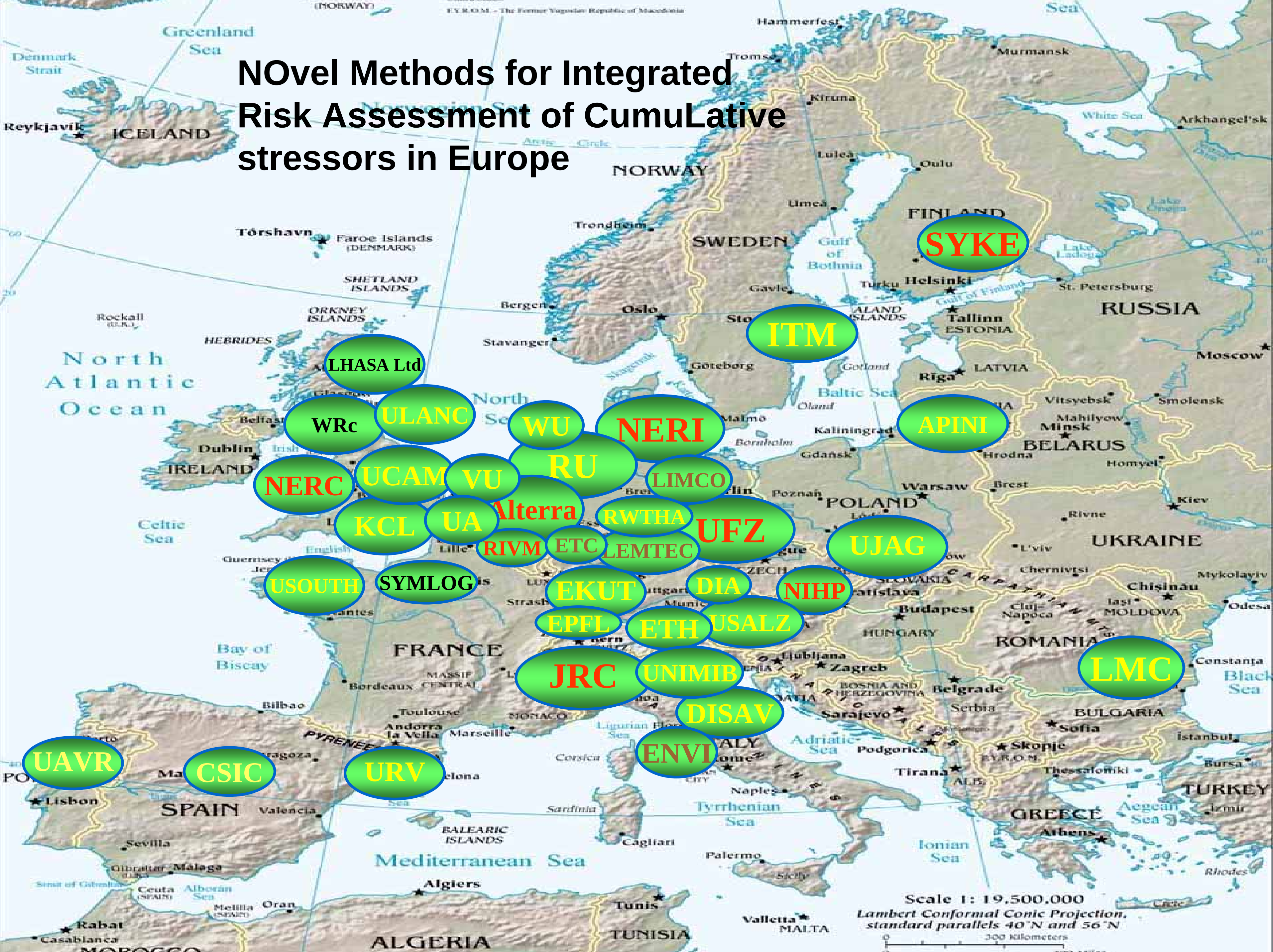
Novel Methods for Integrated Risk Assessment of Cumulative Stressors in Europe

NoMiracle - Integrated Project

co-ordinated by Hans Løkke NERI, Denmark


NOvel Methods for Integrated
Risk Assessment of CumuLative
stressors in Europe
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SYMLOG
EKUT
DIA
NIHP
EPFL
ETH
USALZ
JRC
UNIMIB
LMC
DISAV
ENVI
UAVR
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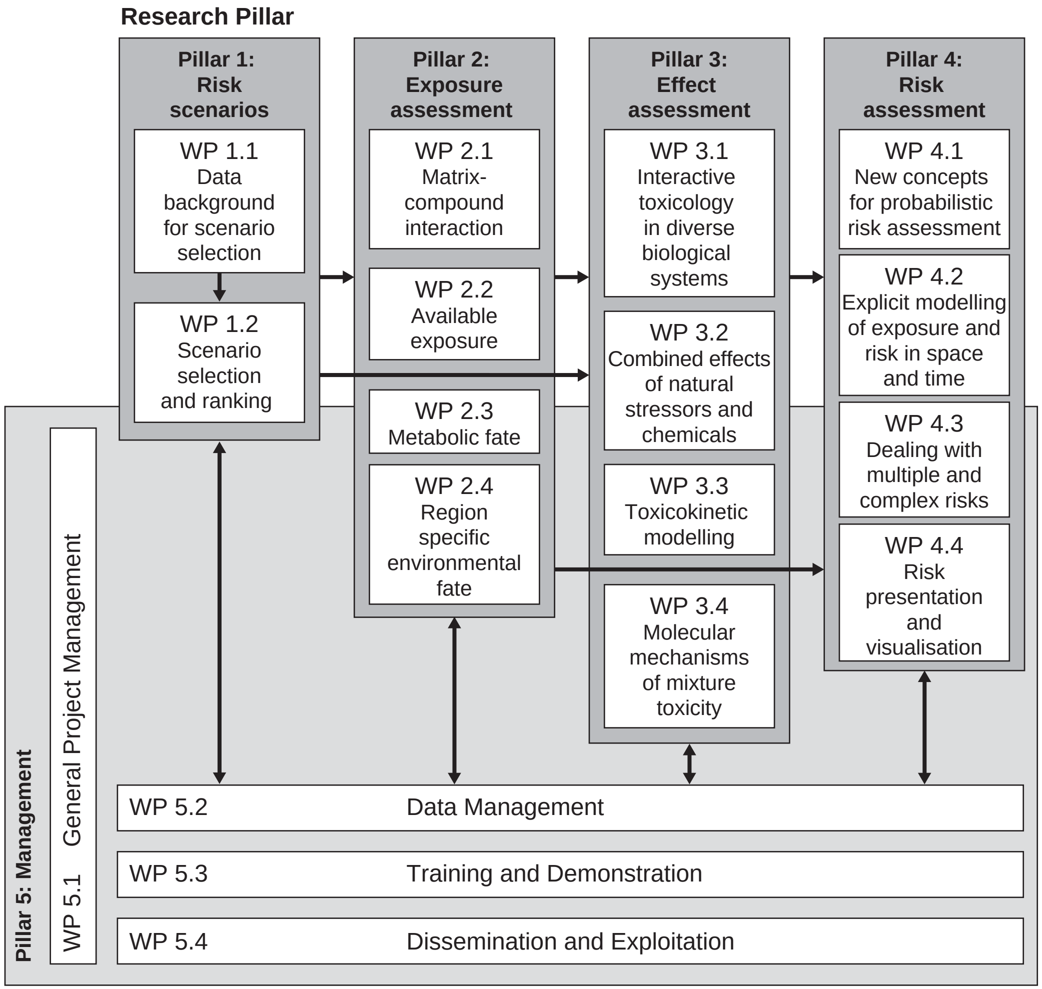
Scale 1: 19,500,000
Lambert Conformal Conic Projection,
standard parallels 40°N and 56°N

NOMIRACLE activities and their components



Science & technology objectives

- To develop new methods for assessing the cumulative risks from combined exposures to several stressors including mixtures of chemical and physical/biological agents

2. To achieve more effective integration of the risk analysis of environmental and human health effects
3. To improve our understanding of complex exposure situations and develop adequate tools for sound exposure assessment
4. To develop a research framework for the description and interpretation of cumulative exposure and effect

Science & technology objectives

5. To quantify, characterise and reduce uncertainty in current risk assessment methodologies, e.g. by improvement of the scientific basis for setting safety factors
6. To develop assessment methods which take into account geographical, ecological, social and cultural differences in risk concepts and risk perceptions across Europe
7. To improve the provisions for the application of the precautionary principle and to promote its operational integration with evidence-based assessment methodologies

The project deals with molecules designed for provoking significant interactions with biological structures

