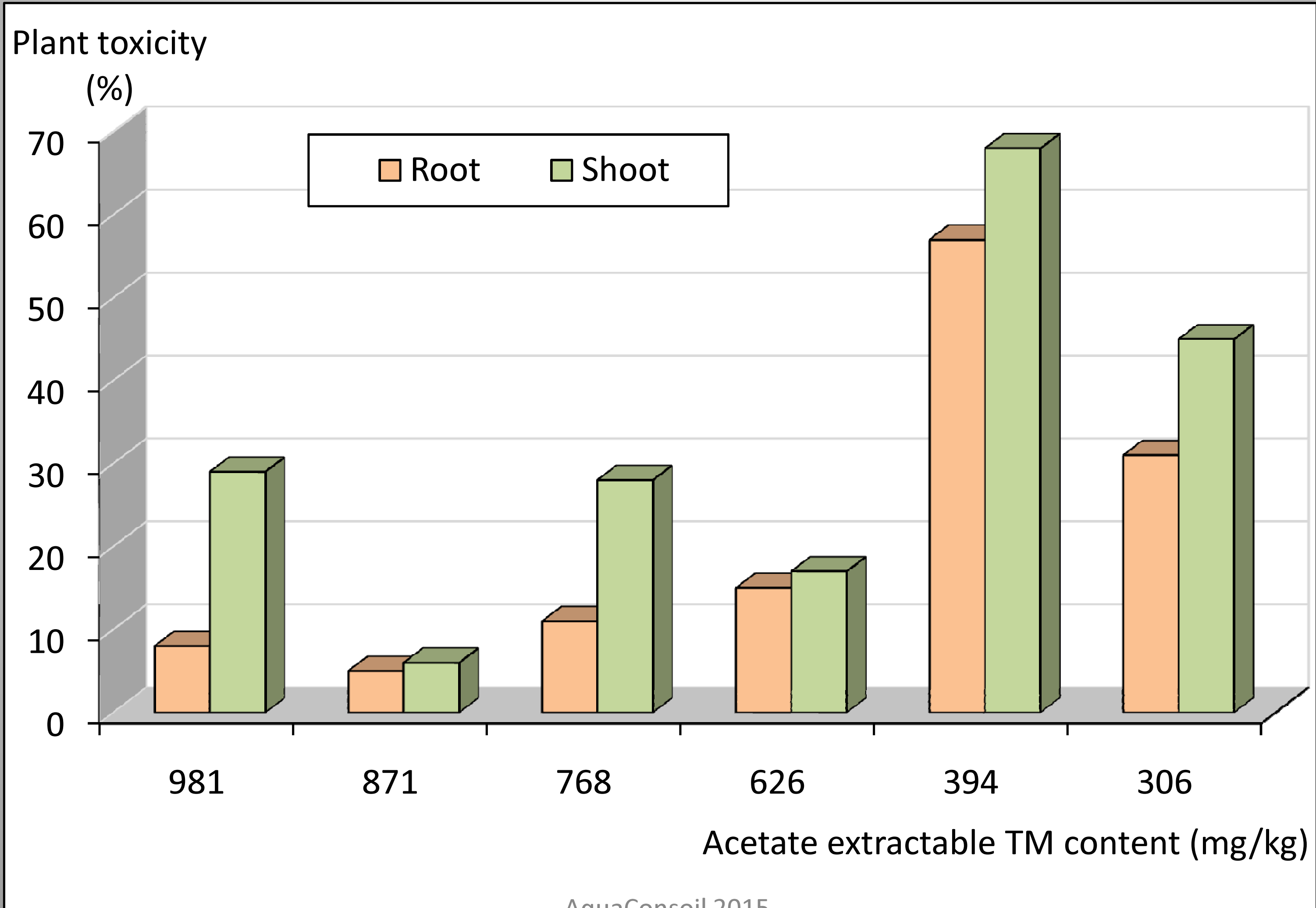
Flotation tailing covered by soil without isolation.

| Layer | pH | Total metal content (mg/kg) | | |
|---|---|---|---|---|
| | | Zn | Pb | Cu |
| Black soil | 4.7–5.2 | 603 | 186 | 72 |
| Grey tailing | 7.0–8.0 | 31,858 | 4,971 | 2,450 |

| Test method: | *Azotobacter agile* dehydrogenase | *Sinapis alba* root & shoot growth | *Alliivibrio fischeri* luminescence |
|---|---|---|---|
| Black soil | Very toxic | Toxic | Very toxic |
| Grey tailing | Non-toxic | Slightly toxic | Non-toxic |

| Bioaccumulation Species | Zn (mg/kg) | Cu (mg/kg | Cd (mg/kg |
|---|---|---|---|
| *Achillea millefolium* | 255 | 17 | 2.4 |
| *Agrostis sp.* | 410 | 32 | 6.3 |
| *Carex sp.* | 355 | 55 | 3.0 |
| *Echium vulgare* | 607 | 45 | 5.0 |
| *Phalaris canadiensis* | 145 | 4.0 | 0.5 |
| *Phragmites australis* | 768 | 41 | 0.7 |
| *Populus sp.* | 1158 | 13 | 19.5 |
| *Silene alba* | 694 | 50 | 2.6 |
| *Silene vulgaris* | 506 | 21 | 4.6 |
| *Tussilago falifara* | 569 | 39 | 8.8 |
| QC for forage* | 150–200 | 15–50 | 1.0 |
| for vegetable** | 20 | 10 | 0.5 |

# Plant toxicity compared to chemically measured metal content at a flooded site



# Evaluation and interpretation of direct toxicity test results and their use in risk management

Endpoints:
- effective soil/groundwater dose (sD/sV)
- no effect dose or volume (NOEsD, NOEsV)

Representation of the ecosystem:
- 3 or more testorganisms from minimum of 3 trophic levels
- average of three representative effect
- the smallest of three testorganisms
- effect distribution of more (7 or more) testorganisms and reading an optional value from the distribution curve

Target toxicity:
- average or smallest NOEsD of the three effect values
- $EsD_5$ or $EsD_{20}$
- any value of the distribution curve

RCR = risk characterization ratio = measured toxicity/target toxicity

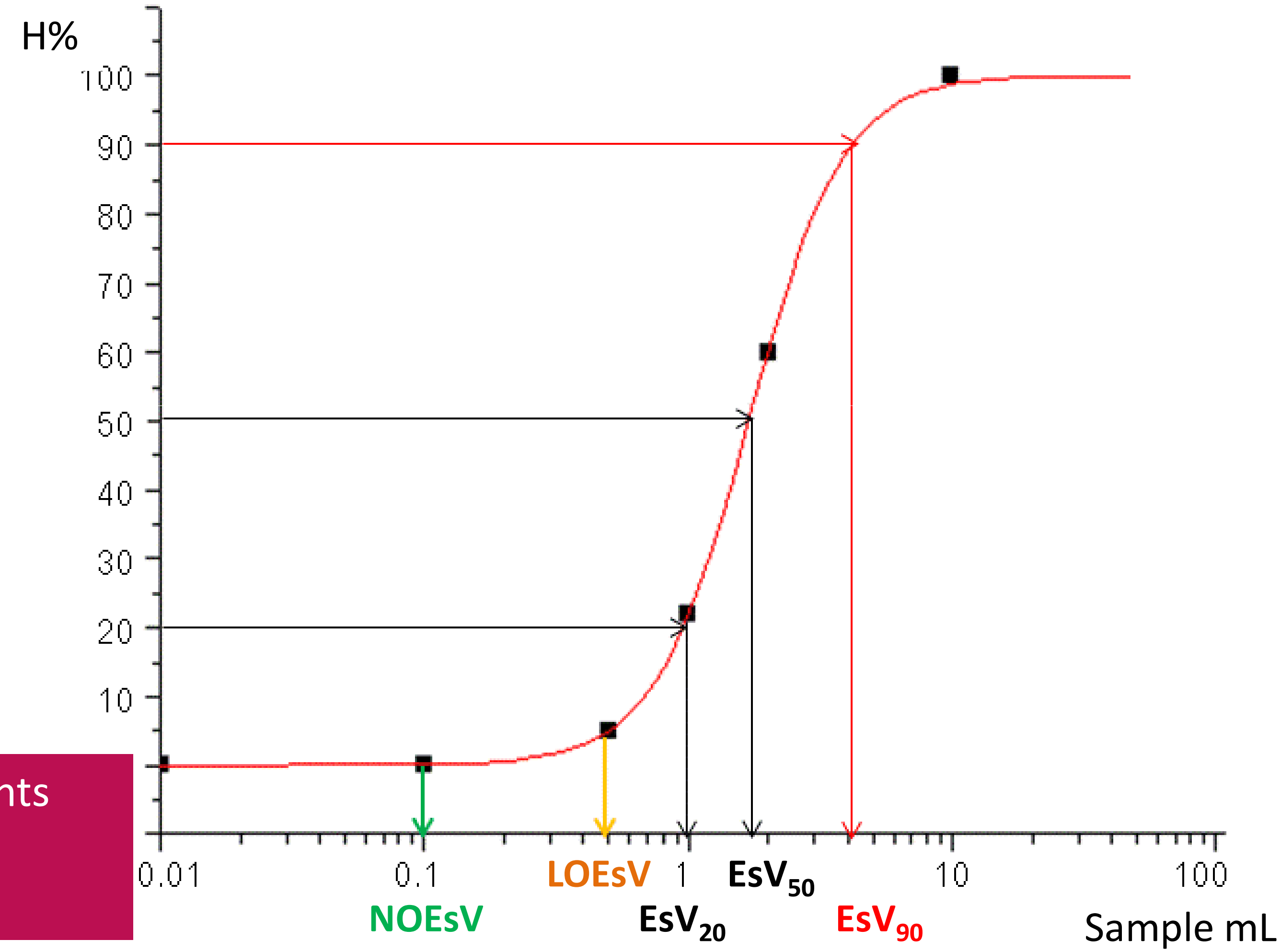
Risk assessment: excess risk or RCR based on comparison with the ref.

Risk-benefit assessment!

Risk reduction measure: based on excess risk, RCR and other, e.g. socio-economic values.

