# ECO24 – Prediction of Non-Extractable Residues Using Structural Information ('Structural Alerts')


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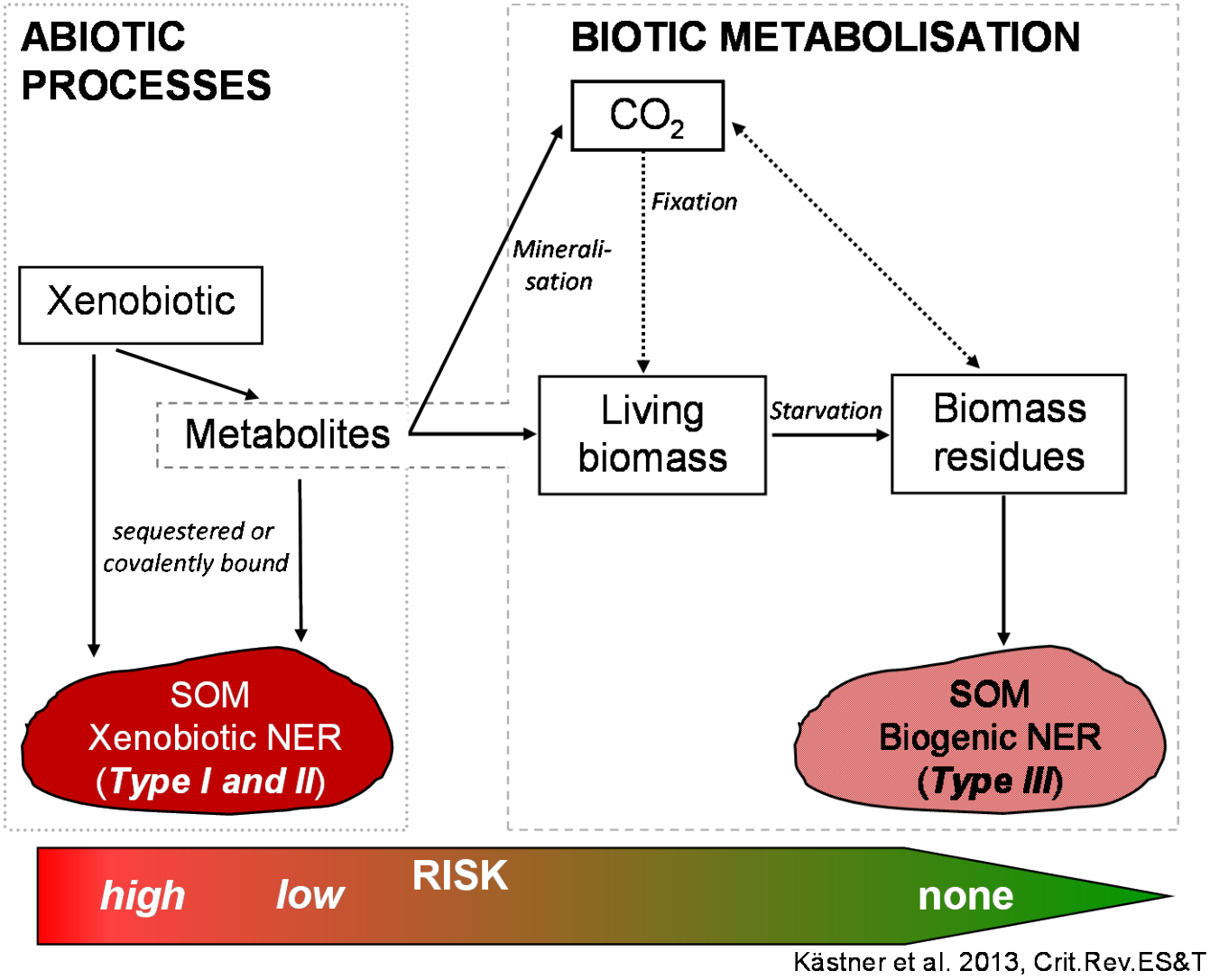
# Non-Extractable Residues (NER)

## Environmental Fate of Xenobiotics in Soil and Sediments

- **Partitioning**
- **Abiotic degradation** and **transformation**
- **Biotic** degradation and transformation
- Non-extractable residues (**NER**)

# Types of NER

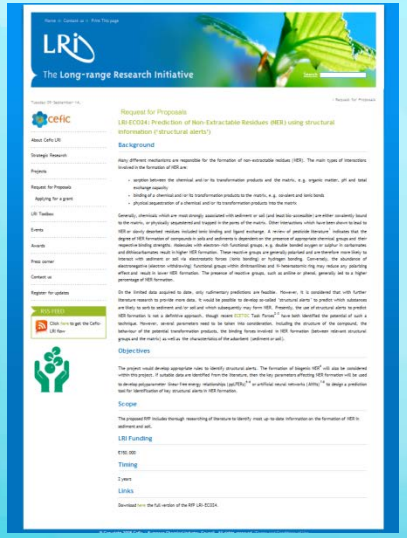


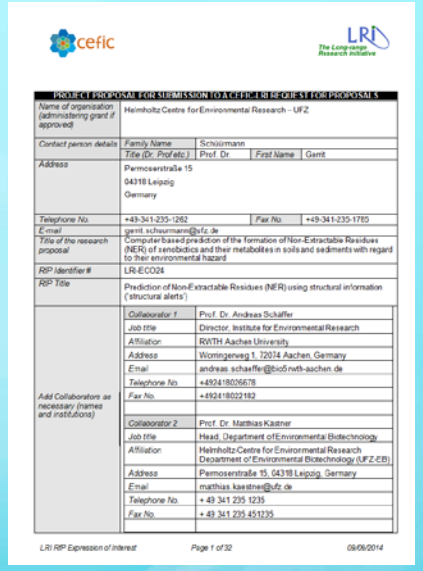

Kästner et al. 2013, Crit.Rev.ES&T

# CEFIC-LRI ECO 24

## Background

- Dependence on **chemical structure**
- Only **rudimentary predictions** feasible yet




## Objectives

- Develop rules to identify **structural alerts** for NER formation
- Consider also **biogenic** NER
- If suitable, **key parameters** to be modelled quantitatively e.g. by Abraham (LSER)

⇨ **Computer tool for predictions**

# First Data Set

- Scientific literature:
  Almost **one source** only
- **141 Values** for **96 different compounds**
- Mainly from **EFSA dossiers until 2008**
- **NER** formation %, **$CO_2$ formation** %

