Characterization of non extractable residues for their risk assessment in soil with special regard to pharmaceuticals

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Non extractable residues (NER) in plants and soil are defined as chemical substances that remain in soil or sediment matrix if extracted by methods which do not significantly change the chemical nature of these residues or the structure of the matrix.

These non extractable residues are considered to exclude fragments recycled through metabolic pathways leading to natural products.

In accordance to: Roberts 1984 adopted by the IUPAC
Possible binding forms and corresponding extractability of chemicals in soils

<table>
<thead>
<tr>
<th>Soil</th>
<th>dissolved in aqueous phase</th>
<th>sorbed</th>
<th>covalent bonding</th>
<th>physical entrapment</th>
<th>biogenic fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Parent compound</td>
<td>- Metabolite</td>
<td></td>
<td>- Parent compound</td>
<td>- Metabolite</td>
<td>- Parent compound</td>
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<td>- Metabolite</td>
<td></td>
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<td>- Metabolite</td>
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<td>- Metabolite</td>
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<tr>
<td>- CO₂</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>extractability</th>
<th>readily</th>
<th>extractability</th>
<th>heavily</th>
<th>non extractable</th>
</tr>
</thead>
<tbody>
<tr>
<td>bioavailability</td>
<td>not matrix destroying</td>
<td>matrix altering</td>
<td>matrix destroying</td>
<td></td>
</tr>
</tbody>
</table>

Eschenbach & Oing, 2013
Significance of NER for risk assessment

NER: operational definition (non extractability)

Types of NER due to processes of formation

- (bio) available
- remobilisation

hazardous potential

- stable fixation
- no release of original substances or metabolites

safe sink
Background of the study

• For biocides the legislative directives refers to the quantification (98/8/EC) and for pesticides to the characterization of NER (91/414/EEC)

• For human and veterinary pharmaceuticals the formation of NER is not mentioned in the relevant directives (exception: veterinary medicinal products in manure (guide line EMA/CVMP/ERA/430327/2009, 14. March 2011)).

• At present NER is considered mainly as substance dissipation with no regard to the formation processes (NER-types). If NER is considered to be available overestimation of risk

• The potential environmental hazard of these NER should be assessed: some fractions are stable others are potentially remobilizable

• Currently no standardized and accepted analysis technique for NER characterization or assessment is available

• To involve the characterization of NER in the regulatory context the development of a general accepted extraction approach is necessary

➢ Survey to develop a sequential extraction scheme for the assessment of NER
Aim of the survey: Approach for NER risk assessment

1) Extraction methods to separate extractable and non-extractable fractions;
   Methods to extract NER
   ➔ Quantity of NER

2) Extraction methods to characterize NER to derive their remobilization/hazardous potential;
   Methods to characterize NER
   ➔ Quality of NER
Commonly used extraction methods to quantify extractable fraction & NER

- Cold shake extraction with organic solvents or buffer solutions
- Ultra sonic extraction with organic solvents or buffer solutions
- Extraction by Soxhlet
- HTD (high temperature distillation)
- ASE (accelerated solvent extraction)
- SFE (supercritical fluid extraction)
- MASE (microwave assisted extraction)
### Determination of NER of pharmaceuticals (selected examples)

<table>
<thead>
<tr>
<th>Method</th>
<th>Compound</th>
<th>NER (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold Shake</td>
<td>Sulfamethoxazole</td>
<td>&gt; 70 %</td>
<td>ECETOC TR. No 118</td>
</tr>
<tr>
<td>Soxhlet</td>
<td>Sulfadiazin</td>
<td>84 - 88 %</td>
<td>Junge et al., 2011</td>
</tr>
<tr>
<td>ASE</td>
<td>Diflocaxin</td>
<td>74 %</td>
<td>Junge et al., 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 - 65%</td>
<td>Rosendahl et al., 2012</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>30 %</td>
<td>Girardi 2011</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>88 %</td>
<td>Girardi 2011</td>
</tr>
<tr>
<td>MASE</td>
<td>Sulfadiazin</td>
<td>&gt; 45 %</td>
<td>Förster et al., 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-30 %</td>
<td>Müller et al., 2013</td>
</tr>
</tbody>
</table>
Quantity of NER: Methods to separate extractable fractions and non-extractable residues