# Inhibition rate as function of groundwater volumes

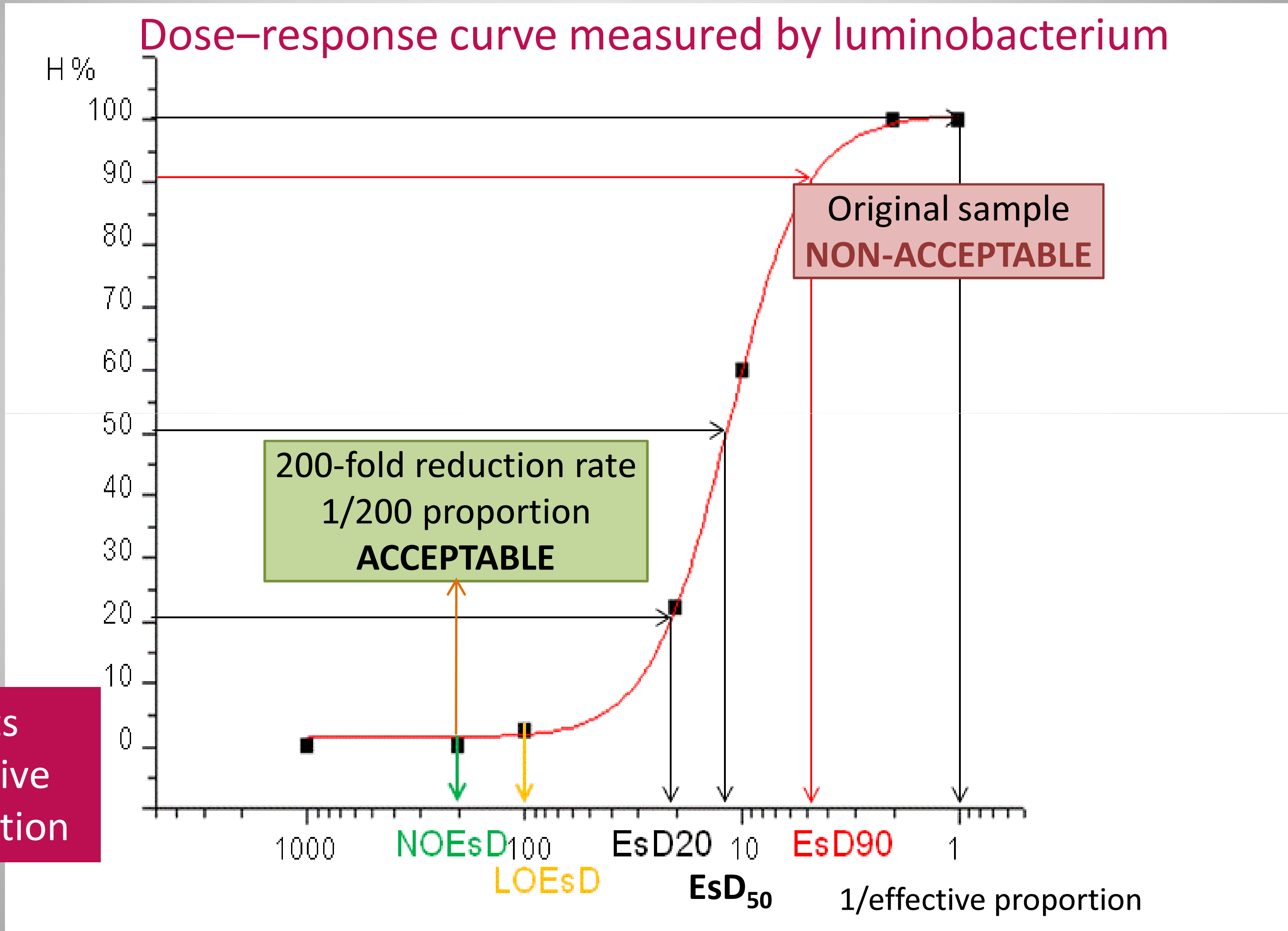
Dose–response curve measured by *Tetrahymena*




Test end points given in sample mL

No effect volume → No-effect dilution = Necessary rate of dilution/removal/clean-up

# Inhibition rate as the function of soil sample mass and the test end points




Test end points given in effective sample proportion

No effect dose → Reduction rate to 'no effect' = Necessary rate of removal/clean-up

Target = $EsD_{20}$ → reduction to 20% inhibition rate → Necessary rate of removal/clean-up

# Artificial cover layers of red mud deposites

Average samples from historical cover layers of 6 reservoirs.
Site specific criterion: not more than 20% inhibition

| Soil sample | Collembola lethality | | Effective sample proportion (SP) | Excess risk |
|---|---|---|---|---|
| | *Inhibition rate [%] of the original sample* | *Sample dose causing 20% inhibition $LsD_{20}$ [g]* | *SP causing $LsD_{20}$ [%]* | *How many times of the reference SP* |
| 1 | 70 | 1,2 | **6** | **17** |
| 2 | 68 | 6,3 | 32 | 3 |
| 3 | 13 | >20 | >100 | 0 |
| 4 | 28 | 17,0 | 85 | 1.2 |
| 5 | 23 | 19,0 | 95 | 1.1 |
| 6 | 40 | 15 | 75 | 1.3 |

Next steps: more testorganisms and more detailed assessment, ecosystem assmnt, hot spot identification, comparison to chemical analytical results

# New artificial soil to cover red mud deposites

## Assessment of the mixtures before emplacement

| Soil sample | Soil proportion resulting 20% inhibition (SP%) | | | | Average SP | RCR | RCR no wheat |
|---|---|---|---|---|---|---|---|
| Code | Bacterial luminescence | Mustard shoot growth | Wheat shoot growth | Collembola growth | % | Sample SP/ reference SP | Sample SP/ reference SP |
| 7 | 23 | 68 | 44 | 5,5 | 35 | 3 | 3 |
| 8 | 4 | 4,4 | 1,2 | 3,5 | 3,3 | 30 | 25 |
| 9 | >100 | 54 | 2,2 | 38 | 49 | 2 | 1.5 |
| 10 | >100 | 4,0 | 2,8 | 2 | 27 | 4 | 3 |
| 11 | 85 | >100 | >100 | >100 | 96 | 1 | 1 |
| 12 | >100 | >100 | >100 | >100 | 100 | 1 | 1 |
| 13 | 38 | 80 | 20 | >100 | 60 | 2 | 1.4 |
| 14 | 23 | 64 | 25 | 2,5 | 29 | 3 | 3 |
| 15 | 22 | 64 | 25 | 9 | 30 | 3 | 3 |
| Cover/0.2 m | >100 | >100 | >100 | 85 | 96 | 1 | 1 |
| Cover/0.4 m | 79 | >100 | >100 | 90 | 92 | 1 | 1 |
| Cover/0.6 m | >100 | >100 | >100 | >100 | 100 | 1 | 1 |

# Comparison of DTA and chemical analytical results

| **Soil sample** | **RCR** based on DTA | RCR based on chemical analysis of EPH, PAH, M10 | |
|---|---|---|---|
| Code | How many times of target $EsD_{20}$ | How many times of SQCecol | How many times of SQCindust |
| 7 | 3 | 2 | <1 |
| 8 | 30 | 10 | 3 |
| 9 | 2 | 2 | <1 |
| 10 | 4 | 3 | 1.5 |
| 11 | 1 | 1 | <<1 |
| 12 | 1 | 1 | <<1 |
| 13 | 2 | 1 | <1 |
| 14 | 3 | 3 | 1.1 |
| 15 | 3 | 3 | 1.2 |
| Cover/0.2 m | 1 | 1 | <<1 |
| Cover/0.4 m | 1 | 1 | <<1 |
| cover/0.6 m | 1 | 1 | <<1 |

**Interpretation:**
Agreement between chemical andecotox ass.: both + or both –;
Disagreement: + / -
+E /-C: not analysed;
-E/+C: not toxic or not bioavailable;
Common quantitative ground is: excess risk (RCR)

# Toxicity equivalencing method

**Advantages**

Toxicity  results can be converted into concentrations;

The toxicity of any mixture with uncertain availablity can be expressed in concentration of the reference material. Our references: Cu  for metals and 4 chloro-phenol for organics;

DTA results can be fit into the chemical model based ERA and ERM;

Makes the ecotox results understandable for non-ecotoxicologists;

Its application is recommended for exploration and non-targeted screening in a tiered assessment;

The shape of the dose–response curve is also informative.

Comparison of testorganisms to reference may also be informativ.

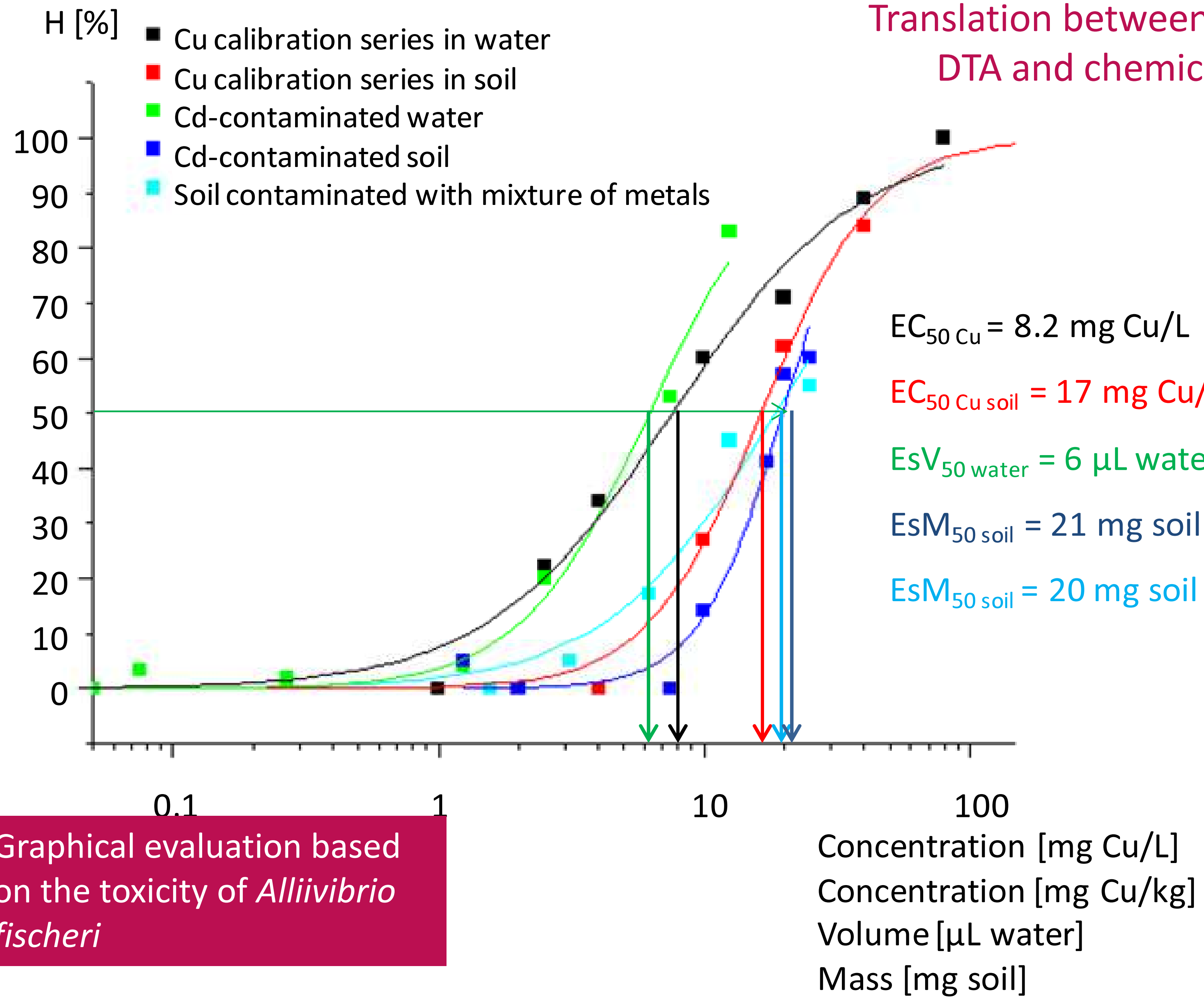
**Disadvantages**

Characterize  only the scale of toxicty of other substabces than reference;

