The development of procedures to establish the toxicity of non-extractable residues (NER) in soil. ECO25

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LRI Long-Range Research Initiative

cefic

NER, Non Extractable Residue

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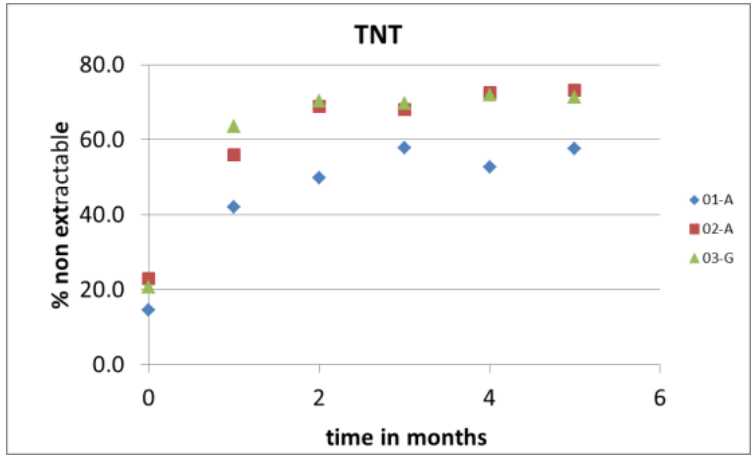
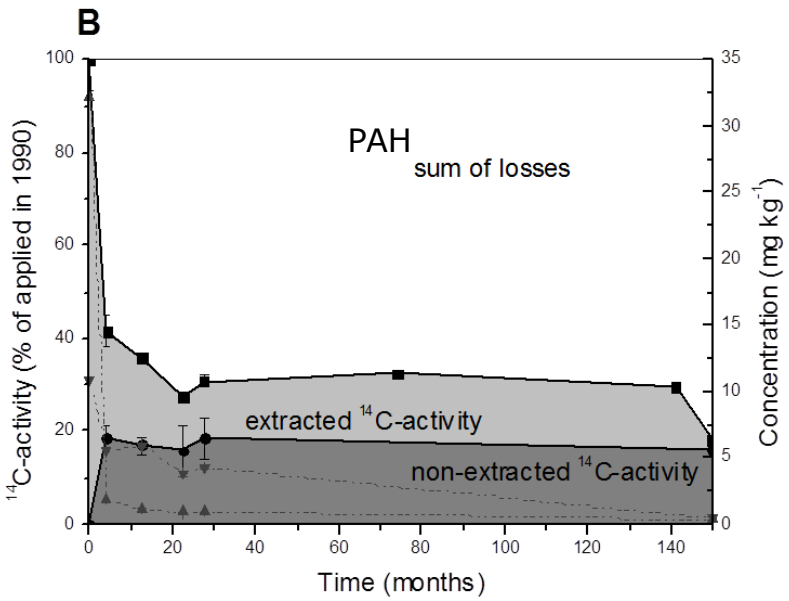
- Formation and presence of NER
  - Association of the parent chemical or breakdown product. Mineral (e.g. carbonates) and/or organic matter
  - incorporation of carbon into microbial or plant biomass
- NER can only be established using ^{14}C-radiolabelled/^{13}C compounds (e.g. mandatory in pesticide regulation)
- **Not measurable** in real life





NER, Assessment

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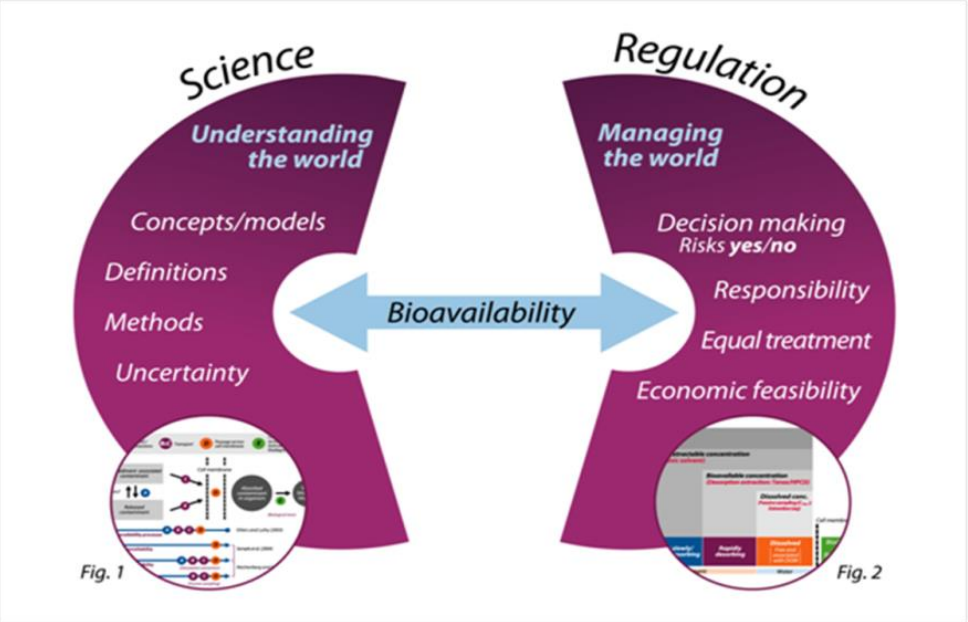
- Ecotoxicological relevance of Non Extractable Residues (NER) in soil
  - Is NER formation a **detoxification** process?
  - Or is it a **hidden hazard**?

