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

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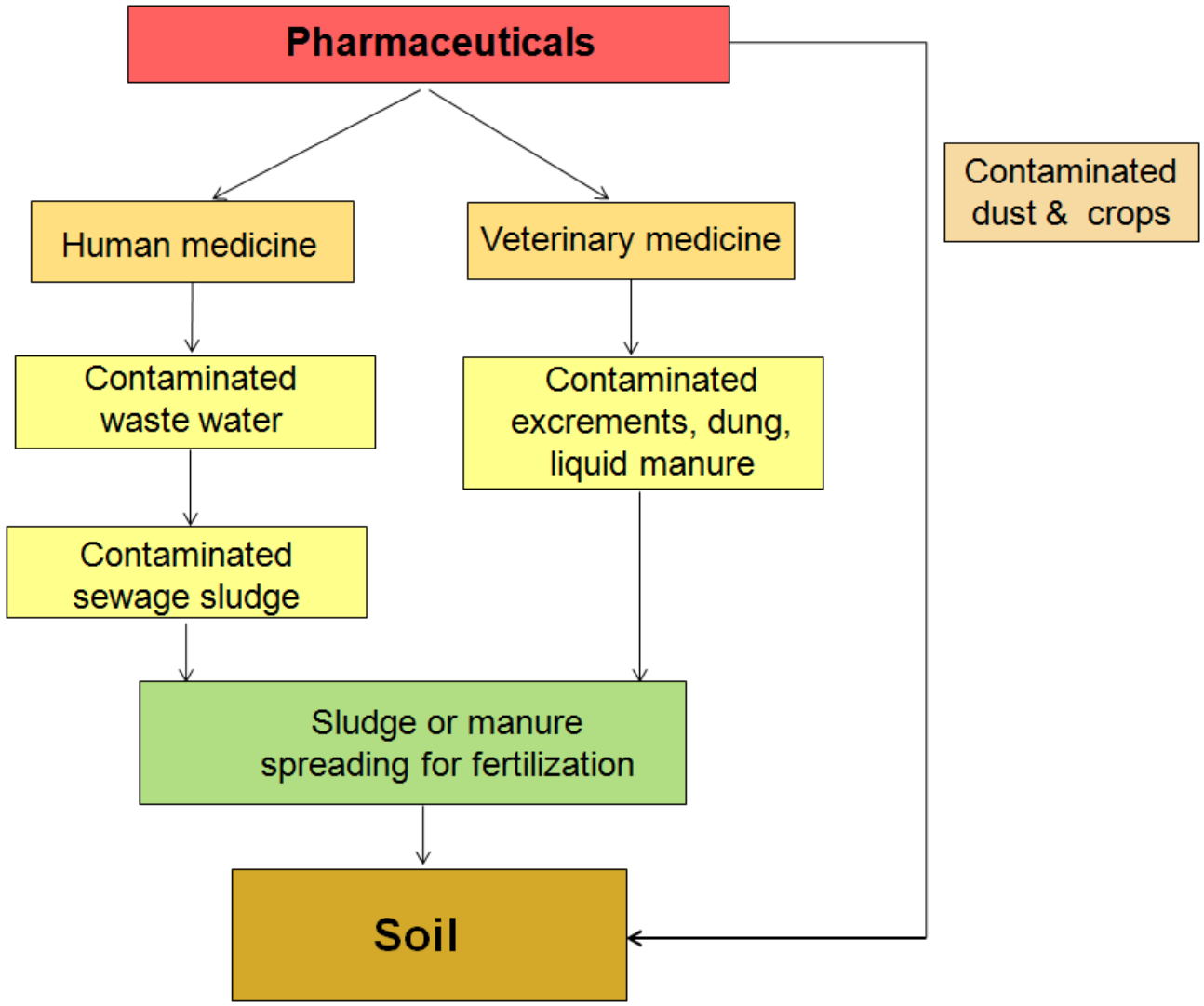
Sources of environmental contamination