Environ. Sci. Technol. 2008, 42, 1045–1054

## Formation of Pesticide Nonextractable (Bound) Residues in Soil: Magnitude, Controlling Factors and Reversibility

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The analysis of the coherent data on nonextractable (bound) residues (NER) from the literature and EU pesticide registration dossiers allows the identification of general trends, in spite of the large variability and heterogeneity of data. About 50% of the pesticides reviewed exhibit a low proportion of NER (less than 30% of the initial amount) while only 12% of pesticides have a proportion of NER exceeding 70%. The lowest proportion of NER was found for dinitroanilines (<20%), and the largest value was obtained for carbamates, and in particular dithiocarbamates. The presence of chemical reactive groups, such as aniline or phenol, tends to yield a larger proportion of NER. NER originating from *N*-heteroatomic ring were found to be lower than those from phenyl-ring structures. Among the environmental factors affecting the formation of NER, microbial activity has a direct and significant effect. Concerning the NER uptake or their bioavailability, consistent data suggest that only a small percentage of the total amounts of NER can be released. The analysis of NER formation kinetics showed that incubation experiments are often stopped too early to allow a correct evaluation of the NER maturation phase. Therefore, there is a need for longer term experiments to evaluate the tail of the NER formation kinetics. Still, the heterogeneity of the NER data between pesticides and for specific pesticides calls for great care in the interpretation of the data and their generalization.

### Introduction

The first research activities on pesticide nonextractable residues (NER) in soils, initially referred to as "bound residues", were initiated in the mid-1970s (*1*, *2*), whereas earlier work had been conducted on plants or vegetal constituents. Definitions or position statements on bound residues appear periodically in the literature (*3*–*6*). The last consensual definition for bound residues was provide by Führ (*7*): "bound residues represent compounds in soils, plants, or animals which persist in the matrix in the form of the parent substance or its metabolite(s) after extraction. The extraction method must not substantially change the compounds themselves or the structure of the matrix".

Beyond the conceptual definition of bound residues, operational definitions depend upon the techniques used for its quantification. Classical analytical chemistry techniques cannot be used because most techniques are based on an earlier extraction of the residues, and bon residues are nonextractable residues (NER) by definition. In this context, NER benefit from an operational definition which is dependent on extraction techniques, incubation times, and other experimental conditions used for soil incubation. It should be noted that this technical dependency is of the same nature as that for the measurement of total extractable residues. The only technique which does not rely on a preceding extraction and which allows a quantification of NER relies on the use of isotopes, usually $^{14}C$. NER are typically studied in incubations of soils treated with pesticides labeled with $^{14}C$. After incubation for a target duration, soils are exhaustively extracted. The unextracted radioactivity remaining in the soil, the operational definition of NER, is measured by liquid scintillation counting of the total $^{14}C-CO_2$ recovered after combustion of the soil samples. Due to the destructive nature of the approach, it cannot determine whether NER measured are the intact pesticides, their metabolites, or $^{14}C$ recycled in microbial biomass or in soil organic matter. For this purpose, additional techniques and laborious protocols are needed to allow a partial degradation of the soil structure or constituents with liberation of $^{14}C$ labeled compounds originating from the $^{14}C$-pesticide (*8*). Also the use of the stable isotopes $^{13}C$ and $^{15}N$ allowed to give information on NER nature and their formation mechanisms (*9*–*12*).

In pesticide risk assessment procedures, NER are usually accounted for the description of dissipation kinetics. Dissipation constants or half-lives (DT50s) are composite parameters resulting from the amalgamation of various dissipation processes, each of which having a specific kinetic. NER formation is typically considered both as a process contributing to pesticide dissipation and as a process decreasing pesticide availability, thereby provoking a transient pesticide stabilization which may lead to a subsequent slow release. The first consequence of the NER formation is a decrease in availability of pesticide residues with a concurrent increase of persistence in soil. The concepts and hypotheses used to explain the formation of NER in soils will have a direct influence on the way they should be described in pesticide fate models. If NER are considered as a true dissipation process, they could be described as an irreversible sink. In contrast, if NER formation is considered as a

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# xenoNER vs. bioNER

## Experimental Determination

- IUPAC: **NER** only **type I and II** (xenoNER)
- **Type III** (bioNER) assumed to provide **no risk**
- **Experimental** approach (radio-labelled): **Includes type III**

## Model Assumptions

- **Total** (= analyzed) NER: **Sum** of **xenoNER** and **bioNER**
- **Biomass** formation depends on **growth yield** and **$CO_2$ formation**
- Compound independent **low** (= conservative) **growth yield**

## Estimation Model for xenoNER Formation

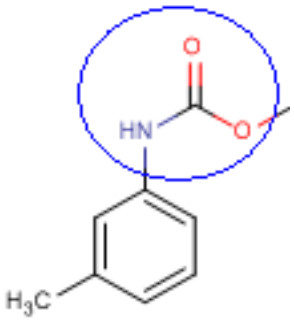
- **Total NER** from **experiments**
- **$CO_2$ formation** (mineralization) from **experiments**

# Preliminary Structural Alerts

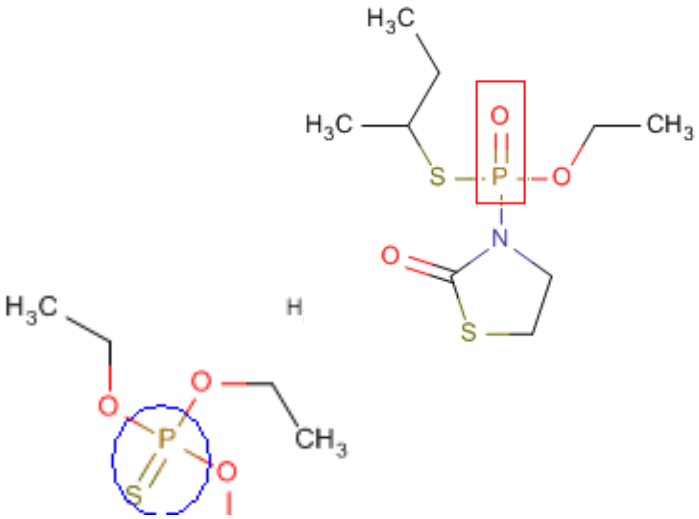
**12 structural characteristics** only

**Examples:**

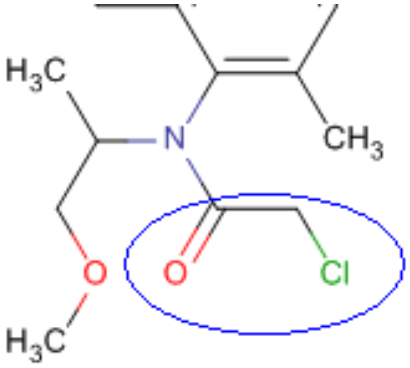
**⇧ High NER**
**O=C(-N)-O**



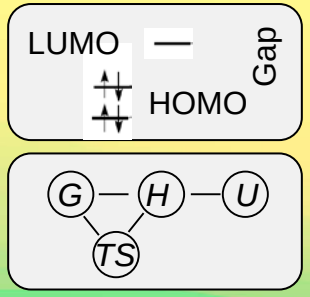

**⇩ Low NER**
**P=[O,S]**




**⇙ Not high NER**
**ClCC=O**



# Quantumchemical (QC) Alternatives?




## Parameters

- **Reactivity** related: HOMO, LUMO, Gap
- **Thermodynamic** properties: *U*, *H*, *G*, *TS*

$$A + O_2 \Rightarrow AO_2$$

## Subjects for Thermodynamic Properties

- **Compounds**
- Mineralisation **balance**
- Mineralisation **products** (net, gross)