- Understandable and measurable. Approach of Ortega-Calvo et al, 2015



NER, Chemical Measurements

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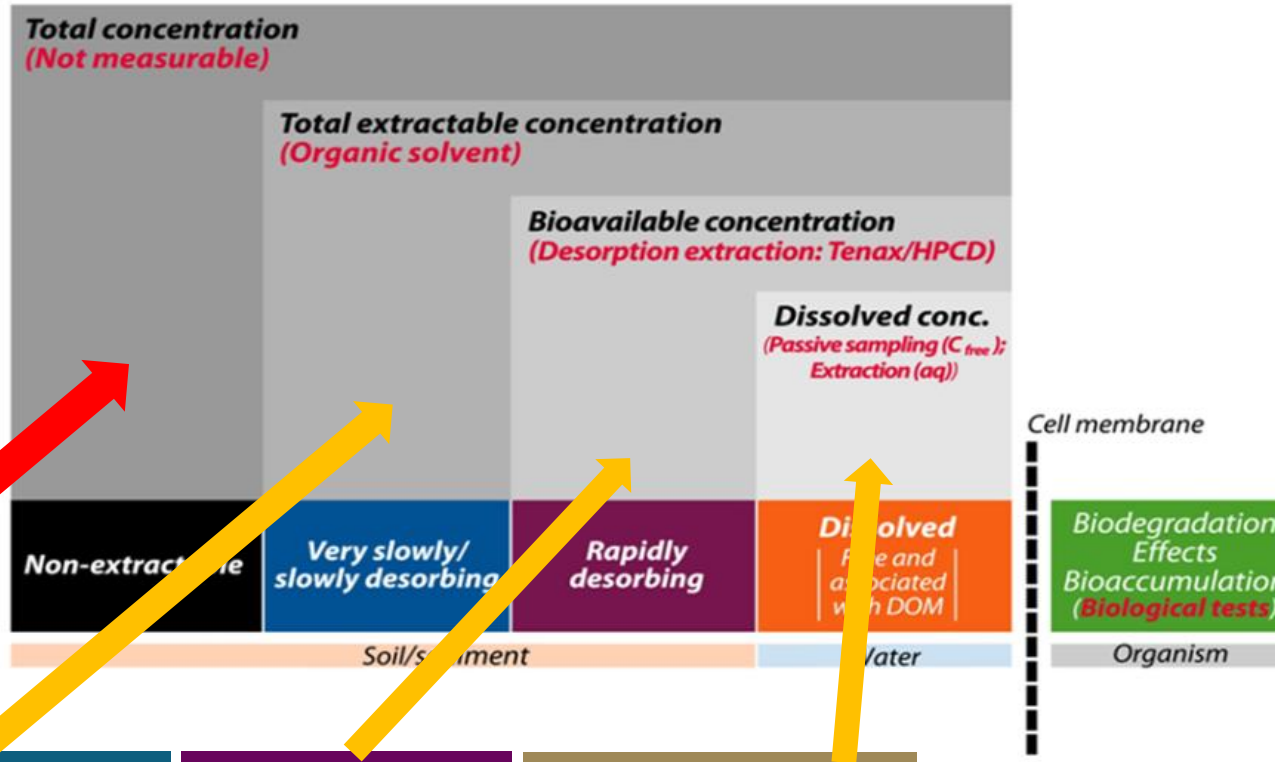

^{14}C-study

(%)

^{12}C = not measurable

Solvent (e.g. ASE)

$TENAX_{7days-1day}$=

Very slowly desorbing

(%; g/kg)

TOTAL

$TENAX_{1day}$=

Slowly desorbing

(%; g/kg)

Potentially bioavailable

$CaCl_2$-extract.

(g/l)

