Science For A Better Life

Revealing the nature of bound residues in soil remains a challenge

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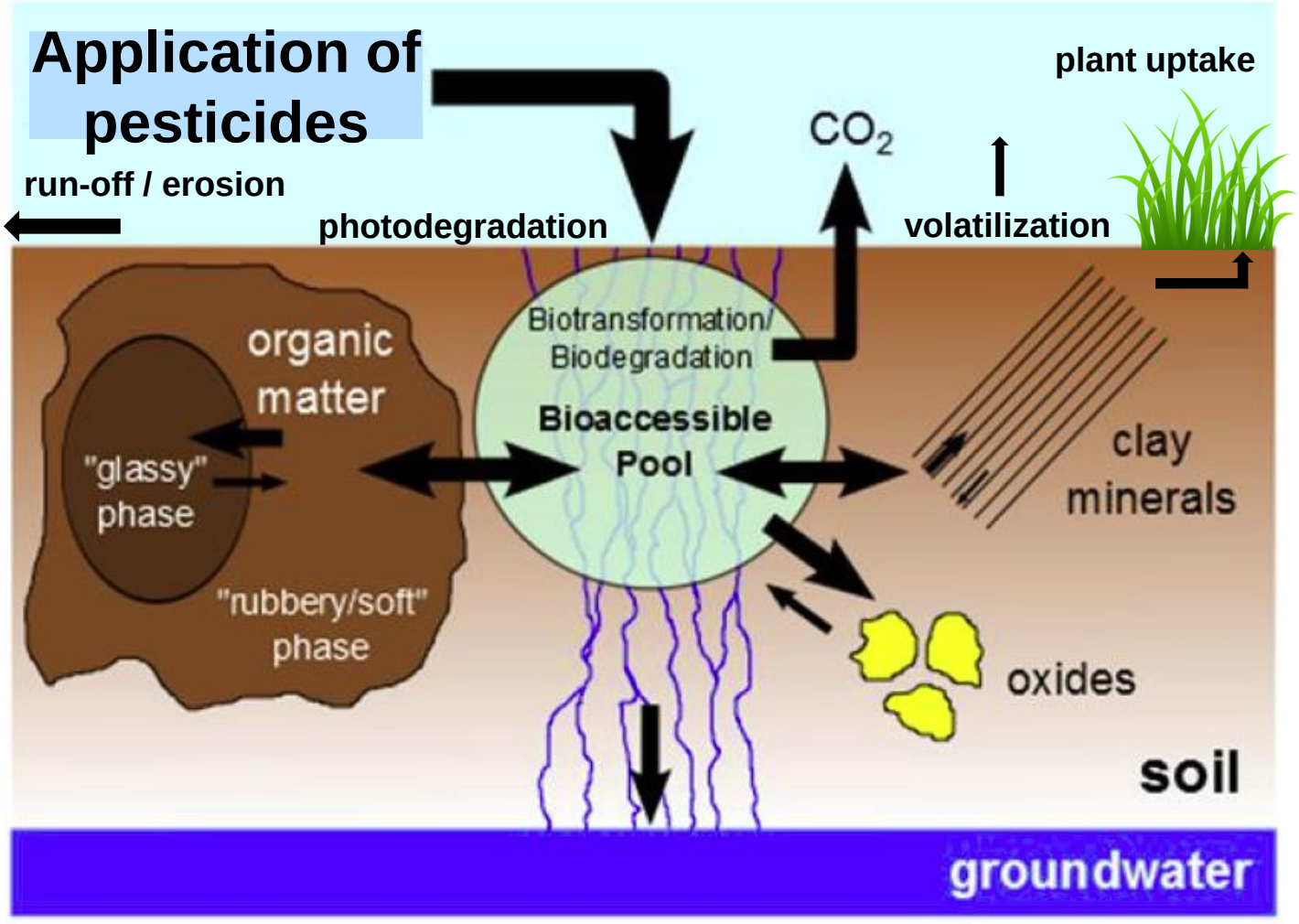
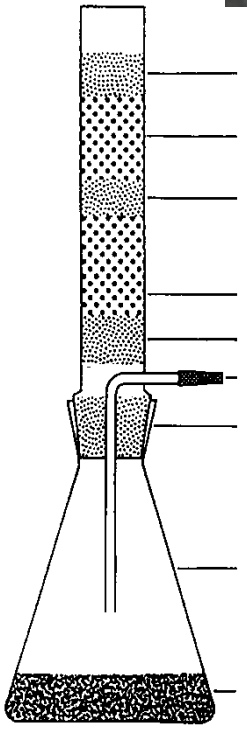
Fate of pesticides in soil



Aerobic soil metabolism study (OECD 307)



Quartz glass wool

Soda lime for adsorption of CO_2 from atmosphere

Quartz glass wool

Soda lime for adsorption of released CO_2

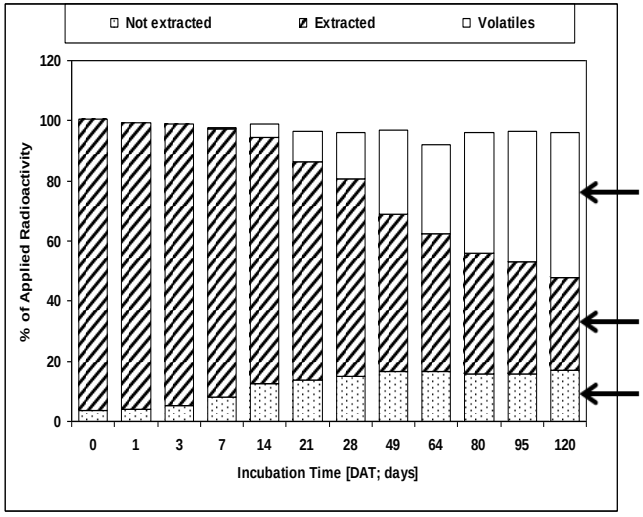
Quartz glass wool

Inlet tube (sealed during incubation)

