Workshop: Significance of Bound Residues in Environmental Risk Assessment 14-15 October 2009, Brussels

Workshop Report No. 17

EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

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European Centre for Ecotoxicology and Toxicology of Chemicals

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Workshop: Significance of Bound Residues in Environmental Risk Assessment

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1. SUMMARY

Thirty-eight leading experts in environmental fate, ecotoxicity and environmental risk assessment, participated in a two-day workshop in Brussels, Belgium, to review the current state of knowledge regarding 'bound residues' (BR) in the context of environmental risk assessment (ERA). The workshop also identified areas of this science that require further research. It was the hope of the workshop that by identifying gaps in the science, a common framework could be proposed that would successfully address bound residues in environmental risk assessments. Steps were taken to bridge differences in interpretation of terms such as 'non-extractable residues' (NER) and BR, such that debate could move forward with a common understanding. As a focal point of the workshop, gaps in the science and in the risk assessment paradigm were addressed during plenary and syndicate sessions, resulting in constructive scientific debate engaged by those from industry, academia and the regulatory areas.

The four workshop objectives of reviewing extraction procedures, developing guidance on the use of NER in ERA, identifying research gaps and drafting Request for Proposals (RfPs), were successfully met during the two days. The knowledge gaps and resulting RfPs are highlighted in a proposed risk assessment scheme. The scheme is envisioned by the steering committee as a plausible framework, once the science has been developed, to adequately address all of the gaps. Keys to the framework are:

- Developing a validated approach to characterising NER and BR and their 'bioavailability'.
- Developing an ecotoxicological testing and assessment approach that addresses both parent compound and potential transformation products within extractable residues (ER), NER and BR. The approach will also need to consider any residues potentially released or bioaccumulated.

The primary outcome of the meeting was agreement on a number of research activities that, if funding was available (e.g. through the CEFIC Long-range Research Initiative), would help improve the current approaches to the environmental risk assessment of bound residues.

It is anticipated that, once the research is completed (as suggested by the RfPs), this proposed framework should be re-visited to assess its potential usefulness in environmental risk assessment and PBT assessment.

2. DEFINITIONS

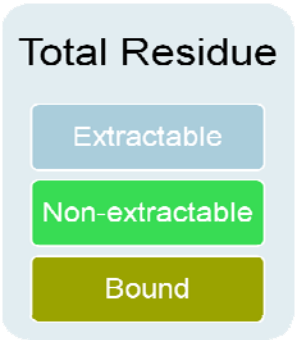
Most current definitions addressing bound or non-extractable residues are focussed on the nature of the extraction procedure and its ability to remove a substance from a matrix. These definitions focus on the degree of partition between the free and bound fractions but do not always consider the reversibility of any adsorption and how this might change with time. Furthermore, there is little consideration given to the relevance of such extraction procedures for determining bioavailability either for degradation or impact assessment. In this report, the following definitions have been used to try to address this issue and ensure a common understanding of the terminology used during the workshop.

Extractable residue: A residue that is extractable using 'mild' extraction methods. This may include aqueous and cold solvent extraction using methods without excessive added energy. These residues are either freely available, or only weakly adsorbed to the matrix, are considered to be bioavailable and must be considered in any impact / risk assessment.

Non-extractable residue: A residue that is not extractable using 'mild' extraction methods, but extractable under harsher conditions. These conditions may include solvent extraction using methods such as refluxing, microwaves or accelerated solvent extraction (ASE). These residues are strongly associated with the matrix, however they may be potentially reversible; but the partitioning is very much in favour of 'binding' to components of the matrix. Therefore, for risk assessment purposes, this matrix associated fraction is unlikely to be available to indigenous organisms.

Bound residue: A residue that is tightly associated with the solid matrix, often forming covalent (or similar) bonds. These residues usually cannot be released from the matrix or can only be released under extreme conditions where the integrity of the substance and/or matrix is likely to be affected. Such residues are often indistinguishable from the natural organic material e.g. humus in soil. These residues are not available for either degradation or available for indigenous organisms and should not be considered in any impact / risk assessment.

ER, NER and BR can be represented by the following figure based on Zarfl *et al* (2009):



ER, NER and BR are defined on an operational basis, which is to say that they depend specifically on the methods used to extract the chemical(s). In addition, it is only possible to detect BR using methods such as isotopic labelling.

Bioavailable (based on Semple *et al*, 2004): "Is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time" e.g. available now (no constraints).

Bioaccessible (based on Semple *et al*, 2004): Is available to cross an organism's cellular membrane from the environment, if the organism has access to the chemical. However, the chemical may be either physically separated from the organism or only bioavailable after a period of time, i.e. available, but not within reach from a given place and/or time (constrained).

Depletion: Removal of a chemical from an environmental compartment. This includes such mechanisms as degradation (including [but not limited to] hydrolysis, photolysis and biodegradation), partitioning and volatilisation.

3. BACKGROUND

The significance of bound residues in environmental risk assessment

Bound residues (BR), including non-extractable residues (NER), are an important factor in PBT assessment and risk assessment of chemicals. Precautionary risk assessments usually assume 100% bioavailability, i.e. all of the chemical present is available for degradation or to have potential toxic effects on the biota. This precautionary approach generally overestimates the exposure concentration by the amount that is not available and therefore overestimates the level of risk to biota in the environment. It is also well documented that chemicals that are irreversibly bound to solids are less degradable and less toxic than the total residue would predict. Even though it is a position that has been recognised by ECPA (2000), and referenced by REACH (2008) and OECD 308 test guidance (2002a), there is no agreed guidance on how to determine what is available or not, and how it should be considered in the risk assessment. As a result, it continues to be debated from a scientific and regulatory point of view (see *Environmental Pollution*, 133, Special Issue, 2005).

Although there is a need to define what is meant by 'bound' in the context of chemical residues in soil, sediment and biosolids, for the purpose of this report the definitions in the previous section have been used. Bound material will be unavailable and therefore depleted from the system. There is also a need to link extraction techniques (e.g. different solvents) to mechanisms of binding and whether this is sufficient to define how much of the total chemical present is bioavailable.

Understanding the mechanisms of binding, and what types of analytical methods are needed to identify such mechanisms, would assist in performing better predictions of which chemical-solid-environment combinations may lead to non-extractable residues.

Guidance on the assessment of NER and their bioavailability in environmental risk assessment (and PBT assessment) is lacking. This workshop will address these concerns and identify future regulatory and research needs.

4. WORKSHOP OVERVIEW

4.1 Workshop structure

The workshop organising committee invited 38 stakeholders representative of industry, academia and regulatory authorities. Their expertise encompassed environmental fate, ecotoxicity and risk assessment areas and their 'bound residue' experiences were related to either the development of respective data sets, interpretation of such data and/or application of data to environmental risk assessments. The workshop began with detailed presentations from the respective stakeholders followed by breakout group discussions (syndicate sessions); these were followed by plenary sessions to share the findings from the syndicate sessions.

4.2 Process

The main tools used for achieving the aims of the workshop were syndicate sessions which addressed specific questions aimed at capturing the current understanding of the science, identifying the different perspectives for interpreting the data and applying it in environmental risk assessments, and for identifying areas where there are scientific gaps and where further research is needed. It was not the objective of this workshop to achieve consensus, but to identify areas of research to help bridge some of these differing views and to fill some of the scientific gaps necessary to clarify our overall understanding of NER / BR. The recommendations from the workshop propose a framework for considering the risk of NER / BR in the context of the science needed to move this debate forward.

4.3 Workshop objectives

The workshop focused on four objectives:

- Review extraction procedures and identify how residues extracted under each regime should be evaluated.
- Guidance on the use of NER and bioavailability in environmental exposure and effects assessment.
- Identify research to address knowledge gaps in these proposed methodologies.
- Draft research plans including potential funding opportunities, collaborations and timelines to develop improved environmental exposure and effects assessment of NER.

5. REPORTS FROM THE SYNDICATE SESSIONS AND RESEARCH TOPICS

The exposure assessment of bound residues syndicate session was split into two parallel groups due to the number of delegates involved (Syndicates 1-A and 1-B). These syndicates addressed the same set of questions.

Syndicate 1-A [1]*: Exposure assessment of bound residues*

Questions for consideration:

1. *How should bound residues be defined / characterised?*
2. *What extraction methods are considered suitable / unsuitable?*
3. *How relevant are they to the conditions experienced in the environment?*
4. *Are all bound residues equivalent in exposure as determined by these characterisation and extraction methods?*
5. *How suitable are current biodegradation tests for the assessment of bound residues and persistence?*
6. *Gaps and research needs associated with the above.*

Q1 - How should bound residues be defined?

- Often they are operationally defined by the extraction methods used in a sediment or soil fate test. Such bound residues could include:
  - Non-extracted parent.
  - Non-extracted degradates.
  - Parent or degradates that are covalently integrated into soil organic materials, including both labile and refractory organic carbon (Note: Such covalent bonds can be labile e.g. ester or stable e.g. ether).
  - It is usually not possible to distinguish among these types of residues.

- It is critical that the extraction schemes are theoretically sound (based upon chemical first principles) and experimentally validated (e.g. spike recovery experiments).
  - Regulators need to be provided with the theoretical rationale behind an extraction scheme and the data supporting its proof of principle.

- It is important to realise for risk purposes:
  - The bioaccessible fraction is not simply equal to 1 minus the fraction unextracted.

[1] Participants in syndicate group 1-A are listed in Appendix G.

- An ideal circumstance would be if there were systems whereby it could be determined what is in the pore water and what would be bioaccessible to particle feeders, using some type of digestion fluid relevant to the digestive track of deposit feeders.

Q2 - What extraction methods are suitable?

- Extraction methods need to consider the chemical characteristics of the parent and degradates, the matrix, and target organisms being protected.
- They need to be theoretically sound (based upon chemical principles) and experimentally validated (e.g. spike recovery experiments).
- Often a sequential series of extractions involving solvents with a gradation of polarities is a good approach since it is likely to recover the broadest range of parent forms and types of degradates.

Q3 - How relevant are they to conditions in the environment?

- It is important to realise that exhaustive approaches used to characterise residual chemical species in soil or sediment are very different, more extreme and reflect higher exposure to that experienced by organisms. For example, some larvae of beetles have high pH gut sections (above pH 10) that may be considered a quite strong alkaline extraction medium for humic substances, e.g. Lemke *et al* (2003).

Q4 - How suitable are current biodegradation tests for assessing persistence and formation of bound residues?

A number of concerns were identified which affect the value of the data generated in current biodegradation tests and how the data should be interpreted, including:

- Unrealistically high dosing.
- Introduction pathways of substance do not simulate in situ conditions.
- Matrix effects are not accounted for.

These tests could be improved to provide greater realism. These steps include:

- Make dosing more realistic. For example, chemicals that enter soil on sludge should be dosed onto sludge and veterinary pharmaceuticals that enter soil in manure should be dosed into manure. These sludge or manure mixtures should then be added into the test soils.