The shape of the dose–response curve of the reference  may differ from the sample.

# Equivalencing

## Translation between the results o DTA and chemical analysis




$EC_{50\,Cu}$ = 8.2 mg Cu/L

$EC_{50\,Cu\,soil}$ = 17 mg Cu/kg

$EsV_{50\,water}$ = 6 µL water

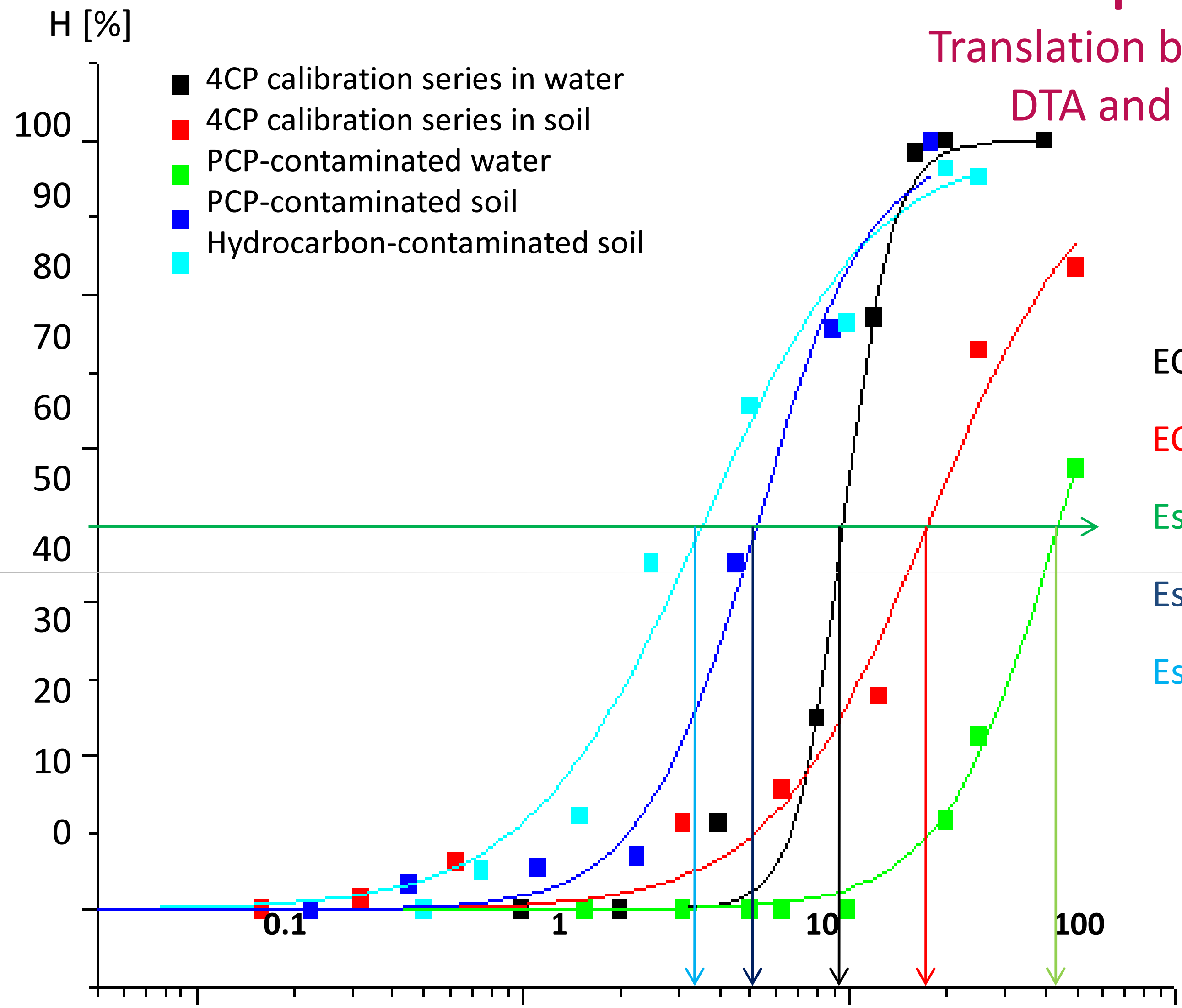
$EsM_{50\,soil}$ = 21 mg soil

$EsM_{50\,soil}$ = 20 mg soil

Graphical evaluation based on the toxicity of *Alliivibrio fischeri*

# Equivalencing

## Translation between the results of DTA and chemical analysis




$EC_{50\ 4CP}$ = 9.64 mg 4CP/L

$EC_{50\ 4CPsoil}$ = 19.20 mg 4CP/kg

$EsV_{50\ water}$ = 41.67 µL water

$EsM_{50\ soil}$ = 4.90 mg soil

$EsM_{50\ soil}$ = 3.55 mg soil

Concentration [mg 4CP/L]
Concentration [mg 4CP/kg]
Volume [µL water]
Mass [mg soil]

Graphical evaluation based on the toxicity of *Alliivibrio fischeri*

# Advantages and uncertainties in DTA of soil

Beneficial, because characterize the toxicity and risk of environmnetal samples without specifying the actual contaminants and interactions with high environmnetal realism.

Application: – The methods and tool are available
- – Evaluation and interpretation is not yet well established
- – Professionals, thinking mechanically according to the chemical model, also understand ecotoxicity results.
- – It can be directly linked to risk management and decision making, and this way to the more efficient risk management of contaminated land.

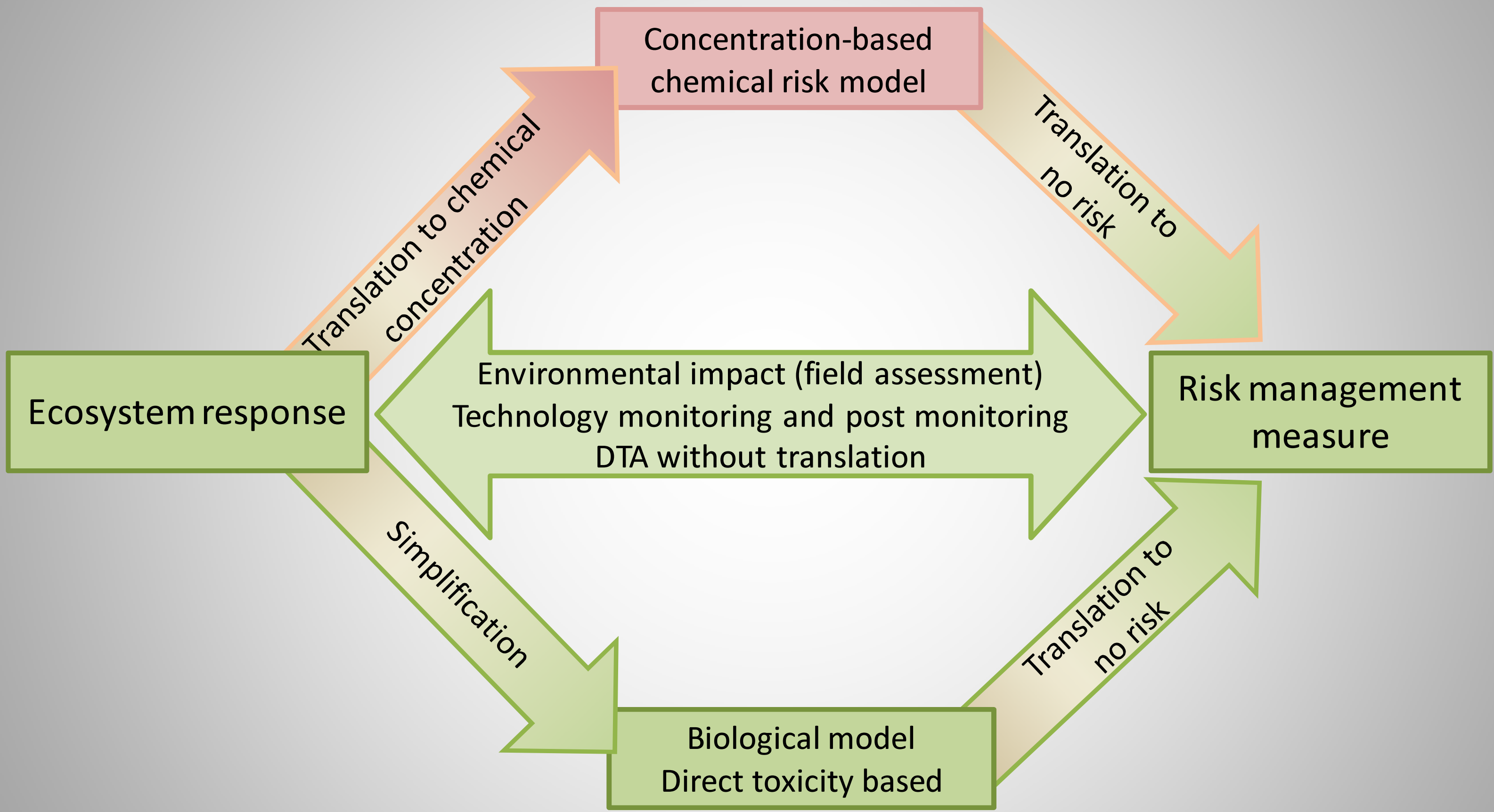
Measurement and evaluation:
- – Inhibition rate or dose–response function can be measured
- – Single species or more species can be applied in paralel
- – Multispecies test methods / microcosms can be applied

Uncertainties:
- – Environmental variability and sampling;
- – Uncertainties of the test methods, very few standard methods;
- – Uncertainties in interpretation.