Polyurethane foam for absorption of volatile organic compounds

Erlenmeyer flask, 300 mL

Soil (usually 100 g dry weight)

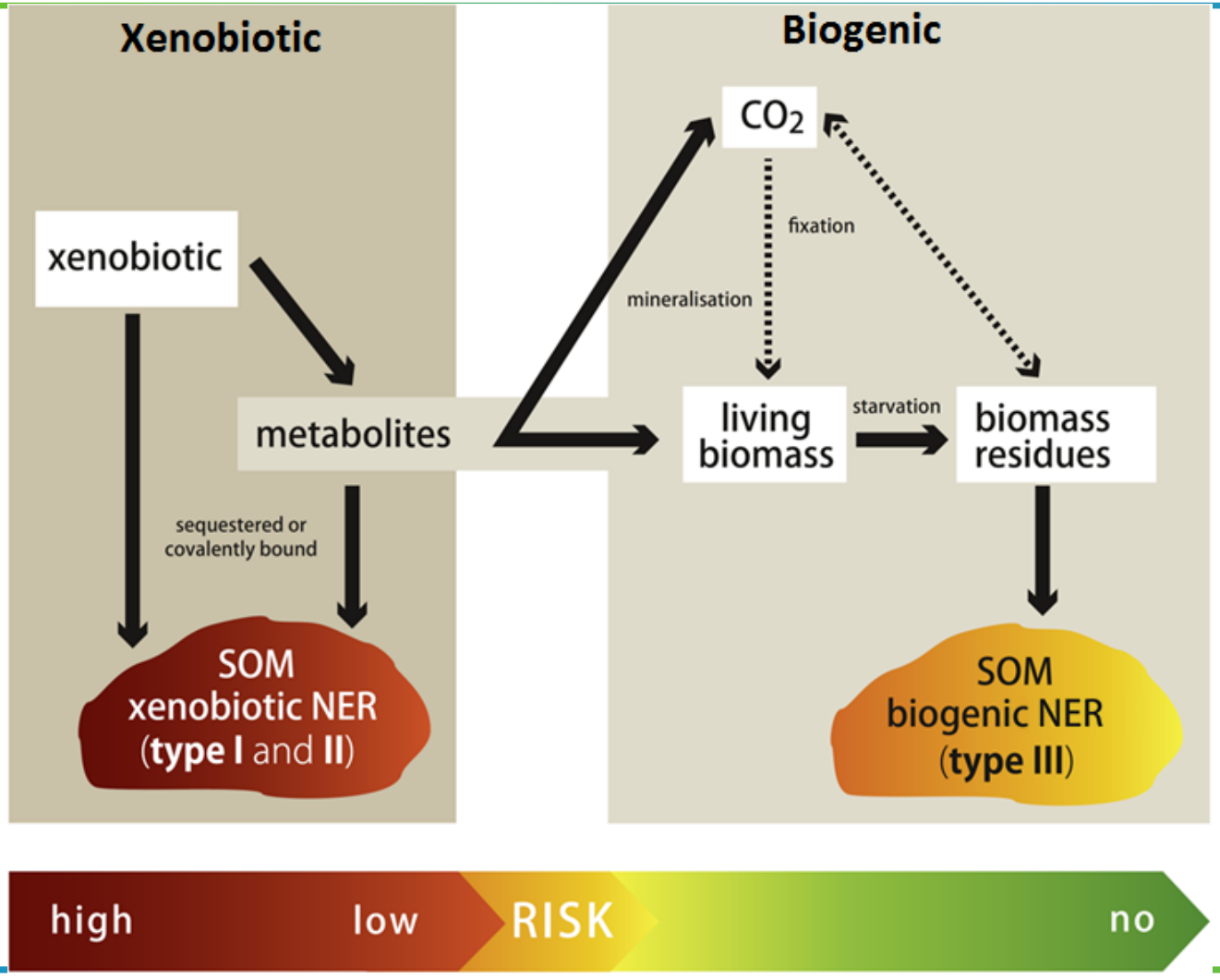


Example soil extraction scheme

Extraction without changing the nature of the compounds,
No acids or bases used

Solvent	Duration	Temperature
acetonitrile / water (4:1)	3 x 10 min	room temperature
acetonitrile / water (1:1)	1 x 10 min	70°C