- Appreciate that labile organic matter on which the chemical may enter soil will disappear over time as it degrades, which could release previously bound residues.
  - This question increases the importance of accurately simulating mode of entry, and running the studies long enough for such remobilisation due to carbon turnover to be evaluated.
  - Normal processes that occur in the field including freezing-thawing, drying-wetting, ploughing and bioturbation could release previously bound residues.
  - These processes should be investigated or simulated in existing tests to verify their importance for risk assessment.

- The design of the current OECD 308 water-sediment test includes a static ratio of approximately 3:1 (v/v) between water and sediment. This small ratio shifts equilibrium mass distribution towards the sediment phase, compared to natural conditions, which may result in unrealistically high levels of bound residues.
- More dynamic test systems (e.g. flow through) may overcome these issues.

Q5 - Gaps and Research

- Increased validation of laboratory test results with higher tier field work (e.g. lysimeters and field plots). Note: This is more practical for soil than sediment.
- Develop methods to simulate disturbances (e.g. freeze-thaw, ploughing, drying-wetting etc) in laboratory tests and evaluate their effect.
- Develop models and collect supporting data to relate organic carbon turnover from sludge, manure, straw, roots etc., to binding and release of chemicals.
- Develop guidance on best practices to serve as a starting point for extracting chemicals and degradates from soil and sediment, based upon their chemical characteristics.
- Fractionate soil from fate tests into size aggregate fractions (e.g. sand, silt, clay) to evaluate what constituents contain the bound residues. This may provide indications of the type of residue and the mechanism of binding. This could be combined with bioaccessibility / bioavailability testing with soil / sediment organisms.

Syndicate 1-B [2]: Exposure assessment of bound residues

Questions for consideration:

1. *How should bound residues be defined / characterised?*
2. *What extraction methods are considered suitable / unsuitable?*
3. *How relevant are they to the conditions experienced in the environment?*
4. *Are all bound residues equivalent in exposure as determined by these characterisation and extraction methods?*
5. *How suitable are current biodegradation tests for the assessment of bound residues and persistence?*
6. *Gaps and research needs associated with the above.*

Q1 - How should bound residues be defined / characterised?

This depends on what the data is to be used for.

- Research or standardised testing?
- Total toxic potential (→ rigid extraction) or ERA (→ mild extraction).

Q2 - What extraction methods are considered suitable / unsuitable? Are all bound residues equivalent in exposure as determined by these characterisation and extraction methods?

- Mild extraction (mimicking natural conditions, but on the safe side).
- More rigorous extraction (e.g. acid or base).
- More rigorous extraction (e.g. organic solvent).
- Accelerated solvent extraction.
- Combustion (→ $^{14}CO_2$ → BR).

Q3 - How relevant are they to the conditions experienced in the environment?

- Many specific conditions require additional caution, e.g. ingestion of sorbed chemicals by organisms and exposure following 'extraction' in the gut.
- Leaching of sorbed chemicals, e.g. after desorption or by colloid facilitated transport.

[2] Participants in syndicate group 1-B are listed in Appendix G.

Q4 - How suitable are current biodegradation tests for the assessment of bound residues and persistence?

- Complexity in the environment should not preclude pragmatic standardised procedures (and guidance for interpretation) in biodegradation simulation tests.

Q5 - Gaps and research needs associated with the above

- Guidance for appropriate extraction procedures for soil and sediment studies (which cover both hydrophilic and hydrophobic chemicals).
- Validation of the environmental interpretation of extraction procedures (e.g. are 'mildly extracted fractions' correlated with 'bioavailable fraction'?).
- Effects of changing dry and wet conditions on bioavailability.
- Studies of effects of sorbed chemicals on a battery of organisms representing different routes of exposure (which should include effects of ageing).

Syndicate 2-C [3]: Depletion mechanisms and modelling of bound residues

Questions for consideration:

1. *Scientific understanding of the mechanisms of binding that lead to bound and non-extractable residues:*
   - *Key literature and/or authors.*
   - *Identify key mechanism for bound and non-extractable residues, covalent binding, partitioning, hydrogen, ionic, etc.*
   - *Role of functional groups (NH_2, -NH, -N-, COOH, other).*

2. *Evaluation of models that may predict:*
   - *The extent and nature (mechanism) of bound residues based on structure, physico-chemical properties, or other.*
   - *Reliability or accuracy of such models; if not, what other weight of evidence is needed before assigning such mechanism?*
   - *The microbial biotransformation products (MBP) of test materials that are subsequently incurred in the binding process and contribute to the non-extractable residues.*
   - *Reliability or accuracy of such models; if not, what other weight of evidence is needed before assigning such structures?*

3. *Gaps and research needs associated with the above.*

Q1 - Scientific understanding of the mechanisms of binding that lead to bound and non-extractable residues

1. Key literature and/or authors

- There was no important literature identified beyond the papers provided with the workshop material.

2. Identify key mechanism for bound and non-extractable residues – covalent binding, partitioning, hydrogen, ionic, etc.

- Diffusion into micropores has to be distinguished from interactions with the matrix. Diffusion is a transport process. Translocation into micropores cannot explain non-extractability without additional sorption by interactions with the matrix. There seems to be a *continuum* of increasing forces for sorption caused by different interaction mechanisms and available binding sites.

[3] Participants in syndicate group 2-C are listed in Appendix G.

- The question was raised, if extraction procedures are insufficient because of the selected mass-to-volume ratio, then does this over-estimate the amount of material that cannot be extracted (NER)? If NER was only a matter of partitioning then a sequential extraction should result in 100% recovery. This is not always achievable in practice because of other interactions between a chemical and the matrix.
- It is commonly accepted that microbial action can be involved in formation of NER. In this case, this could also be a matter of enzymatic catalysis of covalent bond formation. This can be further explored by investigating if there is BR and/or NER formation under sterile conditions.
- Categorisation according to mechanisms needs to take into account substance properties. To date, there are no general rules that describe the extractability of all sorts of compounds from all matrices. Is there direct experimental evidence for single processes occurring under certain conditions? A first categorisation could be based on the following:
  - Covalent binding: From the literature, it is concluded that interactions between nucleophiles and electrophiles play an important role. Reactive groups (nucleophiles) may enhance covalent bond formation, but it could also be an acid / base interaction. Adducts can be formed by Michaels addition, Schiff base formation, etc. Is this a reversible process? Compounds that are H-donors and/or H-acceptors can interact with respective counterparts of the matrix by stronger H-D/A forces. Many bonds are probably stable, but under changing environmental conditions reaction patterns may change and it cannot be completely ruled out that covalent bonds may break again.
  - Hydrophobic interactions: For example, polycyclic aromatic hydrocarbons (PAH) may be subject to physically defined occlusion in a diffusion type process. Additional interactions with the matrix, mainly van der Waals forces which are generally weak, do not fully explain why it is difficult to extract the compounds from solid matrices such as soil or sediment.
  - Ionic bonding: These are formed via electrostatic forces. This mechanism is pH-dependent and the pKa value has to be taken into account. For example, acid dyes form large amounts of non-extractable residues even under harsh extraction conditions. The binding strength of electrostatic forces extends into the range of covalent bonding in terms of energy.
  - Other possible mechanisms that could play a role are ligand exchange (metal binding), charge transfer complexes or uptake by organisms.
    Note: Some (many) compounds can be subject to more than one of the listed mechanisms!

3. Role of functional groups (NH_2, -NH, -N-, COOH, other)

This is directly linked to substance properties, because they are defined by the functional groups of the substance. Nucleophilic / electrophilic properties could be used as indicators for reactivity. Polarisability, H-D/A properties and ionisation properties (pKa) are important indicators for specific interactions.

Q2 - Evaluation of models that may predict:

1. The extent and nature (mechanism) of bound residues based on structure, physico-chemical properties, or other

To the syndicate's knowledge, there was no complete model available for this purpose. However, for single aspects promising approaches are available that should be tested for their applicability under consideration of the application domain (training set of the model).

- Set up of a rule based system for factors that most likely enhance / enable non-biological binding of compounds to solid matrices – this could be done for covalent binding by defining 'alert groups'.
- Polyparameter linear free energy relationship [pp-LFER] approach (Abraham, 1993) is valid for van der Waals and H-donor / acceptor forces. It could possibly also be used for complexes, but is not yet validated. Solute descriptors for a large number of chemicals are available. Phase / solvent descriptors have not yet been determined for the full set of environmental phases, but are available for a number of solvent / solvent systems and for selected water / solid or air / solid systems (e.g. humic acids).
- Kinetic modelling of data sets is possible and can deliver important information. For example, interpretation of the results of water-sediment test (OECD 308, 2002a) is often difficult, because the test is unnecessarily complicated. Transport from water to sediment dominates 'loss' in the water phase. Kinetic modelling allows for identifying first order rate constants for the individual processes, e.g. degradation in the sediment phase or formation of NER. This gives an idea of the time scale on which processes occur.
- QSPR (Quantitative Structure-Property Relationship) approaches or fragment methods can also deliver information for categorisation of chemicals: CATABOL and other computer programs such as UM-PPS (University of Minnesota Pathway Prediction System, free software) may identify possible degradation pathways; EPISUITE/BIOWIN (free software) gives an idea of primary degradation half-lives.

2. Reliability or accuracy of such models; if not, what other weight of evidence is needed before assigning such mechanism?

QSPR methods are critical with respect to the application domain (training set). Since there are no explicit models available, nothing can be said about reliability or accuracy to date.

3. The microbial biotransformation products (MBP) of test materials that are subsequently incurred in the binding process and contribute to the non-extractable residue

Known MBP (from biotransformation information), when available, should be included and assessed with respect to their contribution to NER formation. However, this is a very complex issue and current predictions are generally unreliable.

Q3 - Gaps and research needs associated with the above

- Observed NER formation should be evaluated against solute descriptors (pp-LFER).
- Reference matrices should be defined (standard soil, humic acid, etc) to allow for better comparison of studies.
- Physico-chemical properties (attractive forces) should be used first to explain the observations within a tiered approach. Outliers that cannot be explained by the physico-chemical properties have to be identified and need further exploration using alternative approaches (e.g. electrostatic forces).
- Better models to predict MBP may help to explain NER/BR formation, where this cannot be explained by parent or known MBP properties.

Syndicate 3-D [4]: Effects assessment of bound residues

Questions for consideration:

1. *Current scientific understanding - what are the key influences on bioavailability?*
2. *How suitable are current ecotoxicity methods for assessing effects of bound residues in soil, freshwater and marine sediments?*
3. *What improvements / modifications are needed?*
4. *How can total residue / bioavailable concentration be related to observed effects?*
5. *What are suitable tests that account for different exposure routes (ingesters versus non-ingesters)?*
6. *What soil / sediment species should be included in a testing strategy and how should different trophic levels be addressed?*
7. *Are there any* in vitro / in situ *tests that can be used to assess bioavailability of soil / sediment residues and therefore preclude effects testing?*
8. *What research is needed to answer these questions?*

Q1 - Current scientific understanding – What are the key influences on bioavailability?

