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

J. Harmsen (Wageningen UR-NL), D. Hennecke (Fraunhofer-DE), K. Hund-Rinke (Fraunhofer-DE), J. Laehr (Wageningen UR-NL), J. Deneer (Wageningen UR-NL), B. Hubesch (CEFIC-BE), M. Galay-Burgos, C. Eadsworth (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).

Soil 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 into 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. 1171)
• Development of interim guidance for the inclusion of non-extractable residues in the risk assessment of chemicals (ECETOC Technical Report No. 1181)

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?

BASIS OF APPROACH

• Treat characterised fresh soils with test item
• Determine soil toxicity at T0 determined via battery of bioassays
• Incubate soil for ageing period (months) under controlled conditions
• Determine ecotoxicity of soil at Tm 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 extractability with time

METHODS

DETERMINING BIOAVAILABILITY

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 CaCl2 + 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

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

Soil microflora (ISO 17155)

Avoidance test E. andrei (ISO 17512-1)

DATING TOXICITY – STUDY WITH NON-LABELLED TNT

• 1 characterised fresh soil (1-A) treated: TNT at 120 mg/kg dwt
• T0 – Whole-soil toxicity (Total Extractable + NER) tested via Microtox assay
• Analytical characterisation of soil distribution – Potential vs Actual Bioavailable
• Soil incubated for 5 months (Tm) at 20°C, 40% MWHC
• TM – Whole-soil toxicity tested via Microtox assay
• TM – Whole-soil non-destructively extracted with Tenax® leaving soil intact with NER
• Extractable but non-bioavailable residue
• TM – Tenax® extracted-soil toxicity tested via Microtox assay

Figure 3: Toxicity in soil bioassays with respect to distribution of TNT in different soil fractions at T0 and Tm

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 14C-TNT

• Whole-soil toxicity of TNT significantly reduced from T0 to Tm
• Soil toxicity by Microtox reduces to zero following Tenax® extraction even at Tm
• Tenax® extract fraction represents the bioavailable fraction impinging 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

CONCLUSIONS FROM MASS BALANCE STUDY WITH 14C-TNT

• TNT forms 70 – 80% NER over 6 month incubation period
• Whole-soil toxicity of TNT significantly reduced from T0 to Tm
• Soil microflora bioassay requires adaptation for determination of NER toxicity

Figure 5: Distribution of radioactivity in different fractions at 0 and 6 months

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* 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