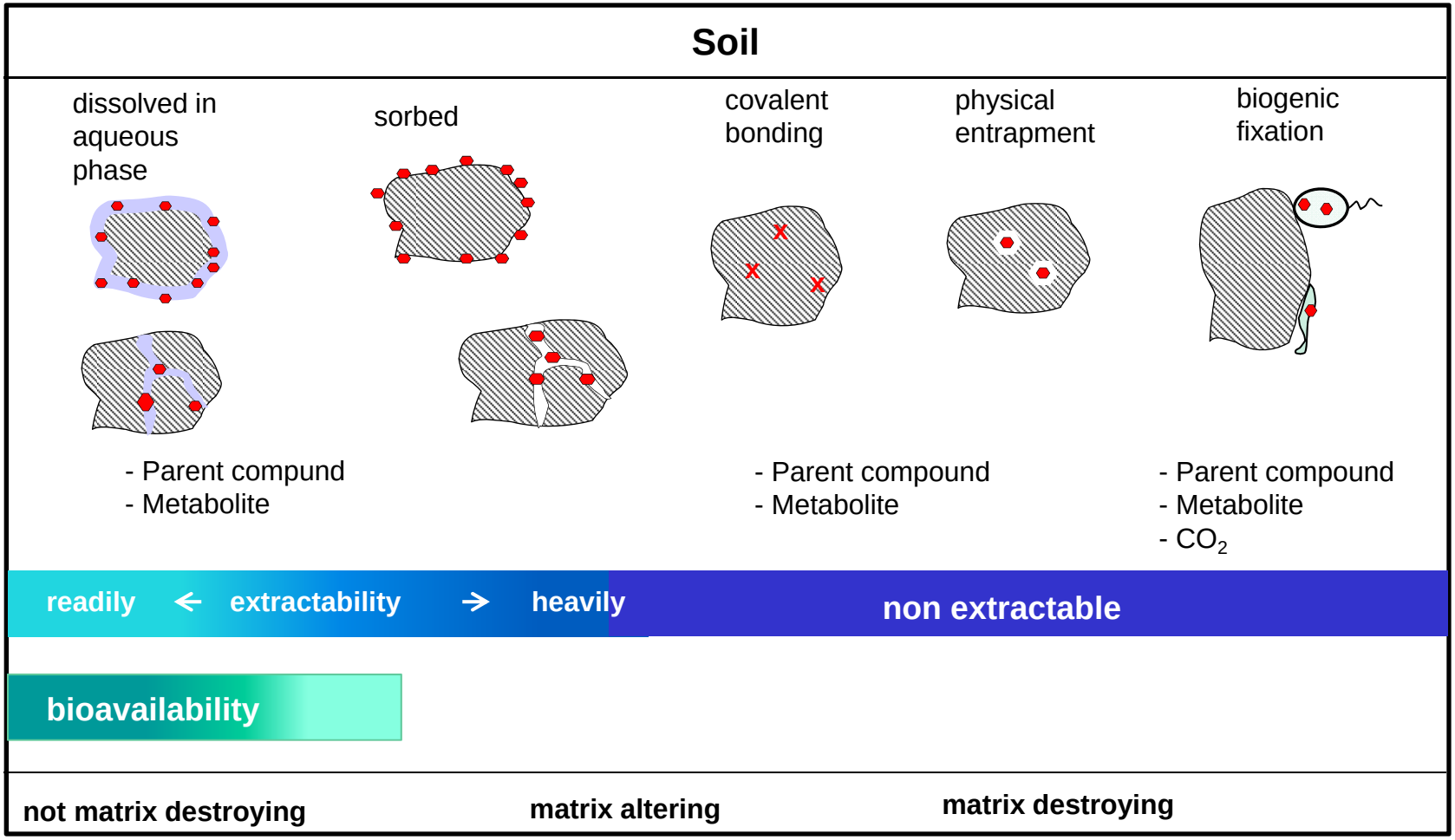
Definition non extractable residues (NER)

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



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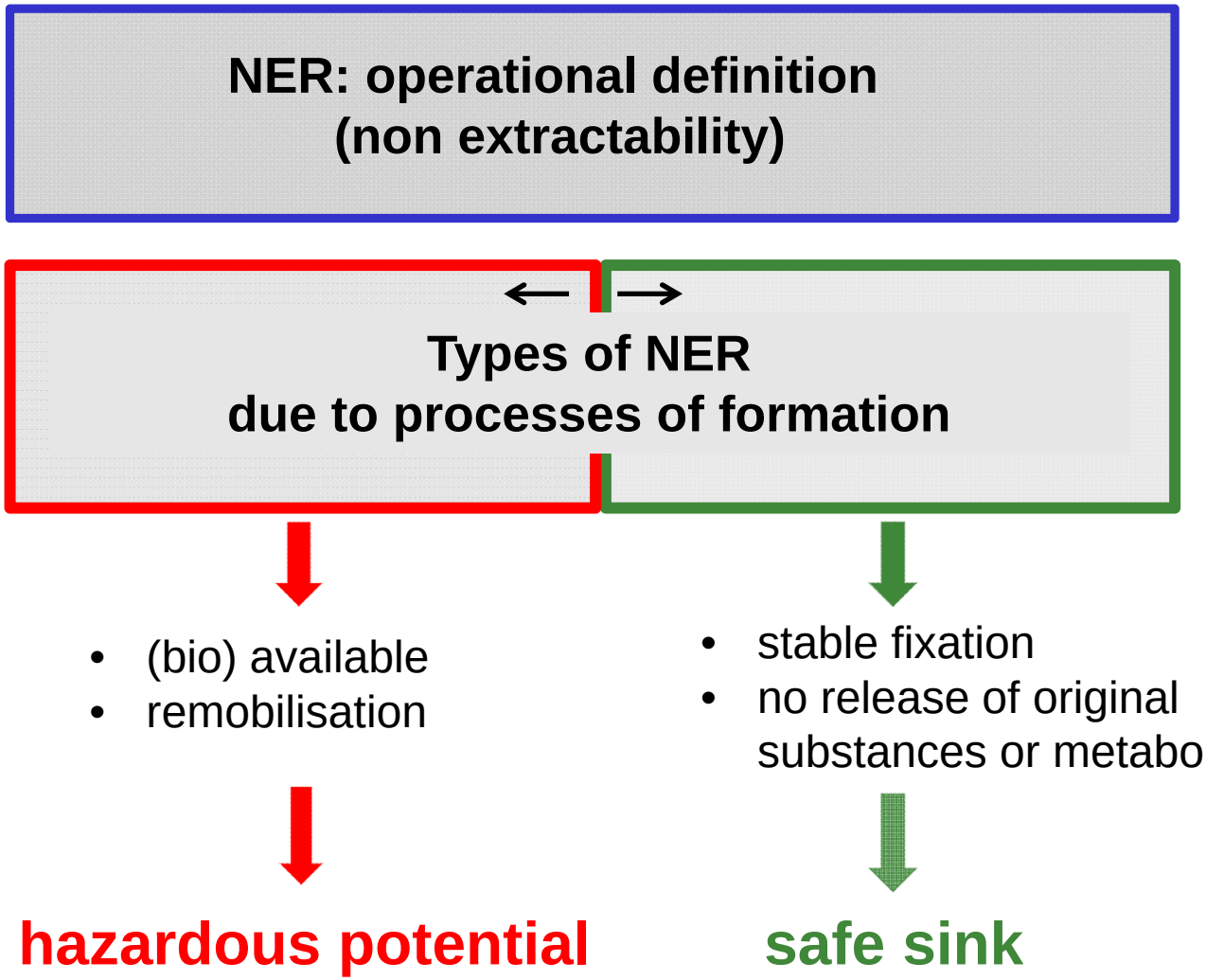
Significance of NER for risk assessment