- Current understanding is limited. The freely dissolved concentration model (see presentation, Appendix E) seems to work well for describing the bioavailability of hydrophobic compounds as the freely dissolved concentration is a good descriptor of the chemical activity of these compounds in soil and sediment. Whether this is the case for a broader range of compounds remains to be seen.
- There is a need for more understanding of the sorption / binding mechanisms of polar and reactive chemicals in relationship to their bioavailability.
- There is also a lack of understanding of the effects of bioturbation, plants and digestion of organic matter on NER and BR.
- Because of the lack of mechanistic understanding there is a need for empirical approaches to the testing of the bioavailability and effects of chemicals in soils and sediments.

Q2 - How suitable are current ecotoxicity methods for assessing effects of bound residues in soil, freshwater and marine sediments?

- Current methods are not very suitable due to, for instance, the use of artificial sediments with short exposure to chemicals (comparable NER/BR to those in real sediments?), the preference for organisms exposed via the water phase (such as chironomids) and the short duration of the tests.
- There is a need to develop higher tier methods to test ecotoxicity of NER.

[4] Participants in syndicate group 3-D are listed in Appendix G.

Q3 - What improvements / modifications are needed?

- Better choice of organisms, including ingesters.
- Protocols to include NER/BR in persistence and toxicity testing.
- Standardised methods to produce NER/BR.

Q4 - How can total residue / bioavailable concentration be related to observed effects?

- Unknown for many chemicals (non-polar hydrophobic chemicals may be an exception as sorption to black carbon seems to be important for these chemicals).

Q5 - What are suitable tests that account for different exposure routes (ingesters versus non-ingesters)?

- These need to be developed (see above).

Q6 - What soil / sediment species should be included in testing strategy and how should different trophic levels be addressed?

- Ingesters such as *lumbriculus* should be included.

Q7 - Are there any *in vitro* / *in situ* tests that can be used to assess bioavailability of soil / sediment residues and therefore preclude effects testing?

- Explore the potential of chemical methods (such as SPME [solid phase micro-extraction]) to characterise the bioavailability of a wide arrange of compounds in a wide range of sediments under realistic conditions.

Q8 - What research is needed to answer these questions?

- Mechanisms of binding of polar and ionic chemicals and consequences for bioavailability.
- Conditions under which NER contribute to the effects of chemicals.
- Effects of digestion, organic matter turnover and soil / sediment management on NER and their bioavailability.
- Long term effects of NER/BR (multi-generation studies).
- Validation of freely dissolved concentration model for a wide range of chemicals.
- Comparison of effects with artificial sediment and natural sediment.

Environmental risk assessment of NER and development of a NER testing strategy

Questions for consideration:

1. *When would bound residues be considered a risk? When would bound residues not be considered a risk?*
2. *At what point in the risk assessment would one consider / evaluate bound residues?*
3. *What additional testing (fate and effects) would be appropriate?*
4. *What considerations are important in the risk assessment?*
   - *Is it important to understand the mechanism of binding – would this affect the way the hazard / risk is assessed?*
   - *Should bound residues be considered part of the overall depletion rate?*
   - *How should different rates of dissipation / degradation from a study with different sediments be used in risk / hazard assessment?*
   - *How should discharge / release pattern and rate be compared to the binding capacity of the receiving matrix (overloading binding capacity)?*
   - *What specie(s) is best to assess the potential effects of NER?*

Syndicate 4-E [5]: PECs

Q1 - Defining the route of exposure and resulting NER environmental compartment

- Veterinary products – Residues in manure are applied to soil resulting in a potential for soil NER. Externally applied veterinary product (e.g. ectoparasiticide) may also result in soil exposure and subsequent potential for NER.
- Plant protection products – Direct or spray application to soil resulting in a potential for soil NER and sediment NER from over spray or run-off.
- Human pharmaceuticals, personal care and industrial products – Sewage treatment effluent discharge into receiving waters or irrigation uses resulting in a potential for sediment NER. Soil NER from land application of sludge biosolids.

Characterisation of extractable and non-extractable residues

- It is important to realise that the bioavailable (accessible) fraction of parent and/or degradates is not equivalent to maximum amount of extractable material using the most exhaustive methods.

[5] Participants in syndicate group 4-E are listed in Appendix G.

- A reasonable approach would be to include a sequential series of extractions, each of increasing strength. The extracted fraction could be associated with a degree of bioavailability (accessibility). For example:

Extraction condition	Possible solvent	Fraction	Availability
Mild	$CaCl_2$ pH adjusted	Pore water	Available
	Surfactant or chelating	Leachable	
	Organic solvent series	Leachable?	
Severe	ASE, soxhlet	NER	Not available.

- The OECD 308 water-sediment (OECD, 2002a) study could be easily adapted to utilise this approach. Test chambers containing sediment and water could be mixed for a specified period of time and the water phase could be considered as the 'available' fraction based on the natural partitioning between the water and sediment phase. This methodology may only address short-term formation of NER and not the longer-term (100-day exposure) aged residues where one typically observes high levels (>50%) of NER.
- Extracted fractions could be analysed to determine levels of parent and/or degradates present.

NER need to be evaluated in terms of risk management; triggered when PEC/PNEC >1 or perhaps using the criteria of >70% NER (but not using the water-sediment ratio of the OECD 308 test) and <5% mineralisation. Possibly subjected to further analysis to determine NER fraction associated with sand, silt, clay, for example. This approach would require a framework or guidance document for extraction methodologies and clear definitions of the individual soil / sediment fractions.

NER could be characterised for the potential release of residues by the gut of sediment organisms using a 'simulated' gut fluid (pH, digestive enzymes, other). Need to identify relevant conditions for various sediment organisms.

Effects on microorganisms and higher organisms can be modelled or determined experimentally. Effects testing possible on:

- Extracted matrix (NER remaining) or
- OECD 308 study (OECD, 2002a) with chironomid (or other organism) exposure starting at 100 days to account for NER and any biotransformation products present (however, a possible ammonia build-up could affect sediment organisms and this would have to be addressed).

Biodegradation Test Methods

Current OECD 308 water-sediment (OECD, 2002a) test method over-estimates bound residue as the 3:1 ratio of water to sediment does not represent 'natural' conditions (needs greater amount of water). As a result, current findings are more of an artifact of the system than what would be observed. The static design of the test also does not represent natural conditions by not accounting for the effects of flow velocity and sediment dynamic. Researchers (Kunkel and Radke, 2008) studying the impact of flow have found a significant increase in rates of biodegradation of anionic pharmaceutical with river water and sediment in a bench-scale annular flume at two different hydraulic boundary conditions (flat sediment surface versus moving sediment) due to improved mass transfer kinetics.

Also, the EMEA technical guidance document (EMEA, 2006) does not account for degradation rates observed in sediment in the calculation of the predicted environment concentration (PEC) for sediments.

Q2 – Gaps and Research

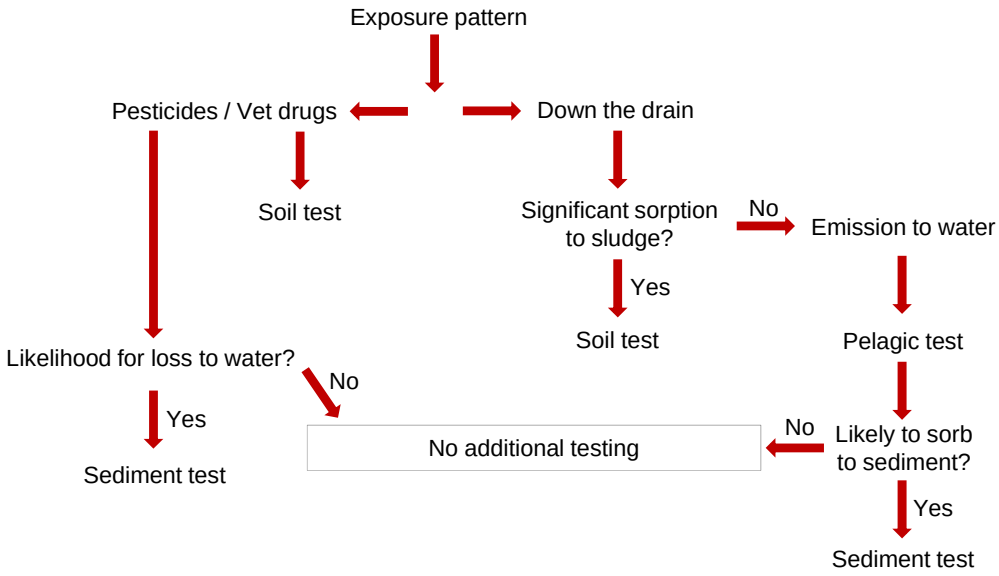
- Develop guidance for extraction methods: Selection of solvents, pH adjustments, other additives (chelators) and sequential series of extractions. Associate the extractable fractions (available, leachable, NER, etc) with both a level of bioavailability (accessibility) and appropriate test organism(s) for predicted environmental compartment.
- Characterise the mechanism of binding between test substance (key functional group responsible for binding) and the key functional groups in the matrix fractions (sand, silt, clay, organic matter). Determine the level of bioavailability (accessibility) and relative risk of each fraction.
- Improve existing biodegradation test methods (OECD 308/307, 2002a,b) to better simulate environmental conditions. This would include the route of introduction and how test substance is dosed (e.g. veterinary medicines – manure amended soil), land use practices such as ploughing and natural occurrences such as drying and wetting. In addition, evaluate the use of moving or fluid bed sediment water systems for determining sediment degradation rates.
- Develop screening methods for assessing NER/BR and determining the bioavailability of ER and NER.
- Understand / characterise conditions that may 'perturb' natural soil and result in unanticipated release or enhanced formation of residue:
  - Freezing and thawing of soil matrix.
  - Application / tilling of manure into soil.
  - Wetting and drying.
  - Other.

Syndicate 4-F [6]: NER PEC testing strategy

It was agreed that the first steps were to collate relevant physico-chemical data for the substance and to consider the environmental exposure pattern(s):

Figure 1: Identification of test strategy related to use patterns

The following figure shows that the type of testing is influenced by the product used and the route of exposure.



