Advances in the Development of Procedures to Establish the Toxicity of Non-Extractable Residues (NER)

MP 084
Monday 07 Nov. 2016

J. Harmsen (Wageningen UR-NL), D. Hennecke (Fraunhofer-DE), K. Hund-Rinke (Fraunhofer-DE), J. Lahr (Wageningen UR-NL), J. Deneer (Wageningen UR-NL), B. Hubesch (CEFIC-BE), M. Galay-Burgos, C. Eadsforth (Shell-GB), C. Finnegan (Unilever-GB), G. Roberts (GR Consulting-GB), G. Sanders (Givaudan-CH)

Introduction

A Historical Debate

Four decades of discussion around the bioavailability and ecotoxicological relevance of Non Extractable Residues (NER)

DETOXIFICATION PROCESS or ***HIDDEN HAZARD***

What is NER ?

NER is challenging to quantify and even more difficult to characterise. The formation and presence of NER may be attributable to :

- ***Association of the parent chemical with mineral and/or organic matter***
- ***Association of a breakdown product with mineral and/or organic matter***
- ***Mineralisation and incorporation of carbon in to microbial biomass***

What do the Regulators think of NER ?

ECHA Chapter R.7b: Endpoint Specific Guidance*

"The formation of NER should not be confused with degradation"
"NERs may potentially be re-mobilised ..... and thus pose a potential risk"
"Ultimately, it is the bioavailability of the residues which is actually of environmental significance"

Regain of Interest

Two recent ECETOC Task Forces were set-up

- Understanding the relationship between extraction technique and bioavailability (ECETOC Technical Report No. 117†)
- Development of interim guidance for the inclusion of non-extractable residues in the risk assessment of chemicals (ECETOC Technical Report No. 118†)

CEFIC ECO25 LRi Project

A multi-year project research programme (ECO25) sponsored by CEFIC (European Chemical Industry Council) will shed light on the ecotoxicity of NER :

Are NER toxic ?
To what extent are they toxic ?
What mechanisms are related to their toxicity ?

Soil Toxicity – Mass Balance Study with ^{14}C-TNT

- 3 characterised fresh soils (1-A, 2-A, 3-G) treated : U-ring^{14}C-TNT at 120 mg/kg dwt
- Mass balance measured in freshly spiked soils (T_0) and soils incubated for 6 months (T_{6M}) at 20°C, 40% MWHC
- Whole-soil toxicity (total Extractable + Non-Bioavailable + NER) tested via soil bioassays and Microtox for dissolved fraction
- ^{14}C-distribution profile vs bioavailability established

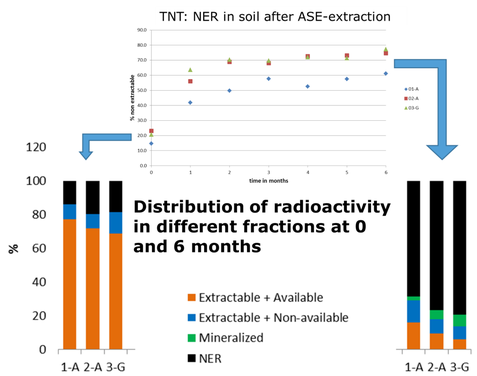


Figure 3 : Toxicity in soil bioassays with respect to distribution of TNT in different soil fractions at T_0 and T_{6M}

Toxicity of soil reduces over time

Time 0

Soil	Earthworm Avoidance (% effect)	Microtox (% effect)
1-A	100	93
2-A	100	79
3-G	100	79

Time 6 months

Soil	Earthworm Avoidance (% effect)	Microtox (% effect)
1-A	0	22
2-A	0	2
3-G	6	3

Conclusions from Mass Balance Study with ^{14}C-TNT

- TNT forms 70 – 80% NER over 6 month incubation period
- Whole-soil toxicity of TNT significantly reduced from T_0 to T_{6M}
- Soil microflora bioassay requires adaptation for determination of NER toxicity

Soil Toxicity – Study with Non-Labelled TNT

- 1 characterised fresh soil (1-A) treated : TNT at 120 mg/kg dwt
- T_0 - Whole-soil toxicity (Total Extractable + NER) tested via Microtox assay
- Analytical characterisation of soil distribution – Potential vs Actual Bioavailable
- Soil incubated for 5 months (T_{5M}) at 20°C, 40% MWHC
- T_{5M} - Whole-soil toxicity tested via Microtox assay
- T_{5M} - Whole-soil non-destructively extracted with Tenax® leaving soil intact with NER + Extractable but non-bioavailable residue
- T_{5M} - Tenax® extracted-soil toxicity tested via Microtox assay