- **Pesticides**
- **Biocides**
- **Pharmaceuticals**

but also includes some

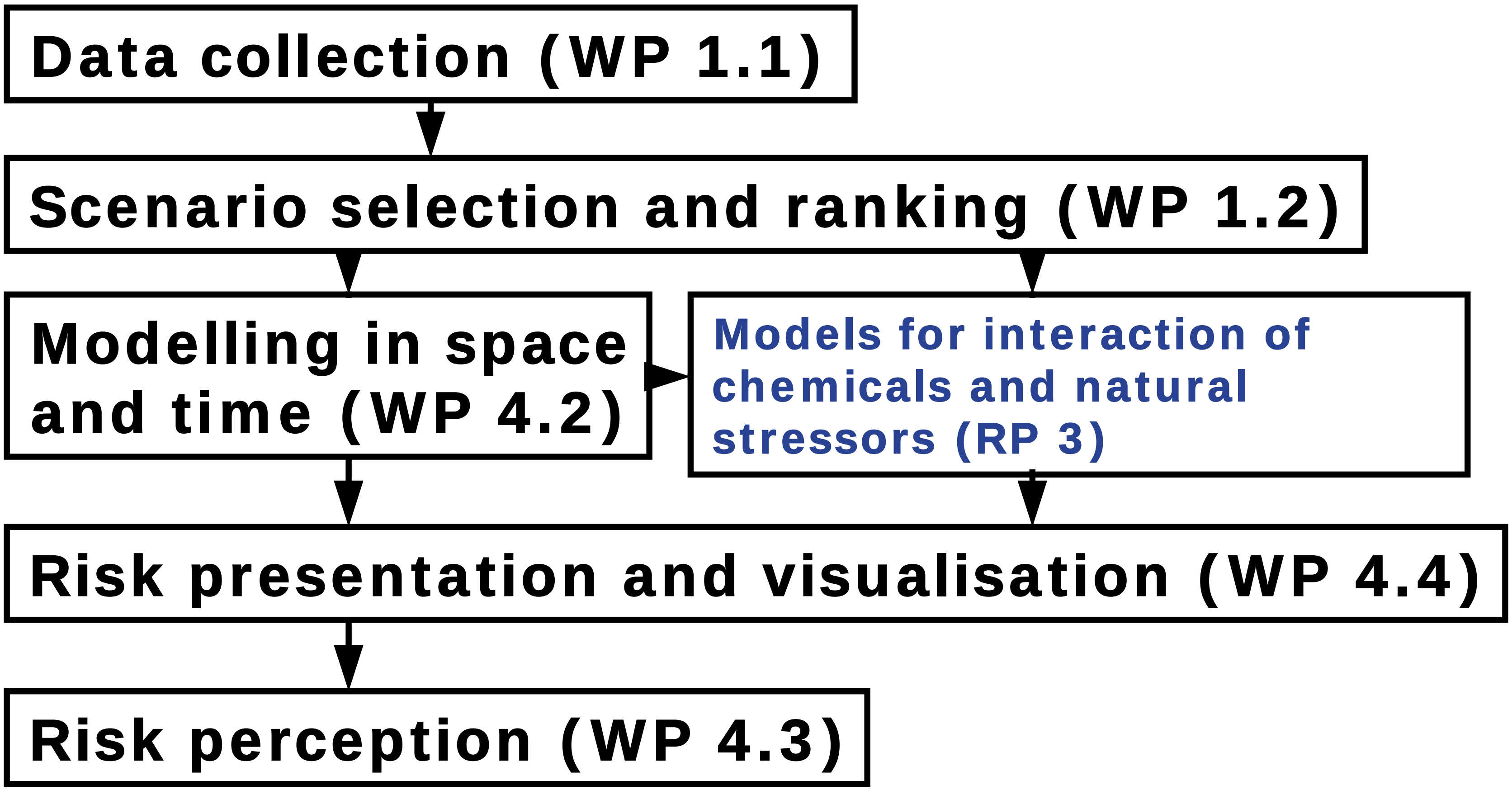
- **VOCs and semi-VOCs**
- **selected chemicals with baseline or reactive mode of action**
- **metals, in particular Ni**

NOMIRACLE supports:

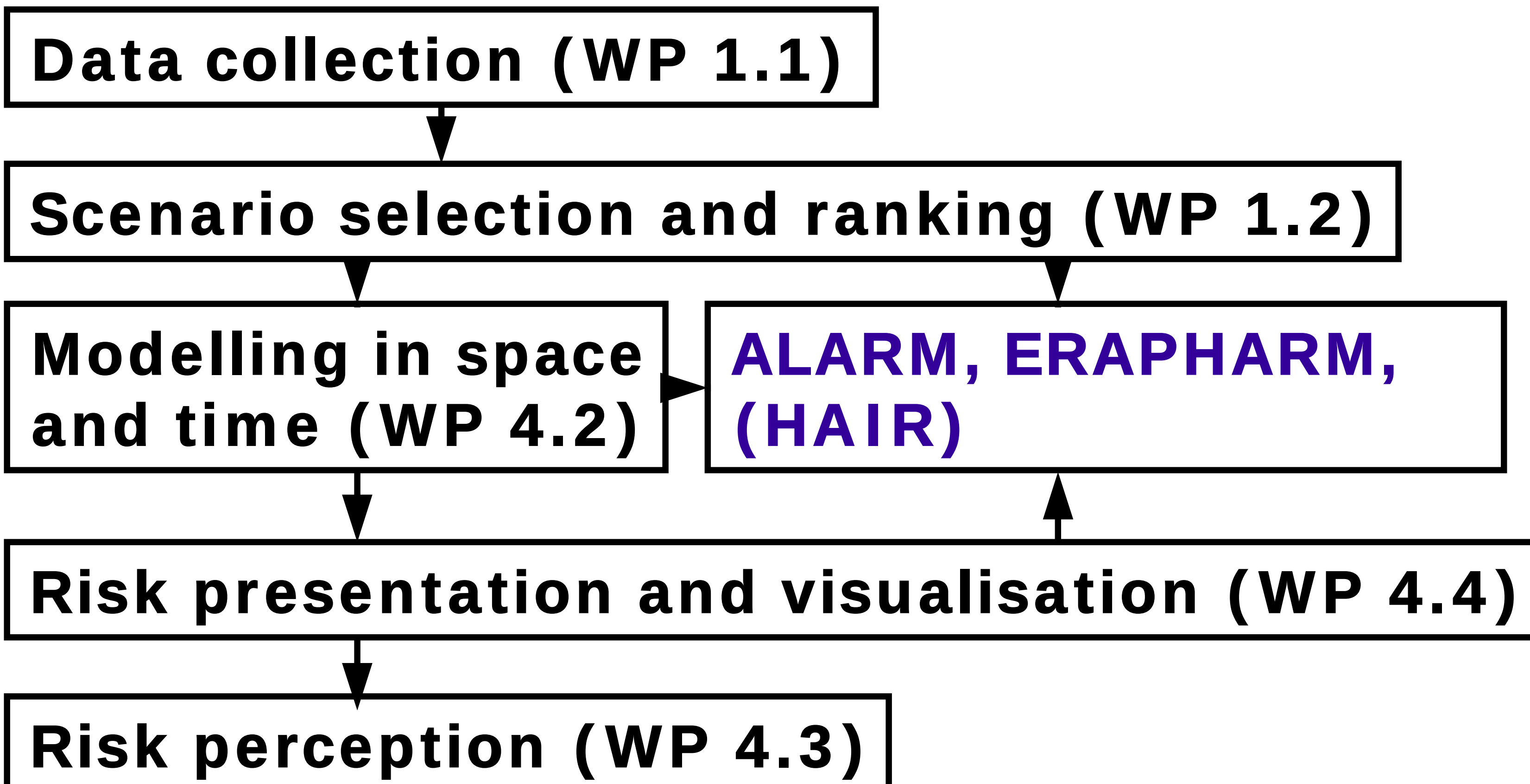
- The European Environmental and Health Action Plan (Action No 7)
- Thematic Strategy on the Sustainable Use of Pesticides
- Plant Protection Directive 91/414/EEC
- Biocide Directive 98/8/EEC
- Pharmaceutical directive
- Strategy for Soil Protection
- Strategy for Waste Reduction and Recycling
- (REACH)

Research pillar 1: Scenario selection

- **WP 1.1 Establishment of data background for scenario selection (Leader: Alberto Pistocchi)**
- **WP 1.2 Scenario selection and ranking (Leader: Peter B. Sørensen)**


Data collection (WP 1.1)
Scenario selection and ranking (WP 1.2)
Modelling in space and time (WP 4.2)
Models for interaction of chemicals and natural stressors (RP 3)
Risk presentation and visualisation (WP 4.4)
Risk perception (WP 4.3)

Collaboration with other FP6 projects:

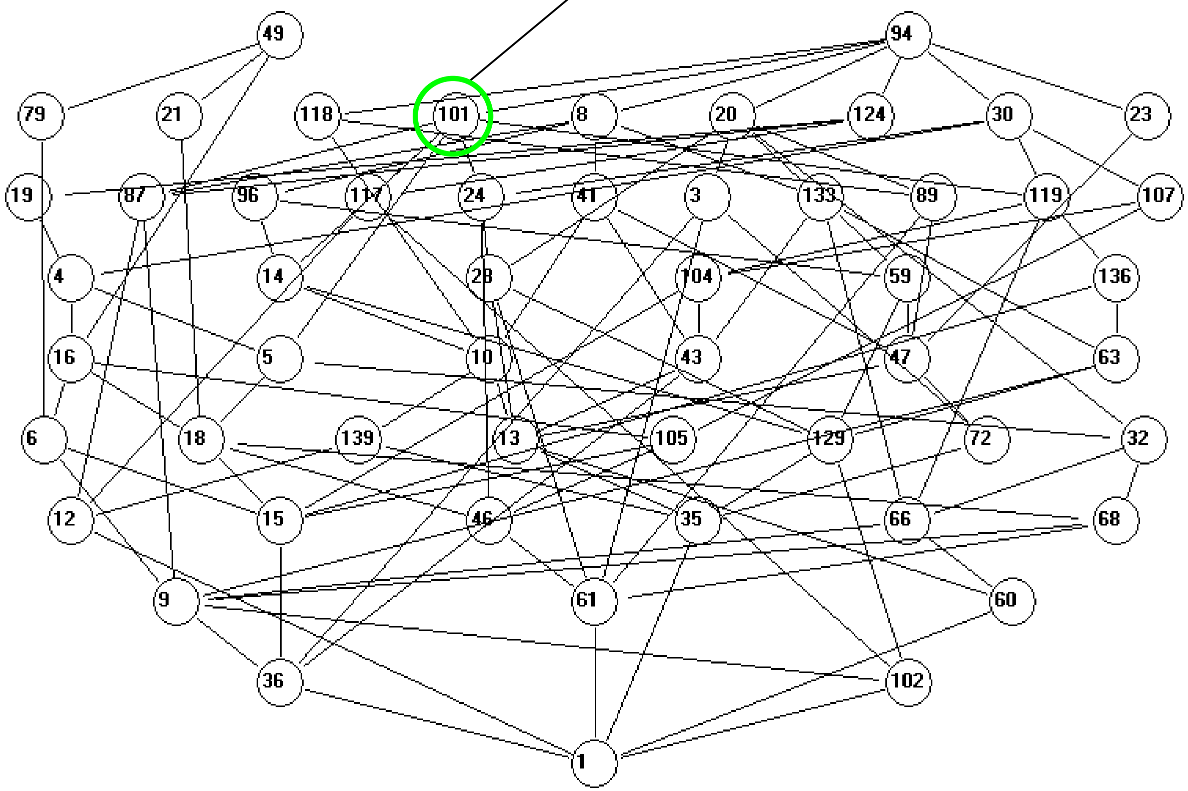


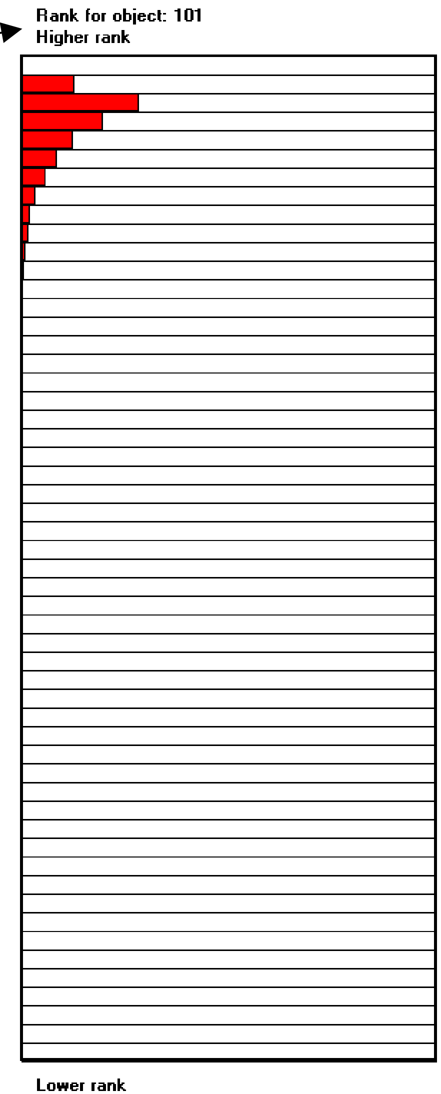
WP 1.2: Application of multi criteria methods in scenario selection

Multi-attribute uncertainty

10 criteria case

Id 101
Methyloxirane
Cas no. 75-56-9





Research Pillar 2: Exposure Assessment

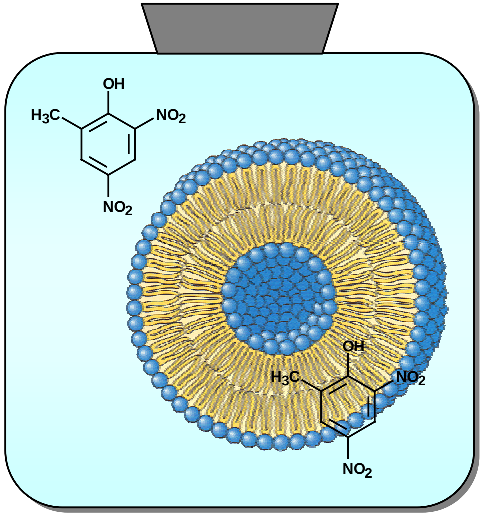
- **WP 2.1: Matrix-compound interaction (Leader: Gerrit Schüürmann)**
- **WP 2.2: Available exposure (Leader: Philipp Mayer)**
- **WP 2.3: Metabolic fate (Leader Ovanes Mekenyan)**
- **WP 2.4: Region-specific environmental fate (Leader: Mark Huijbregts)**

WP 2.1: Experimental Determination of Membrane-Water Partitioning

NOMIRACLE
WP 2.1

Reference substances

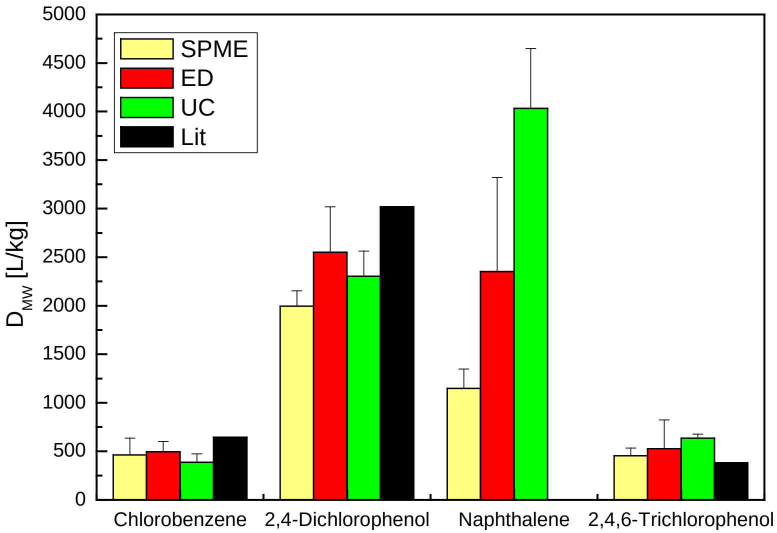
UFZ Leipzig
Germany
(Dabitz et al.)



$$K_{mw} = \frac{c_{membrane}}{c_{water}}$$

Mimics bio-partitioning of organic compounds

Membrane surrogate: Small unilamellar phospholipid vesicles
Experimental methods: SPME/ED/UC + GC/LC analysis

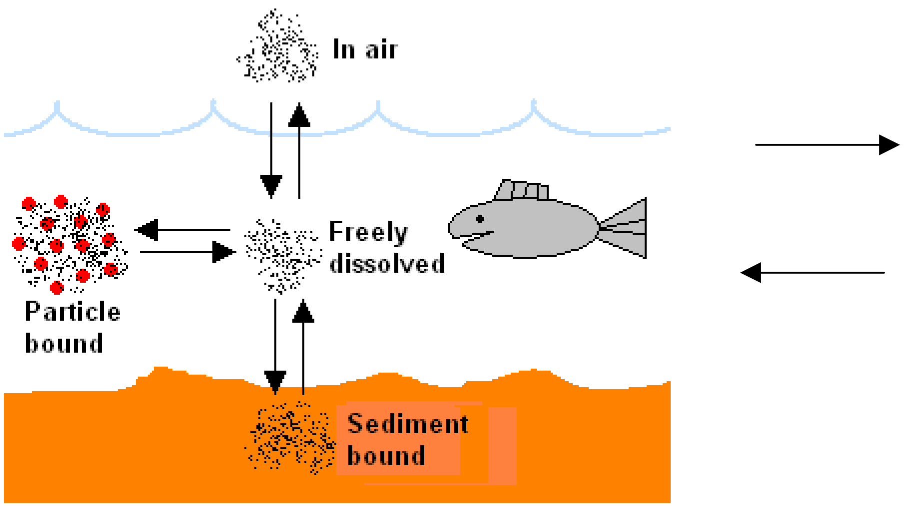


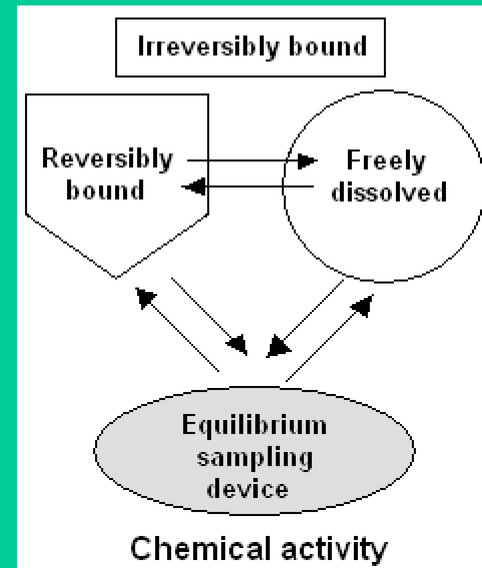
- SPME and ED yield comparable results, but
- SPME shows a higher sensitivity and is more time-saving and cost-efficient

Model for K_{mw}

WP 2.3
WP 2.4

WP 2.2: Parameterization of ”bioavailability” into three well defined exposure parameters