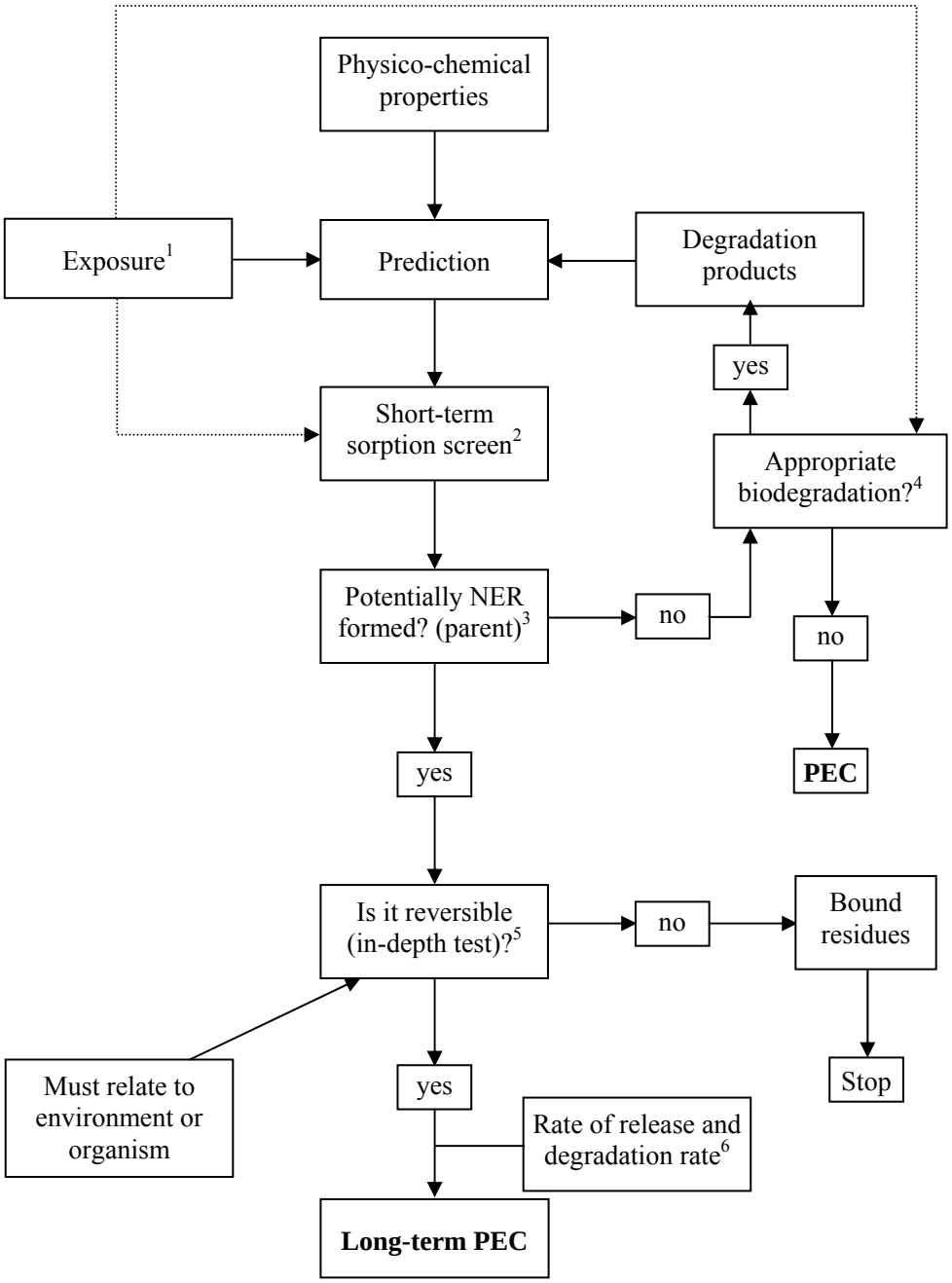
Research questions

- What would the short-term sorption screens look like? How could the data be interpreted?
- How would you address the reversibility of the partitioning?
- How could you address the formation of degradation products? What type of test would be appropriate and would you always need to identify them?
- What would a long term test to establish long term steady state (desorption and degradation) look like?
- How could these tests be related to appropriate ecotoxicity tests?

[6] Participants in syndicate group 4-F are listed in Appendix G.

Figure 2: PEC testing flow chart for bound residues and NER

The subsequent PEC testing flow chart was developed, keeping in mind the relevant exposure pattern.



Notes to Figure 2.

The syndicate clarified what they considered to be NER and BR. NER is a reversible binding, whereas a BR is irreversible binding and includes formation of covalent (or equivalent) bonds.

[1] Exposure considerations (Figure 1) must be taken into account when determining the PEC, considering the formation of NER/BR. For example, terrestrial (soil) tests are not appropriate for a water-soluble (non-sorptive) substance which is discharged down the drain, where the environmental exposure will be via sewage treatment and the aquatic environment.

[2] Appropriate short-term sorptive screening tests do not currently exist for all environmental compartments and read across from one matrix e.g. soil, to another e.g. sewage biosolids, is not appropriate for most chemicals. The environmental exposure should guide the selection of the appropriate test.

[3] Initially the parent should be considered. If during the evaluation, degradation products are formed then they too should be assessed for their partitioning behaviour.

[4] Appropriate biodegradation testing should consider the environmental compartment (exposure) that is relevant. For example if the terrestrial compartment is exposed, then a suitable soil test should be used, not an aquatic test such as OECD 301, 309 or 310 [OECD 1992a, 2004a, 2006, respectively] (unless scientifically justified). Suitable triggers for quantifying the formation of degradation products should be considered (e.g. >10% of parent at the end of the test). It may not be necessary to identify the degradation products if they are non-toxic (e.g. see Syndicate 4-G – soup testing). If suitable methods are available, degradation products may be predicted using validated QSARs.

[5] To differentiate between NER (reversible) and BR (non-reversible) binding, some in-depth investigation will be required. Whilst the details have yet to be developed, from a risk assessment perspective, this distinction is important. BR would not be bioavailable and therefore of no concern. NER, on the other hand, could be bioavailable and therefore requires further investigation. It is important to relate this back to the environment being exposed and the indigenous organisms present in the appropriate compartment.

[6] When NER is formed, the long-term PEC could be estimated from the rate of release under realistic (or as a more conservative scenario, extreme) environmental conditions and knowing the rate of degradation of the NER fraction. Degradation products in the NER fraction should be considered using predictions and/or suitable testing approaches.

Syndicate 4-G: PNECs

Topics for discussion

- Matrix influence on degree of bound residue formation / release (e.g. pH).
- Release over time.
- Release as a different chemical.
- Build-up over time.
- Differences among organisms (e.g. different ingestion / digestion conditions).
- Direct toxicity versus secondary poisoning.
- Mixtures.

What are the appropriate tests?

- Emphasis on organisms that consume soil / sediment.
- NER and BR formation dependent on matrix, which requires consideration in selecting test matrix / material.
- Time – depending on mechanism of residue formation and release, partitioning equilibration alone may not be sufficient to represent NER and BR in nature.
- If current chronic tests are sufficient for assessing effects of parent in the absence of NER/BR, are they therefore sufficient for assessing effects of NER/BR?

Research questions

- What is the potential for a pairing of accumulation test with OECD 308 test for chemicals (OECD, 2002a) with appropriate characteristics?
- There is a need for an ecotoxicity test to assess the extent of NER bioavailability and potential release of NER as related to period of exposure, relevant environmental conditions (temperature, moisture, weather), changes in conditions (e.g. seasonal variation; freeze / thaw conditions) and various land management practices (e.g. tilling; irrigation). Is it possible to develop such a test? Is it possible to develop a screen (laboratory / in-field) for such a test such that rapid regulatory decisions can be made without full term studies?
- Can increasing the exposure concentration be used to simulate effects of multiple years of residue formation?
- Can the freely dissolved chemical paradigm be used to assess NER, their effects, and connect to the PEC? Can deviation from this paradigm be used as an indicator that bound residues are creating a novel effect?
- Can sequential extraction approaches be used to create relevant samples for ecotoxicity testing, either by testing the fractions or testing the matrix that remains?
- Ecotoxicity of mixtures of parent and/or degradation products (present as ER/NER/BR) e.g. soup testing.

Syndicate 4-H: PNECs

Chemical

Unavailable / available.

Risk Assessment

Tested the parent compound. → We know how to do that.

Concern is the fate issue: What to do with the bound residual?

Prove the predicted risk is the true risk under field conditions.

1. Prove that the bound residue is truly bound:
   - Need to do sequential extractions to prove that it is truly bound.
   - Various solvent systems sequentially.
   - Solvents are much like soil organic C.

2. Do not know what the bound residue is:
   - Cannot do analytical.
     a) Assume it is parent:
        - A lot is known about the properties of the parent; in particular the degradation rate.
        - Model: Simulate the PEC for various scenarios; realistic worst case.
        - Do a series of release rates and compute PEC.
     b) Is it degradate?
        - Could ecotoxicity tests be performed at the same time as the degradation test, thereby addressing the ecotoxicity / bioavailability of the parent and/or degradation products?

Syndicate 5-I: Environmental risk assessment of bound residues

1. *How can we develop an integrated testing strategy that includes the risk of bound residues?*
2. *What would be the overall strategy? Does it address the bioavailability of NER?*

Screen

For industrial chemicals where there is a need to assess high numbers of chemicals, an initial screening step was felt valuable in helping to maintain procedural efficiency. Conventional approaches can be taken to discriminate chemicals that present no concern and those that require further work to evaluate whether they are likely to be ecotoxic under proposed conditions of use. The first step is to simply assume that 100% of the chemical is available, i.e. there is no reduction in bioavailability and the inherent ecotoxicity could be expressed in proportion to the total exposure concentration. A conservative assumption on the duration of emission to the environment is also necessary to ensure a worst case estimation of PECs for chemicals that might be persistent. For example, repeated / continuous annual emissions for, say, a 20-year period was felt adequate in estimating an appropriate 'steady state' PEC.

A useful next step in the risk assessment process would be to use physico-chemical data and potential structural alerts, based on knowledge of functional groups and interactions with environmental matrices, to identify the potential for strong binding. Such knowledge has not yet been developed. An investigation into the possibility of such an approach could be made by a team including computational chemistry experts working with environmental chemists experienced in residue extraction techniques. This idea was developed as one of the workshop RfPs (Appendix H).

It was considered unlikely that readily biodegradable chemicals would present residue issues due to the parent structure, but that biodegradation products could, e.g. in the cases where parent structures degrade to more hydrophobic structures.

The chart (Figure 3) could be merged with the PEC assessment chart produced by Syndicate 4-F to produce an integrated approach addressing both fate and effects.

Subsequent to the workshop, the organising committee have merged these into an overall risk assessment scheme (Figure 5 in Chapter 6).

In-depth assessment

As a first step it is necessary to obtain inherent ecotoxicity data for the chemical, i.e. determined under conditions favouring maximum bioavailability in water.

Determining the empirical ecotoxicity of NER/BR may require that the chemical is present in a representative matrix at relevant concentrations. This may mean that chronic exposures are required.

It should be advantageous to model / predict expected environmental concentrations prior to ecotoxicity testing. Predictions could be compared to chemical extraction procedures in order to verify predictive methods based on structural rules (functional groups).