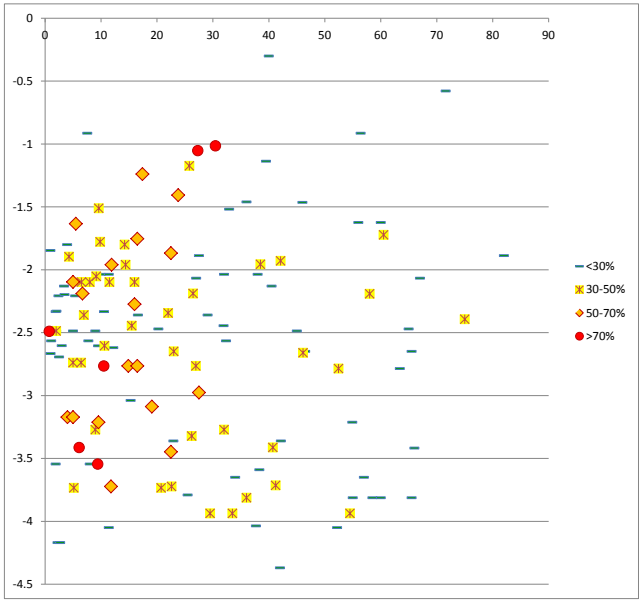
$$\frac{A}{x}$$

## Normalisation

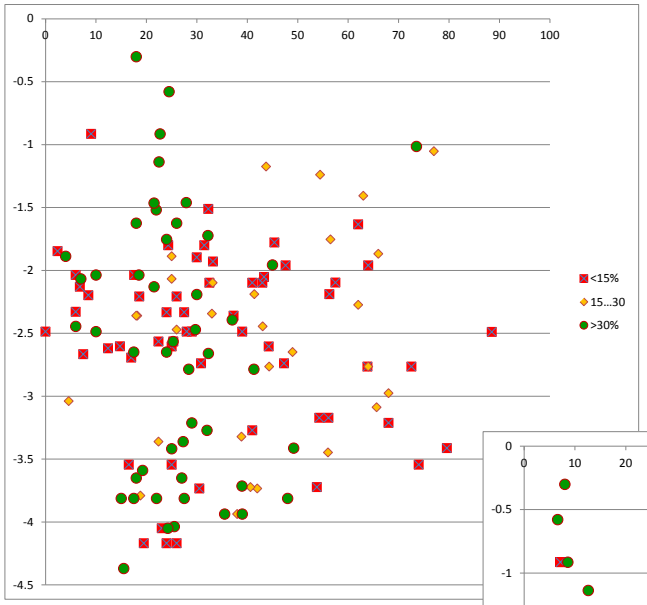
- Per **molecule** of the **compound**
- Per **C atom** of the compound
- Per **molecule** of **products** (net, gross)