-Chemical activity
-Accessibility
-Diffusive conductivity

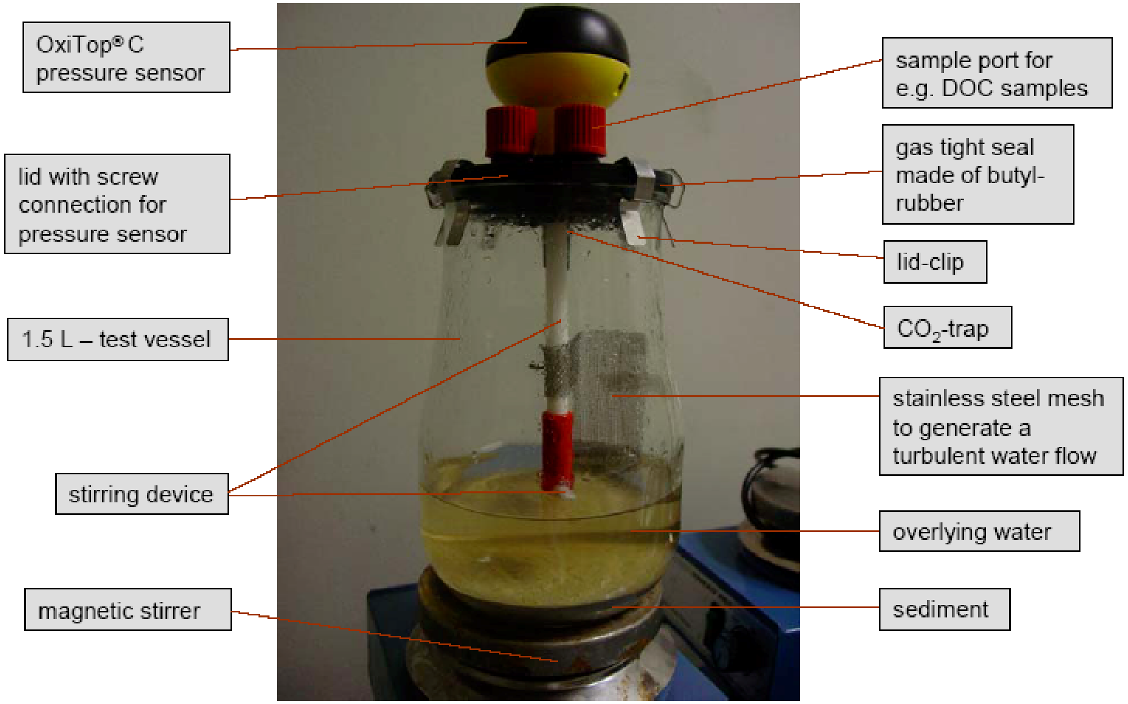
RESULTS

<u>Metals</u>: Differences between field vs lab results were evaluated

<u>Nonpolar organics:</u> Chemical activity and accessibility were proven useful for risk assessment of polluted soil

<u>Polar organics:</u> Sampling and analysis techniques were developed for the freely dissolved phase

WP 2.3: New experimental and theoretical and experimental methods for estimating degradation



A new water-sediment test system designed to provide realistic degradation rates for polar compounds

- Also, new computational methods for degradation of polar chemicals in soils have been developed based on the well-known model, CATABOL

WP 2.4 Region-specific Fate Framework

A. Development of a spatial explicit (1x1 km) and temporal explicit (month) European fate model within GIS

-> Model framework and data input has been succesfully established

B. Comparison and validation of 4 fate models with varying spatial detail

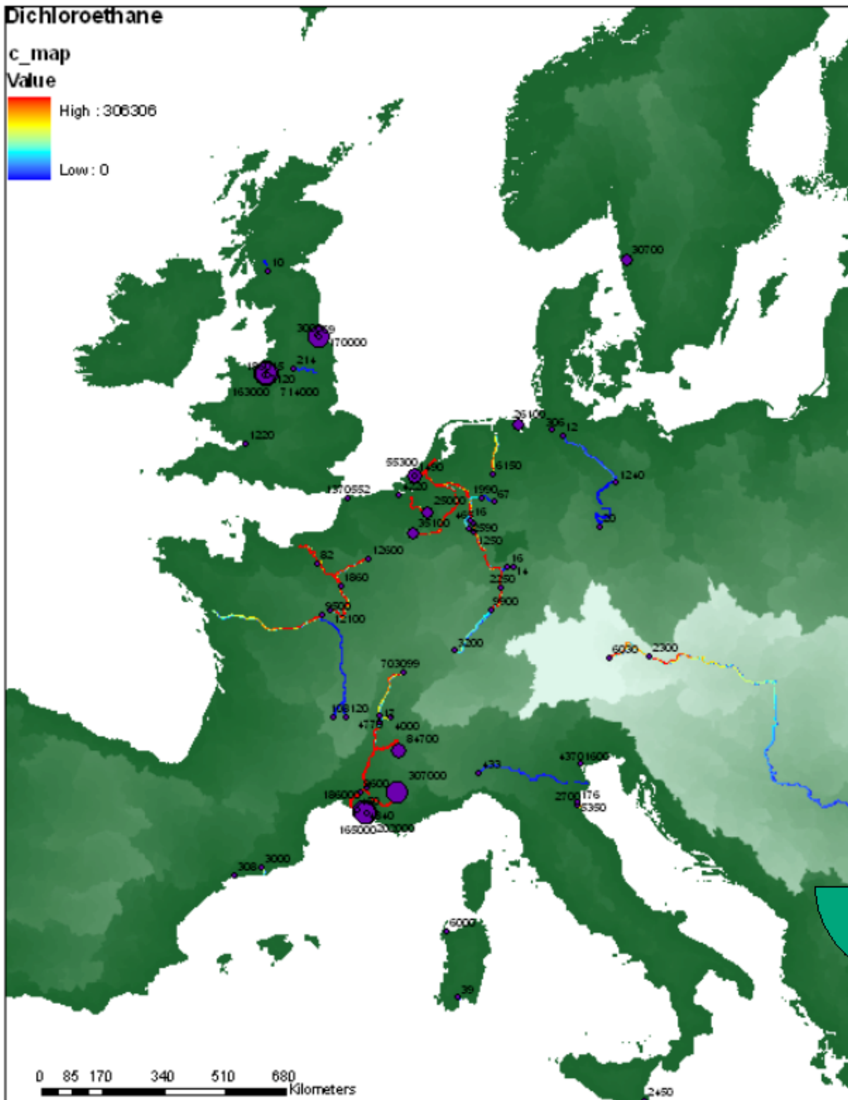
-> Spatially differentiation in modelled POP concentrations equal or larger than uncertainties due to chemical property estimations or differences in models

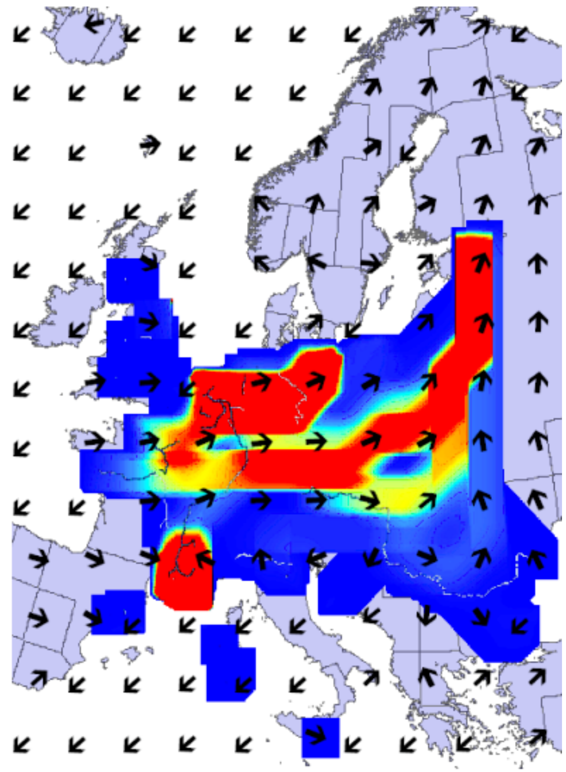
-> Model results appears to particularly underestimate POP concentrations in soils, freshwater and sediment compared to measured data

C. Evaluate the suitability of neural algorithms to describe multimedia behaviour

-> The trained neural network models were capable to predict partitioning for the chemicals in the test set with high accuracy.

WP 2.4: Development, application and evaluation of European-scale exposure models (dichloroethane)



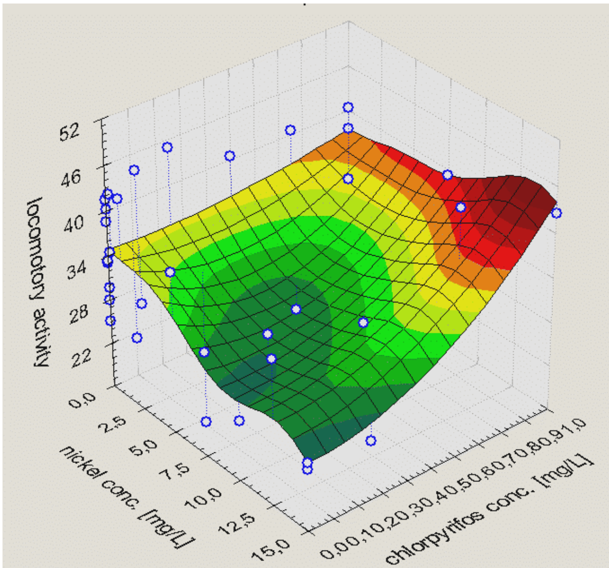


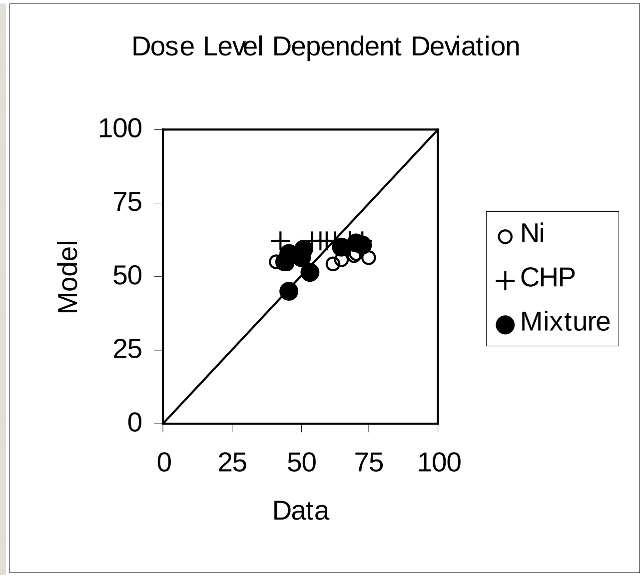
WP.2.4 utilises data from WPs 2.1, 2.2. and 2.3