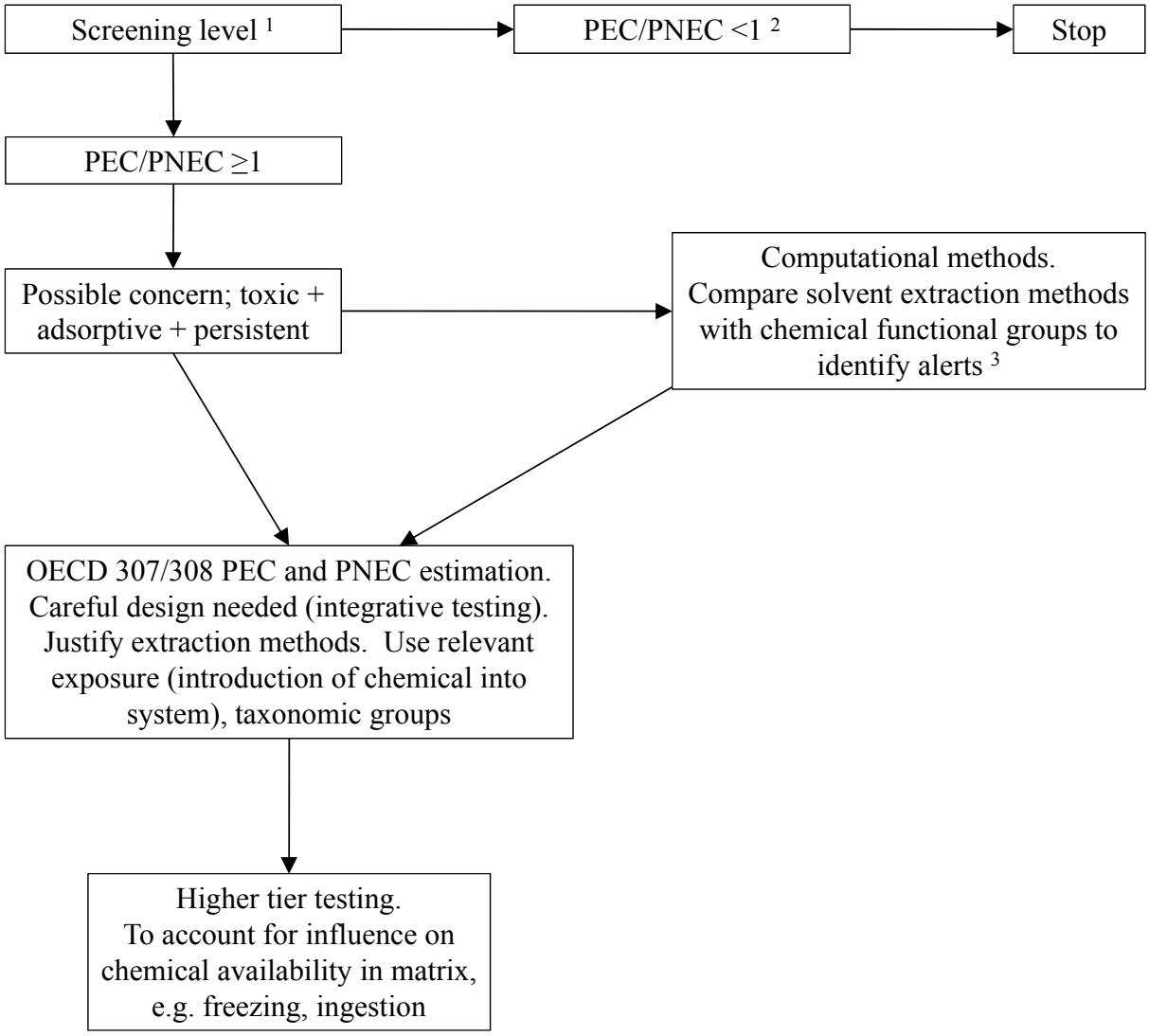
Simulation studies

A next step could be to run a simulation study such as OECD 307 (2002b) or OECD 308 (2002a). A key aspect of the study would be to justify the selection of chemical extraction methods used to demonstrate whether or not the chemical is extractable. An important question arose over the predictive value of chemical extraction techniques and whether even the most conservative methods can predict future increases in bioavailability resulting from matrix changes or from ingestion. There were diverging views on this.

There is existing guidance on aspects of the study design including use of natural and representative soils or sediments, chronic exposure to organisms from relevant taxonomic groups. Studies should be designed for specific requirements, i.e. they should not be prescriptive protocols.

If necessary a further tier of testing may be undertaken to assess the influence of changes in environmental conditions on bioavailability, e.g. freeze / thaw cycles, changes in moisture content.

Figure 3: PNEC testing flow chart for NER



[1] If readily biodegradable do conventional risk assessment – at least for parent

[2] Worst case assumptions: 100% available; 20-year emission (steady state)

[3] Alerts could also be transformation products with different sorption properties

Syndicate 5-J: Environmental risk assessment of bound residues

Development of a decision tree for considering the bioavailability of bound residues in chemical assessment

A flow diagram for considering 'bound residues' in chemical assessment was developed (Figure 4). Additional important concepts are captured below, which have some overlap with Syndicate 4-F.

The decision tree was structured with the intent of developing a weight of evidence for chemical assessment endpoints as well as for supporting testing strategies. It was also structured to consider the interaction of various elements with each other (chemical profiling informing solvent extraction testing). It commences with chemical profiling for binding potential that can be understood largely from chemistry (e.g. known binding mechanisms such as covalent and non-covalent interactions). Chemical profiling alone cannot take into account all possible substrate interactions therefore a second tier involves characterising the extent to which a chemical is bound to a substrate considering both laboratory extraction methods and simulated or *in vivo* gut bioaccessibility testing. At this stage it is important to anticipate the likely environmental substrate(s) to which the chemical may come into contact (e.g. biosolids spread on soil). This step also involves anticipating the potential for increased bioavailability under field conditions, such as physical disturbances from agricultural practices or bioturbation.

The consideration of how a substance enters the environment (mode of entry) is then taken into account because this will determine the likely partitioning media and will thus be important for considering exposure media and corresponding hazard based tests. The approach then suggests that for the hazard side of chemical assessment (i.e. inherent ecotoxicity as well as persistence and bioaccumulation), the extent to which 'binding' of a chemical to a substrate influences its bioavailability and thus the impact on the property or endpoint in question should be examined. For persistence, for example, do strongly bound chemicals influence the kinetics of biodegradation and thus persistence as defined by regulations (DT_{50} and single media half-life criteria) and if a chemical is not 100% bound, how should biodegradation rate be determined? When considering bioaccumulation, binding affinity can help explain the lack of observed assimilation efficiency in dietary fish and mammalian testing for very poorly metabolised substances. For ecotoxicity testing, it may be important to create test exposure conditions that simulate the build-up of strongly bound residues over a long time period (i.e. dosing at predicted 20-year concentration levels).

It was agreed that the process illustrated in the PEC flow diagram developed under Syndicate 4-F was a good starting point for considering bound residues for the exposure side of the risk equation. Syndicate 5-J's interpretation of this diagram was that the flow diagram answers question of the impact of bioavailability on exposure concentrations. If chemicals are irreversibly

bound then a PEC may be set at the bound fraction concentration, because it cannot be easily known if this will remain in the longer term or upon uptake into organisms. If a substance is not very strongly 'bound' then biodegradation rate and metabolite characterisation become important and may influence the PEC concentration as well as the need to consider metabolites in exposure assessment.

Finally, evidence generated during chemical profiling, bound residue characterisation and laboratory and/or field testing are combined and weighted to prove or disprove a hypothesis set on the impact of bound residues for chemical assessment endpoints or to support a line of reasoning in a testing strategy.

Additional issues discussed

The concept of 'bound residues' is important not only for risk assessment (i.e. PEC/PNEC) but also for the hazard assessment of chemicals (PBT, vPvB).

- Probably the most important question for considering the concept of 'bound residues' in chemical assessment is determining with a high degree of certainty that non-extractable residues remain so in the longer term under expected environmental conditions.
  - This relates to our ability to determine what is truly 'bound' under laboratory conditions (i.e. do repeated extraction routines still yield chemical residues however small that yield may be? If so, can we consider the chemical to be still bioavailable?), as well as our ability to anticipate mechanisms in the field that may lead to increased bioavailability of NERs (e.g. physical disturbance / bioturbation).
  - Evidence of biotransformation probably means the parent chemical is bioavailable, but what about the binding capacity of transformation products and thus conclusions on the bioavailability of the substance as a whole?

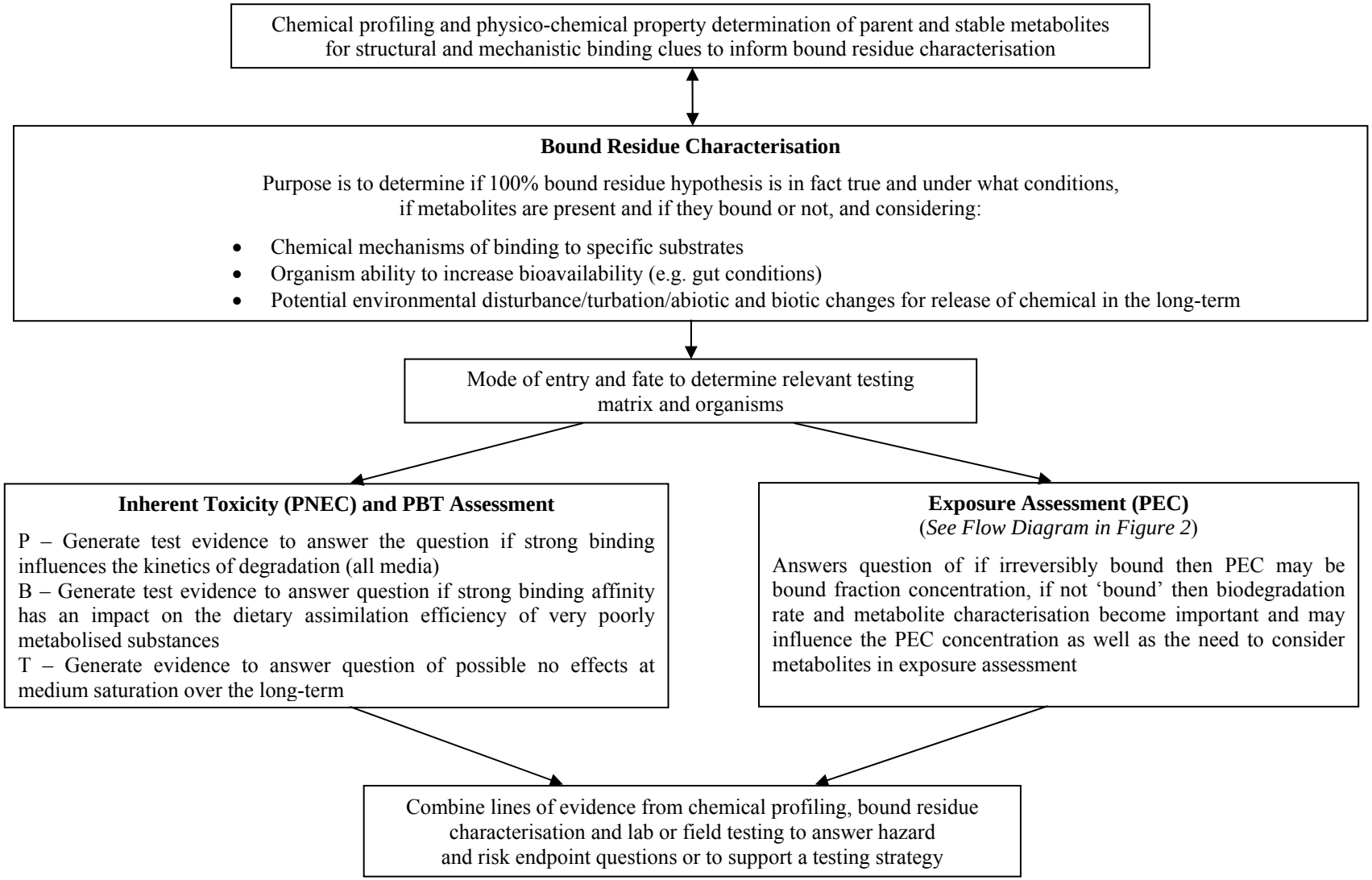
- The concept of 'bound residue' as appropriate terminology to describe the concept of chemical and non-chemical interactions of a substance with a substrate for use in risk assessment was also questioned (i.e. the terminology suggests a non-reversible process when in fact it may be reversible in the longer term). Terms such as weak, moderate and strong binding affinity were suggested.