QC Model: semiempirical

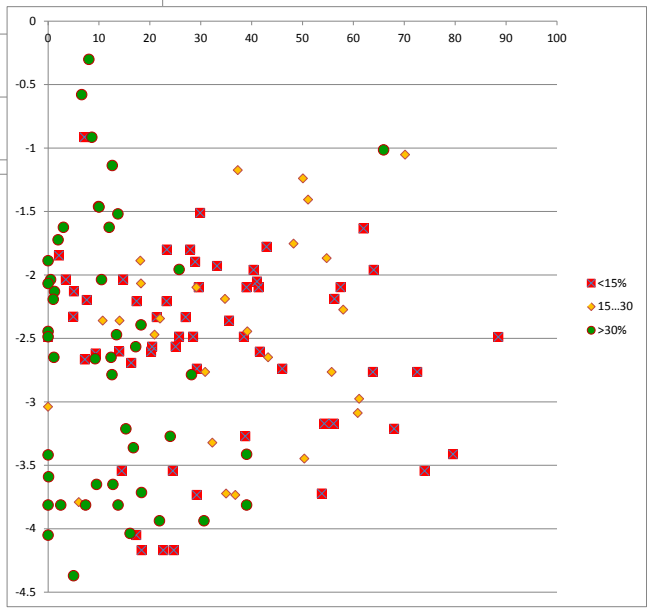
# Many of the Plots Look Like That …




% $CO_2$




% NER

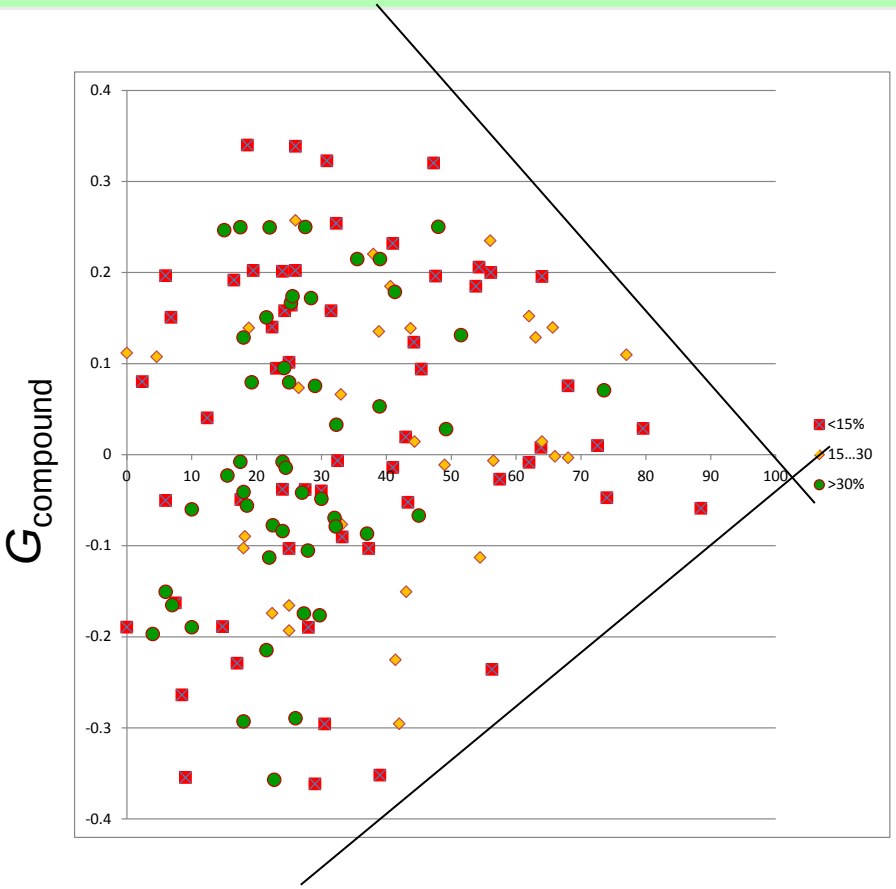



% xenoNER

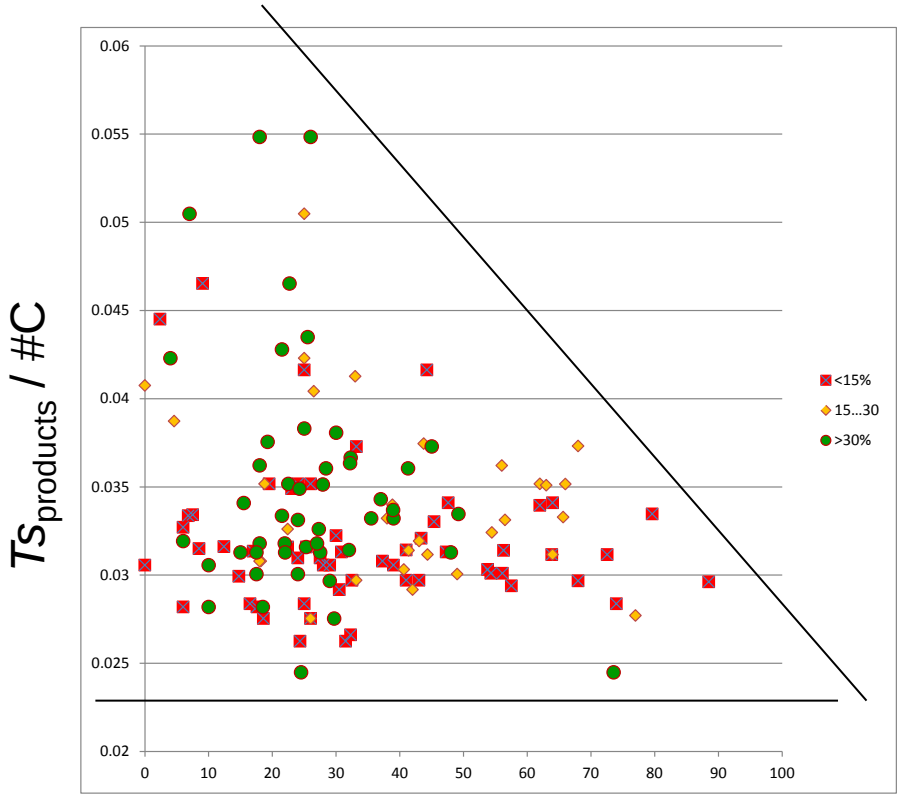
**Example: *G* of the mineralisation products per molecule of the compound**

# … BUT Some Look Like That

## *G* of the compound per molecule




## *TS* of the products per C atom




% NER

# Should We Go On With That?

## Pro

- Deriving some (even vague) **thresholds** possible
- Could be **combined** with **structural alert**

## Contra

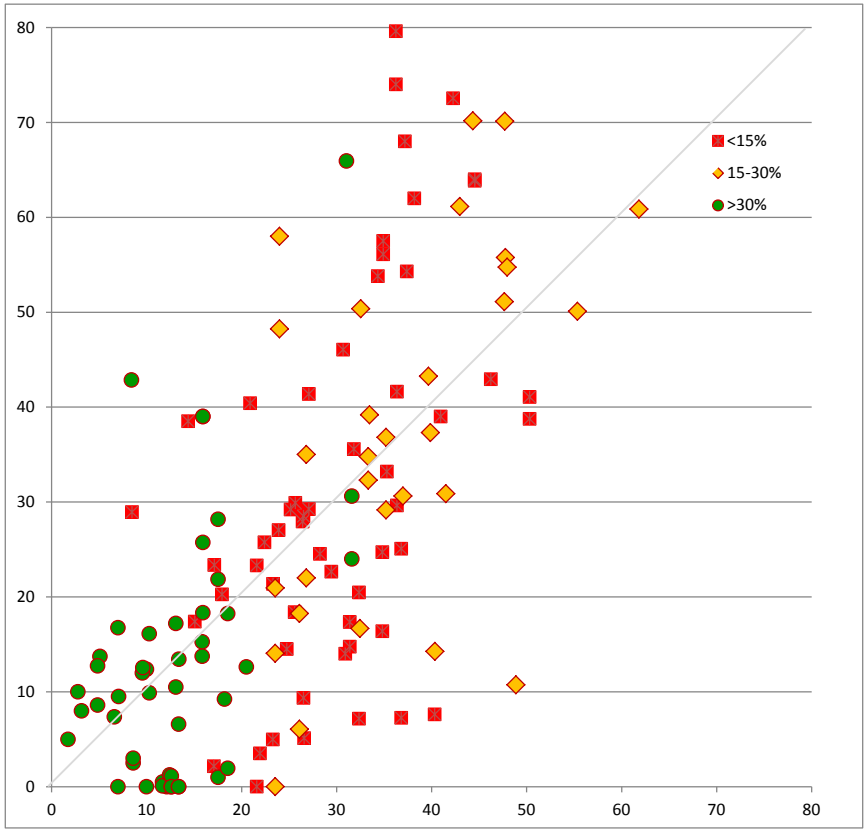
- **Benefits** still **limited**
- Actual QM model values depend on method ⇨ **Translation** to other method introduces **additional uncertainty**