Actually bioavailable

NER, Ecotoxicological Methods

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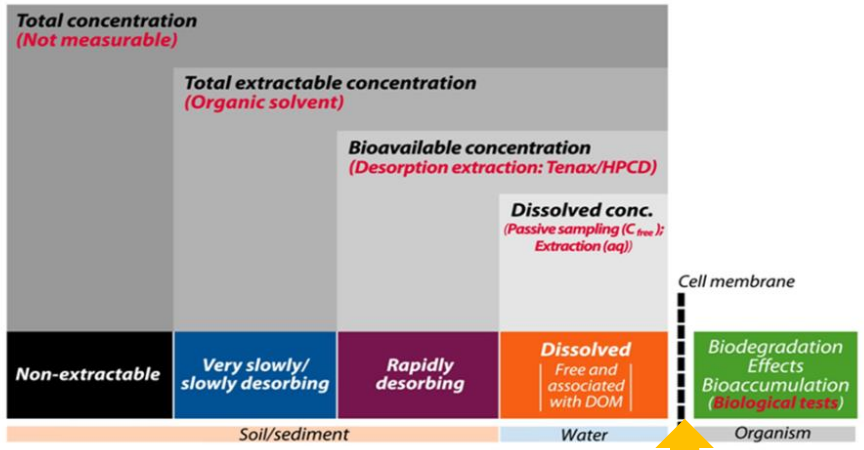
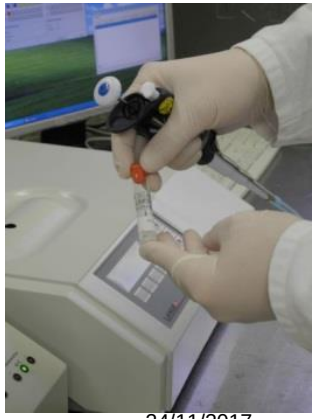
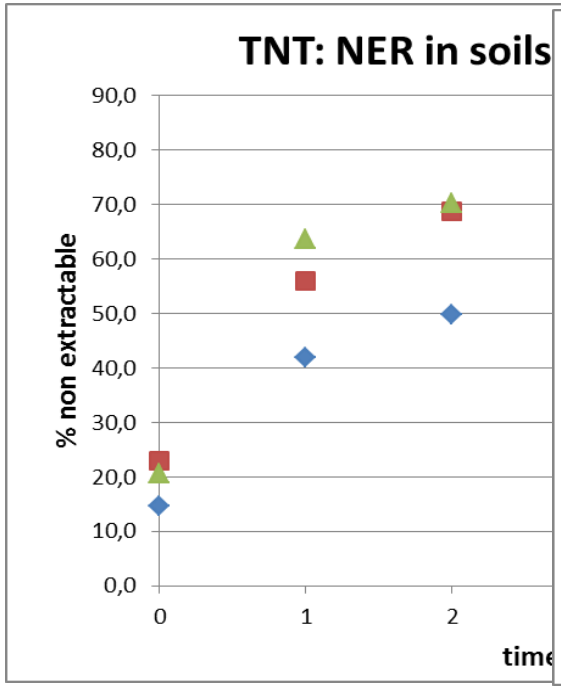
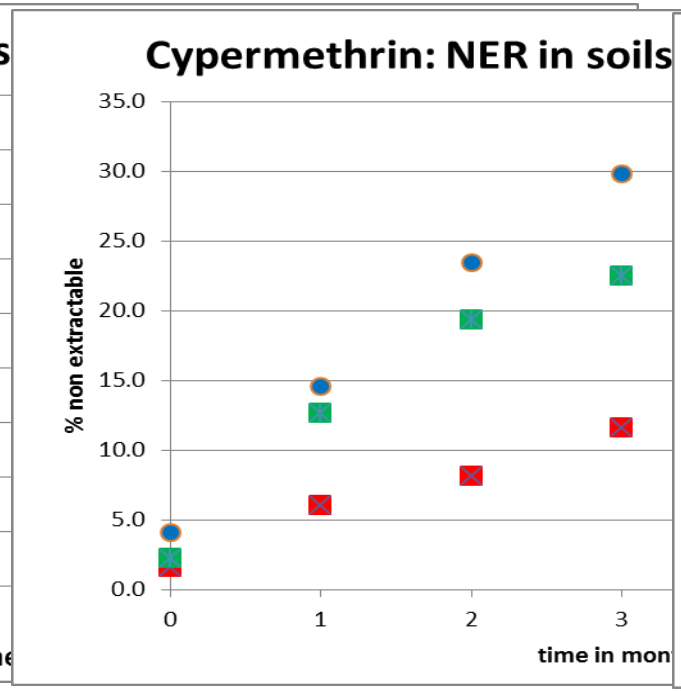


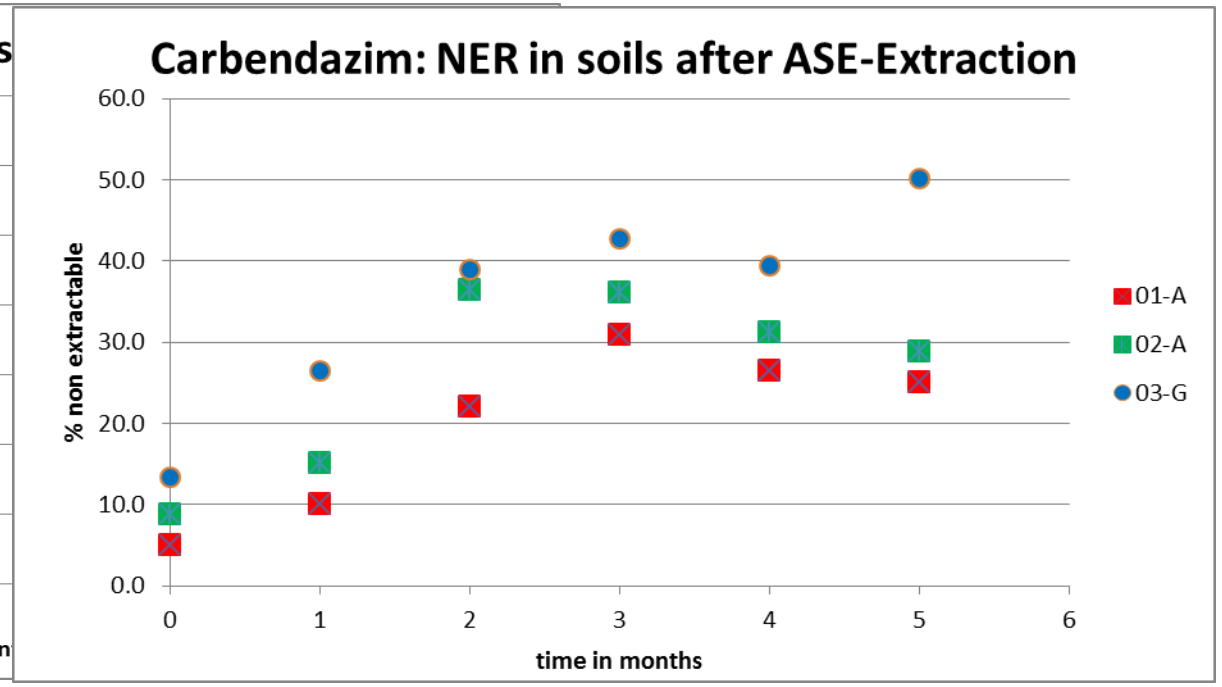
- Short term experiment. $t_{effect} < t_{NER\text{-}formation}$
- Chemical must be toxic (toxicity NER-chemicals is limited)
- Test on spiked (fresh and aged) soil and after removal potential bioavailable fraction
  - Spiking and Removal of Bioavailable Fraction without influence on toxicity (e.g. use of solvent)
- Earth Worm Avoidance, Microtox, Daphnia, Ammonium oxidation