- Results of significant 'binding' can be used to help inform which tests for hazard and risk assessment are needed and how they should be conducted.
  - Results of residue extraction tests can be used to explain the observations made in other standard tests used in risk assessment.
    - For results showing low biodegradation when structurally speaking it might be expected that the chemical is susceptible to biotransformation.

  - For fish dietary biomagnification (BMF) tests and biota-soil or biota-sediment accumulation factor (BSAF) tests to help explain low tissue residue values and dietary assimilation efficiency.
  - Evidence of significant NER may lead one to conclude that ecotoxicity tests involving a solid phase should also be considered *a priori* for risk assessment or to help explain no effects at saturation (e.g. benthic macroinvertebrate and/or oligocheate and/or earthworm, etc, tests for organisms that ingest sediment or soil).

- It was not suggested that evidence of significant 'binding' be used to directly negate hazard or risk assessment results but rather in a weight of evidence to explain chemical fate and ecotoxicity results both measured and predicted.
- It was suggested that a battery of solid phase tests is preferred to a single solid phase test (e.g. to address trophic differences and interspecies variability).
- For soil and sediment, often ecotoxicity results from exposure to the bioavailable pore water phase of a chemical. Concentrations of highly sorbed chemicals in the guts of invertebrates are often, but not always, at equilibrium with exposure media (i.e. BMF = 1, BSAF = 1). It was suggested that whole soil and sediment toxicity tests address the potential ecotoxicity of bound residues and non-bound residues in a holistic manner and can address this issue.

Figure 4: Consideration of bound residues in chemical profiling and/or hazard classification



Conclusions from the syndicate discussions

The following needs emerged from the syndicate sessions:

- Extraction strategies that clearly identify available and non-available fractions.
- An ecotoxicity testing strategy for NERs that addresses the potential effects of residues that may be released by soil / sediment ingesting organisms, in addition to the parent compound, and their transformation products that may also be present.
- Increased understanding of binding mechanisms and predictive methods to quantify and characterise NER.
- Knowledge base for how environmental changes (ploughing, seasonal variation and freeze-thaw cycles) may impact the behaviour of NER and BR.

Risk assessment paradigms that were discussed in the syndicates presented an iterative process. The risk assessment could begin with 'worst-case' (conservative) assumptions, accumulating scenarios with relatively simplistic models or data. If a potential risk was suggested, then a more refined risk assessment using data developed from appropriate screens, simulations and environmental monitoring could be performed. Such a paradigm was considered applicable to the risk assessment of NER and BR. However, the current methodologies were considered inadequate and would require research prior to adoption into regulatory schemes.

A total of nine requests for research proposals (RfPs) were suggested during the workshop (see Appendix H), these fall into the four major themes (RA1-RA4) detailed in Table 1.

Table 1: Research topics and proposals for how to address [7]

Research area	Description	Action
RA1: Extraction strategies	Non-extractable residues are currently characterised by a pragmatic extraction approach by determining whether they are extractable or not. This extraction approach has been historically implemented with various solvents under varying conditions and not necessarily linked to the properties of the chemical, nor the matrix. There is a need to develop a standard framework for extraction methods and to associate the extractable fractions (leachable and NER) with both a level of bioavailability (accessibility) and appropriate test organism(s) for the appropriate environmental compartment. It is recognised that the development of new methods to screen bioavailability of such fractions may be needed to validate this association. This framework would support a consistent interpretation of the data and provide a transparent basis for assessing the potential risk of NER.	Develop guidance document for extraction schemes in soils and sediments based on functional groups and provide scientific rational for approaches recommended. (RfP 1). A one year targeted activity (Ecetoc TF). Review current literature to assess if chemical methods from extraction schemes compare to uptake in organisms. (RfP 3). A one year literature review followed by a workshop to consider recommendations for further research.
RA2: Ecotoxicity and environmental fate testing for NERs including mixtures and accumulation	Ecotoxicity testing strategy for NERs that addresses the potential effects of residues that may be released by soil / sediment ingesting organisms, in addition to the parent compound, and their transformation products that may also be present. Risk assessment of bound residues could begin with 'worst-case' (conservative) assumptions. If a potential risk was suggested, then a more refined risk assessment could be performed. However, the current methodologies were considered inadequate and would require research prior to adoption into regulatory schemes. Improvements to existing biodegradation and effects testing methodologies including addressing the route of introduction, how the test substance is dosed, the dynamics of the compartment and the effect of perturbation of the natural soil / sediment on the behaviour of bound residues.	Methods are needed to determine the potential ecotoxicity of NER, as an input to regulatory risk assessment. Ecotoxicity of NER will be evaluated by comparing ecotoxicity in tests with no NER formation (parent chemical only), with NER only (ER has been removed from the test matrix); and both ER and NER. Soil and sediment dwelling organisms including those with gut segments of high humic matter extraction potencies (e.g. alkaline pH) should be considered in the choice of the appropriate organisms for the testing strategy. Assessment of ecotoxicity in the context of measured / predicted freely dissolved chemical concentration will be used to both compare potencies of different exposures, and to determine whether the freely dissolved paradigm is suitable for evaluating potential effects of NER. Because the regulatory concern over NER stems from NER formation in tests like OECD 307/308 (OECD 2002b,a, respectively), test procedure development should provide for NER formation in a similar manner. Research proposal to be developed (RfP 9).

[7]These research areas are listed in the order RA1 – RA4, that being the order in which the Organising Committee have prioritised this research.

***Table 1: Research topics and proposals for how to address** (cont'd)*

Research area	Description	Action
RA3: Predictive methods to quantify and characterise NERs	It was generally recognised that binding of organic and inorganic compounds to environmental matrices may entail a number of mechanisms. There is a need to better understand the overall process, and a need for deterministic methods that better characterise the individual mechanisms, as well as mechanisms collectively. Such work would characterise the mechanism of binding between a test substance (having identified the key functional group(s) responsible for binding) and the key functional group(s) in the matrix (e.g. sand, silt, clay, organic matter and/or dissolved organic matter). With the development of that knowledge base, better predictive models would be achievable.	Characterisation of the bonds between a compound and an environmental matrix. Research proposal to be developed (RfP 2). Develop simple and rapid assays to evaluate irreversible binding of chemicals to soil and sediment constituents. Relate binding characteristics to underlying mechanisms. Research proposal to be developed (RfP 4). Develop understanding of major mechanisms of non-covalent and covalent binding to soil and sediment matrices. Develop structural rules for differentiating between high and low extent of non-extractable residues. Research proposal to be developed (RfP 5).
RA4: Impact of environmental changes on NER	There is a need to develop more understanding of how environmental changes (land management, seasonal variation and freeze-thaw cycles) may impact the behaviour of NER and BR.	Develop better understanding of the impact of environmental changes (temperature variation, humidity variation, changes in redox conditions in response to flooding) on the availability / release of bound residues. Define plausible critical conditions where environmental change would impact the bioaccessibility / bioavailability of bound residuals of chemicals; provide plausible mechanisms for the occurrence of this change; relate results to eventual changes in risk assessment strategies. Research proposal to be developed (RfP 6). Develop better understanding of how changes in land use (such as minimal tillage or fertilisation, or increased chemical application, crops or afforestation / deforestation) affect the non-extractable pool of chemicals in soil. Will a better understanding of soil organic matter pool dynamics be key to understanding the non-extractable pool dynamics? Research proposal to be developed (RfP 7). How do changes in land use affect the non-extractable pool of chemicals in soils? For one plausible and relevant change in land use (for example change from tillage to non-tillage agriculture and increased of agricultural chemicals or removal of flood defence to allow temporary flooding of land) investigate systematically and how the non-extractable pool of chemicals will be affected and under what conditions the bioavailability / bioaccessibility of the non-extractable pool of chemicals in soil will change. Research proposal to be developed (RfP 8).