Research Pillar 3: Effects assessment

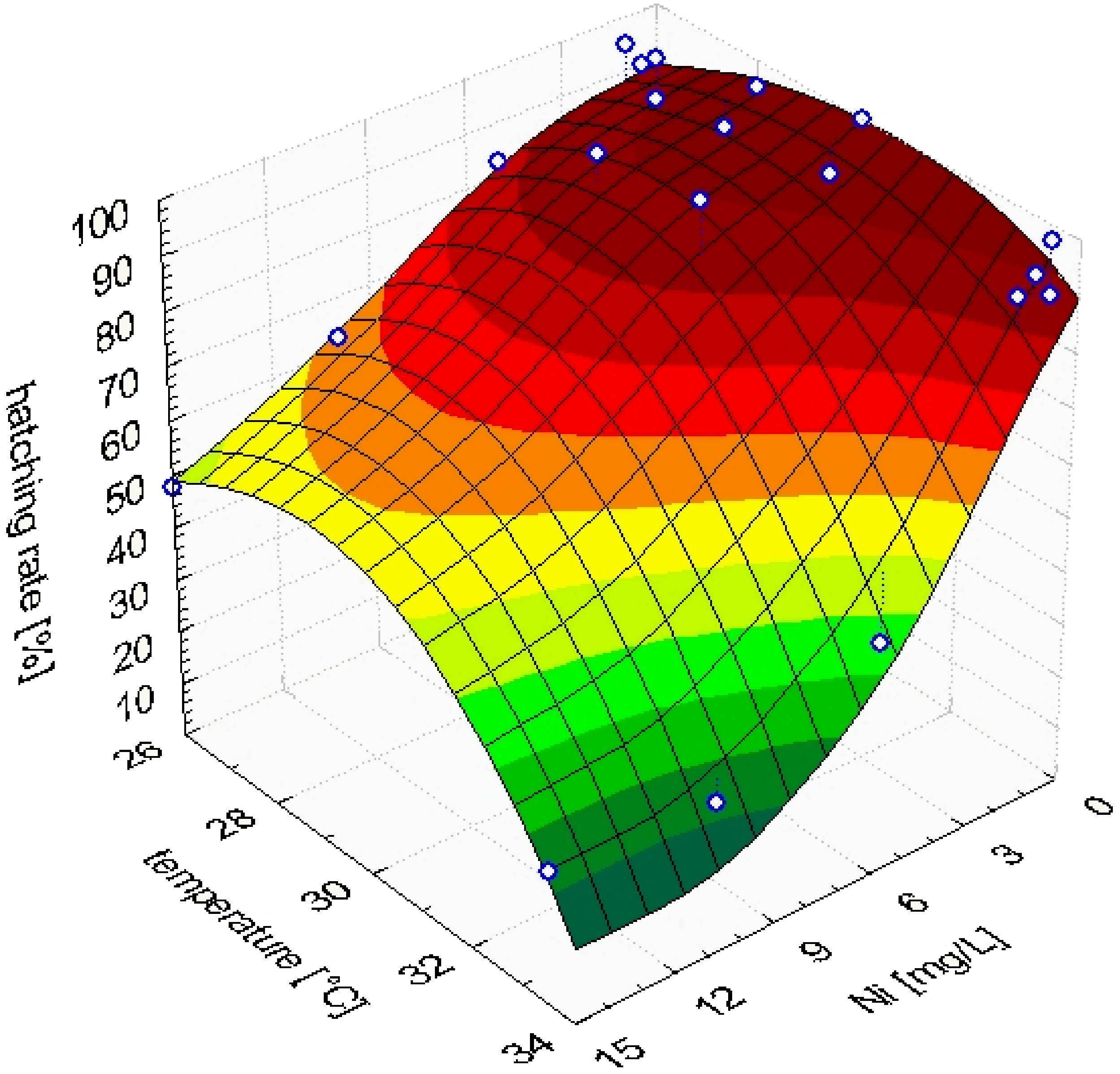
- **WP 3.1 Interactive toxicological effects in diverse biological systems (Leader: Almut Gerhardt)**
- **WP 3.2 Combined effects of natural stressors and chemicals (Leader: Martin Holmstrup)**
- **WP 3.3 Toxicokinetic modelling (Leader: Kees van Gestel)**
- **WP 3.4 Molecular mechanisms of mixture toxicity (Leader: Aldo Viarengo)**

Effects of Ni and chlorpyrifos in young larval stages of *Danio rerio* (2 hrs. exposure) (WP 2.1)

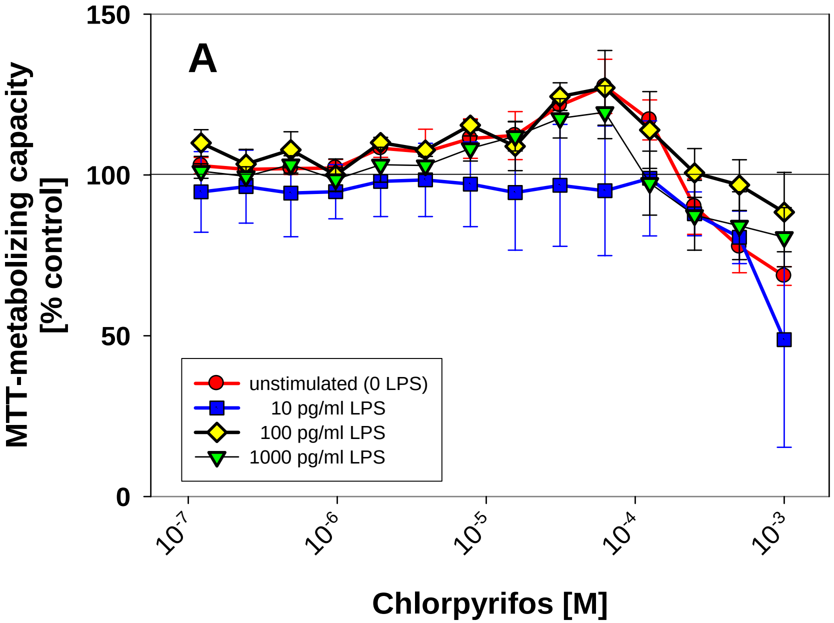


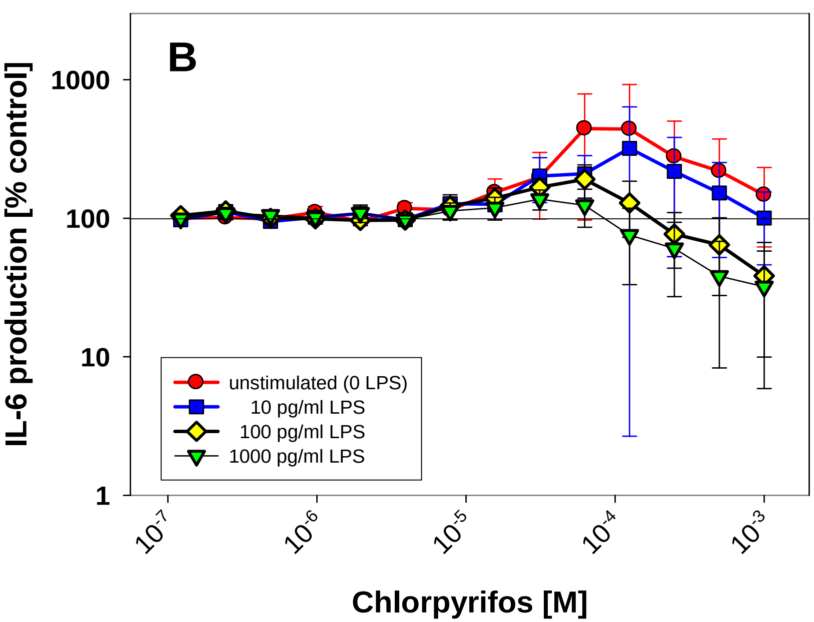


<u>Left hand side</u>: Locomotory activity of *Danio rerio* larvae five days after fertilization in tests with nickel chloride,
chlorpyrifos and binary mixtures of the substances;
mean of 8 - 13 larvae per data point, data of the second hour measurement;
<u>Right hand side</u>: model fit for dose level dependent deviation for single substance and mixture data