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 seperate 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**

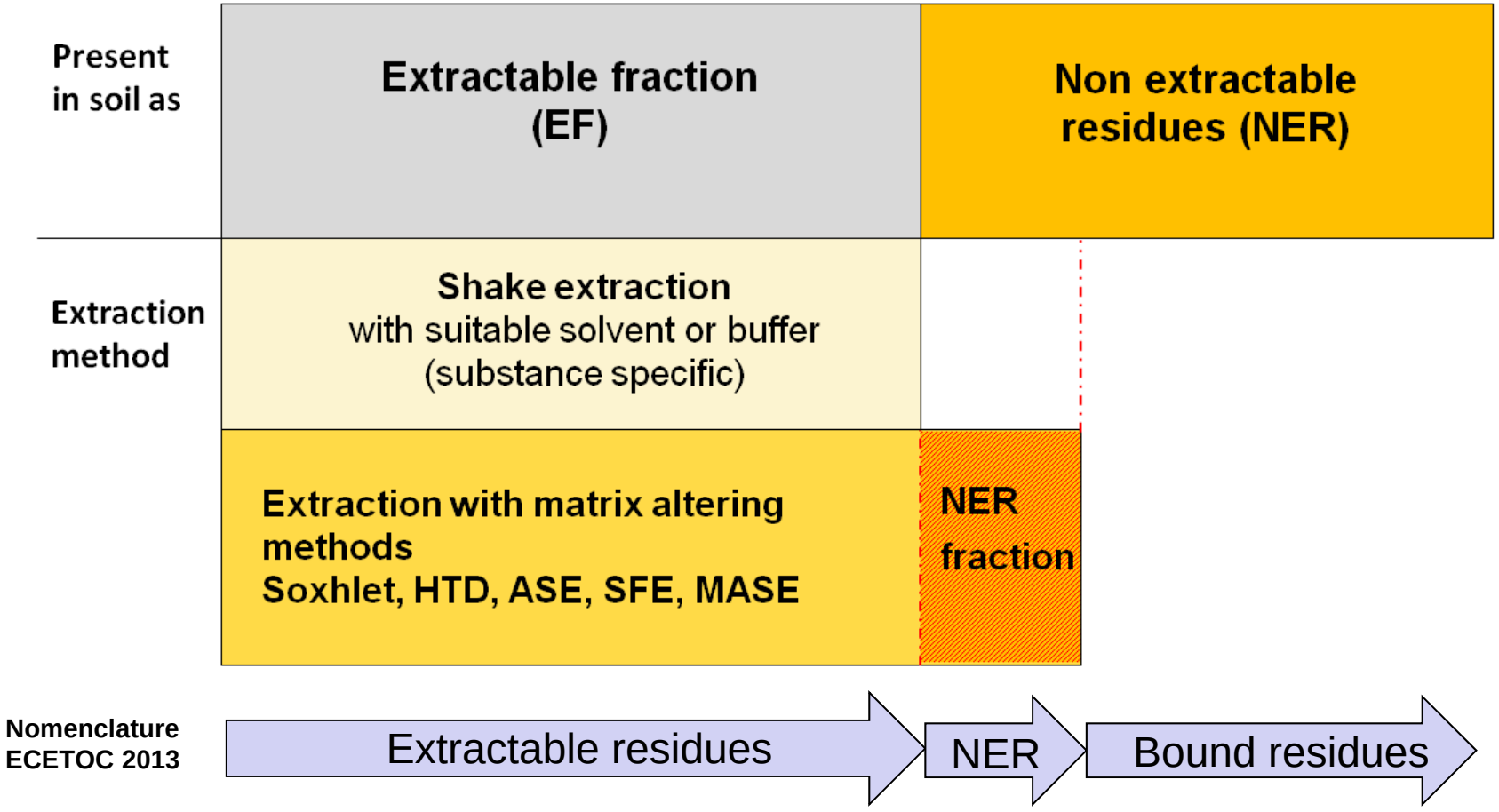
Commonly used extraction methods to quantify extractable fraction & NER