6. CONCLUSIONS AND RECOMMENDATIONS INCLUDING RISK ASSESSMENT SCHEME TO ADDRESS BOUND RESIDUES

Understanding the issues surrounding the phenomenon of bound residues continues to stimulate considerable debate. There are contrasting views about whether such residues are truly irreversibly bound, immobilised and non-biologically available or whether their long-term behaviour is impossible to predict. The discussions from the syndicate sessions identified a number of areas that require a greater scientific understanding if the assessment of bound residues and their respective fate and effects in the environment is to be improved. Nine RfPs were drafted during the workshop. Although not given a priority during the workshop, in the process of writing this report, the authors sought an indication of the priority for the work from the workshop participants. From this information the following areas were identified where further research would be considered to significantly improve the understanding of the potential risks associated with bound residues:

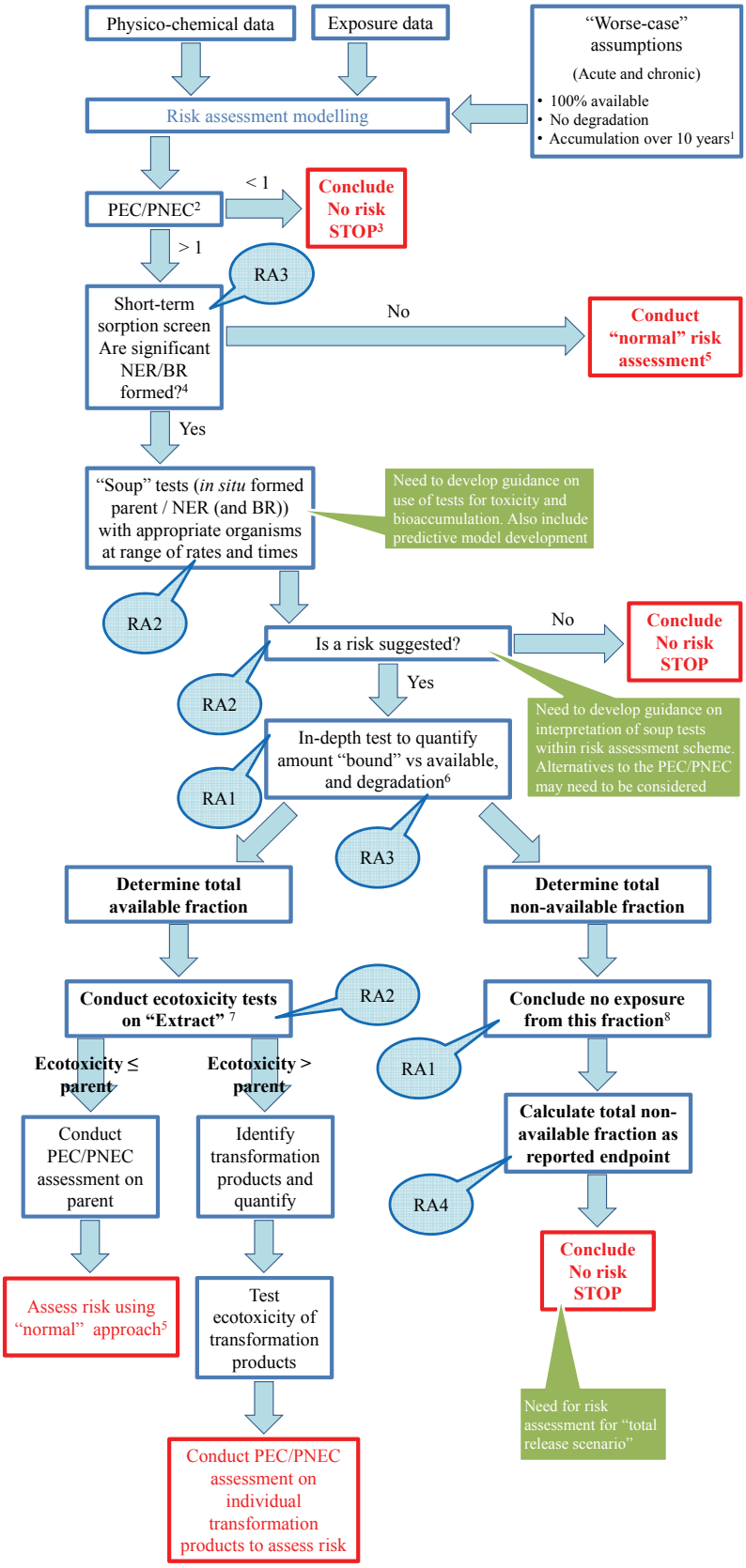
- **Understand the relationship between extraction technique and bioavailability.** Non-extractable residues are currently characterised by a pragmatic extraction approach by determining whether they are extractable or not. This extraction approach has been historically implemented with various solvents under varying conditions and not necessarily linked to the properties of the chemical, nor the matrix. There is a need to develop a standard framework for extraction methods and to associate the extractable fractions (leachable and NER) with both a level of bioavailability (accessibility) and appropriate test organism(s) for the appropriate environmental compartment. It is recognised that the development of new methods to screen bioavailability of such fractions may be needed to validate this association. This exercise would support a consistent interpretation of the data and provide a transparent basis for assessing the potential risk of NER. It is recommended that an ECETOC TF be commissioned to develop a framework for intelligent extraction strategies.
- **Understand the chemical mechanisms underlying NER formation.** It was generally recognised that binding of organic and inorganic compounds to environmental matrices may entail a number of mechanisms. To better understand the overall process, there is a need for deterministic methods that better characterise the individual mechanisms, as well as mechanisms collectively. Such work would characterise the mechanism of binding between a test substance (having identified the key functional group(s) responsible for binding) and the key functional group(s) in the matrix (e.g. sand, silt, clay, organic matter and/or dissolved organic matter). With the development of that knowledge base, better predictive models would be achievable.
- **Develop tests to determine the potential ecotoxicity of NER as related to realistic environmental conditions.** Combining existing fate and effects testing were suggested as one means of accomplishing this, as well as improving existing test methods. By doing so, appropriate test methods could be designed to represent how the residues are introduced into

the environment (e.g. veterinary medicines – test substance applied in manure amended to soil), dynamics of the compartment (e.g. flow through system) and the effect of perturbing the natural soil / sediment which may result in an unanticipated release (e.g. ploughing, drying-wetting and freeze-thawing). Such research would be helpful in interpreting the risk of NER and BR. The use of 'real' sediment and soil matrices for ecotoxicity testing could address the potential concern for parent compound, their transformation products, the bound residue and any residue that may be potentially accumulated and/or released over time.

- **Understand the potential for NER release or changes in bioavailability in response to aging or environmental change.** To systematically investigate how the non-extractable pool of chemicals in soil or sediment could be affected by any foreseeable environmental perturbation. To examine under what conditions the bioavailability / bioaccessibility of the non-extractable pool of chemicals in soil or sediment could change and hence increase the potential risk to non-target organisms.

The feedback from the syndicate and plenary discussions, including the knowledge gaps and the proposed research projects have been used to develop a framework outlining a possible approach for advancing and improving the risk assessment of NER (Figure 5). It is anticipated that as the research (outlined above) is completed this framework would be re-visited to assess its potential usefulness in environmental risk and PBT assessments.

Figure 5: Risk assessment scheme to address non-extractable residues



Notes to Figure 5.

RA1 – RA4 refer to the research areas described in Table 1 (see Conclusions from the syndicate discussions)

[1] The use of 10 years is assumed as a realistic worst case. Precedent for this is from REACH (ECHA, 2008 in Table R16-10, p 47).

[2] The current agreed safety factors for calculation of all PNEC values should be applied in this assessment.

[3] This is assumed to account for any potential transformation products. A transformation product would need to be significantly (approximately ten times) more ecotoxic than parent or bioaccumulate to present an increased risk.

[4] A screen using realistic environmental matrices should be developed. Methodology, e.g. OECD 106 (2000) could be modified / developed. Guidance should be developed to define 'significant'.

[5] 'Normal' risk assessment refers to existing regulations, e.g. in the EU, REACH, EMEA, pesticides.

[6] This test should include an agreed framework for extraction methods to relate the behaviour / partition of the chemical to its bioavailability to allow a full assessment.

[7] If extract testing is not practical / desired then you can bypass extract testing and proceed direct to identification and quantification of transformation products to assess risk.

[8] A conclusion of no exposure would be justified on the basis of a robust and agreed extraction framework indicating which fraction was available to organisms. This will need to be supported by suitable data.

ABBREVIATIONS

ASE	Accelerated solvent extraction
BMF	Biomagnification factors
BR	Bound residue
BSAF	Biota-soil or biota-sediment accumulation factor
CAS	Chemical abstracts service
DT_{50}	Disappearance time (50%)
ER	Extractable residue
ERA	Environmental risk assessment
K_d	(Apparent) distribution coefficient
K_{oc}	Organic carbon-water partition coefficient
LOEC	Lowest observed effect concentration
MBP	Microbial biotransformation products
NER	Non-extractable residues
NOEC	No observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic aromatic hydrocarbon
PBT	Persistent, bioaccumulative, toxic
PEC	Predicted environmental concentration
pKa	Acid dissociation constant
PNEC	Predicted no effect concentration
pp-LFER	Polyparameter linear free energy relationship
PPP	Plant protection products
QSAR	Quantitative structure activity relationship
QSPR	Quantitative structure-property relationship
RfP	Request for proposals
SPME	Solid phase micro-extraction
UM-PPS	University of Minnesota pathway prediction system
US EPA	United States Environmental Protection Agency
vPvB	Very persistent, very bioaccumulative

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APPENDIX A: CONSIDERATION OF BOUND RESIDUES IN REGULATORY ENVIRONMENTAL RISK ASSESSMENT – PROBLEMS AND OPEN ISSUES

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It is commonly agreed that the formation of non-extractable or bound residues of chemicals in soil or sediment will have a significant impact on their behaviour in the environment. Consequently, this issue is also addressed in regulatory environmental risk assessment. This paper focuses on the risk assessment of active substances, comprising pesticides, biocides, human and veterinary pharmaceuticals. Depending on the intended uses of those active substances, direct or indirect contamination of environmental compartments could occur. Non-extractable residues could either be formed at the site of application or point of contamination or they could be introduced in a compartment together with matrices like sewage sludge or manure.

In the regulation of chemicals, two legal aspects have to be considered: The required amount and quality of data to assess certain properties must be defined as well as the procedures for evaluating those data and for decision-making. For pesticides, the legislative act currently in force at EU level is Directive 91/414/EEC. Annex II on data and information requirements mentions non-extractable residues in the context of soil metabolism data (route of degradation). Non-extractable residues should be "identified (…), where feasible" and must be "characterised and quantified" when they exceed 70 % after 100 d of incubation in a laboratory experiment on substance degradation in soil, whereupon "techniques and methodologies applied are best selected on a case-by-case basis". Directive 98/8/EC for biocides refers more generally to non-extractable residues by stating that in some cases – depending on the product type – the "extent and nature of bound residues" must be investigated. In contrast, bound or non-extractable residues are currently not mentioned in the relevant directives and guidelines for human (Directive 2001/83/EC, amended by Directive 2004/27/EC) or veterinary (Directive 2001/82/EC, amended by Directive 2004/28/EC) pharmaceuticals. However, manure from animals treated with veterinary pharmaceuticals has meanwhile been identified as a relevant source of environmental contamination. Hence, a recent concept paper on the environmental fate assessment of veterinary pharmaceuticals in manure now also states that non-extractable residues should be considered in the evaluation, but without providing specific guidance.

Decision criteria with respect to non-extractable residues only exist for pesticides and biocides and are contained in the respective Annexes VI of Directive 91/414/EEC and 98/8/EC. By mentioning formation of non-extractable residues in combination with mineralisation, these

criteria are in principle targeted at insufficient degradation of a substance in the environment: No authorisation shall be granted if more than 70% non-extractable residues are formed under laboratory conditions after 100 days while mineralisation remains below 5%. However, active substances fulfilling these criteria could still be approved if it could be scientifically demonstrated that under field conditions "there is no accumulation in soil at such levels (…) that there is an unacceptable impact on the environment" (pesticides) or "there is no unacceptable accumulation in soil" (biocides). Factually, these unless-clauses are often addressed by conventional studies on active substance accumulation in soil; with the consequence that the non-extractability of residues is then no longer in the regulatory focus.