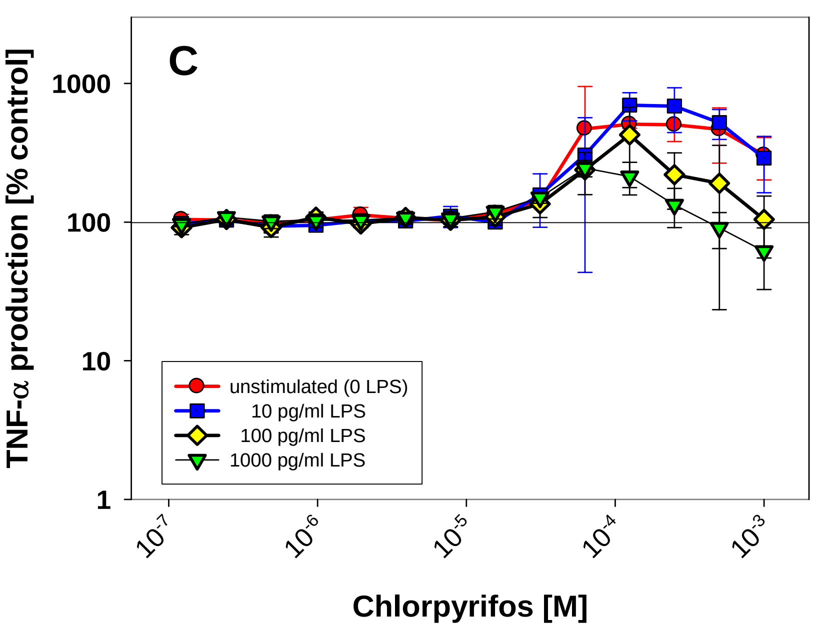
Hatching rate of *Danio rerio* larvae exposed to $NiCl_2$ and different temperatures (WP 3.2)



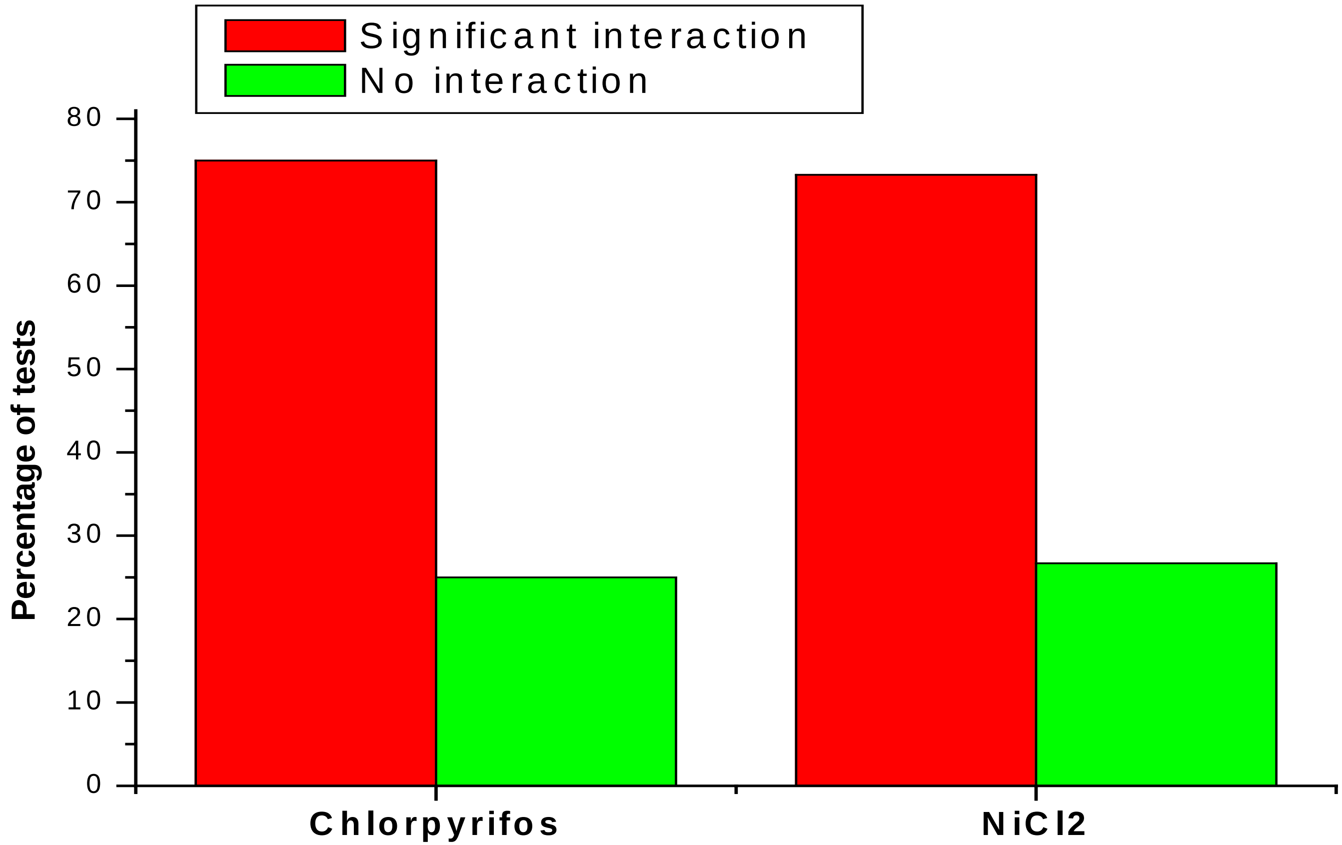


Pathogenes (LPS) and chemicals (WP 3.2)

Effect of different LPS doses on the immune modulation by chlorpyrifos. Shown are means and standard deviation of PBMC of three blood donors for the MTT-metabolizing capacity as measure for cell viability (A) as well as for the production of IL-6 (B) and TNF-α (C) in percent of solvent control. Please note the different scaling which is in log 10 for cytokine production.



Natural stressors and chemicals (WP 3.2)



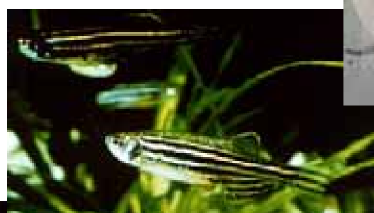
The frequency of tests in which significant interactions between a relevant natural stressor and a toxic chemical was detected was about 75% for both Chlorpyrifos (12 different test systems) and $NiCl_2$ (15 different test systems)

WP 3.4: Molecular mechanisms of mixture toxicity

Model organisms:

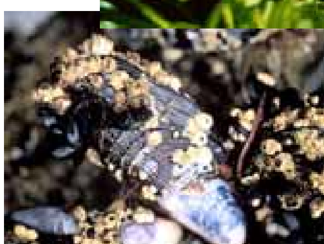
Homo sapiens

(Caroline)



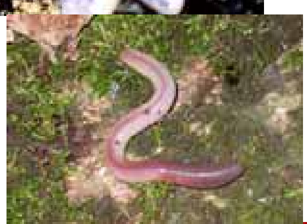
The zebrafish

Danio rerio



The marine mussel

Mytilus spp.



The earthworm

Lumbricus rubellus

The nematode

Caenorhabditis elegans

The social amoeba
Dyctiostelium discoideum

Partners institutions:

DISAV, Alessandria, I

NERC, UK

UFZ, Liepzig, D

WU, Wageningen, NL

King’s College, London, UK

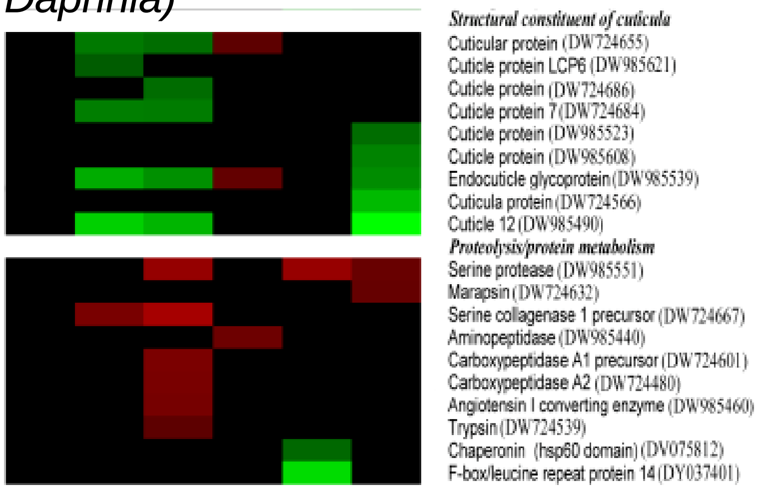
UCAM, Cambridge, UK

EKUT, Tübingen, D

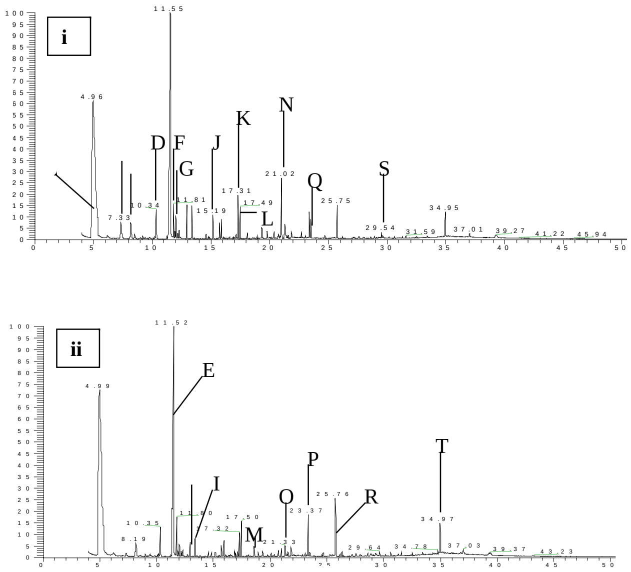
UA, Antwerp, B

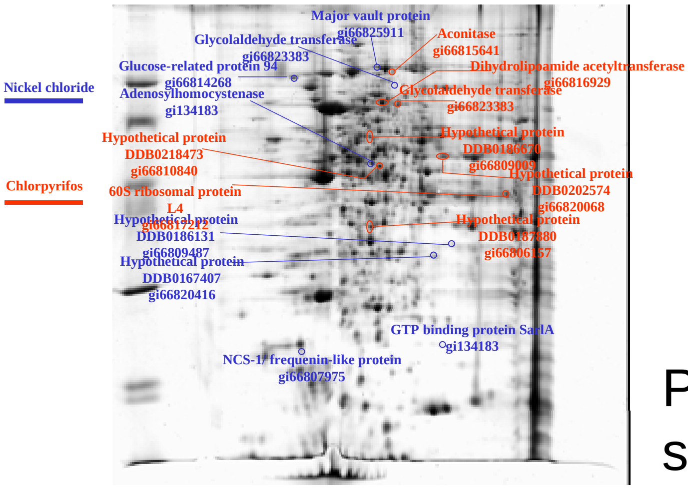
WP 3.4: Unveiling modes of action of prioritized chemicals and mixtures by means of high throughput analyses