Classification of non-extractable residues

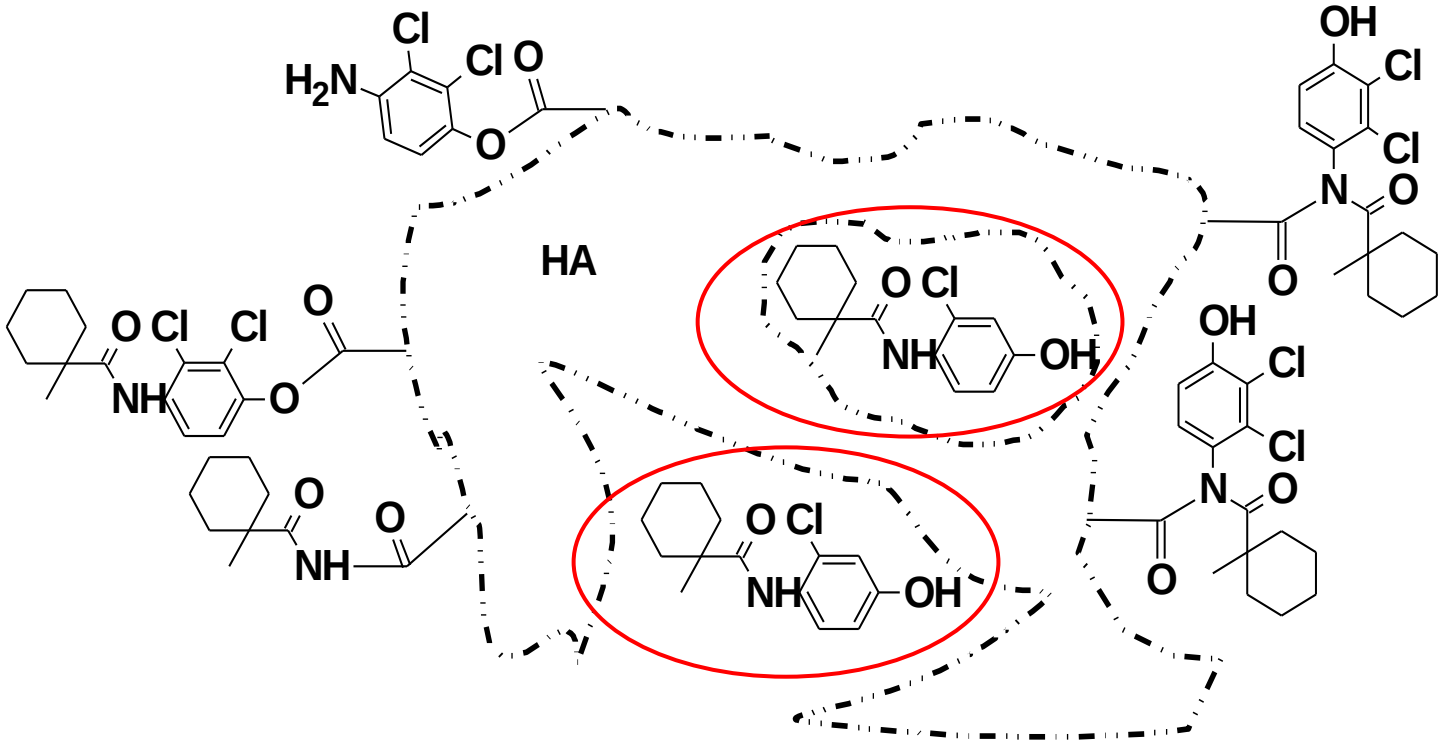


Kästner et al., Schaeffer et al 2014, Critical Reviews in ES&T, Vol 44, Iss 19

Classification of non-extractable residues

NER type I

- Entrapped
- Strongly adsorbed
- No covalent binding



Classification of non-extractable residues

➢ Covalent bound not extractable residues

NER type II

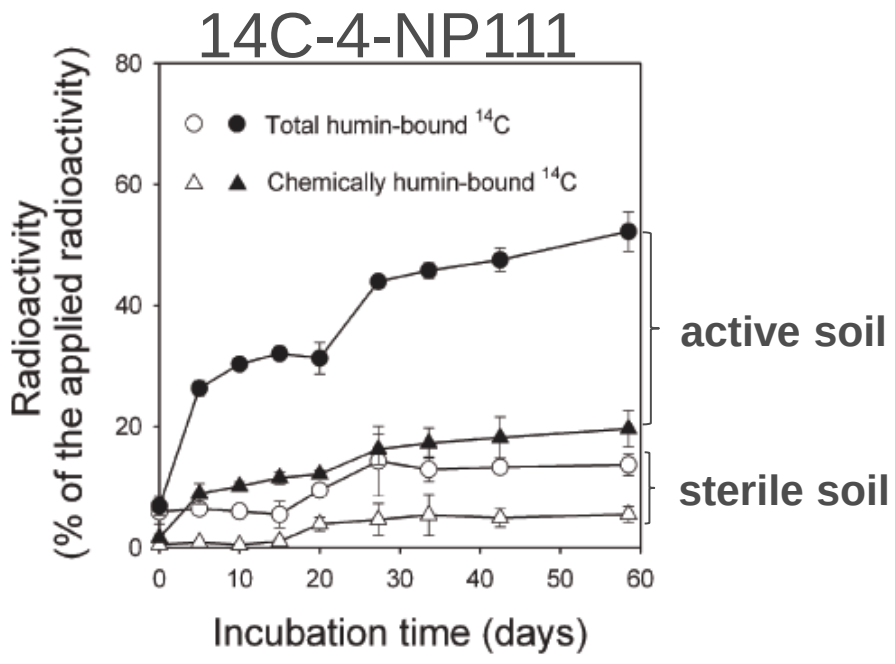


Figure 5. Formation courses of total and chemically humin-bound residues of ^{14}C-4-NP$_{111}$ during incubation in the active (closed symbols) and sterilized (open symbols) rice paddy soils under oxic conditions. Values are means with standard deviations of three replicates.