## Even More?

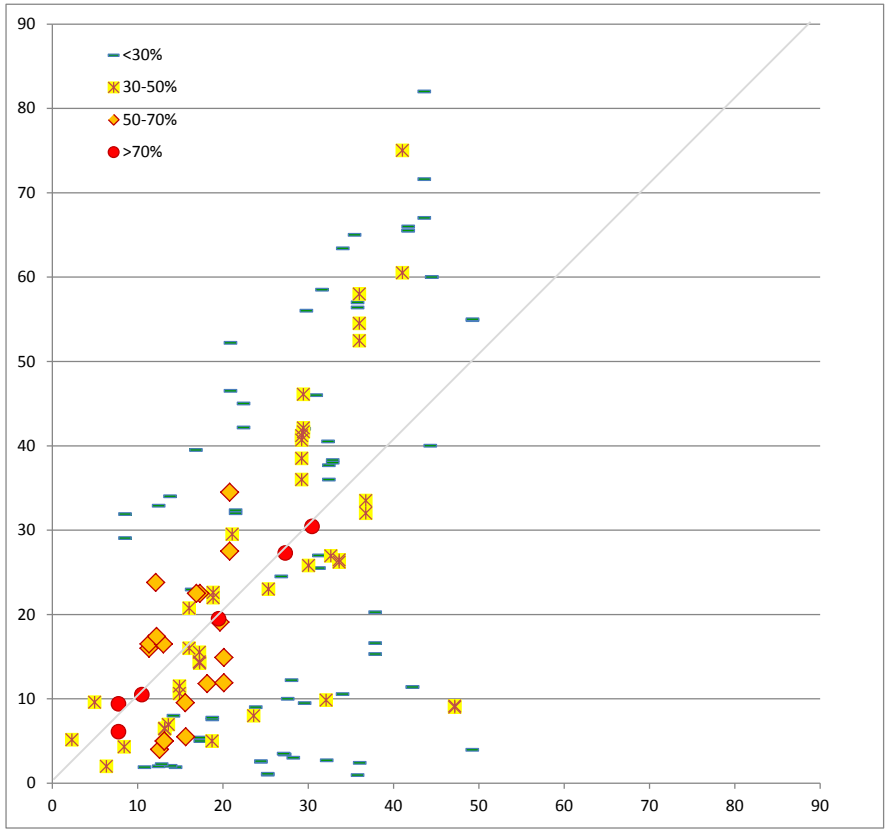
- **Physicochemical**, **molar** and **molecular** properties: additional **uncertainties** and **applicability limitations**

# May Abraham (Equations) Help?

## % xenoNER




## % Mineralisation



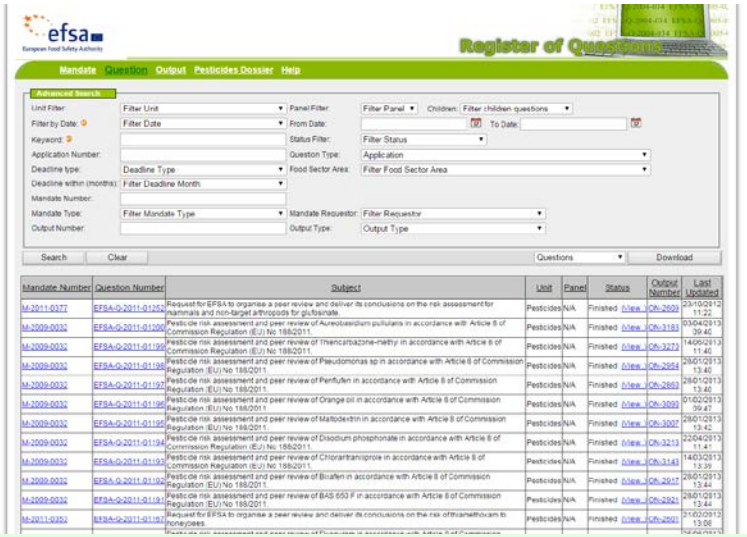

% Experimental

% Calculated

**Yes helpful, but separate equations for separate classes**

**⇨ $CO_2$ class needed for NER/xenoNER prediction an vice versa**

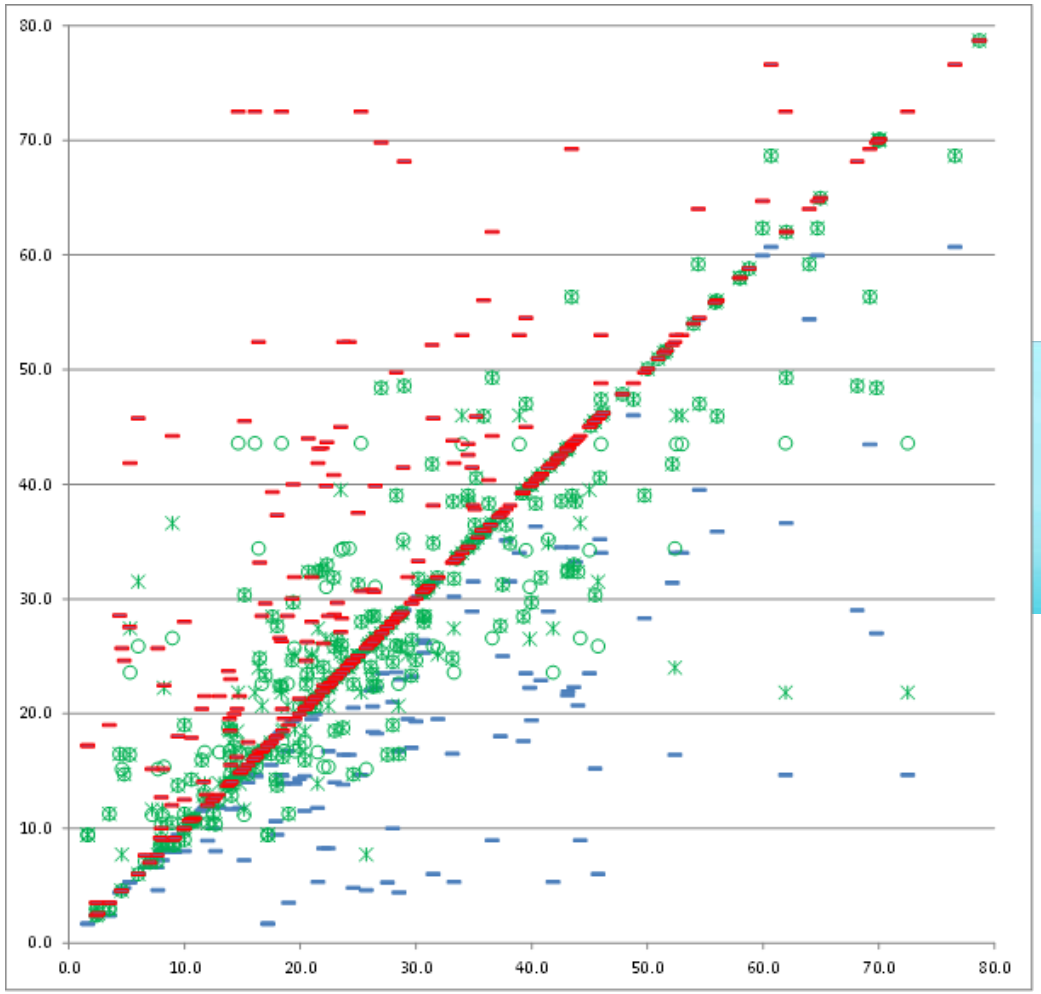
# New Hope: EFSA Dossiers




- **EFSA dossiers** from **2008 to 2015**
- **332 Values** for **189** different **compounds**
- **Mostly** (but not exclusively) **pesticides**
- **NER** formation %, **$CO_2$** formation %
- Only **small overlap** to first set (18 chemicals)