Genomic analysis: Microarray outcome showing differentially expressed genes *(Daphnia)*



Metabolomic analysis: GC-MS showing differentially represented metabolites *(Lumbricus)*



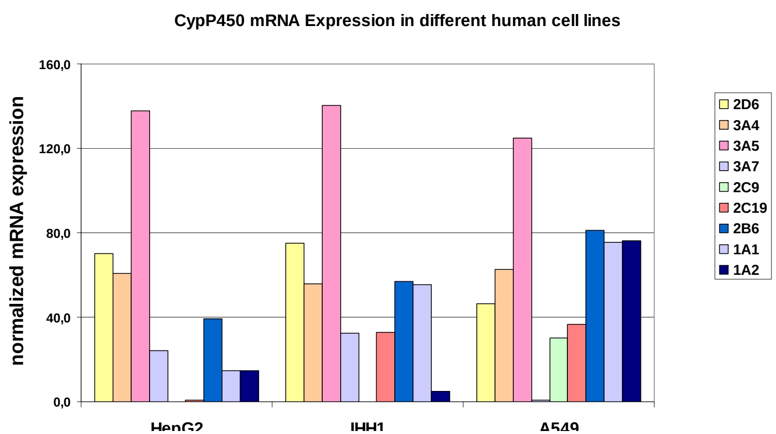


Proteomic analysis: 2DE gels showing differentially expressed protein patterns *(amoeba)*

WP 3.4: Investigation on single critical toxicity parameters

- Lipofuscins (oxidative stress biomarker)
- Acetylcholinesterase activity

- EROD, GST
- Cellular Energy Allocation (CEA) assay
- HSP70
- Histopathology



__Examples:__

Evaluation of biotransformation activities in human cells

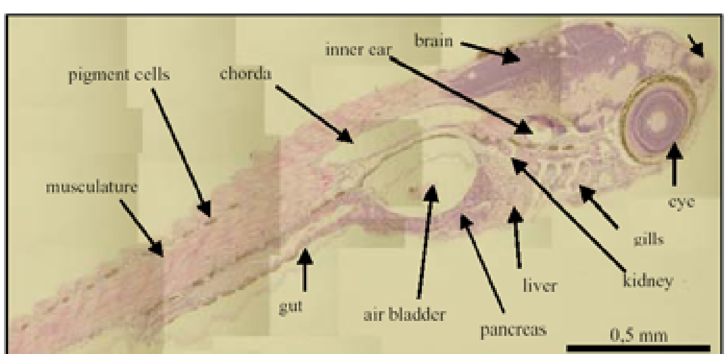


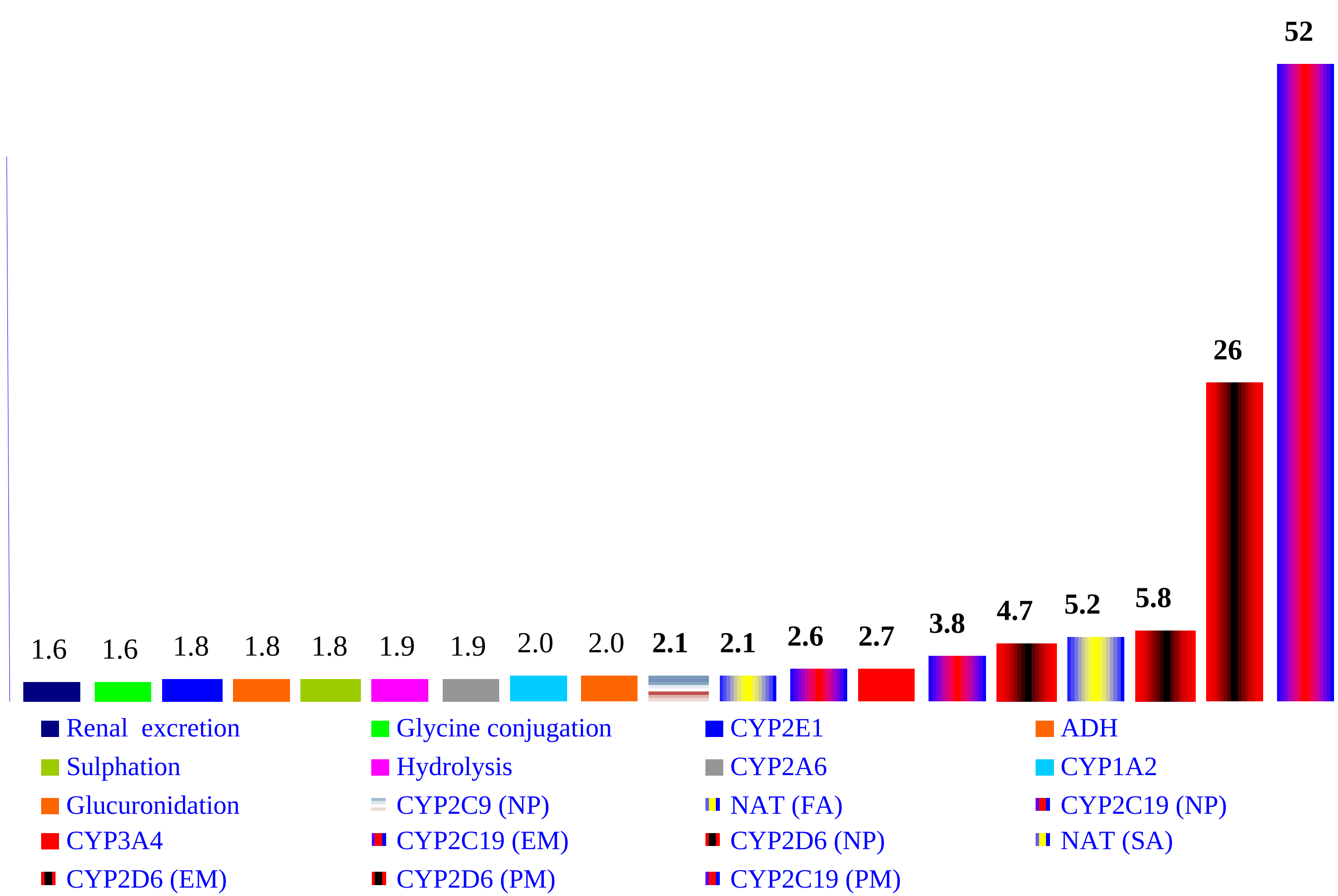
Fig. 1: Sagittal section of a zebrafish larva

Histopathology in zebrafish larvae

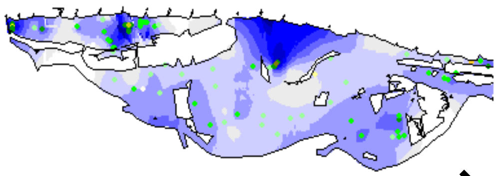
Research Pillar 4: Risk Assessment

- **WP 4.1 New concepts and techniques for propabilistic risk assessment**
  (Leader: Ad Ragas)
- **WP 4.2 Explicit modelling of exposure and risk in space and time**
  (Leader: Uwe Schlink)
- **WP 4.3 Dealing with multiple and complex risks in a management context**
  (Mikael Hilden)
- **WP 4.4 Risk presentation and visualisation**
  (Leader: Joost Lahr)

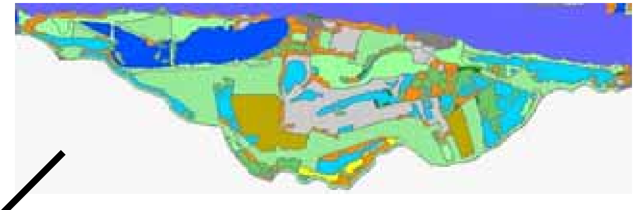
WP 4.1: Science based extrapolation factors