- 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)

Cold Shake:	**Sulfamethoxazole**	> 70 %	*ECETOC TR. No 118*
Soxhlet:	**Sulfadiazin**	84 -88 %	*Junge et al., 2011*
ASE:	**Diflocaxin**	74 % 60 - 65 %	*Junge et al., 2012* *Rosendahl et al., 2012*
	Ibuprofen	30 %	*Girardi 2011*
	Ciprofloxacin	88 %	*Girardi 2011*
MASE:	**Sulfadiazin**	> 45 % 20-30 %	*Förster et al., 2009* *Müller et al., 2013*

Quantity of NER: Methods to seperate extractable fractions and non extractable residues



Modified after:
Eschenbach & Oing , 2013

Quality of NER:
Published methods to characterize NER

Extraction with matrix altering methods

Solvent extraction with elevated temperature, pressure or energy input

Soxhlet, ASE, SFE, MASE, HTD

Extraction via destabilization of SOM and SEC

e.g. Chelating agents

Silylation and SEC

Seq. chemical degradation methods

Immunoassay

Extraction biomolecules

- Fatty acid extraction
- Amino acid extraction
- Amino sugar extraction

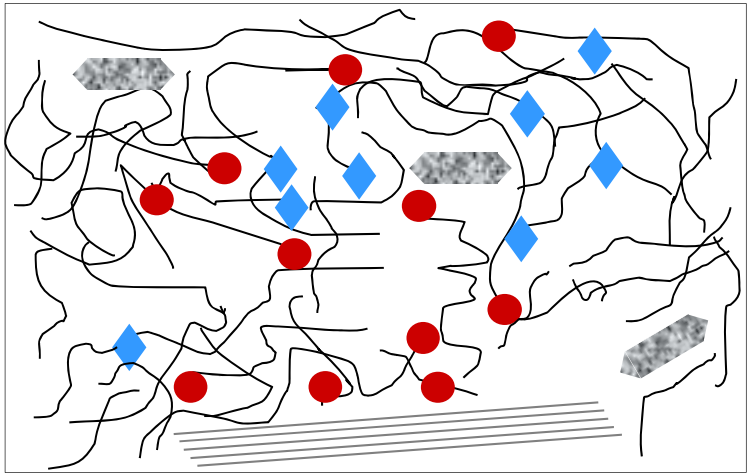
Biomass determination

- Fumigation methods

Physical entrapment

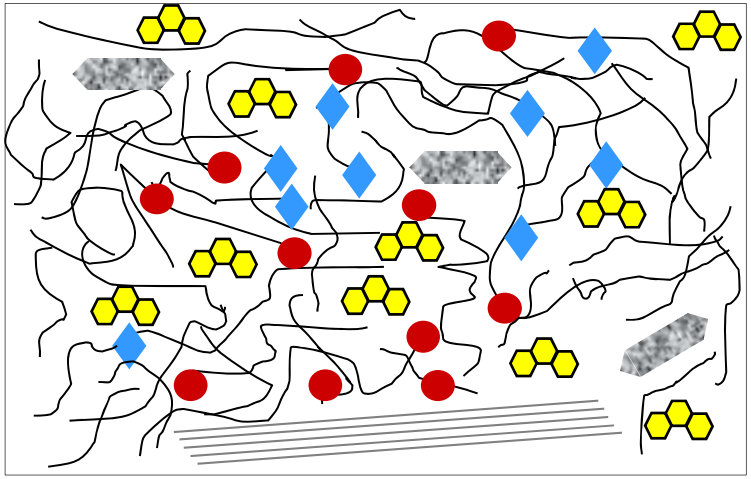
Stabilization of soil organic matter via:

- Polyvalent cations
- Hydrogen bonds
- Organic metal-complexes



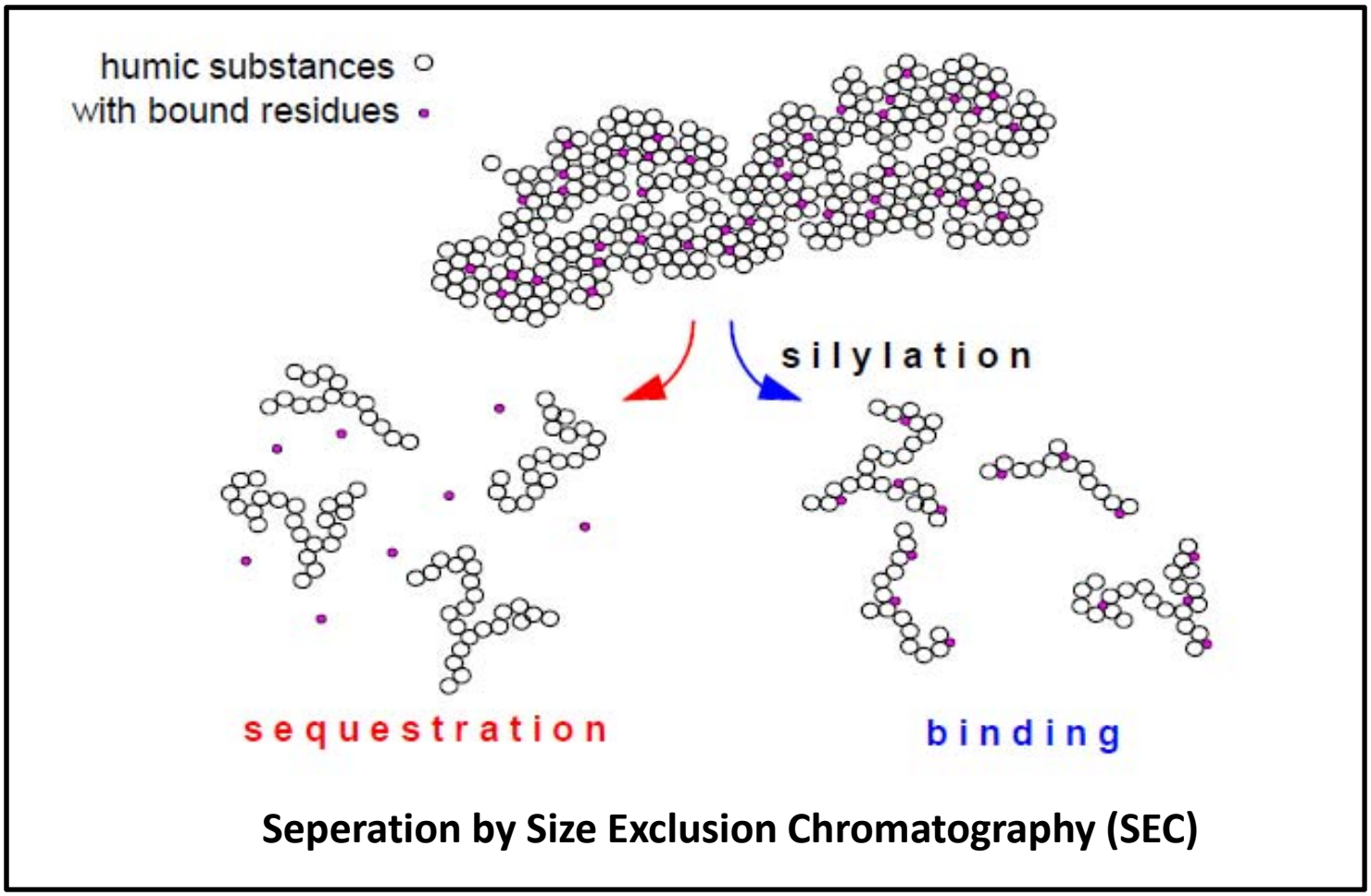
Formation of hydrophobic cavities with the possibility to entrap pollutants

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



Eschenbach et al.

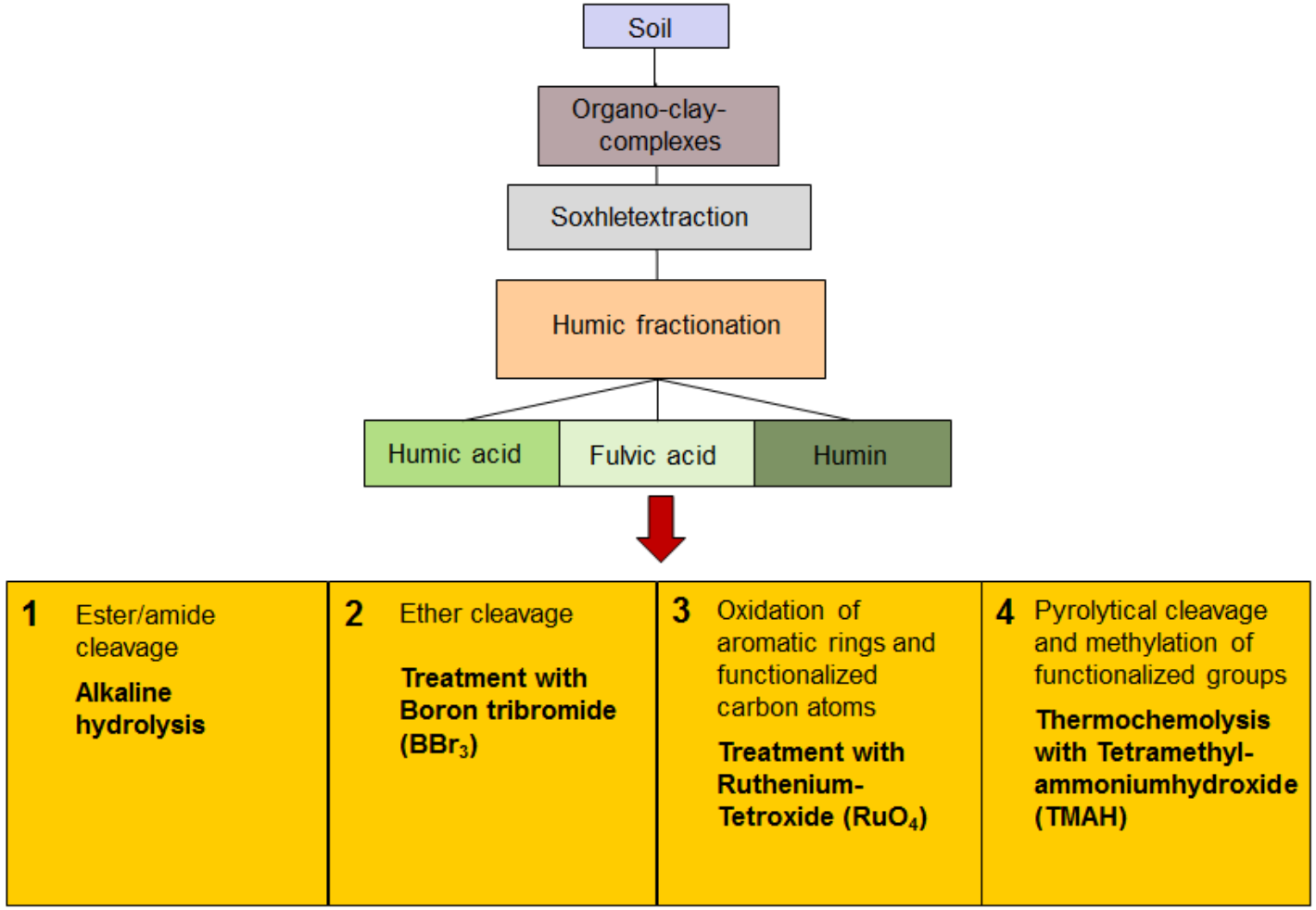
Differentiation of NER-types via silylation