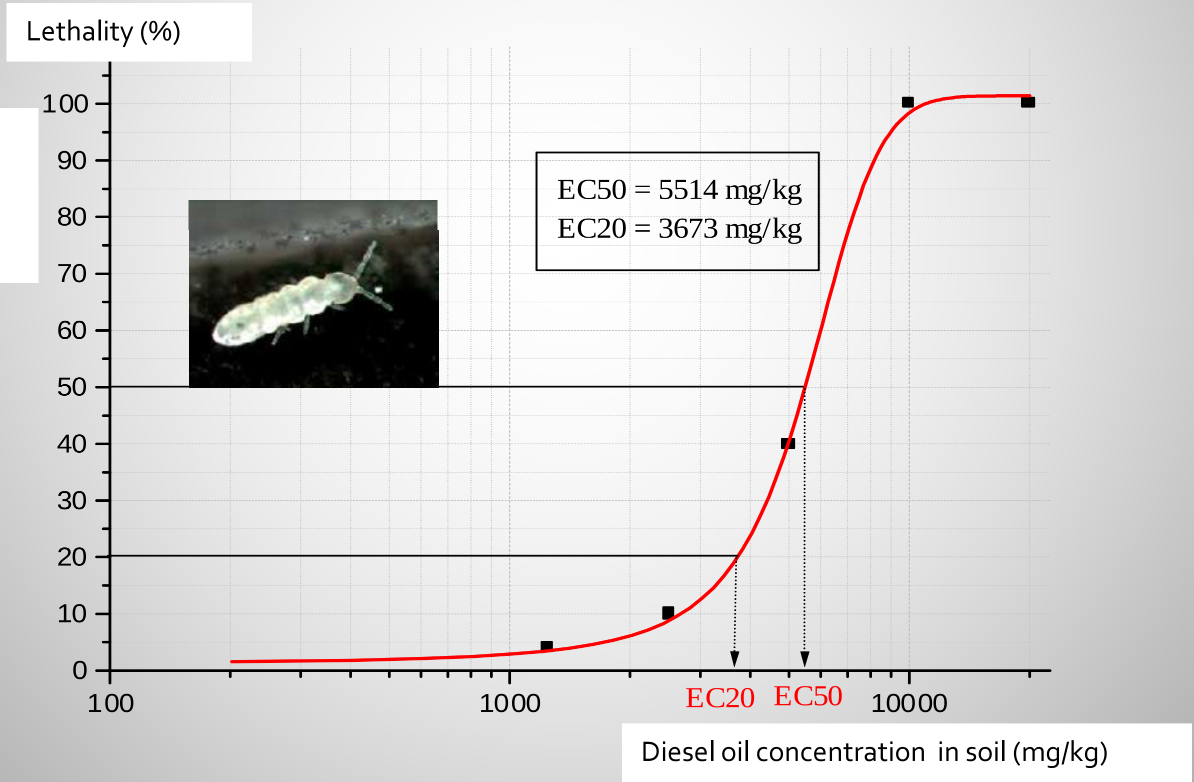
Statistical evaluation:
- – Hypothesis testing for the determination of NOEC;
- – Regression methods for $EC_5$ and $EC_{20}$, $EC_{50}$.

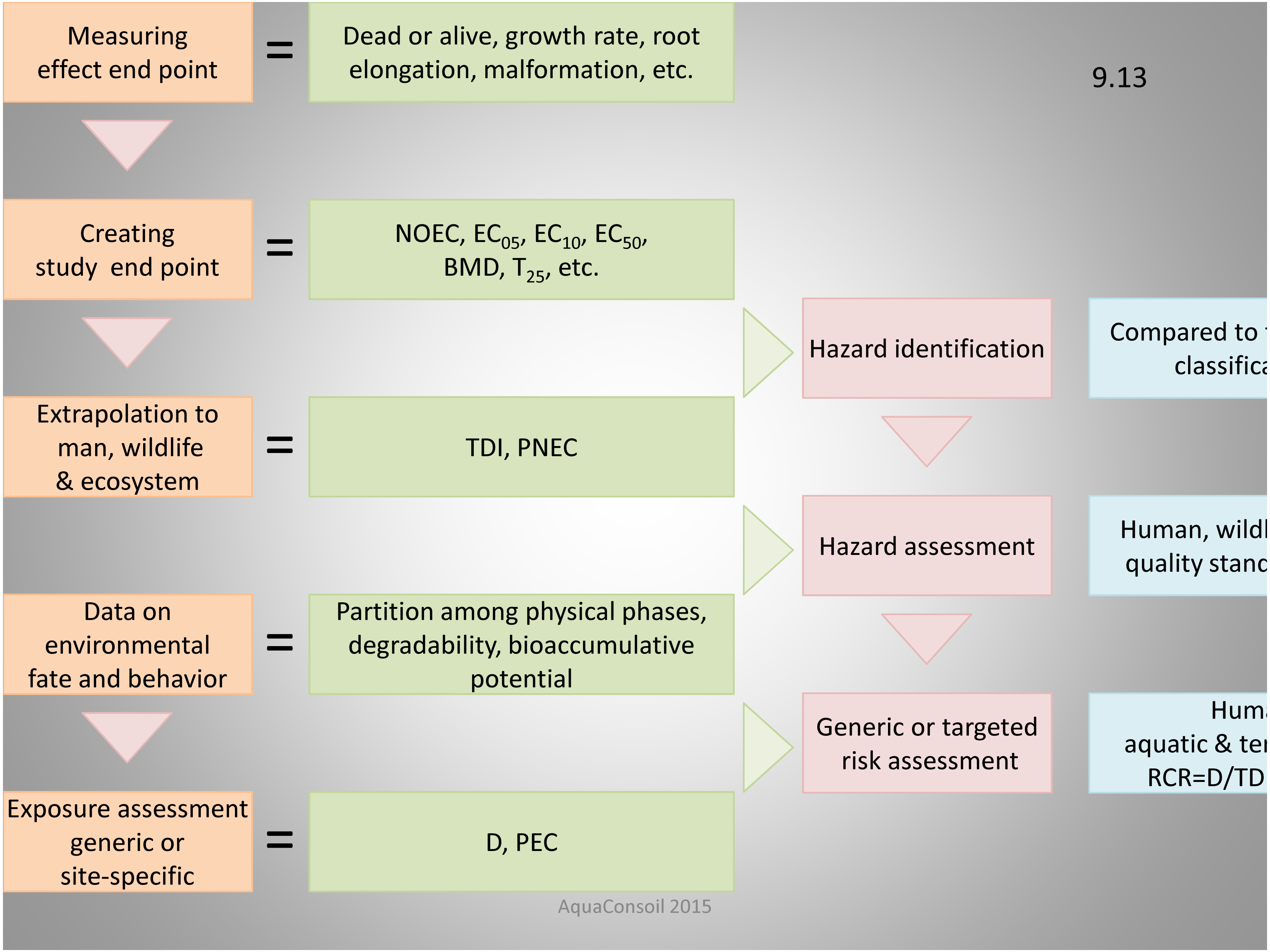
Thank you for your attention!

# Concepts for ecosystem management



An example
Creating a cover layer on a mine waste dump site: made of artificial soil for plant cultivation.
A mixture of debris, organic wastes and komposts, wwtp sludge, fly ash, lime slurry, paper-pulp waste, construction waste, dredged sediment, etc. , in a mixture of continuously changing quality.
Land use: restricted area in a forest
Target: isolation by natural plant coverage
Accepted inhibition: 20%.
Multicriteria analysis for the comparison of quantitative ecotox and chemical analytical data.


Lethality (%)
100
90
80
70
60
50
40
30
20
10
0
EC50 = 5514 mg/kg
EC20 = 3673 mg/kg
100
1000
EC20
EC50
10000
Diesel oil concentration in soil (mg/kg)