# Experimental Data Variability

Aggregations per compound




Individual entries

NER formation in %

| | |
|---|---|
| – | Minimum |
| ○ | Average |
| ✱ | Median |
| – | Maximum |

(similar for xenoNER)

# Why so Different?

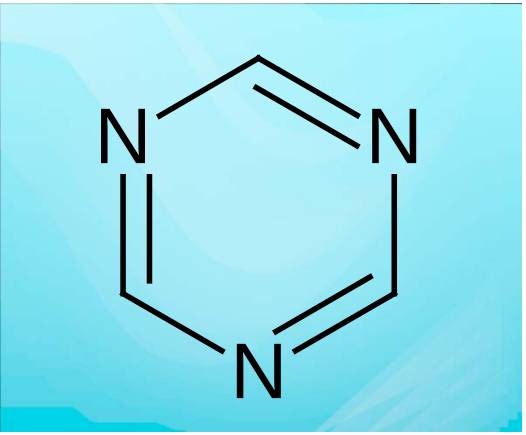
## Experimental Uncertainty

- **Labelling**
- Sufficient **duration**
- Extraction **techniques**
- **Stirring**

## Environmental Variability

- Soil **biological** activity
- Amount of soil **organic matter**
- **Other compounds** in soil
- Pesticides: Dependence on **placement**
- **Temperature**, **pH**

# Prominent Example: *s*-Triazines




**So Much Possible**

- Strong **ionic** bonds
- **Hydrogen** bonds
- **Ligand exchange**
- Hydrophobic **partitioning**
- **Charge-transfer** complexes

⇨ **High** NER forming **potential expected**

**Our Data Set: 14 *s*-Triazines**

- **3** between 30% and 50% (**medium** NER)
- **11** below 30% (**low** NER)

# “Yes We Can” – or “No We Can’t?”

## No We Can’t

- **Complex** influence of **environmental** conditions and **experimental** setup, many different **competing processes**
- **Increasing** and **decreasing** substructures / properties in the **same molecule**

⇨ **No simple** structure based **model** feasible

- **Complex model** (decision tree) possible in **theory**, but modeling would require **many more data**

## Yes We Can

- **Identify** NER **increasing** and **decreasing** substructures / properties
- **Combine** them in **non-linear** manner for **classification**

⇨ **Artificial Neural Network** (ANN) model for quantitative **prediction**

- ANN output not taken directly but **used for classification**

# Some Rules at Least

## Bulk Molecular Properties

- Hydrogen bonding basicity
- Polarity/polarisability
- Size related (characteristic volume, molecular mass)

## Atom Counts

- Carbon in general
- Special account for certain halogen at C

## Increasing Substructures

(in specific environment)

- Carbamate
- Phenol, carboxyle, nitro

## Decreasing Substructures

(in specific environment)

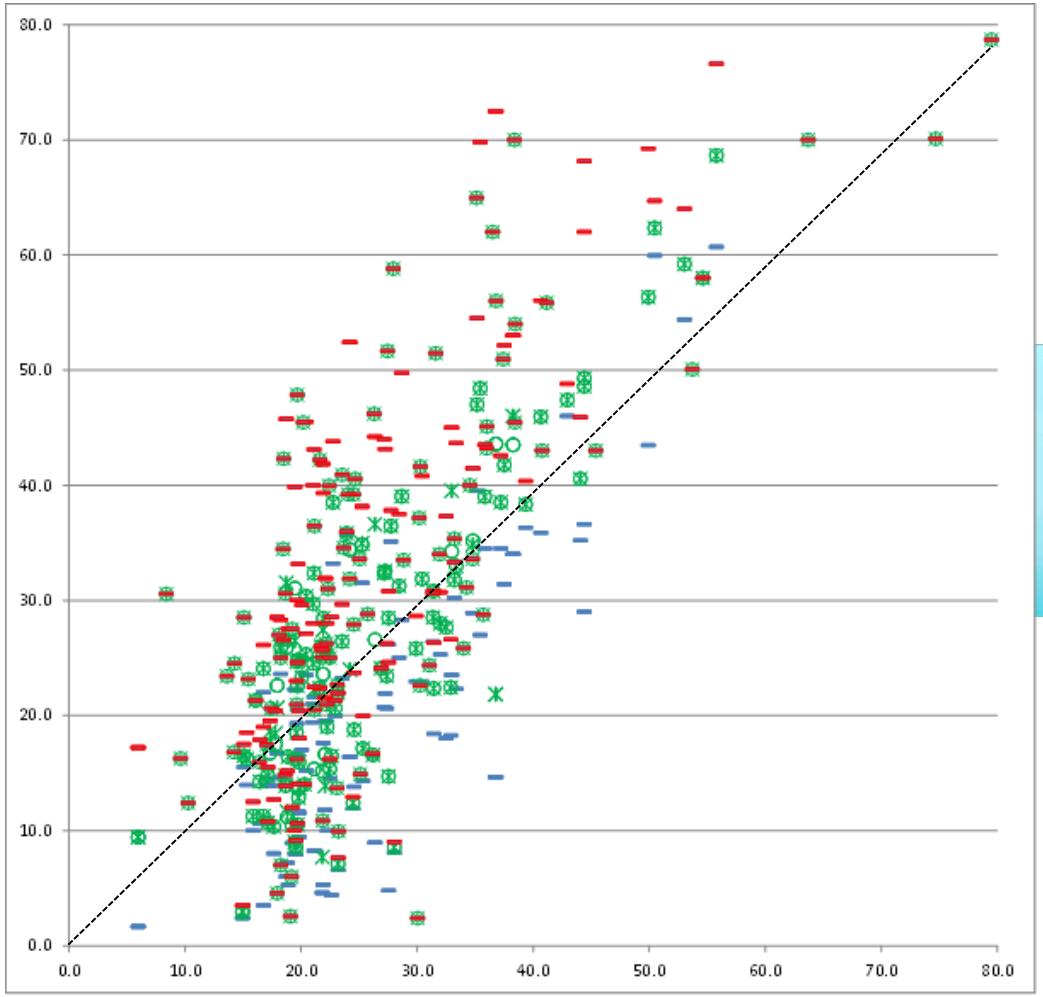
- Carboxyle
- OH, ketone, imine, nitrile, nitro acylhydrazine, certain S and P

## ANN Model

- Aggregated into **10 input descriptors**
- Simultaneous **output** of **NER** and **xenoNER** formation %