Shan (2011) differentiated between

1. total humin bound residues

After silylation all entrapped molecules would have been liberated

2. chemically humin bound residues

After silylation residues still remain bound

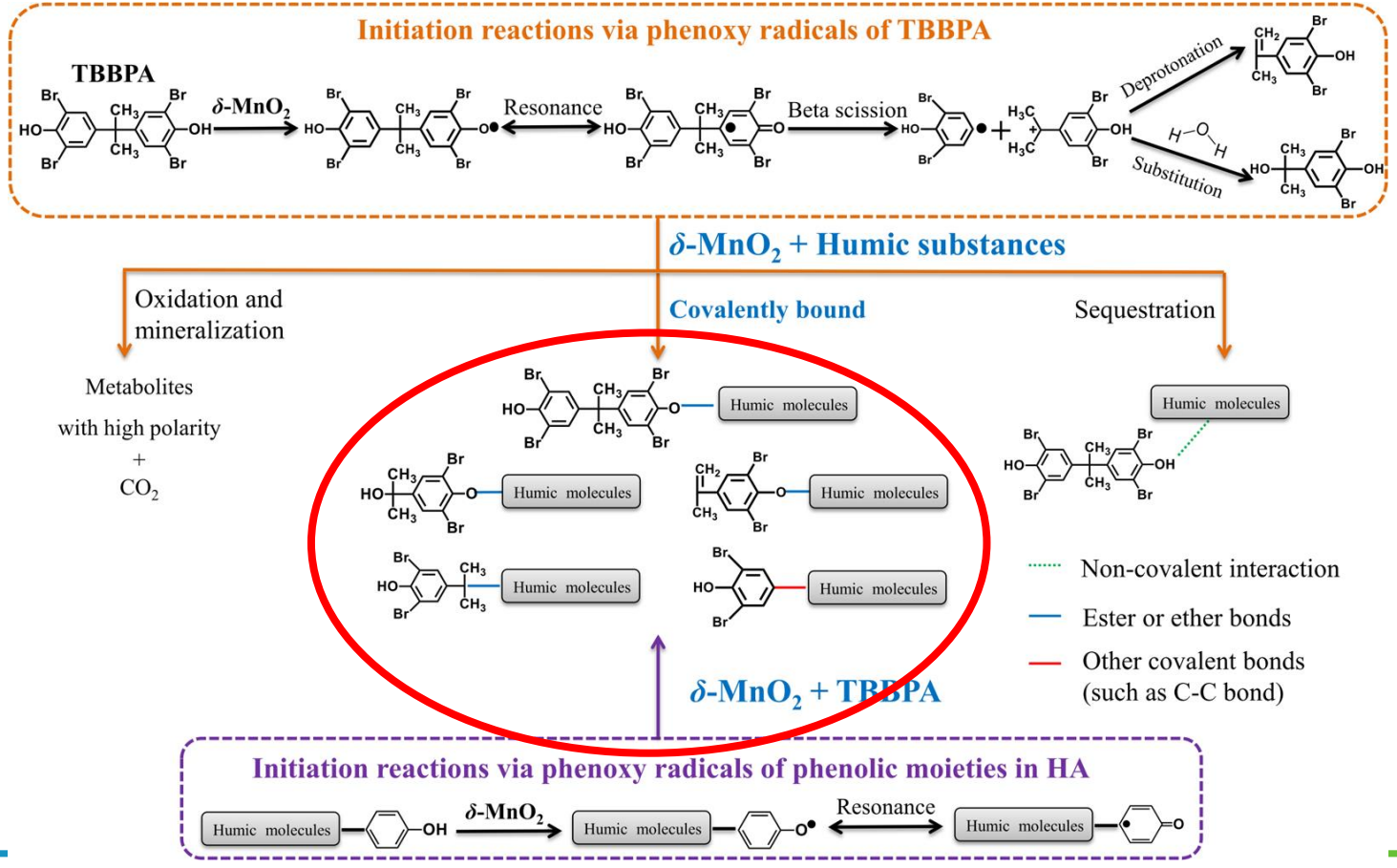
Shan et al., Environ. Sci. Technol., 2011, 45 (19), pp 8283–8289

Classification of non-extractable residues

➢ Covalently bound not extractable residues

NER type II

Tong et al. (ES&T 2016) proposed binding to humic molecules

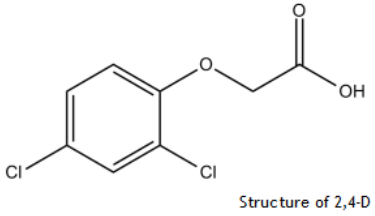


Tong et al., Environ. Sci. Technol., 2016, 50 (12), pp 6257–6266

Classification of non-extractable residues

NER type III

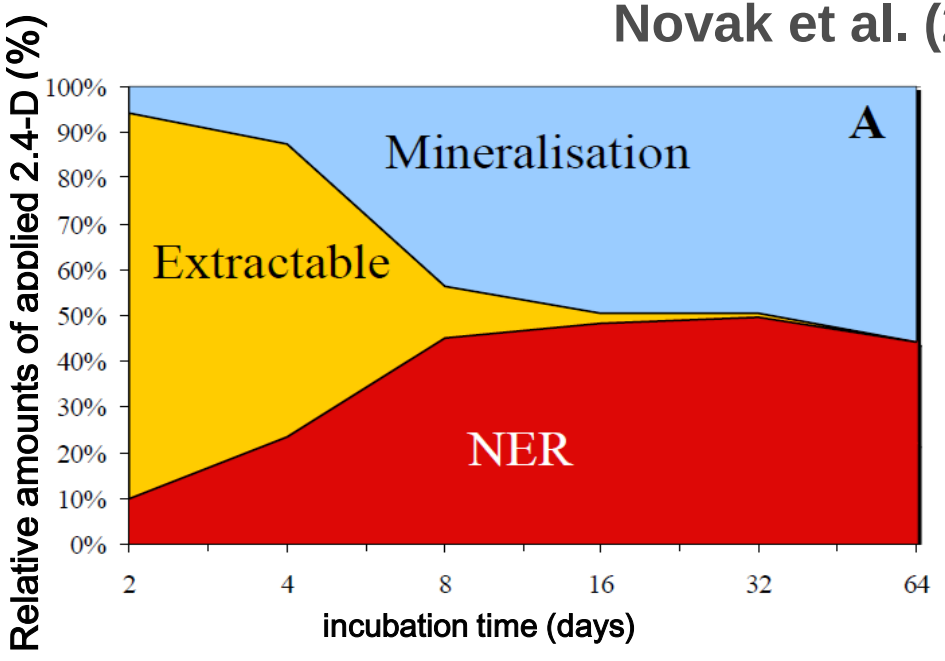
- Biogenic bound residues; fixation of CO2 (and other C2 building blocks into amino acids and fatty acids)