9.13
Measuring effect end point
=
Dead or alive, growth rate, root elongation, malformation, etc.
Creating study end point
=
NOEC, $EC_{05}$, $EC_{10}$, $EC_{50}$, BMD, $T_{25}$, etc.
Extrapolation to man, wildlife & ecosystem
=
TDI, PNEC
Data on environmental fate and behavior
=
Partition among physical phases, degradability, bioaccumulative potential
Exposure assessment generic or site-specific
=
D, PEC
Hazard identification
Hazard assessment
Generic or targeted risk assessment
Compared to t
classifica
Human, wildl
quality stand
Huma
aquatic & ter
RCR=D/TD
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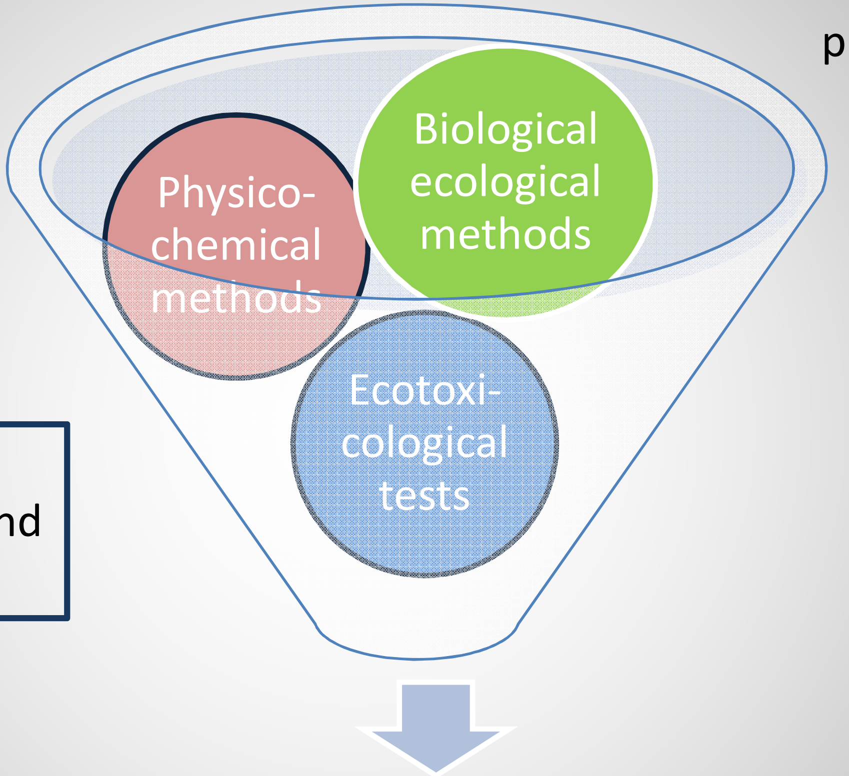
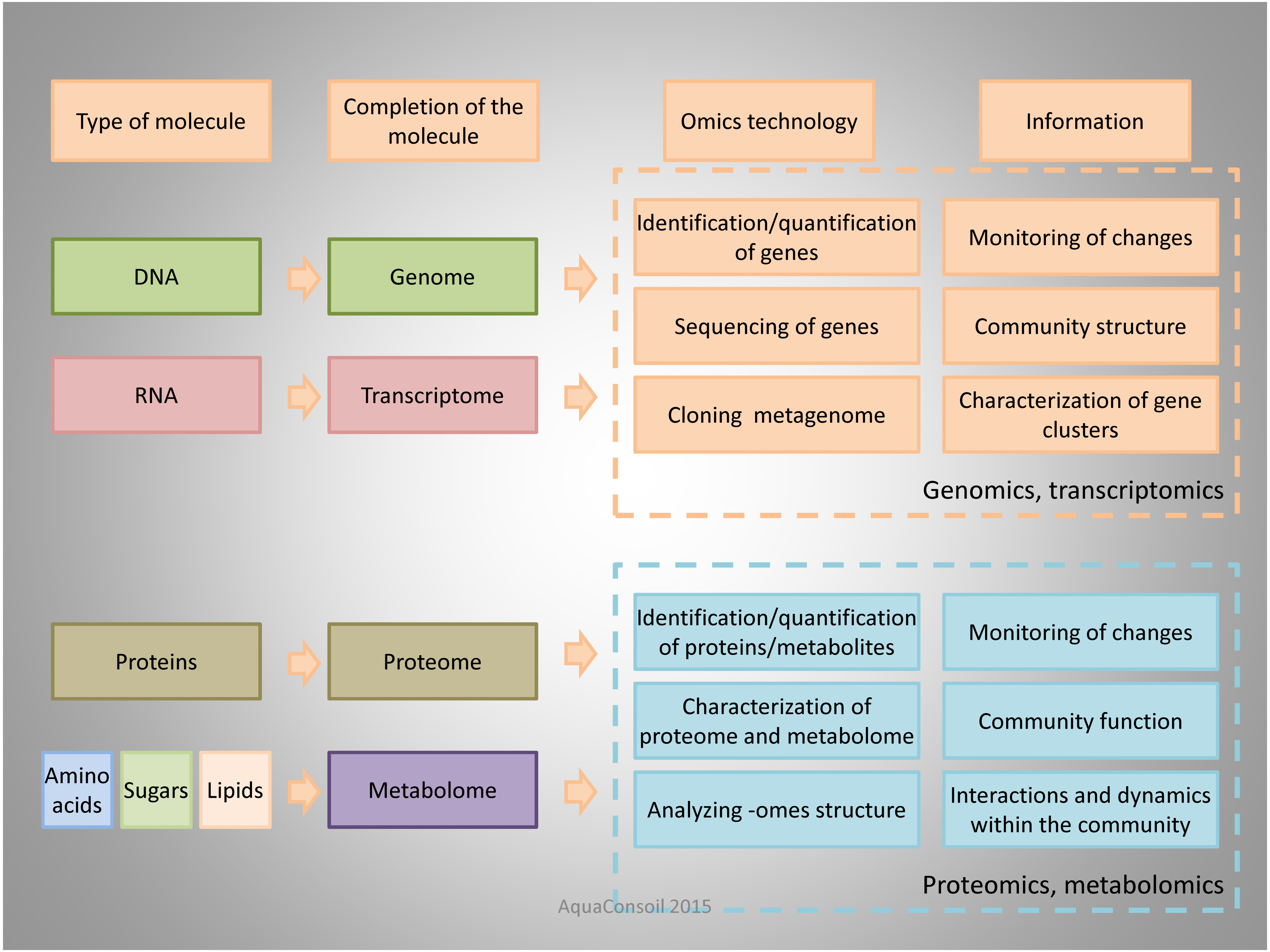
Postremedial phase