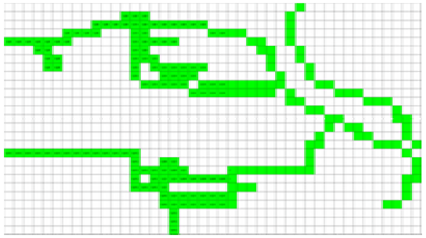
WP 4.2: Random Walk Models



Pollution Map

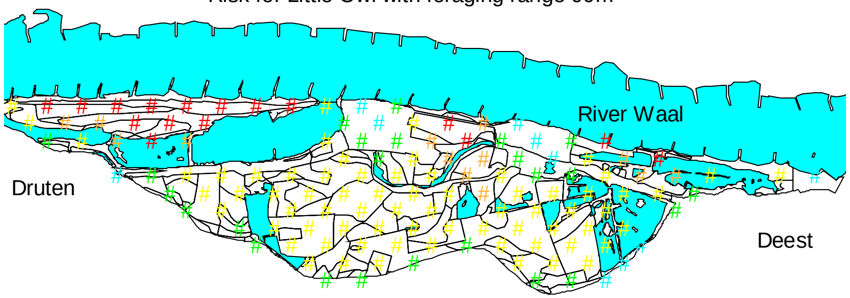


Habitat Map

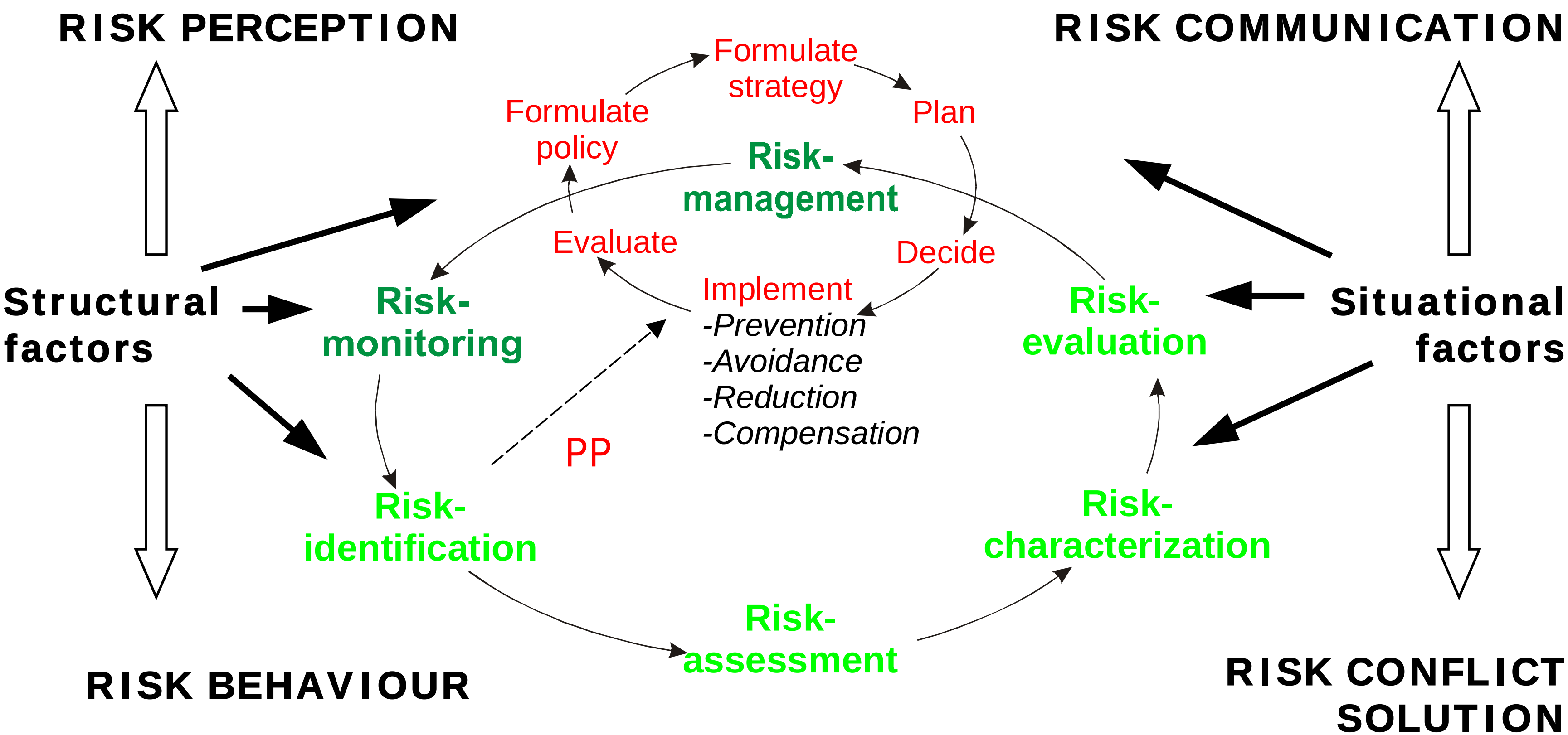


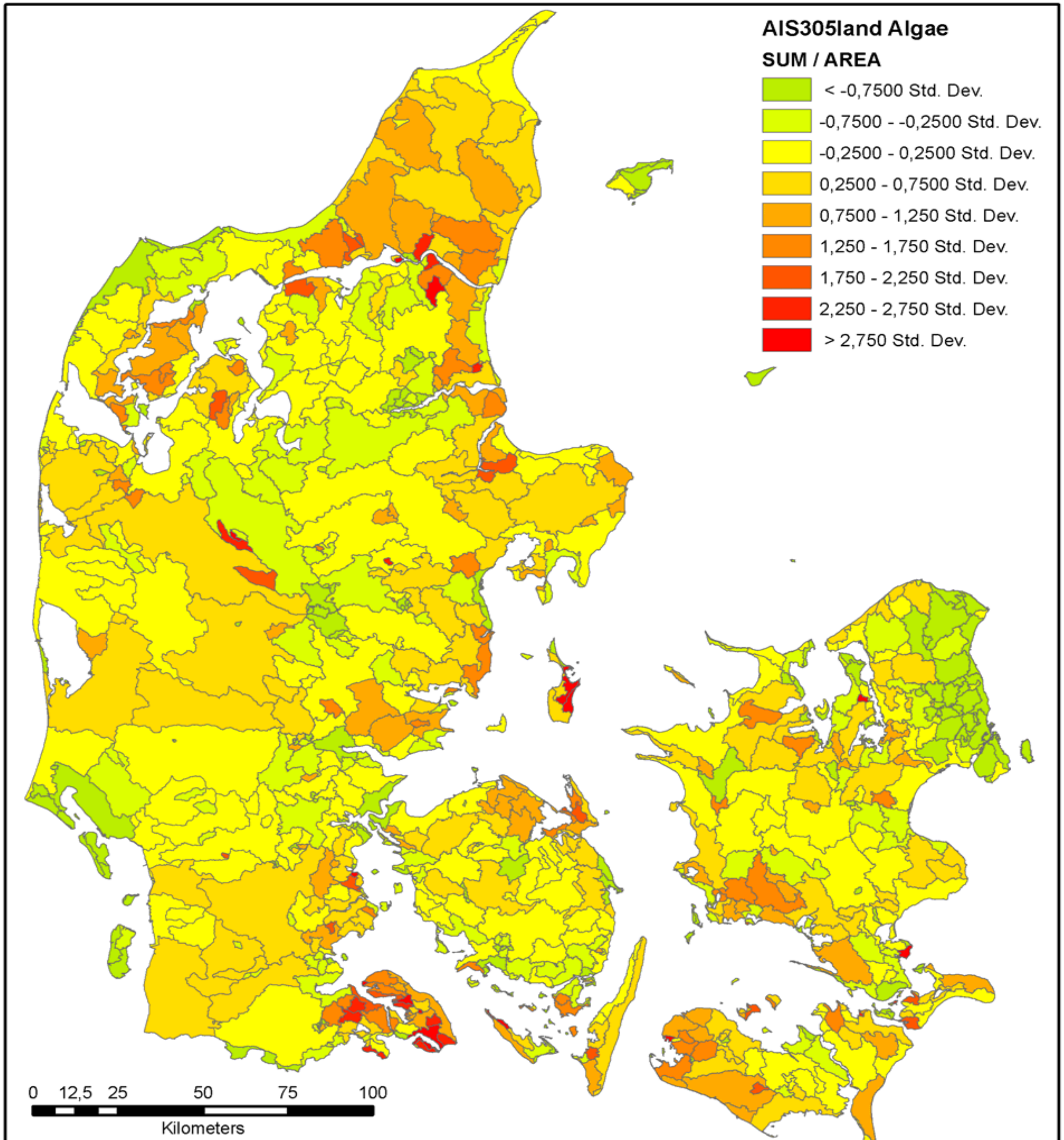
Walking Algorithm

Risk for Little Owl with foraging range 90m



WP 4.3: Risk Management & Science





Risk map for aquatic algae exposed to pesticides used in Denmark

Conclusions – output from NoMiracle

NoMiracle will provide new concepts and methods to deal with existing and emerging chemicals in a real world of cumulative stressors:

<u>Exposure assessment tools</u>

- methods for matrix-compound interactions
- methods to measure available exposure, based on chemical activity and other novel approaches
- methods for metabolic fate
- models for exposure assessment, incl. modelling of exposure and risk in space and time

Conclusions – output from NoMiracle

<u>Integration of human health and environmental methods</u>

- Risk scenarios to identify most likely combinations of chemical and other stressors, and methods to make risk mapping
- Exposure assessment (bioavailability) based on chemical activity
- Mechanistic approach in effects assessment, including uptake mechanisms
- Methods for toxicokinetics - single chemical uptake and interactive effects
- Demand for less use of mammalian test animals; in vitro methods and invertebrate testing in focus
- General biomarkers for human and environment
- New concepts and techniques for probabilistic risk assessment

Conclusions – output from NoMiracle

<u>Development of methods for assessing uncertainty</u>

– separation of true uncertainty and individual variability in predicted risks of human populations from exposure to pesticides through all relevant environmental pathways

– describing the metabolism and preliminary pharmacodynamic data in human subgroups. Derivation of uncertainty factors for subgroups and test species (single chemicals and mixtures)

Conclusions – output from NoMiracle

Models and risk maps:

- Risk presentation techniques
- Spatial aggregation of risks to man and environment
- Multimedia fate and exposure model with varying spatial resolution
- Up-scaling methods based on small scale modelling
- Model for health risks in cities
- Ecological vulnerability analysis
- Development of methods to present and visualise risks