NER-formation, ^{14}C experiment. Three selected chemicals







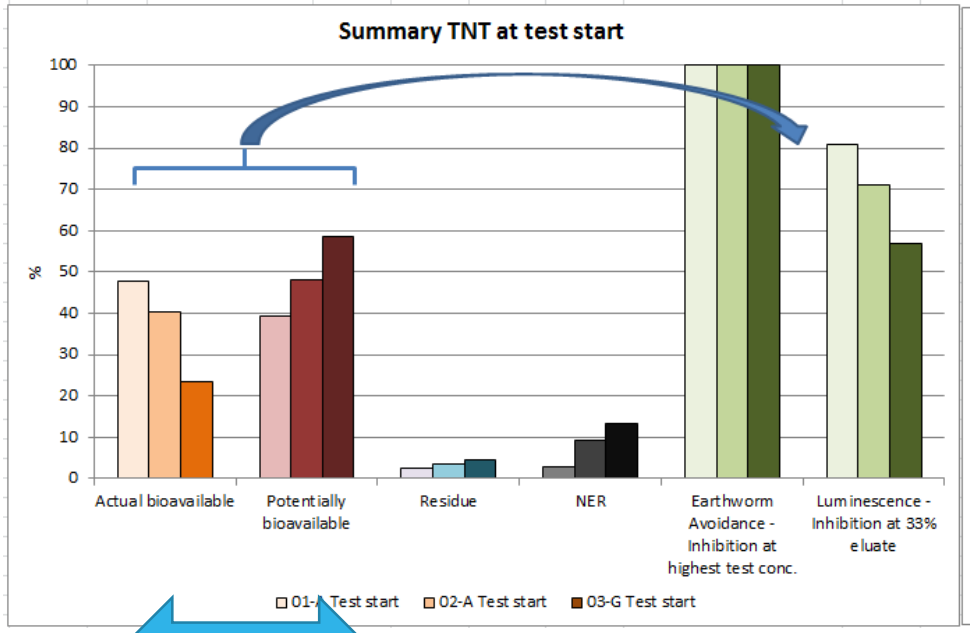
TNT	High NER-formation	60-75%
Cypermethrin	Low NER-formation	15-30%
Carbendazim	Intermediate	25-50%

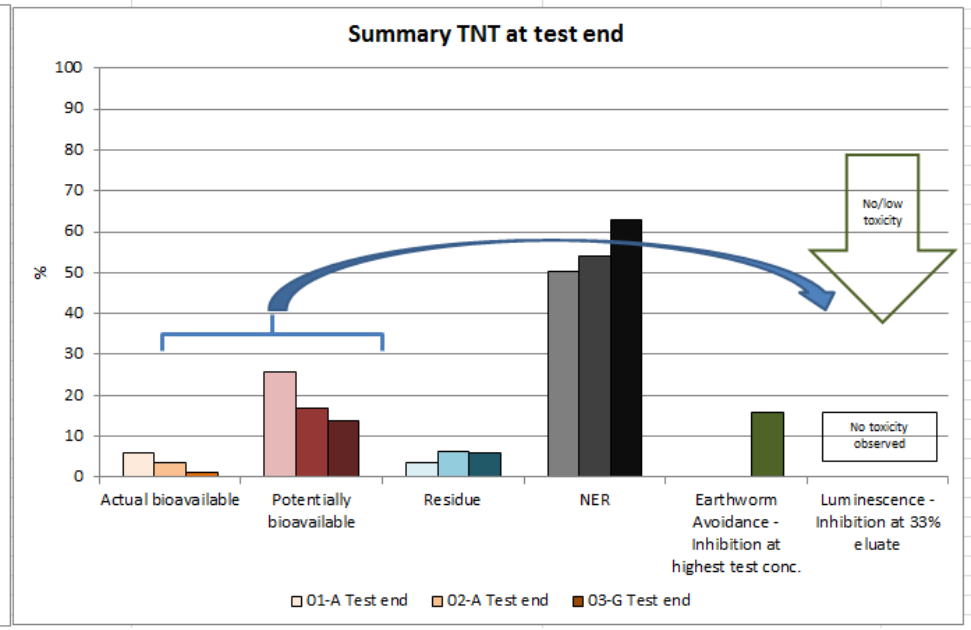
Method development TNT (^{14}C)