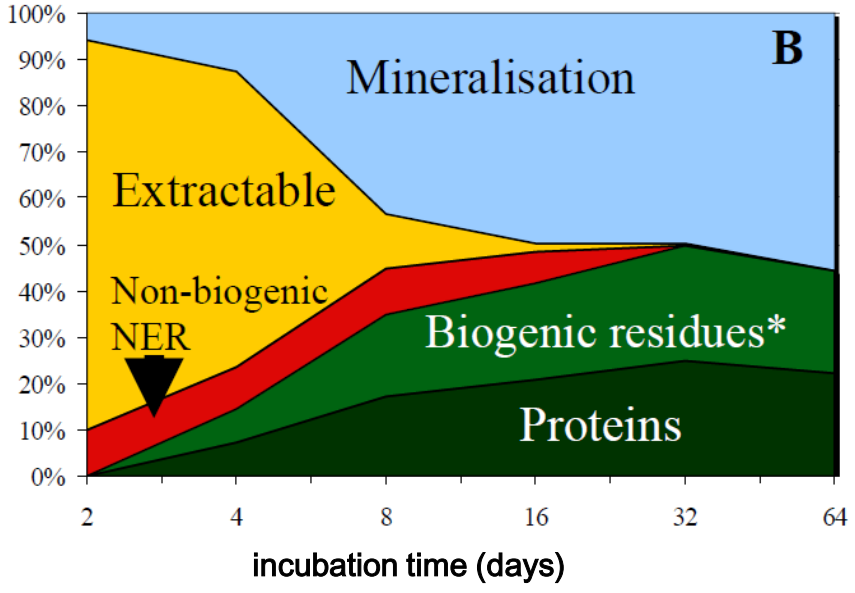


Structure of 2,4-D

→ **CO2** → **Biogenic bound residues**

Novak et al. (2011)





Novak et al., Environ. Sci. Technol., 2011, 45 (3), pp 999–1006

Three types of NER

NER type I

- Entrapped
- Strongly adsorbed

Xenobiotic residues may be still intact and could be slowly released, likely low levels, perhaps already covered by risk assessment?

NER type II

- Covalently bound residues

Limited risk, further risk evaluation unlikely to be necessary

NER type III

- Fixation in biogenic molecules like fatty acids, amino acids, etc.

Loss of active moiety, no risk at all, no risk assessment necessary

Quantitative determination of biogenic NER with [phenyl-UL-^{14}C]-bromoxynil (Poßberg et al., 2016)

Exhaustive (Soxhlet) extracted soil

↓

Hydrolysis with 6M HCl for 22 h at 110°C

Hydrolyzate

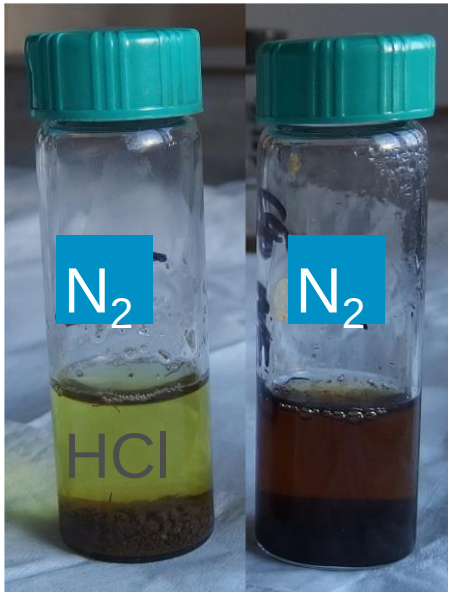
↓

Clean-up with Cation Exchange Resin (Dowex)

AA-Eluate

↓

MeOH/NH_4 acetyl solution

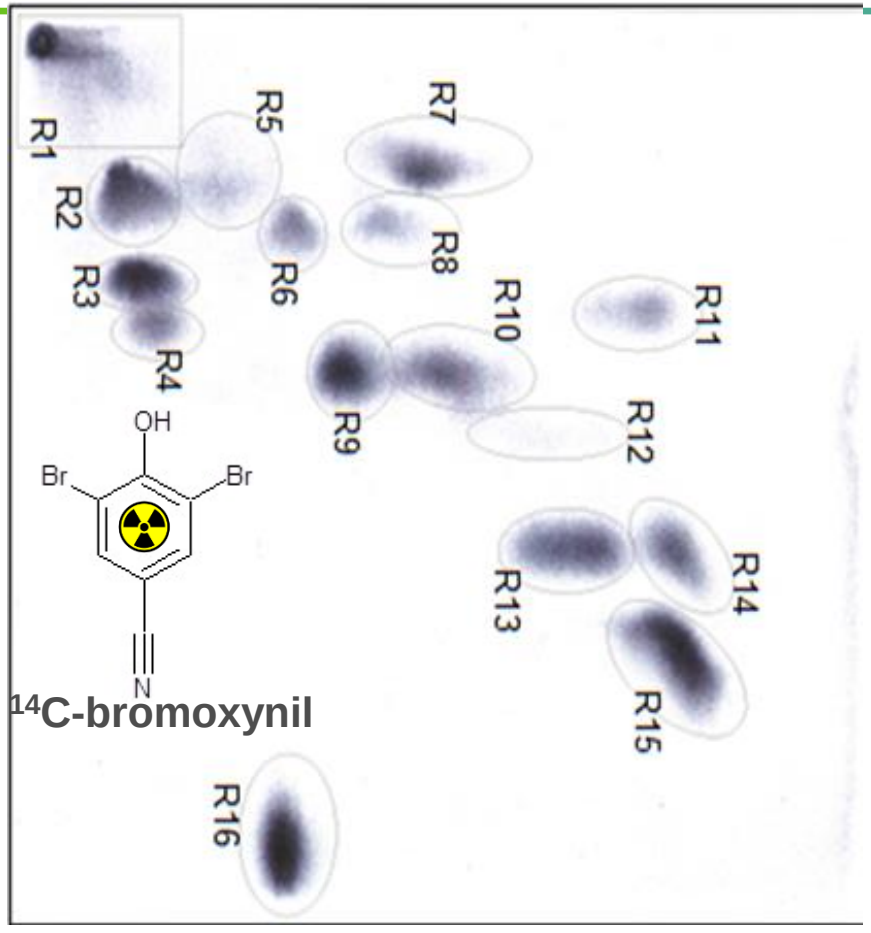


Before and after hydrolysis

2D-TLC (separation) & GC-MS/LC-MS/MS (identification)