Seperation by Size Exclusion Chromatography (SEC)

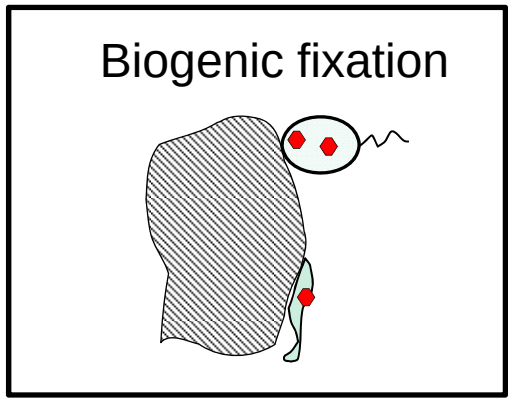
Modified after: Schäffer et al. 2010

Sequential chemical degradation



Modified after: Riefer et al. 2011

Determination of biogenic residues



Fatty-Acid-Extraction

(Miltner et al. 2004, Nowak et al. 2011)

- **PLFA**
- **tFA**

Fumigation-methods (CFE, CFI)

(Soulas et al. 1984, Ghani et al. 1996, etc.)

Peptidehydrolysis

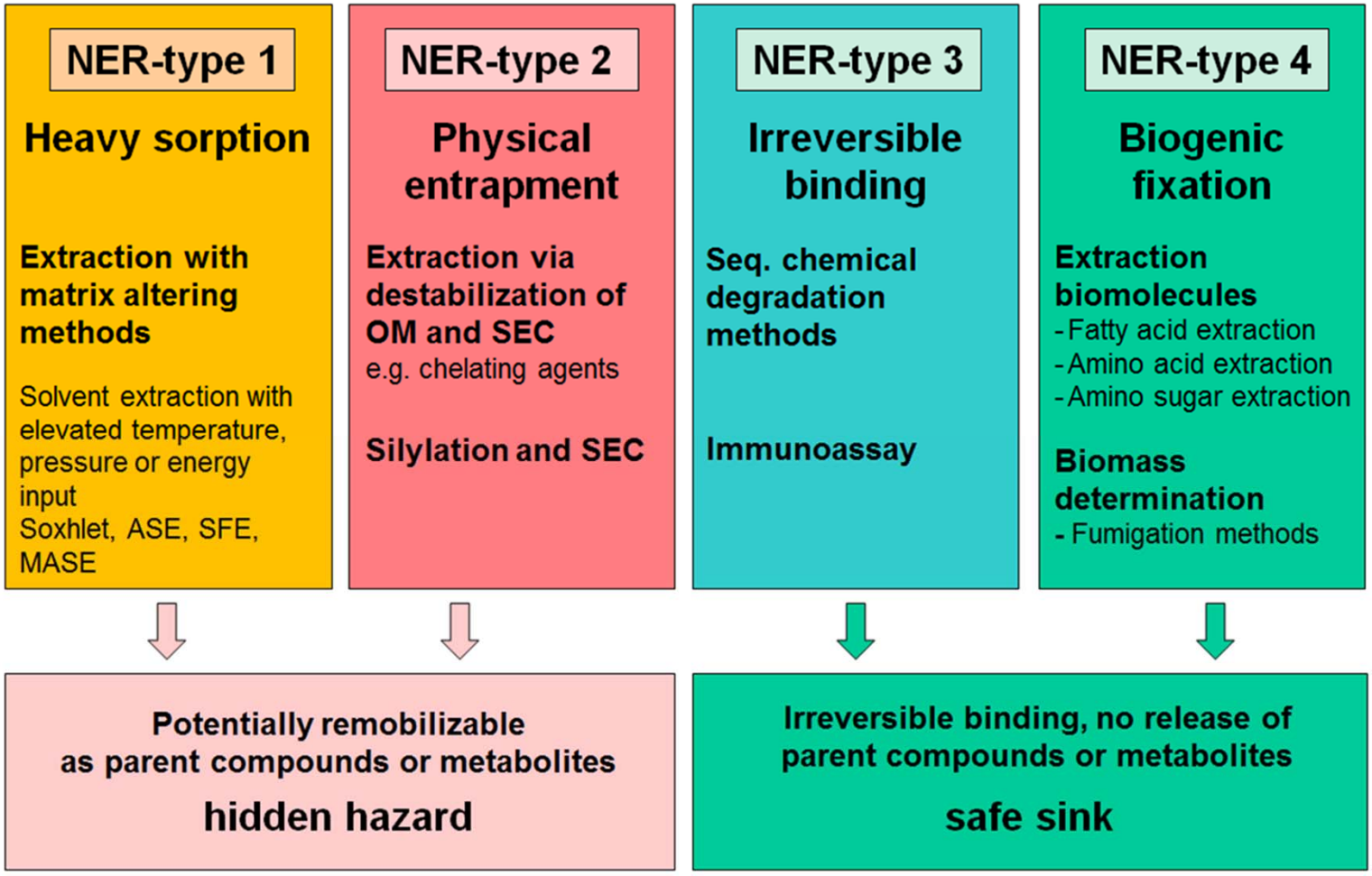
(Nowak et al. 2011)