- TNT has a high water solubility
- High NER-formation during aging
- Aging reduces bioavailability and toxicity
- Easy explainable (continuous NER formation)

chemical

Bio-assay





bioavailable

total

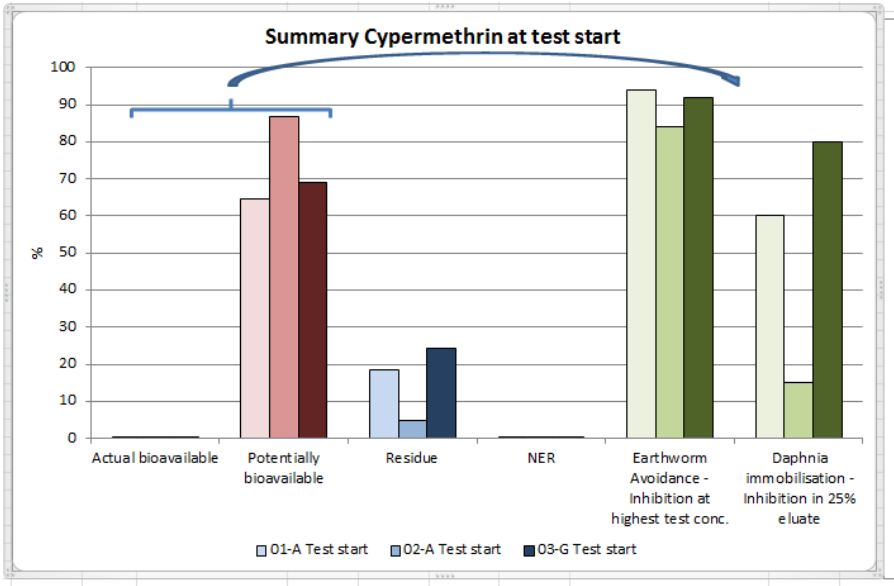
NER

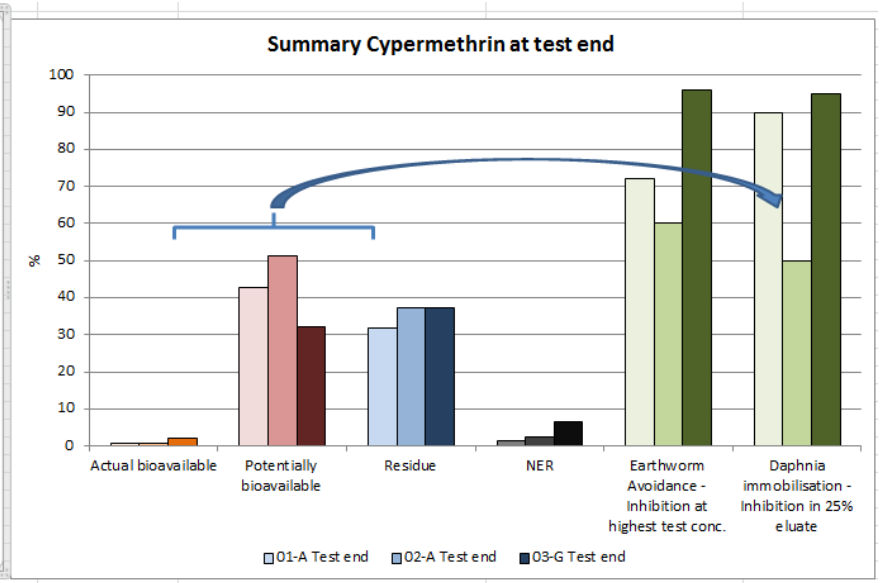
Method development Cypermethrin (^{14}C)

- Cypermethrin has a very low water solubility
- Little NER-formation during aging (this experiment)
- Aging reduces bioavailability and toxicity not/slightly
- Initial concentration very high (120 mg/kg)

chemical

Bio-assay





bioavailable

total

NER

Method development Carbendazim (^{14}C)