Present in soil as

<table>
<thead>
<tr>
<th>Extractable fraction (EF)</th>
<th>Non extractable residues (NER)</th>
</tr>
</thead>
</table>

Extraction method

- Shake extraction with suitable solvent or buffer (substance specific)
- Extraction with matrix altering methods: Soxhlet, HTD, ASE, SFE, MASE

Nomenclature ECETOC 2013

- Extractable residues
- NER
- Bound residues

Modified after: Eschenbach & Oing, 2013

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## Quality of NER: Published methods to characterize NER

<table>
<thead>
<tr>
<th>Extraction with matrix altering methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent extraction with elevated temperature, pressure or energy input</td>
</tr>
<tr>
<td>Soxhlet, ASE, SFE, MASE, HTD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extraction via destabilization of SOM and SEC</th>
</tr>
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<tr>
<td>e.g. Chelating agents</td>
</tr>
<tr>
<td>Silylation and SEC</td>
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</table>

<table>
<thead>
<tr>
<th>Seq. chemical degradation methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoassay</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extraction biomolecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Fatty acid extraction</td>
</tr>
<tr>
<td>- Amino acid extraction</td>
</tr>
<tr>
<td>- Amino sugar extraction</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomass determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Fumigation methods</td>
</tr>
</tbody>
</table>
Physical entrapment

Stabilization of soil organic matter via:

- Polyvalent cations
- Hydrogen bonds
- Organic metal-complexes

Formation of hydrophobic cavities with the possibility to entrap pollutants

Extraction with chelating agents (e.g. EDTA) and SEC

Eschenbach et al.
Differentiation of NER-types via silylation

Separation by Size Exclusion Chromatography (SEC)

Modified after: Schäffer et al. 2010
Sequential chemical degradation

1. Ester/amide cleavage
   - Alkaline hydrolysis
2. Ether cleavage
   - Treatment with Boron tribromide (BBBr₃)
3. Oxidation of aromatic rings and functionalized carbon atoms
   - Treatment with Ruthenium Tetroxide (RuO₄)
4. Pyrolytical cleavage and methylation of functionalized groups
   - Thermochemolysis with Tetramethylammoniumhydroxide (TMAH)

Modified after: Riefer et al. 2011
Determination of biogenic residues

- Fatty-Acid-Extraction
  (Miltner et al. 2004, Nowak et al. 2011)
  - PLFA
  - tFA

- Peptidehydrolysis
  (Nowak et al. 2011)
  - bioAA
  - tAA

- Ammino-Sugar-Hydrolysis
  (Miltner et al. 2004)

- Fumigation-methods
  (CFE, CFI)
  (Soulas et al. 1984, Ghani et al. 1996, etc.)
Advanced NER-type model

**NER-type 1**
Heavy sorption
- Extraction with matrix altering methods
  - Solvent extraction with elevated temperature, pressure or energy input
  - Soxhlet, ASE, SFE, MASE

**NER-type 2**
Physical entrainment
- Extraction via destabilization of OM and SEC
  - e.g. chelating agents
- Silylation and SEC

**NER-type 3**
Irreversible binding
- Seq. chemical degradation methods
  - Immunoassay

**NER-type 4**
Biogenic fixation
- Extraction biomolecules
  - Fatty acid extraction
  - Amino acid extraction
  - Amino sugar extraction
- Biomass determination
  - Fumigation methods

- Potentially remobilizable as parent compounds or metabolites
  - hidden hazard
- Irreversible binding, no release of parent compounds or metabolites
  - safe sink

Eschenbach & Oing, 2013
### Results: Substance specific evaluation of extraction methods

<table>
<thead>
<tr>
<th>NER-type 1</th>
<th>Heavy sorption</th>
</tr>
</thead>
</table>

**Extraction with matrix altering methods**

- Solvent extraction with elevated temperature, pressure or energy input
- Soxhlet, ASE, SFE, MASE, HTD
  - widely-used

Eschenbach & Oing, 2013
# Results: Substance specific evaluation of extraction methods