- **bioAA**
- **tAA**

Ammino-Sugar-Hydrolysis

(Miltner et al. 2004)

Advanced NER-type model



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Results: Substance specific evaluation of extraction methods

NER-type 1

Heavy sorption

Extraction with matrix altering methods

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

– **widely-used**

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Results: Substance specific evaluation of extraction methods

NER-type 2 **Physical entrapment**	
Agents to destabilise OM and SEC e.g. chelating agents – **PAH** – **TNT**	**Silylation and SEC** – **Simazine** – **Anilazine** – **Imazalil** – **Sulfonamide**

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Results: Substance specific evaluation of extraction methods

NER-type 3

Irreversible binding

Sequential chemical degradation method

- **MCPA**
- **Nonylphenole**

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Results: Substance specific evaluation of extraction methods

NER-type 4

Biogenic fixation

Extraction biomolecules	Biomass determination

– Fatty acid extraction
  – 2,4-D
  – Sulfadiazine
  – Ibuprofen
– Amino acid extraction
  – 2,4-D
  – Sulfadiazine
  – Ibuprofen
  – Glyphosat
  – Simazine
– Amino sugar extraction
	– Fumigation-extraction methods
	  – 2,4-D
	  – Sulfadiazine

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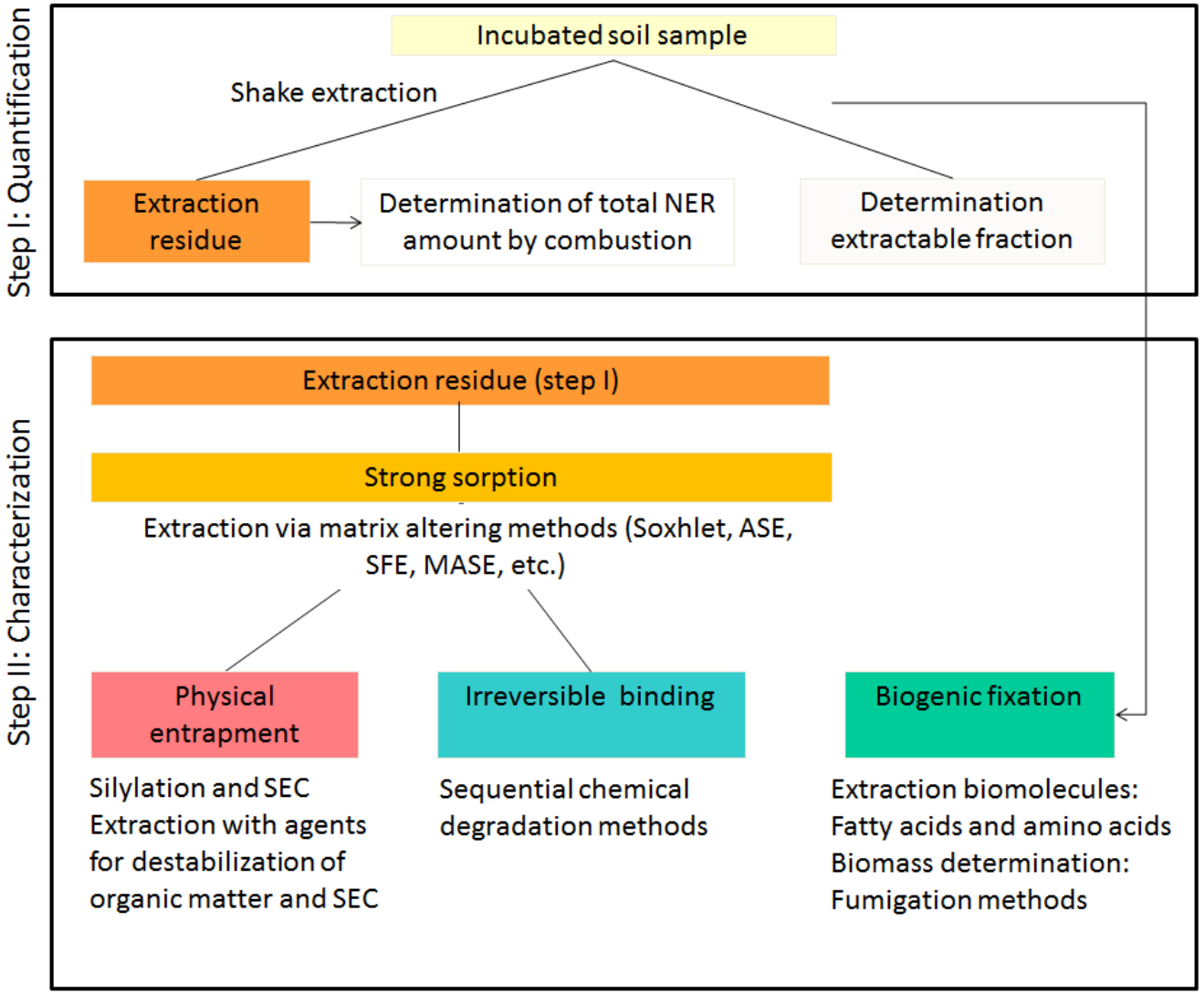
Principle scheme of the preliminary sequential extraction procedure