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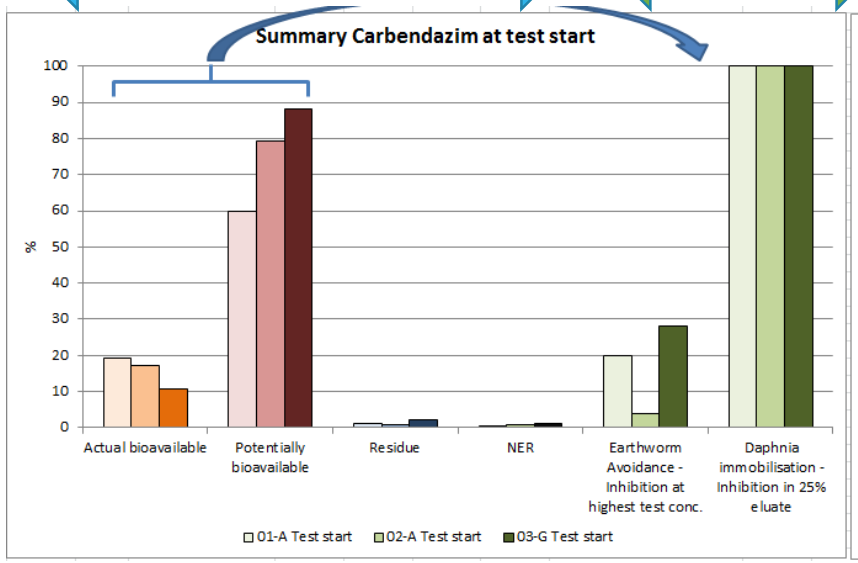
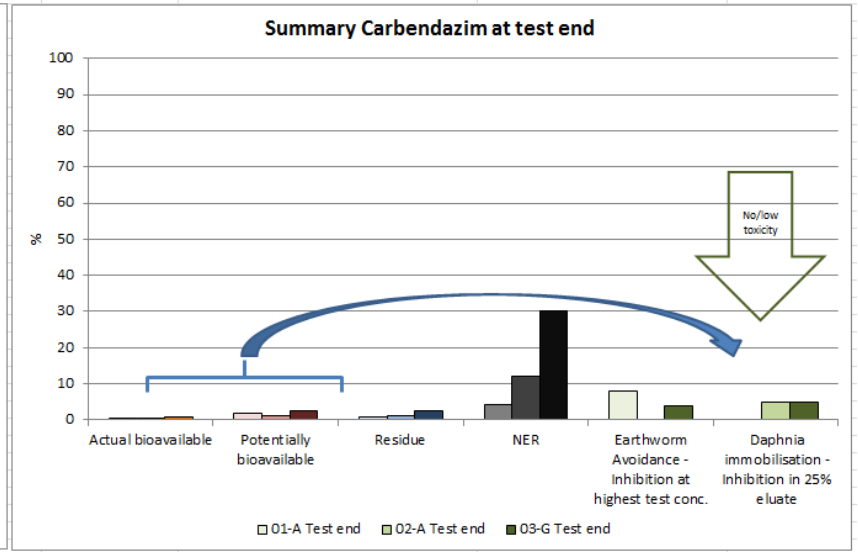
- Carbendazim has an intermediate water solubility
- Rapid degradation after lag phase
- Low NER-formation during aging
- Loss of carbendazim (very bad mass balance)

chemical

Bio-assay





bioavailable

total

NER

TNT- test ready for application

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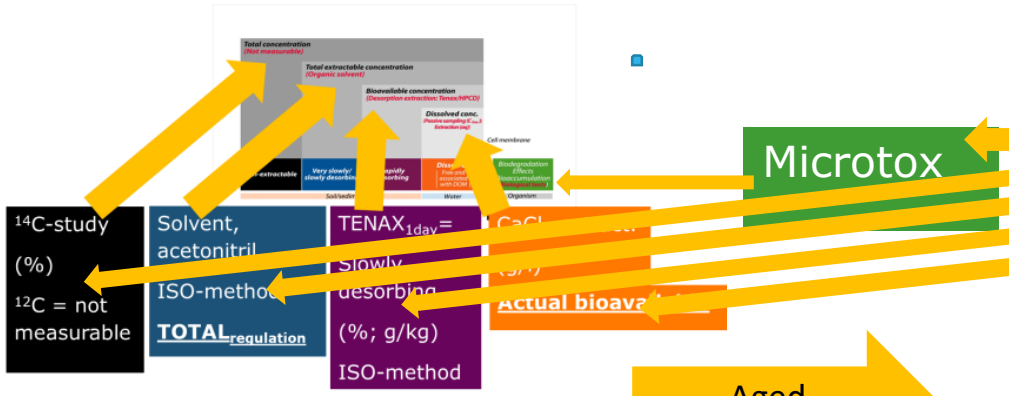
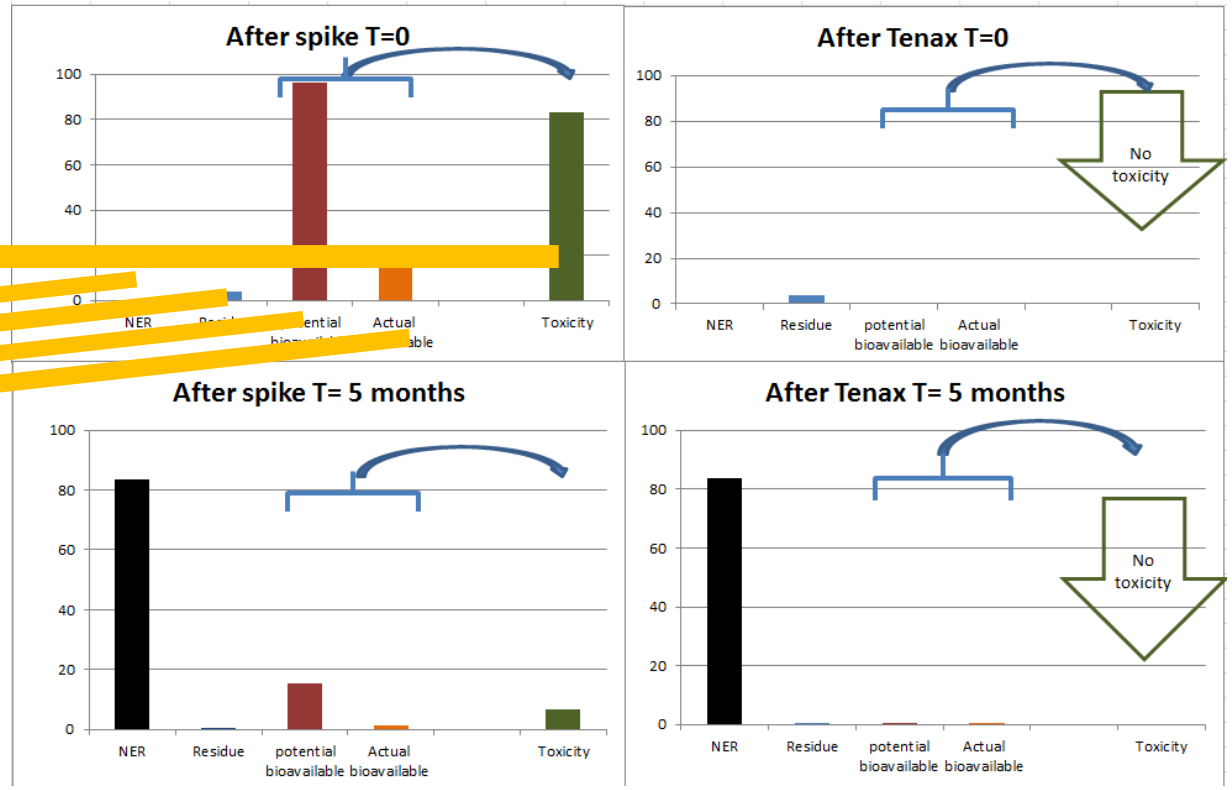