Monitoring of the raw materials and the remediation




Integrated evaluation

Interpretation


Type of molecule
Completion of the molecule
Omics technology
Information
DNA
Genome
RNA
Transcriptome
Identification/quantification of genes
Monitoring of changes
Sequencing of genes
Community structure
Cloning metagenome
Characterization of gene clusters
Genomics, transcriptomics
Proteins
Proteome
Amino acids
Sugars
Lipids
Metabolome
Identification/quantification of proteins/metabolites
Monitoring of changes
Characterization of proteome and metabolome
Community function
Analyzing -omes structure
Interactions and dynamics within the community
Proteomics, metabolomics
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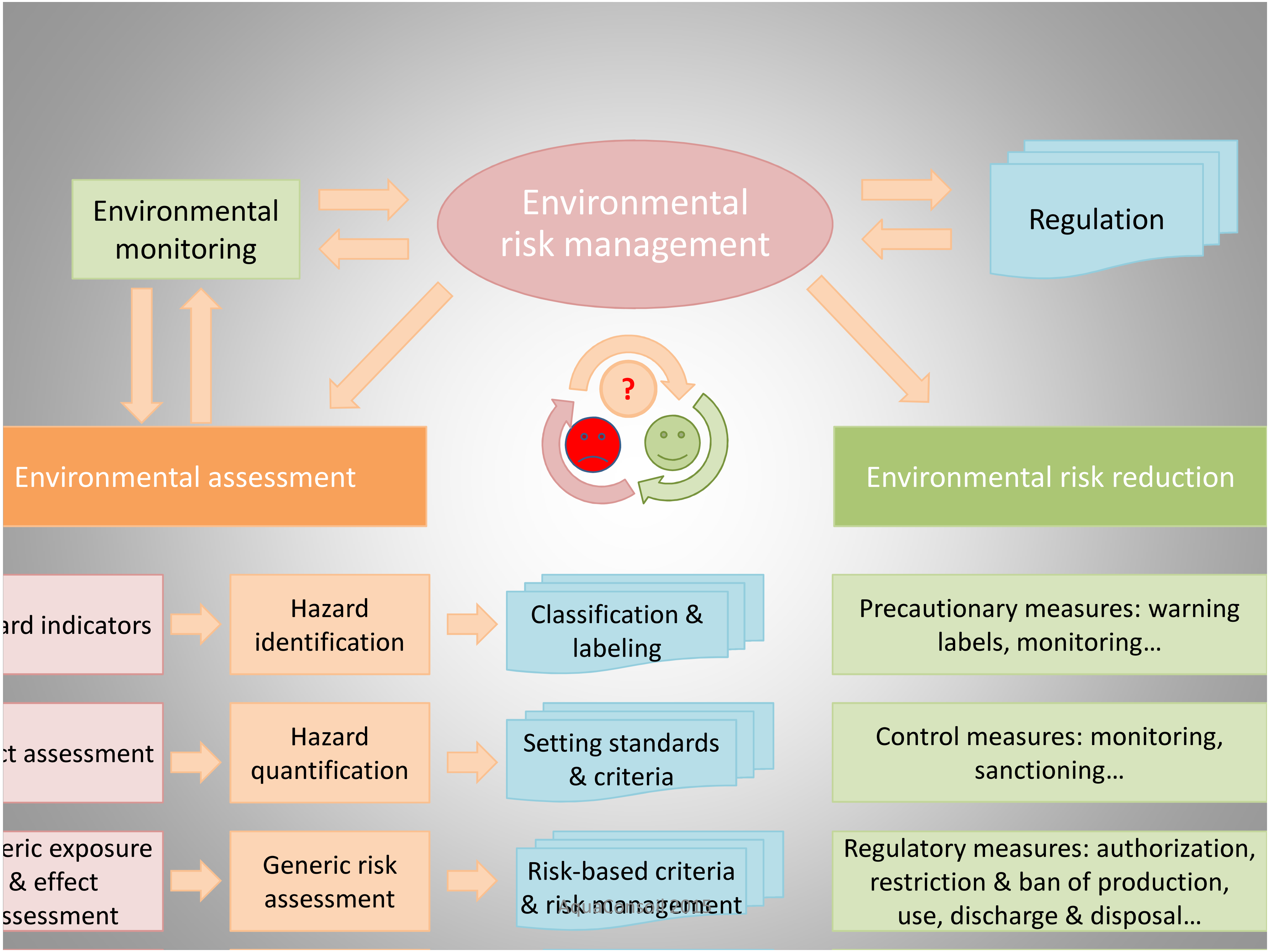
Environmental monitoring
Environmental risk management
Regulation
Environmental assessment
?
Environmental risk reduction
ard indicators
Hazard identification
Classification & labeling
Precautionary measures: warning labels, monitoring…
ct assessment
Hazard quantification
Setting standards & criteria
Control measures: monitoring, sanctioning…
eric exposure & effect ssessment
Generic risk assessment
Risk-based criteria & risk management
Regulatory measures: authorization, restriction & ban of production, use, discharge & disposal…
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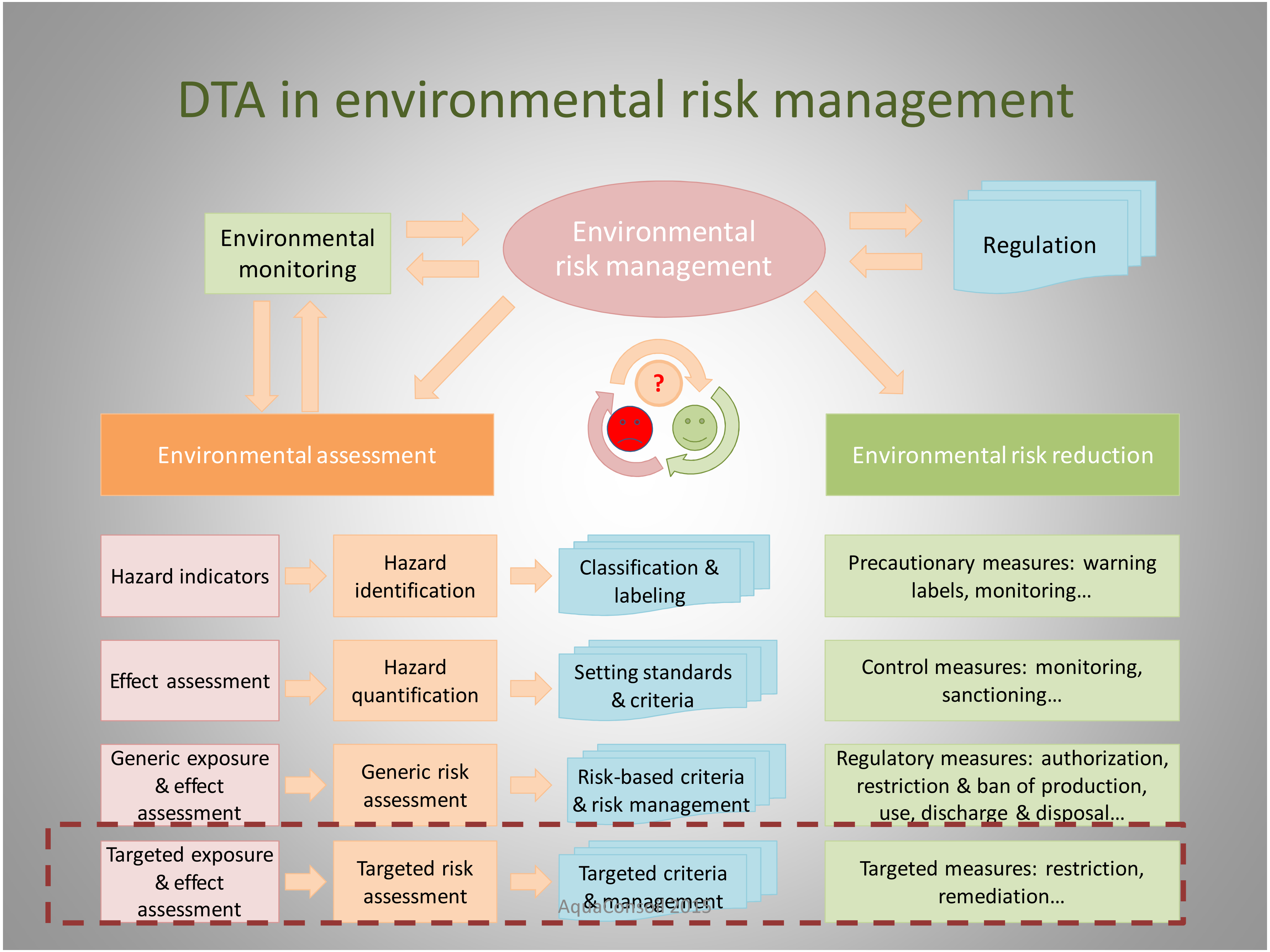
DTA in environmental risk management
Environmental monitoring
Environmental risk management
Regulation
?
Environmental assessment
Environmental risk reduction
Hazard indicators
Hazard identification
Classification & labeling
Precautionary measures: warning labels, monitoring…
Effect assessment
Hazard quantification
Setting standards & criteria
Control measures: monitoring, sanctioning…
Generic exposure & effect assessment
Generic risk assessment
Risk-based criteria & risk management
Regulatory measures: authorization, restriction & ban of production, use, discharge & disposal…
Targeted exposure & effect assessment
Targeted risk assessment
Targeted criteria & management
Targeted measures: restriction, remediation…
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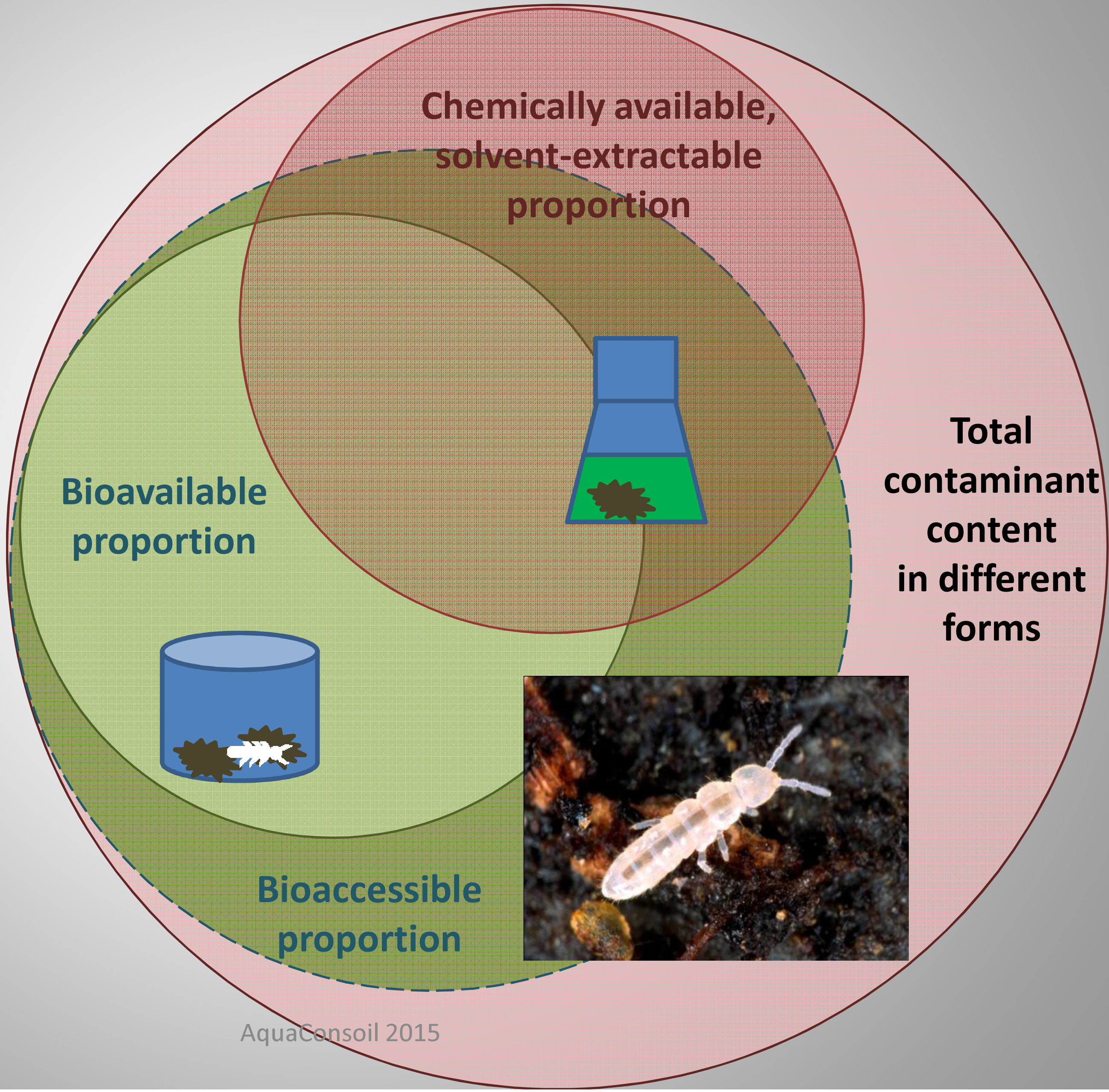
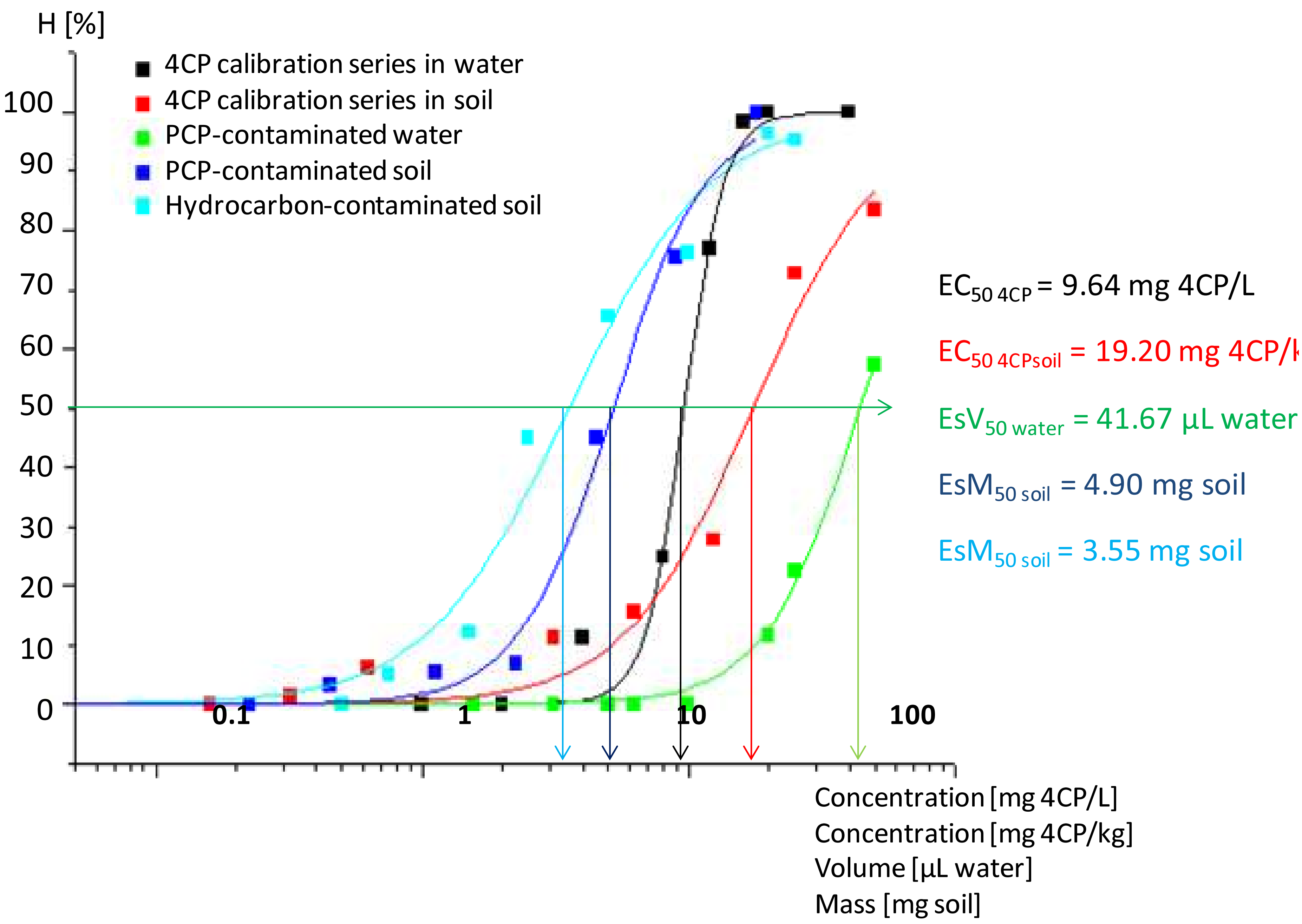
# Chemical and biological availability

Need for DTA in soil testing: bioaccessibile and availablie portion of contaminants in soil greatly differs from the total .