Poßberg et al., Environ. Sci. Technol., 2016, 50 (12), pp 6415–6422

2D-TLC of amino acid eluates from Soxhlet extracted soil incubated with ^{14}C-bromoxynil for 56 days (Poßberg et al., 2016)

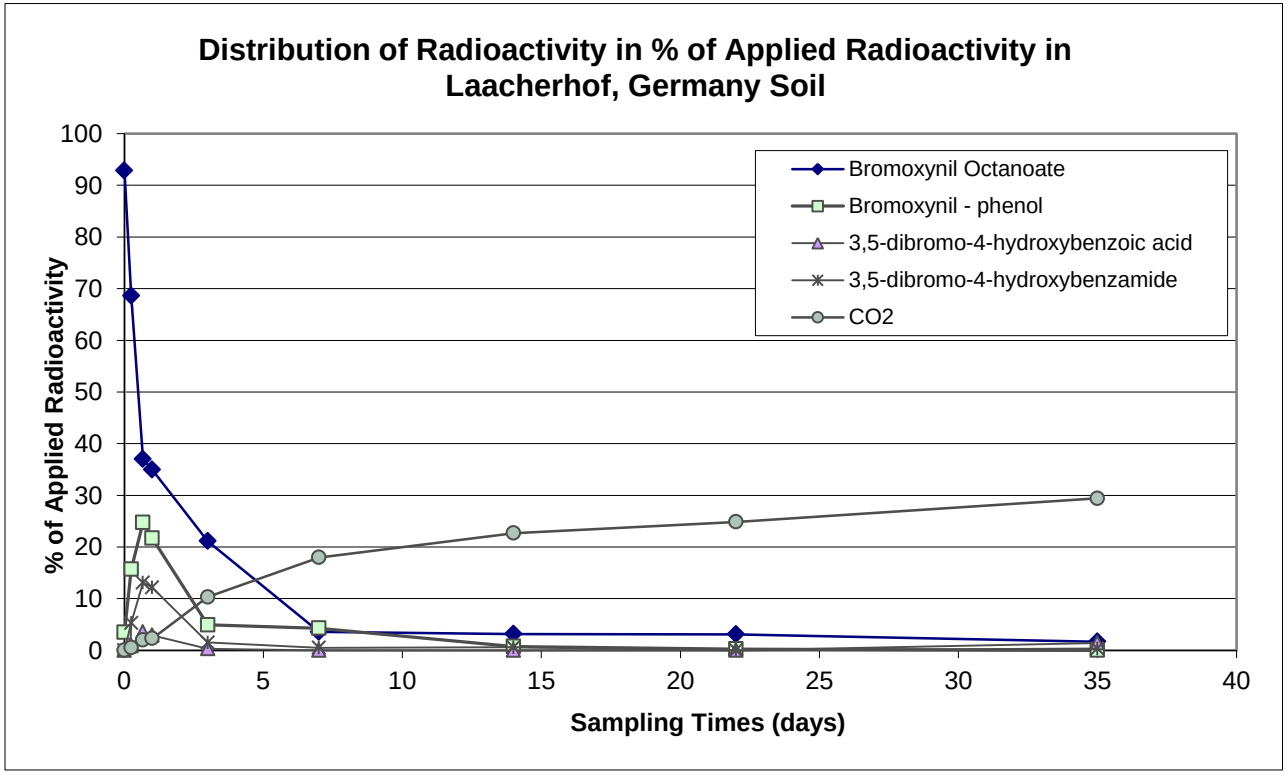


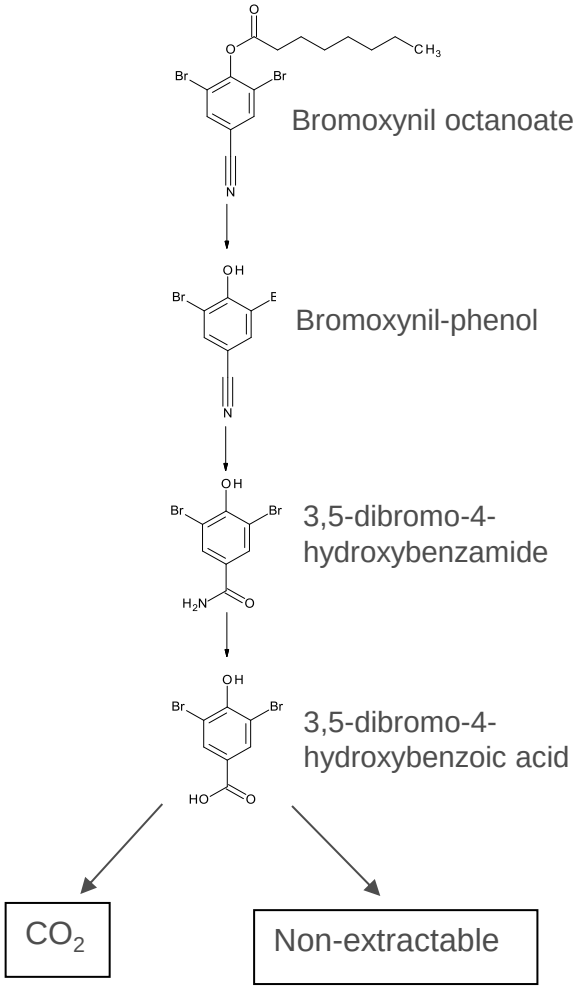
Spot	Substance
R1	Start
R2	Aspartic acid
R3	Glutamic acid
R4	glutamic acid and unknown compound
R5	Unknown compound
R6	Glycine
R7	Unknown compound
R8	Serine
R9	Alanine
R10	Proline
R11	Threonine
R12	Tyrosine
R13	Valine
R14	Phenylalanine
R15	Leucine and isoleucine
R16	Bromoxynil byproduct