Microtox

Aged

Bioavailable part removed with TENAX



Conclusion: Bioavailable part explains toxicity

NER, residue and potential bioavailable in %
Actual bioavailable in mg/l
Toxicity (microtox) in 1/Ec_{20}

Cypermethrin, Concentration level?

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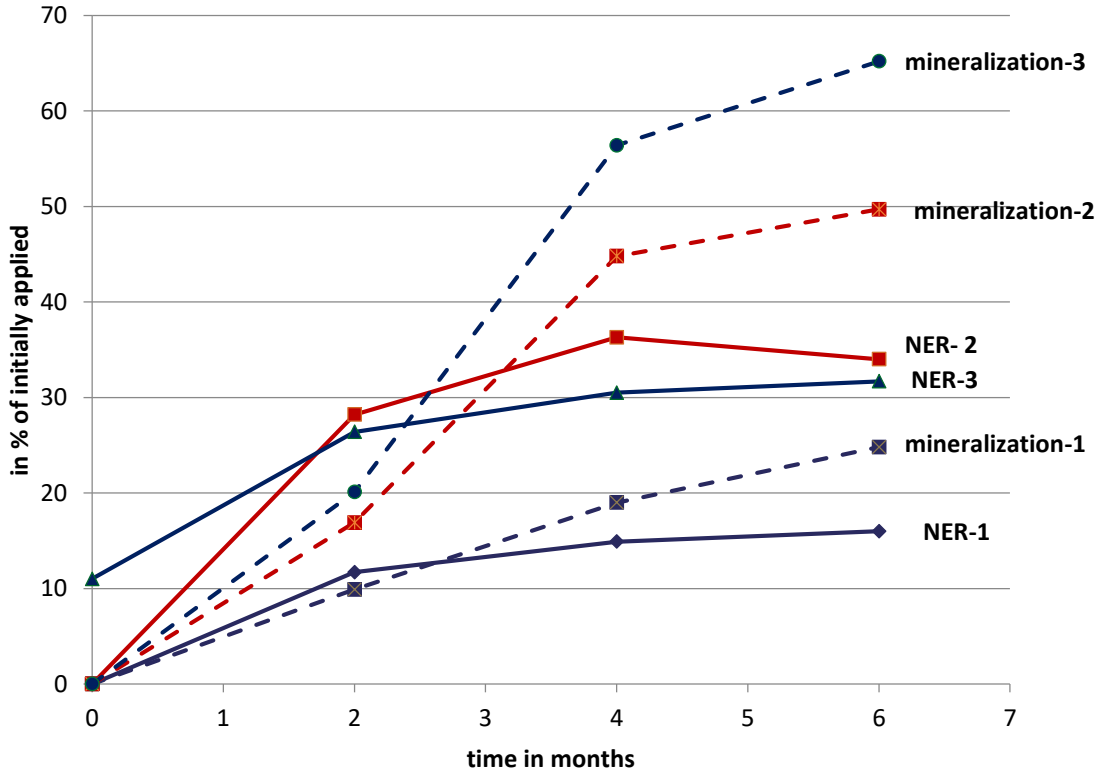
- First experiment. At 120 mg/kg only 2-4% mineralized in 6 months period
- Lower initial concentration of 5 mg/kg



How to explain non-labelled test results in third experiment?