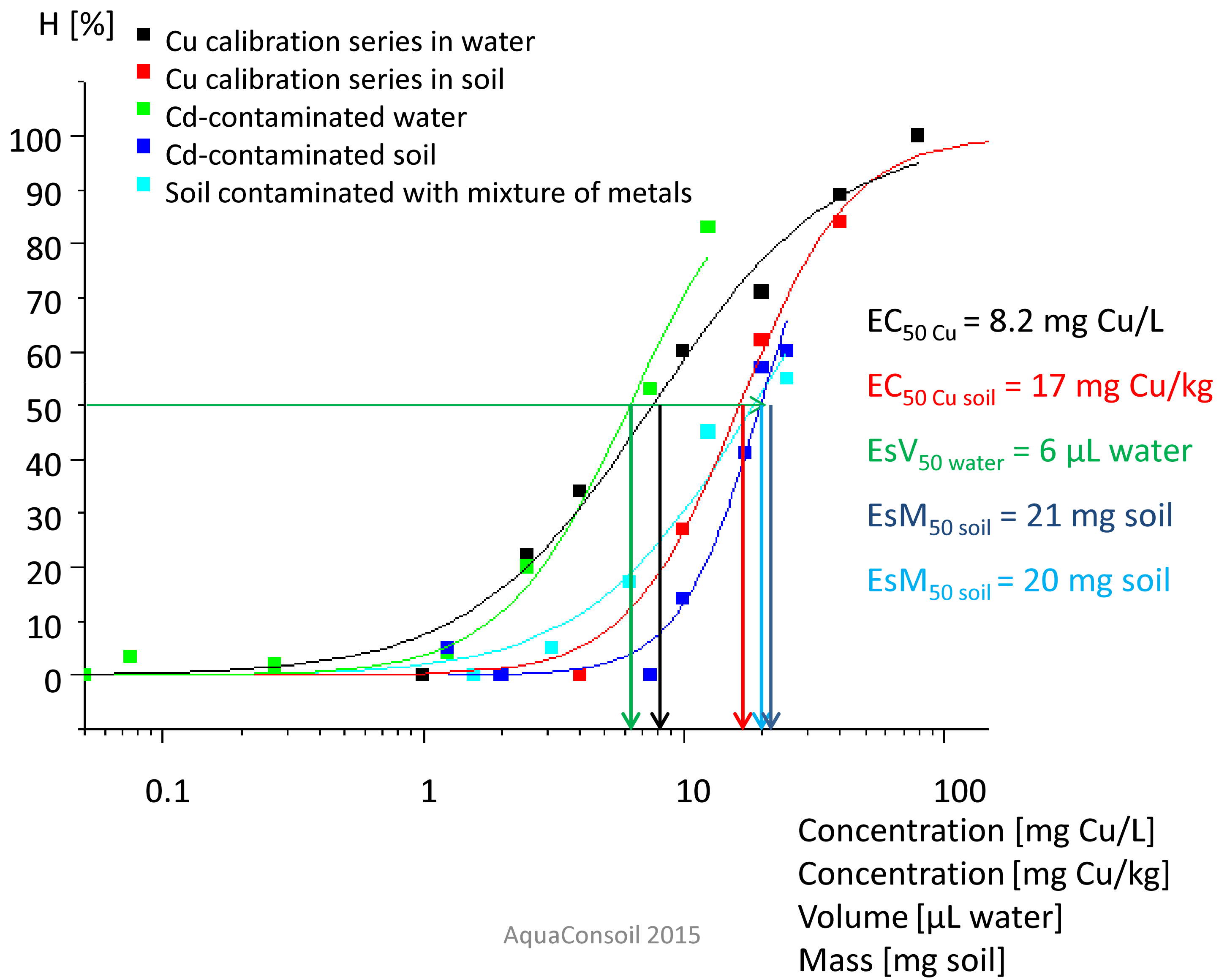
Overlap of chemical and biological methods is casual /random.

This sheme changes from substance to substance.




H [%]
4CP calibration series in water
4CP calibration series in soil
PCP-contaminated water
PCP-contaminated soil
Hydrocarbon-contaminated soil
100
90
80
70
60
50
40
30
20
10
0
0.1
1
10
100
EC50 4CP = 9.64 mg 4CP/L
EC50 4CPsoil = 19.20 mg 4CP/k
EsV50 water = 41.67 µL water
EsM50 soil = 4.90 mg soil
EsM50 soil = 3.55 mg soil
Concentration [mg 4CP/L]
Concentration [mg 4CP/kg]
Volume [µL water]
Mass [mg soil]

# Equivalencing



# Methods, equipments, endpoints

Test type: laboratory, rapid *in situ*, real time, online;

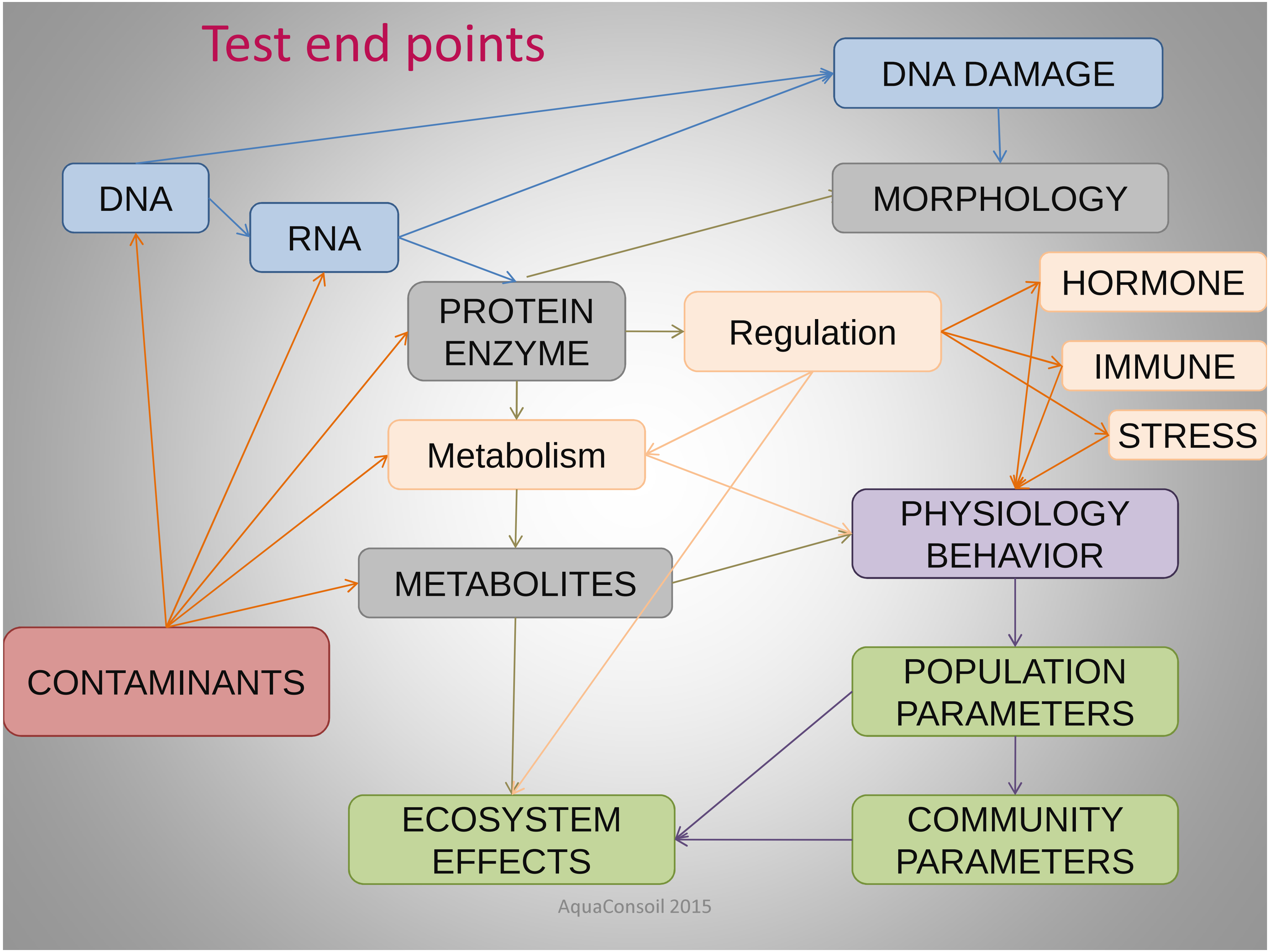
Test set-up: biosensor, bioassay, microcosm, fiel assessment, etc.

Test organisms: soil bacteria, algae, fungi, single cell animals, nematodes, plants, insects (springtails, aunts, pinceb, spiders, woodlice), worms (nematodes, Eisenia sp.), birds, mammals. The soil in whole: metagenom, metatranscriptomics, metabolic activities (respiration, nitrification, sulphate redustion), adaptation, resistance, etc.

Equipment: lab, mobile, handheld, locally deployed or remote sensors with data loggers and telecommunication;

Endpoints: from envir*omics* through metabolic activities to population indicators;

- The properly selected end point should have a diagnostic value and should be in close relationship with the hazardous effect and risk;
- The measured end point should be consistent with the study goal & qualitativeness;
- Direct and indirect effects can be measured, such as genetic, metabolic reproductive, growth or lethal effects;
- The measured end points should represent adequate sensitivity and the response time should be as short as possible;
- High signal/background ratio is desired;
- The implementation, evaluation and interpretation should be easy and practical.