Good separation of amino acids some activity at start
Sum of amino acids: 3.1 % of applied radioactivity (7.3% of NER)

Poßberg et al., Environ. Sci. Technol., 2016, 50 (12), pp 6415–6422

Degradation of ^{14}C-bromoxynil-octanoate in sandy loam





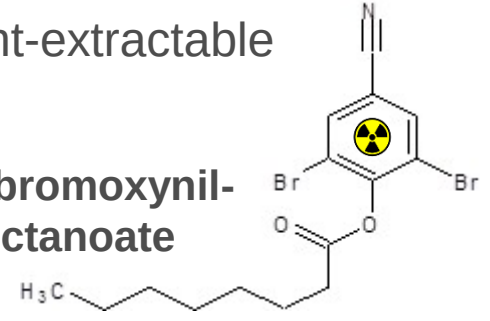
Bromoxynil-octanoate, as well as its metabolites bromoxynil-phenol, 3,5-dibromo-4-hydroxybenzamide and 3,5-dibromo-4-hydroxybenzoic acid degrade rapidly (DT50 < 1 day)

Further investigations close to method developed by Poßberg et al. (2016)

- Study conducted with [phenyl-UL-^{14}C]-bromoxynil-octanoate instead of [phenyl-UL-^{14}C]-bromoxynil
  - Application: 70.4 KBq/g soil, 22.7 µg/g of bromoxynil-octanoate (specific radioactivity 3.10 MBq/mg)
  - Incubation: 100 g of soil for 30 and 60 days, 55% of WHC_{max}
  - Extraction: 3 times cold with ACN:H_2O, 80:20, v:v

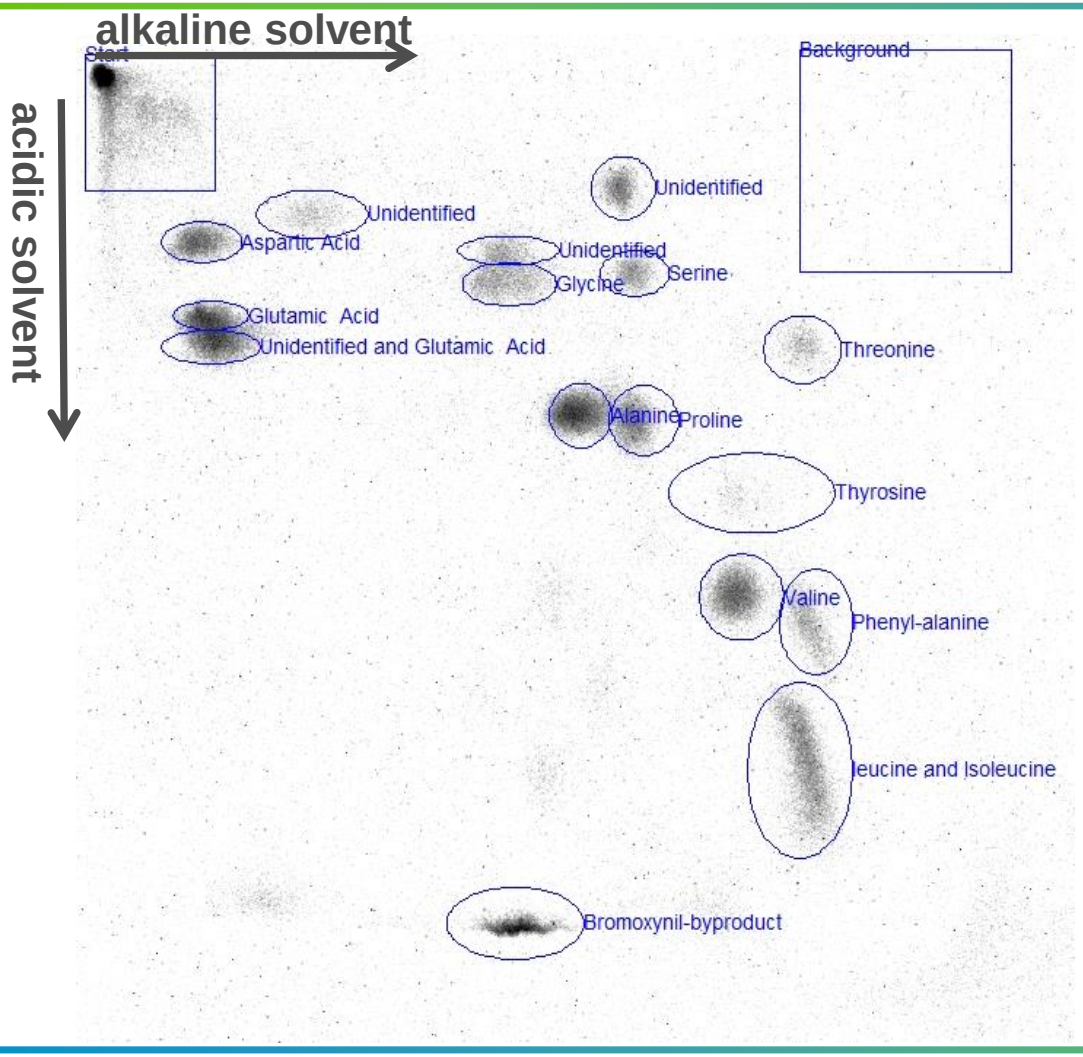
    1 time hot with ACN:H_2O, 80:20, v:v (microwave 70°C)
  - Amino acid hydrolysis: 1 g of soil after exhaustive extraction of the soil
  - ^{14}C-mass balance: determination of $^{14}CO_2$ formation, solvent-extractable residues, total NER and bio-NER