## NER-type 2

### Physical entrapment

<table>
<thead>
<tr>
<th>Agents to destabilise OM and SEC</th>
<th>Silylation and SEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g. chelating agents</td>
<td></td>
</tr>
<tr>
<td>- PAH</td>
<td>- Simazine</td>
</tr>
<tr>
<td>- TNT</td>
<td>- Anilazine</td>
</tr>
<tr>
<td></td>
<td>- Imazalil</td>
</tr>
<tr>
<td></td>
<td>- Sulfonamide</td>
</tr>
</tbody>
</table>

Eschenbach & Oing, 2013
Results: Substance specific evaluation of extraction methods

NER-type 3

Irreversible binding

Sequential chemical degradation method

- MCPA
- Nonylphenole

Eschenbach & Oing, 2013
**Results: Substance specific evaluation of extraction methods**

<table>
<thead>
<tr>
<th>NER-type 4</th>
<th>Biogenic fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extraction biomolecules</td>
</tr>
<tr>
<td></td>
<td>– Fatty acid extraction</td>
</tr>
<tr>
<td></td>
<td>– 2,4-D</td>
</tr>
<tr>
<td></td>
<td>– Sulfadiazine</td>
</tr>
<tr>
<td></td>
<td>– Ibuprofen</td>
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<tr>
<td></td>
<td>– Amino acid extraction</td>
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<td></td>
<td>– Ibuprofen</td>
</tr>
<tr>
<td></td>
<td>– Glyphosat</td>
</tr>
<tr>
<td></td>
<td>– Simazine</td>
</tr>
<tr>
<td></td>
<td>– Amino sugar extraction</td>
</tr>
</tbody>
</table>

Eschenbach & Oing, 2013
Principle scheme of the preliminary sequential extraction procedure

Step I: Quantification
- Incubated soil sample
- Shake extraction
- Determination of total NER amount by combustion
- Determination extractable fraction

Step II: Characterization
- Extraction residue (step I)
  - Strong sorption
    - Extraction via matrix altering methods (Soxhlet, ASE, SFE, MASE, etc.)
  - Physical entrapment
  - Irreversible binding
  - Biogenic fixation
    - Silylation and SEC
    - Extraction with agents for destabilization of organic matter and SEC
    - Sequential chemical degradation methods
    - Extraction biomolecules:
      - Fatty acids and amino acids
      - Biomass determination:
        - Fumigation methods

Eschenbach & Oing, 2013
Demand for research

- Characterization of matrix alteration
- Binding form

Validation of methods for different substances
- Relevance of different SOM
- Verification of reliable quantification

Relation to specific binding form
- Quantification by calculating differences

Verification of reliable quantification

Verification NER-biomolecules / total biomass
- Validation of statistical correlation e.g. to CFE
Summary

- For most substances just the total NER amount or formation rate is determined
- In part matrix altering extractions (Soxhlet, ASE etc.) used for the separation of EF and NER
- Recommendation: Determination NER quantity by exhaustive cold shake extraction with appropriate solvents or buffer solutions (substance specific)
- For the characterization of NER different methods available, often very labor-intensive
- Distinction of 4 NER-types: heavy sorption, physical entrapment, irreversible binding, biogenic fixation
- Currently for most substances of priority no results for characterization of NER are available
- A substance specific general assessment of the hazardous and remobilization potential of NER is not possible yet
- Recommendation of a principle sequential extraction scheme was derived
- There is considerable demand for research to establish a data base for a general suitability of the extraction methods to ensure a hazard assessment
Deficiency and demand for research

- Application of extraction methods for the determination of specific NER-types with more substances
- Systematic studies on determination / quantification of all NER-types for a single substance
- Studies to compare different methods for similar NER-types e.g. silylation – chelating agents, extraction of biomolecules - CFE
- Studies to proof quantitative detection of NER-types
- Development of more simple procedures or quantification by calculating the difference (e.g. irreversible binding)
- Studies on consideration of soils with different properties, their variability and different genesis; climate and land use change; realistic simulation of environmental conditions

Need for systematic scientific studies to clarify open questions and to enable a validation of the specific methods proposed in the sequential extraction scheme
Thank you for your attention

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