- Cypermethtrin, % extracted

	T =0	T= 7 months
1A	96 %	62%
2A	97	62
3A	102	68

- How to explain difference of approx. 35%?
  - NER-formation
  - Mineralization

- Simultaneous ^{14}C is necessary to reduce uncertainty

Carbendazim

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- Total recovery radioactivity after 6 months 7% to 36% only,
  Most of radioactivity disappeared from system
- No volatile trapping (only $^{14}CO_2$), unexpected loss
- Degradation speed depends on soil, remaining radioactivity only partially due to parent

very high uncertainty on NER without ^{14}C-experiments

	soil 01-A		soil 02-A		soil 03-G	
Sample	Extractable	% Parent	Extractable	% Parent	Extractable	% Parent
0d	100.7	89.9	102.1	99.4	101.1	99.4
2M	63.4	85.8	35.5	97.7	36.3	91.7
3M	7.8	49.9	2.5	69.8	25.6	87.7
4M	1.9	21.2	1	59.2	11.9	82.4
5M	1.6	19.4	0.8	20.8	17.8	82.9
6M	1.3	16.1	0.8	20.2	8.2	77.7

Conclusions (technical)

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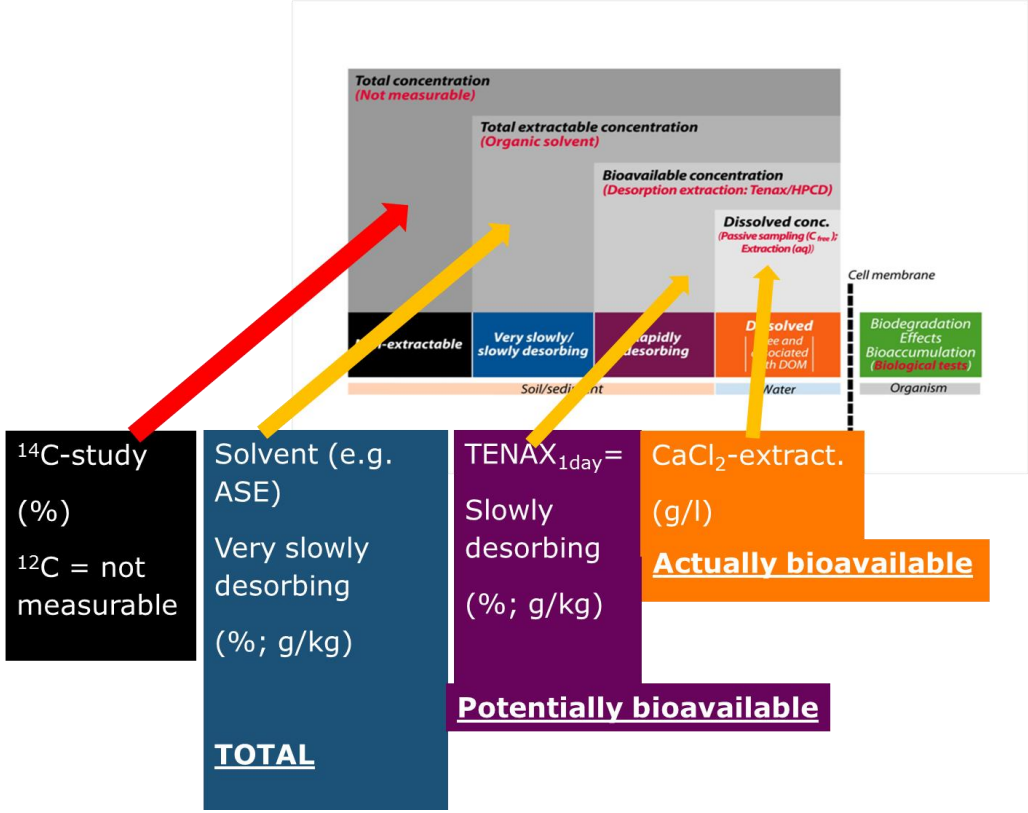
- The approach of Ortega-Calvo (2015) is generally applicable to support assessment NER;
- If fate is known, a test with non-labelled chemical is available and results are well explainable (TNT);
  - Toxicity can be explained by the bioavailable chemical and not by NER;
- Uncertainties in fate (e.g. volatility, biodegradation) result in high uncertainty of NER without labelled experiments;
  - Additional detailed chemical investigations (^{14}C) are necessary to eliminate uncertainties.



Recommendations

9th International Workshop on Chemical Bioavailability in the Terrestrial Environment. Warsaw 5th-8th November 2017

- If chemicals are non-extractable, they are strongly bound and will not cause risks
- In the past scientific communications and unclear definitions have caused uncertainty in regulatory context
- Present scientific “certainties” on bioavailability have to be communicated

This Project

- Make results of this project available for regulators in a proper form
- Define shortcoming of current approaches (technical and regulatory)

Future work

- Show with well designed experiments, including ^{14}C-experments the presence and toxicity of NER. Use general accepted definitions
- If toxicity cannot be shown, define what is necessary to show that NER is really not extractable

Acknowledgement

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THANK YOU!