^{14}C-bromoxynil-octanoate



Poßberg et al., Environ. Sci. Technol., 2016, 50 (12), pp 6415–6422

2D-TLC of amino acids after 30 days (after Dowex purification and after evaporation & taking up in alkaline NH_3 solution)



Good separation of amino acids
Some activity at start

Sum of amino acids:
4.7% of applied radioactivity (15.9% of NER)

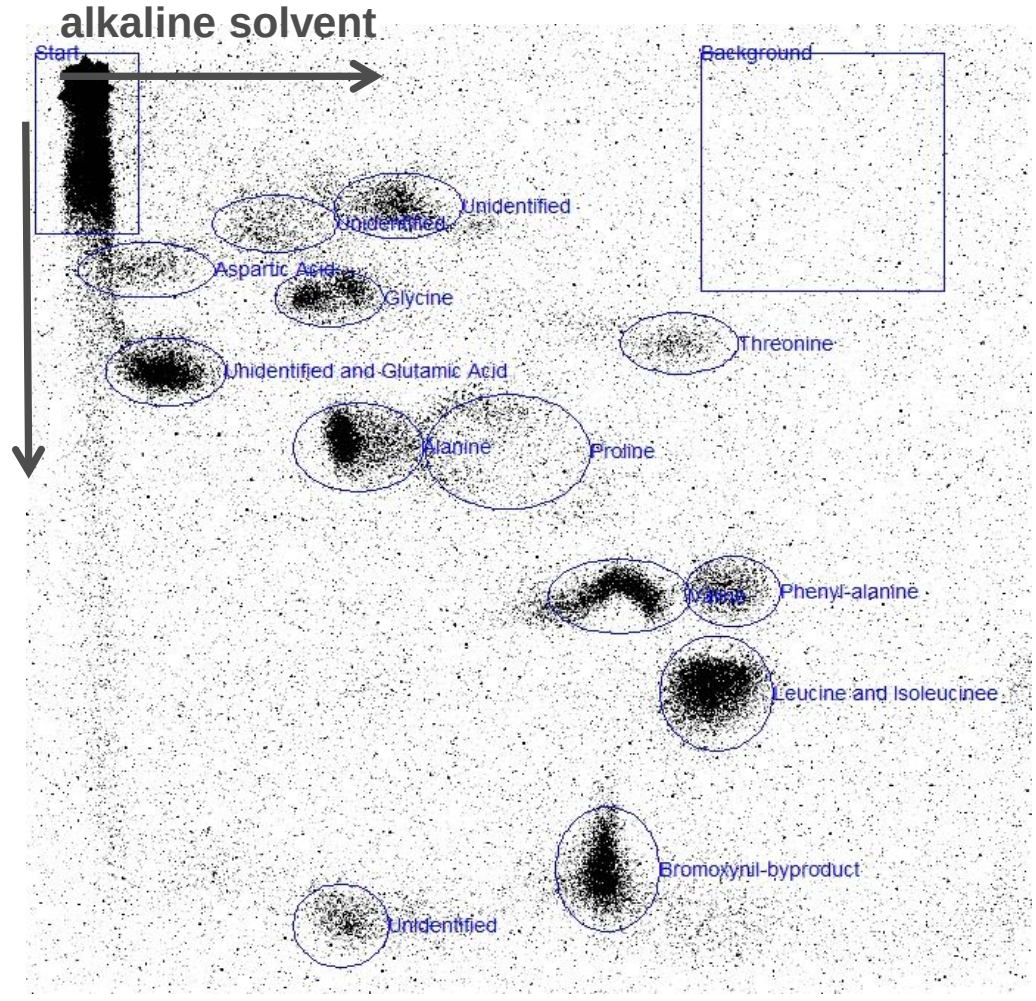
52.3% of applied radioactivity are CO_2

29.3% of applied radioactivity are NER

8.5% of applied radioactivity are extractable

1.) alkaline development
(2 x times with n-butanol-aceton / 25% NH3 aqua)
2.) acidic development isopropanol/formic acid

2D-TLC of amino acids after 30 days (without Dowex purification; 6 m HCl extract after centrifugation directly spotted on TLC plate)



Good separation of amino acids
Some activity at start

Sum of amino acids:
4.7% of applied radioactivity (16.0% of NER)

52.3% of applied radioactivity are CO_2

29.3% of applied radioactivity are NER

8.5% of applied radioactivity are extractable

1.) alkaline development
(2 x times with n-butanol-aceton /25% NH3 aqua)
2.) acidic development isopropanol/formic acid

Summary and conclusions

- In investigations into metabolism of xenobiotics in soil extraction without changing the nature of the compounds present is necessary to ensure valid results.
- As degradation proceeds incorporation into the soil matrix increases (time dependent sorption) and therefore harsher extraction methods are needed.
- As the xenobiotic degrades its atoms become re-used, e.g. uptake of CO_2 and incorporation into microorganisms.
- Non extractable residues can be characterised into three types:
  - Entrapped and/or strongly adsorbed
  - Covalently bound
  - Fixation into natural biogenic compounds
- All chemicals are different

Summary and conclusions

- Bromoxynil has been shown to be mineralised to carbon dioxide and then re-incorporated into natural chemicals.
- For the remaining non-extractable residues even with soil destroying methods like the digestion with 6 molar HCL at 110°C, the main portion of bound residues will remain bound to the soil and therefore it can be concluded that this part is not bioavailable.
- The main part of the “liberated bound residues” of bromoxynil could be assigned to natural amino acids.
- Both results indicate that for bromoxynil bound residues will not contribute to future risks or hazards because they are on one hand not bioavailable or on the other hand they have been converted to natural biogenic residues, like natural amino acids
- Analyses of bound residues remains a challenge; whereas 8.5% of applied radioactivity was extractable; 29% of applied radiactivity remained not extractable of which 4.7% of AR was shown to be amino acids.

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Thank you!