Test end points
DNA DAMAGE
DNA
RNA
MORPHOLOGY
HORMONE
PROTEIN
ENZYME
Regulation
IMMUNE
STRESS
Metabolism
PHYSIOLOGY
BEHAVIOR
METABOLITES
CONTAMINANTS
POPULATION
PARAMETERS
ECOSYSTEM
EFFECTS
COMMUNITY
PARAMETERS
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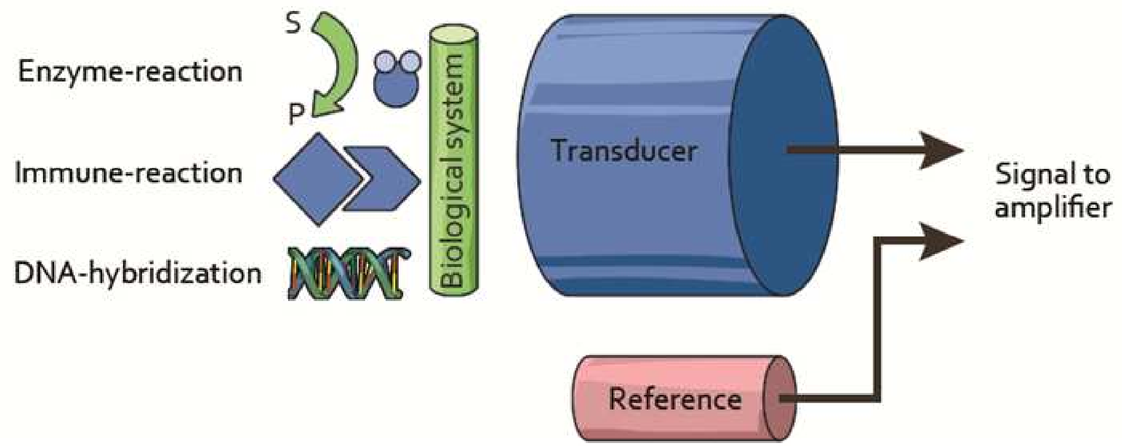
# *In situ* /real-time toxicity measuring methods

*In situ* site investigation may ensure better fit to the specific soil management and greater flexibility in the field work compared to laboratory based solutions.

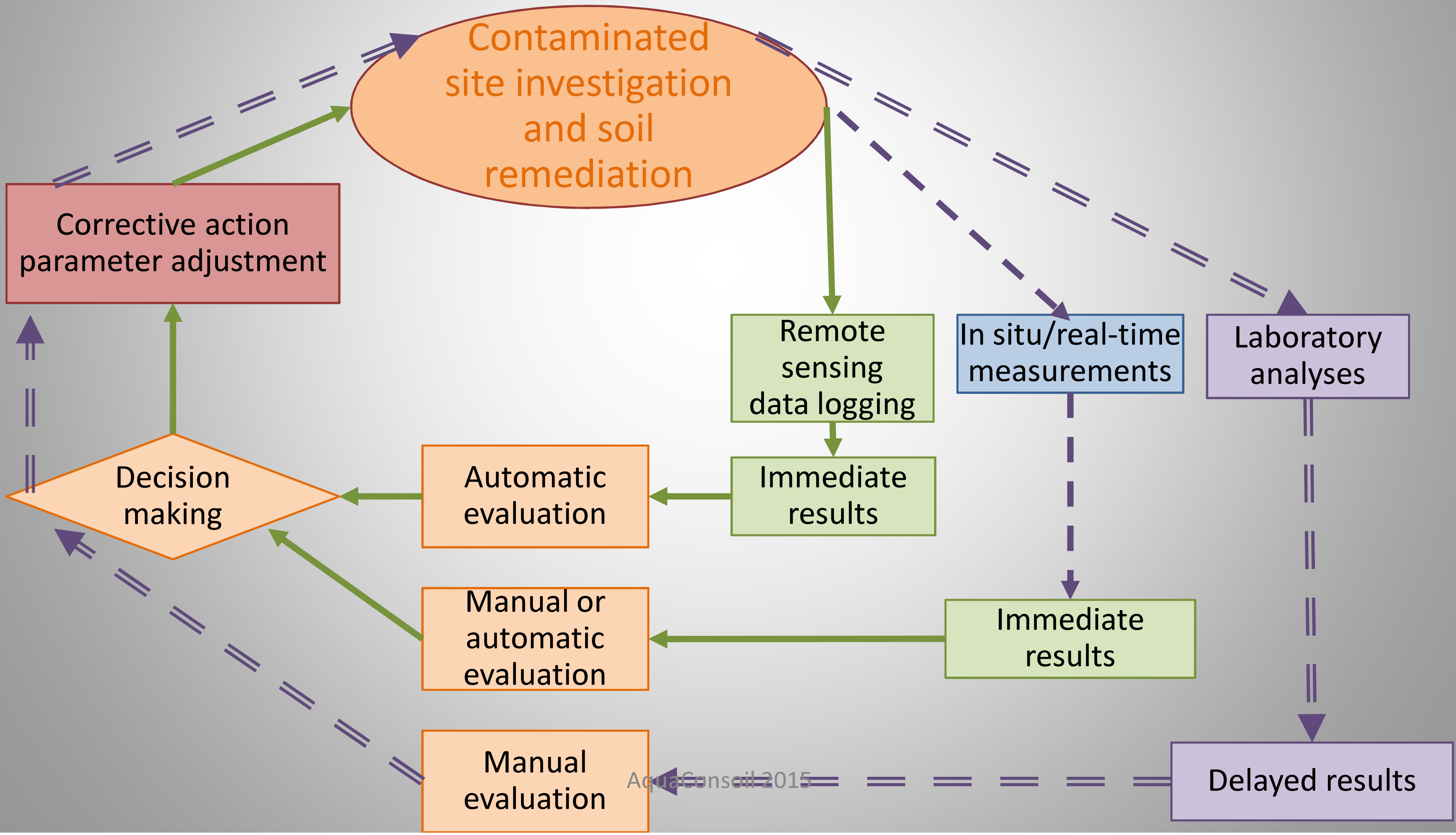
Real-time toxicity values make automatic control and regulation possible.

Some tools for in situ toxicity measurements:

- Mobile versions of laboratory tests, test kits, conserved test organisms;
- Biosensors / microprobes for measuring respiration and contaminant specific biochemical responses, biospecific metabolic products;
- DNA and other omics probes for diversity testing;



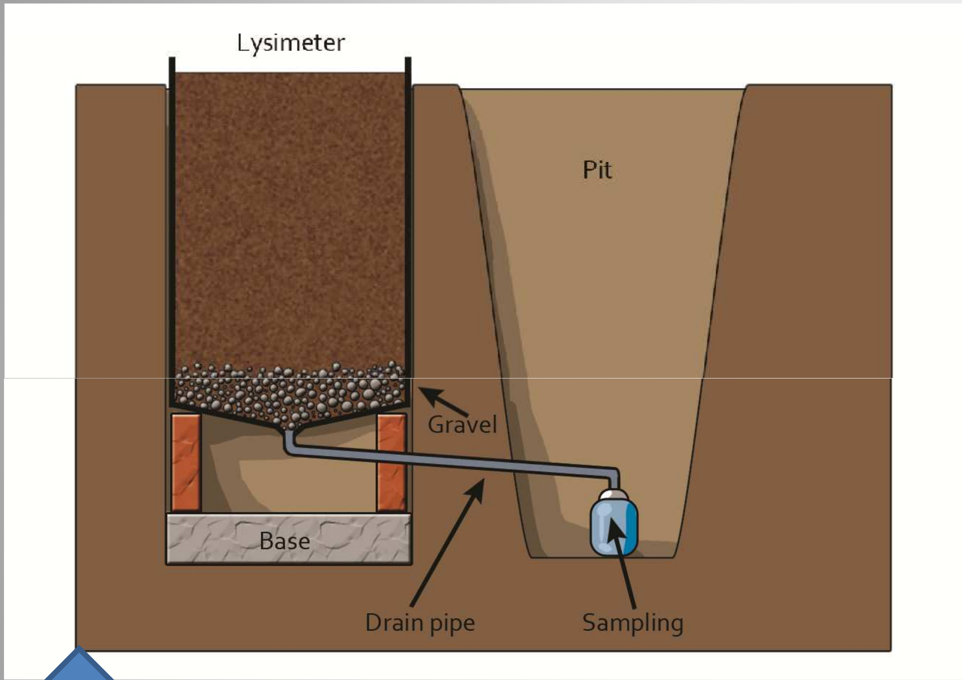
# Laboratory, *in situ* /real-time and remote measurements based on biological response



# Mobile luminometers for measuring bioluminescence *in situ*

Field applicable equipments

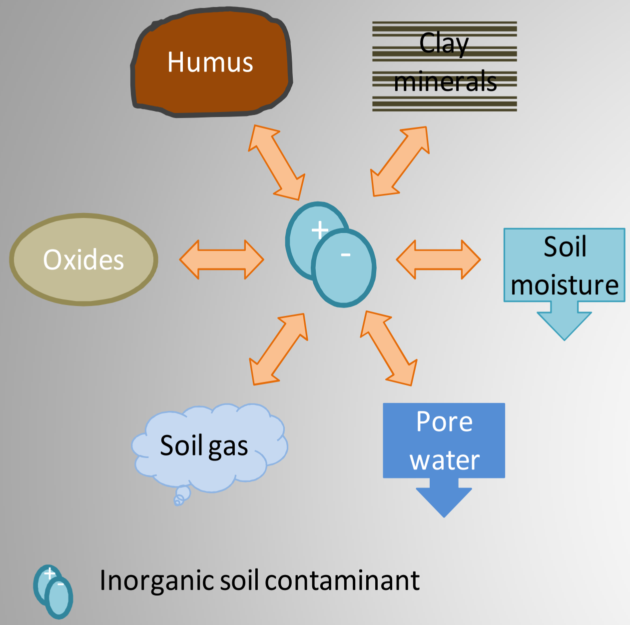
- Caged test organisms;
- Field micro and mezocosms:  cotton strip, litter bag, pitfall traps,  bait lamina, soil lysimeters, avoidance tests, field asessment of species densitiy, diversity.




Underground deployed field lysimeter

Above ground  field lysimeter

# Can chemistry truely model the biological response?




Toxic metals contaminated soil. Approximation of the biological response by:

- sequential multiple metal extraction with separate fractions: several methods resulting 3, 4 or 5 fractions, e.g. BCR, SEE (8 fractions)
- simulations, biomimetic extractants, etc.

But: generalization and interpretation is still fragile

# Plant toxicity compared to chemically measured metal content at a flooded site