# Quantitative “Model” for NER

Experimental Data:
Aggregations per compound




Predictions per compound

NER formation in %

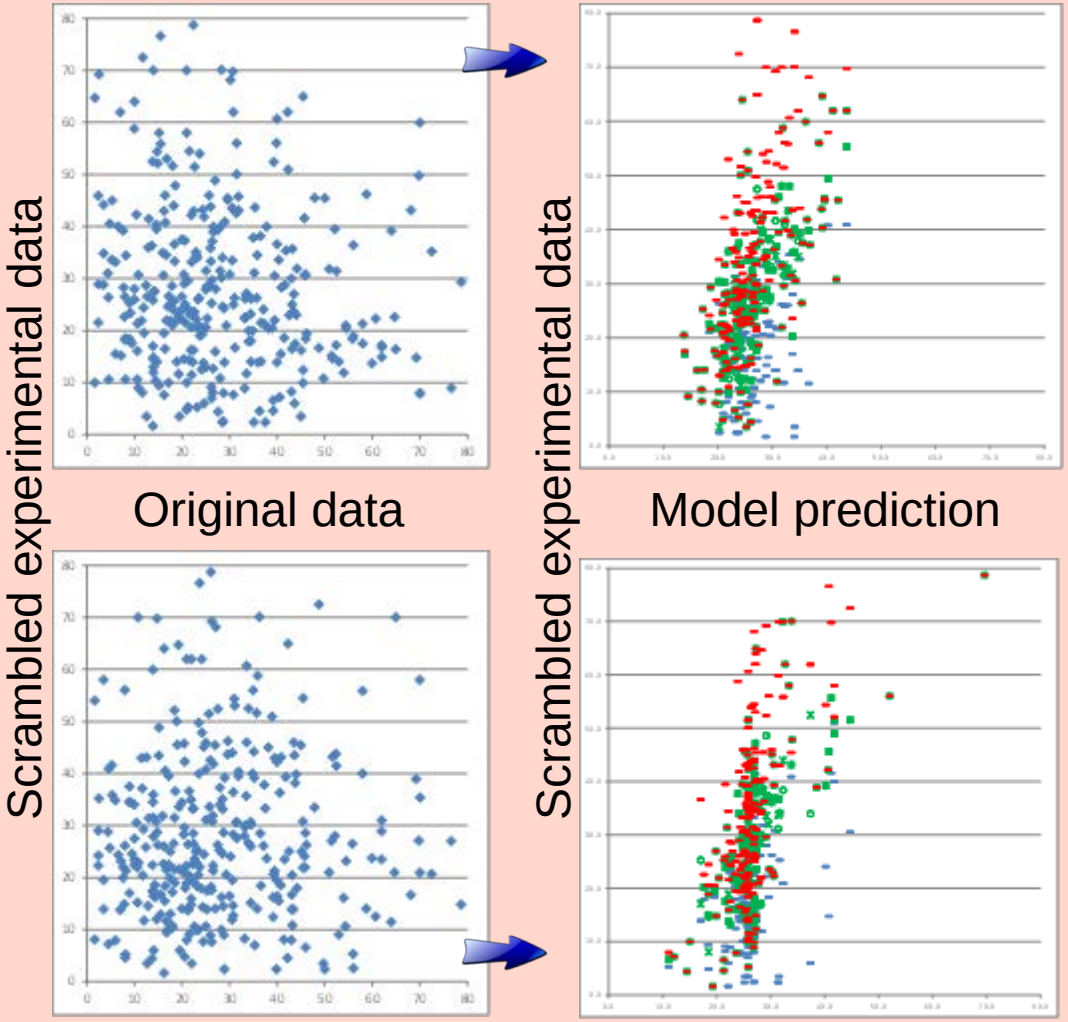
— Minimum
○ Average
✱ Median
— Maximum

(similar for xenoNER)

# But How to Validate?

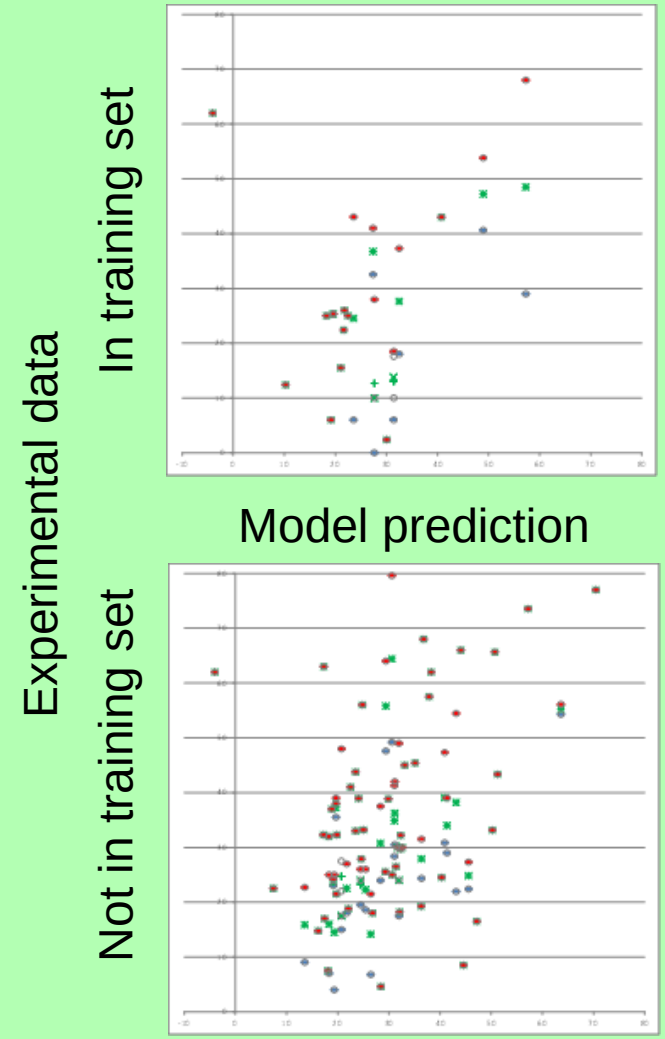
## **Y scrambling** (permutation)