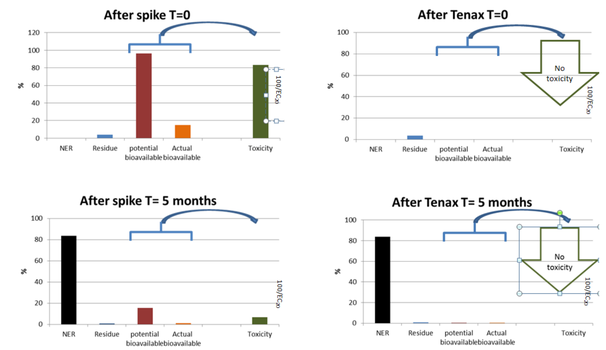


Figure 4 : Distribution of TNT in different soil fractions versus observed soil toxicity by Microtox Assay before and after Tenax® extraction

Conclusions from Mass Balance Study with ^{14}C-TNT

- Whole-soil toxicity of TNT significantly reduced from T_0 to T_{5M}
- Soil toxicity by Microtox reduces to zero following Tenax® extraction even at T_0
- Tenax® extract fraction represents the bioavailable fraction imputing toxic effects

Where do we go from Here ?

- Apply approach to other substances : Carbendazim, Cypermethrin
- Further validation of extraction framework and confirm function of Tenax® extraction
- Refine, validate and implement soil bioassays – existing and new approaches
- Extend concept to investigate the chronic toxicity of NER

Methods

Basis of Approach

- Treat characterised fresh soils with test item
- Determine soil toxicity at T_0 determined via battery of bioassays
- Incubate soil for ageing period (months) under controlled conditions
- Determine ecotoxicity of soil at T_{XM} following ageing using bioassays
- Develop non-destructive extraction technique leaving soil and NER intact

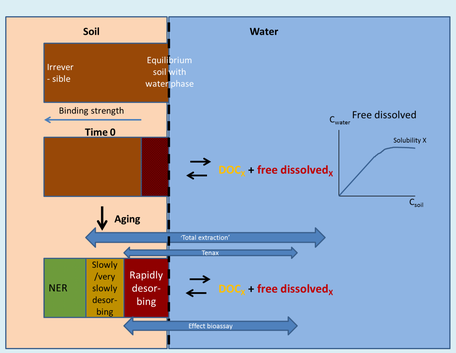


Figure 1 : Conceptual distribution of a chemical substance between the soil and aqueous phase and re-apportioning of extract-ability with time

Determining Bioavailability

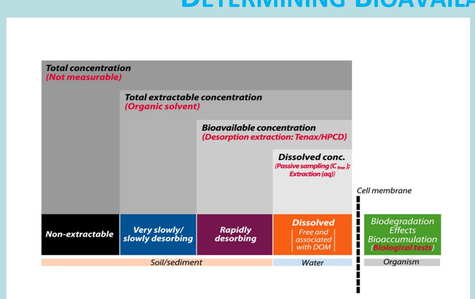


Figure 2 : Bioavailability and NER (Ortega-Calvo *et al*, 2015#)

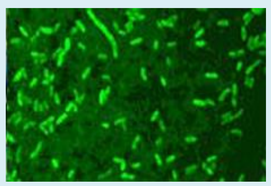
- Use of a standardised extraction procedure differentiating "Bioavailable" fraction (dissolved, readily desorbed) from "Extractable and Non-Bioavailable" and "Non-Bioavailable and Non-Extractable (NER)"
- Bioavailable and Dissolved fractions measured using 0.01M $CaCl_2$ + Tenax®
- The total Extractable and Non-Bioavailable concentration can be extracted and measured using (standardized) solvent extraction methods
- NER of commercial products **cannot** be measured

Establishing Ecotoxicity Effects

- Battery of Bioassays to determine Ecotoxicity effects of soils at T_0 and T_{XM}

Avoidance test *Eisenia andrei* (ISO 17512-1)

Soil microflora (ISO 17155)



Microtox *Vibrio fischeri andrei* (ISO 11348)

* ECHA, Chapter R.7b : Endpoint Specific Guidance, v4.0 Public Draft – June 2016
† ECETOC Workshop Report No. 17, Technical Reports Nos. 117 & 118 are available from www.ecetoc.org/publications
# Ortega-Calvo *et al*. Environ. Sci. Technol., 2015, 49, 10255-10264