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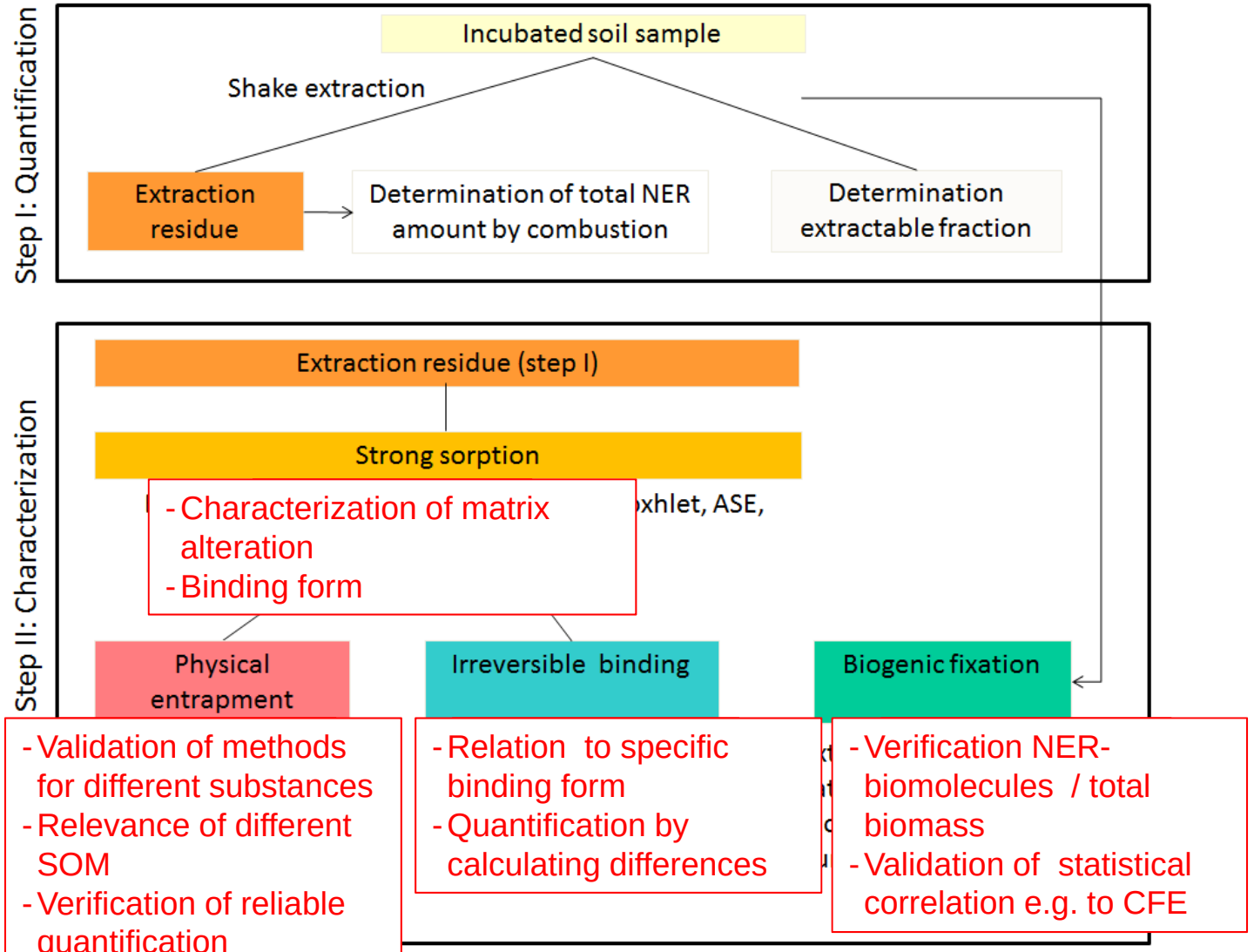

Summary

- 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 reserach

- ➔ 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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