Scrambled vs original  Model from scrambled data




## **External prediction**

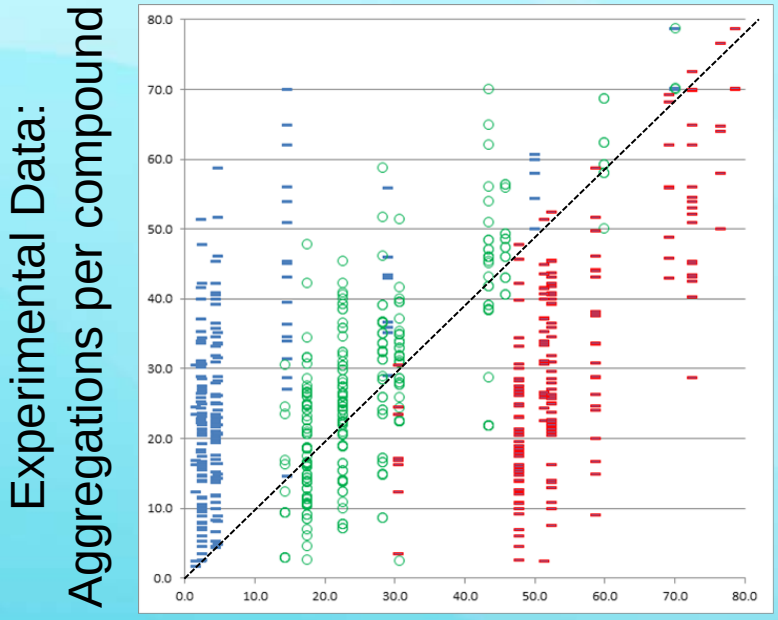
First data set



# To Interpret Calculated Results Properly

- **Output range class** from predicted value
- Min, median etc. from corresponding **class values**

| Model output range | Experimental data range | | | | |
|---|---|---|---|---|---|
| | Minimum | Maximum | Median | Below 30% | Above 50% |
| 0 … 15 | 2% | 31% | 14% | 93% | 0% |
| 15 … 20 | 3% | 48% | 17% | 90% | 0% |
| 20 … 25 | 4% | 52% | 23% | 73% | 1% |
| 25 … 30 | 5% | 59% | 28% | 56% | 5% |
| 30 … 35 | 2% | 51% | 31% | 42% | 3% |
| 35 … 40 | 15% | 73% | 44% | 18% | 39% |
| 40 … 50 | 29% | 69% | 46% | 7% | 39% |
| 50 … 60 | 50% | 77% | 60% | 0% | 100% |
| 60 … 80 | 70% | 79% | 70% | 0% | 100% |




Predicted min, median, max per compound

NER formation in %

— Minimum

○ Median

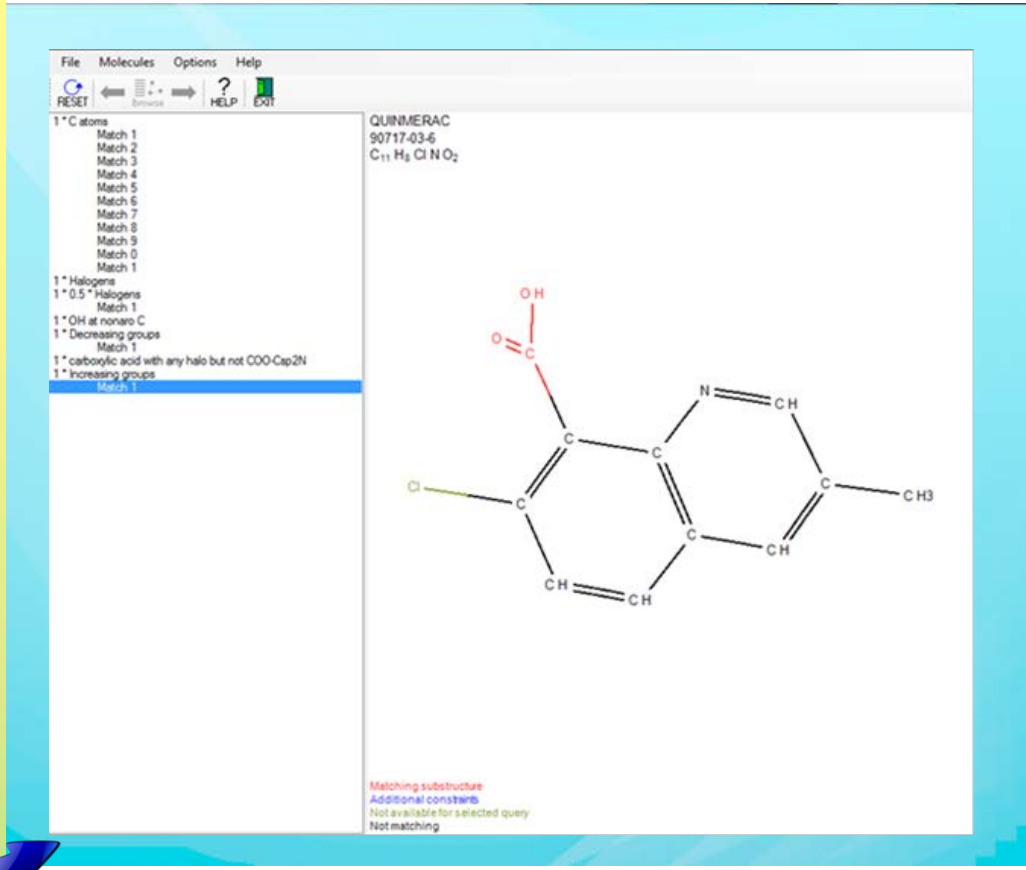
— Maximum

(similar for xenoNER)

# Computer Implementation (ChemProp)

- **ChemProp**: Fully **automated**
- **Manual** input of **Abraham parameters** if **desired**
- **Output** of **class values** (min, median, max, propabilites) for **NER** and/or **xenoNER**
- **Applicability domain** test: **Chemical** space **Physicochemical** thresholds
- Optionally: **Details** **ANN** calculated results **Structural** rules

Available soon for free
**www.ufz.de/ecochem/chemprop**



# So, Can We Really Predict NER Formation?

## NER and xenoNER Formation

- **Complex**, many **different processes**
- **Experimental** data for formation and conditions **still too limited**

## Prediction Feasible?

- **Simple** model **not possible** at all due to complexity
- **Complex** model would require **much more data** / **knowledge**
- **Screening** level estimation **possible**!

# Our Actual Results

## Achievements of This Study

- **Rough estimation** of **xenoNER** formation from NER and $CO_2$
- **Properties** and **substructures** relevant to NER formation
- **Classification** approach

## Suggestions

- **Targeted experimental** studies with **certain compound classes**
- Sophisticated research on **xenoNER** / **bioNER identification**

# Acknowledgements

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**Chemicals in the Environment**
**Integrated Project "Exposome" &**
**Integrated Project "Controlling Chemicals' Fate"**

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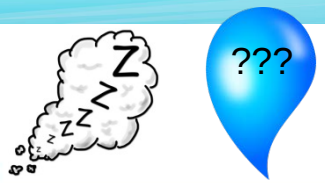
## Thank you for your attention!

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