The actual assessment of non-extractable residues in active substance regulation is based on an operational definition: "Chemical species originating from pesticides used according to good agricultural practice that cannot be extracted by methods which do not significantly change the chemical nature of these residues." Thus, the amount of non-extractable residues depends on the extraction methods used. Since the relevant test guidelines for evaluating substance transformation in soil (OECD 307, 2002b) or sediment (OECD 308, 2002a) do not specify analytical procedures, there is currently no formally standardised approach for determining the amounts of non-extractable residues of different compounds.

In summary, current regulatory schemes for active substances do account for formation of non-extractable or bound residues, but

- Rely on an operational definition (non-extractability);
- do not allow to conclude on the nature of non-extractable residues;
- do not quantitatively link occurrence with potential effects;
- do not encompass all types of active substances.

A number of open issues and questions can be identified, which should be addressed to improve the regulatory risk assessment of compounds forming non-extractable residues. Regarding the definition and nature of non-extractable residues, it should be explored how the operational definition of non-extractable residues can be further developed to include also mechanistic aspects. Recommendations for meaningful extraction sequences are required, which should allow differentiation between different binding types of non-extractable residues and would possibly also allow predictions on their expected behaviour under real field conditions.

It should be investigated how the association of non-extractable residues to humic substance fractions corresponds to their fate and behaviour in the environment. More information is also required on the dynamics of non-extractable residues on a long-term time scale. This would also comprise investigations on the binding capacity of soils or sediments as well as on a possible remobilisation of residues under certain circumstances.

To obtain reliable information on the actual bioavailability of non-extractable residues or on their possible remobilisation by organisms, it will be necessary to perform ecotoxicological tests. Harmonised testing protocols are needed, which could possibly be based on existing testing schemes for assessing ecotoxicological effects.

The core question for a final risk assessment will be whether reliable differentiation is possible between 'potentially critical' (may be remobilised and/or exert effects) or 'uncritical' (irreversibly bound; biologically inert) residues. Criteria are also required for evaluating the bioavailability of non-extractable residues and their possible remobilisation under certain environmental conditions. In this respect, application of sewage sludge or manure to soil, or land use changes, are important topics to be considered.

In summary further development of regulatory schemes will thus require

- Additional information on the nature of non-extractable residues;
- reliable models for predicting the long-term fate of non-extractable residues in the environment;
- reliable models for predicting the interaction between non-extractable residues and organisms in the environment.

APPENDIX B: AVAILABILITY OF ORGANIC CONTAMINANT RESIDUES IN SOIL

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Forschungszentrum Jülich, Germany

On behalf of

Kirk T. Semple
Lancaster Environment Centre, Lancaster University, UK

Risk assessment and regulatory management should minimise the impacts of chemicals on human health and the ecosystem. As contaminants age in soil, they become increasingly unavailable to soil biota, increasingly resistant to desorption and reduced toxicity of the chemical. The observed decline in toxicity and biodegradability as a result of aging and the accompanying sequestration may represent a natural remediation process because, although the chemical is not destroyed, the exposure of susceptible organisms to the harmful effects of the chemical, and thus the risk from it, decreases with time. Sequestration occurs by partitioning into soil organic matter and entrapment of the molecule within soil micropores through sorption and diffusion processes, leading to a reduction in environmental reactivity and mobility of a chemical. Thus, organic matter content will affect the bioavailability / bioaccessibility, extractability and mobility of compounds that bind to organic matter. As a result of this, there is some concern about the long-term fate of highly sequestered or non-extractable residues. However, in discussing the risk of non-extractable / bound residues, Calderbank (1989), noted that "the important matter is not so much how the residue is defined but the question of its biological availability".

Organic solvent extracts are poor predictors of bioavailability and there is compelling evidence suggesting that vigorous or exhaustive extractions will fail to predict declining contaminant availability as compounds age in soil. The difficulty with trying to chemically mimic bioavailability lies with the fact that it can be specific to particular taxonomic groups. The availability of a compound to an organism may depend on a number of factors, including the routes of exposure (e.g. dermal, intestinal, or respiration in animals); trophic status and 'lifestyle', all of which will differ for different flora and fauna living in the soil. In light of this information, it would seem an impossible task to seek a generally applicable chemical means of mimicking bioavailability across all compound classes and soil biota. Fundamentally, what do chemical solvent extractions tell us about biological soil processes? The primary objective for performing these analyses is presumably to provide information on the exposure of living organisms to, and hence the risk from, these chemicals. There is overwhelming evidence to suggest that chemicals become less available for uptake by organisms, for exerting toxic effects, and for biodegradation as they age in soils. Yet the types of chemical analyses required for making regulatory decisions usually fail to show a correlation between extractability and a diminution in bioavailability.

There is remaining uncertainty and concern in sections of the research and regulatory communities about the long term bioavailability / bioaccessibility of anthropogenic chemicals and the potential for their possible future remobilisation from soil.

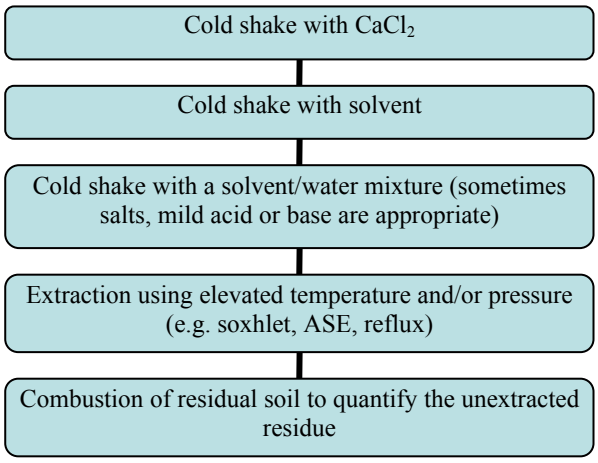
Conclusions

- Bioavailability / bioaccessibility rather than total concentrations is very important when considering fate, behaviour and risk of organic chemicals in soil.
- There is no single value for bioavailability or bioaccessibility – organism-dependent and species-dependent.
- Do non-extractable residues matter?
  - Remobilisation of soil bound residues – long term low level release.
  - Low level long term effects in soil.
  - Long range transport in environment.
  - Persistence per se.
- Is bioavailability / bioaccessibility the key?
  - Calderbank (1989) “the important matter is not so much how the residue is defined but the question of its biological availability”.

APPENDIX C: EXPERIENCES WITH BOUND RESIDUES IN ENVIRONMENTAL FATE STUDIES ON PLANT PROTECTION PRODUCTS

Robin G. Oliver
Syngenta Crop Protection, UK

When extraction methods for soil and sediment studies are developed, both regulatory requirements and operational efficiencies are taken into account. Methods are therefore developed to extract the maximum proportion of the residue possible in the minimum number of extraction steps. A typical extraction method is outlined below:



In a few cases where rapid, strong sorption takes place, extraction methods that destroy the soil matrix have had to be employed. A notable example is the cationic herbicides from the bipyridyl class (paraquat, diquat). The extraction method for these requires extended reflux in strong acids.

Once the pattern of metabolism is established using radiolabelled compound in the lab, an extraction method is developed for enabling parent and key metabolites to be analysed in samples from field studies.

In cases where the unextracted residue accounts for a significant proportion of the applied residue the Schnitzer method is often used to fractionate the organic matter into fulvic acid, humic acid and humin. Less frequently, reagents such as sodium pyrophosphate, dithionite-citrate, and acid ammonium oxalate can be used to provide additional information as to which component of the soil the unextracted residue is associated with.

Extractions using cold shakes with solvent / water mixtures have been correlated with the residue fraction that is bioavailable to soil biota (often earthworms). In many cases particularly where residues in soil have 'aged' such extraction methods overestimate the bioavailable fraction (Alexander, 2000). This is particularly likely in the case of a typical extraction method for a PPP (plant protection products) because of the operation drivers to select solvents that are as efficient as possible. In a normally functioning soil, the organic matter is continually turned over by microbial processes resulting in the formation of less labile material and mineralisation. This natural turnover often results in the slow release and mineralisation of some of the recalcitrant / bound residues in soil.

APPENDIX D: EXPERIENCES WITH THE OECD 308 METHOD – HUMAN PHARMACEUTICALS

Jon F. Ericson *, Richard Murray-Smith[1], Tirso Garcia de Oteyza Feldermann[1], Bob Hannah[2], Andreas Hartmann[3], Birgit Hoeger[3] and Harry Yekel[4]
PhRMA Fate Strategic Initiative Team (SIT)
Astra-Zeneca[1], GlaxoSmithKline[2], Novartis[3], Pfizer and Wyeth[4]*

Five PhRMA companies participated in the review of OECD 308 studies (OECD, 2002a) conducted over the last 2-3 years. Compound specific information (product name, generic name, structure, CAS number) were blinded. Physico-chemical properties and raw data from 36 OECD 308 studies were shared and evaluated to address how the OECD 308 is used in the ERA and what has been learned from the studies in respect to non-extractable residues, extraction techniques, dissipation and biodegradation rates, and observations made when pharmaceuticals are grouped as a cation, anion or neutral.

A high level of sediment residues were observed in many cases by study termination at day 100, with a mean of 71% ±22. Non-extractable residues (NER) were also moderately high, with a mean of 44% ±23. When grouped by their ionic properties, the NER values for cations, neutral and anions were approximately 52%, 33% and 30% respectively. Cations with the highest level of NER also had the most unchanged parent and least amount of metabolites at study termination when compared to neutrals and anions. Anion and neutral compounds had relatively shorter parent degradation half-lives from the water-sediment system (mean of 29 and 30 days respectively) when compared to cations, with mean half-life of 87 days. Dissipation rate for total residues from aqueous phase averaged 15.5 days, with an apparent trend related to log D properties rather than soil / sediment k_d or k_{oc}.

Chironomid sediment toxicity studies (OECD 218, 2004b) available for 18 of the 36 compounds studies generally showed no toxicity for 12 of the 18 (LOEC reported as '> than'). For the other 6 compounds, the mean NOEC was 98 mg/kg. It was not clear how chironomid sediment toxicity is affected by bioavailability of residues via sorption and formation of NER.

A correlation was found between the percentage of parent found in extractable sediment at day 100 to the parent degradation half-life for the water-sediment system as determined from a first order regression of 5-6 time points. This would potentially support an abbreviated 1 time point (day 100) for use as a screen in Tier A testing rather than a high tier OECD 308 study.

Recommendations from the PhRMA Fate SIT include: 1) the use of a worst case PEC/PNEC sediment risk determination in the environmental risk assessment prior to employing high tier OECD 308 sediment biodegradation test, 2) in Tier A screening, consider OECD fate and degradation protocols addressing release through treatment plants rather than through spray drift, e.g. OECD 302 (OECD, 1981a,b; 1992b), 303 and 314 series (OECD, 2001 and 2008, respectively), 3) the need for standardisation of extraction schemes for determination of available and non-extractable residues, and 4) the potential use of an abbreviated OECD 308 study for screening purposes.

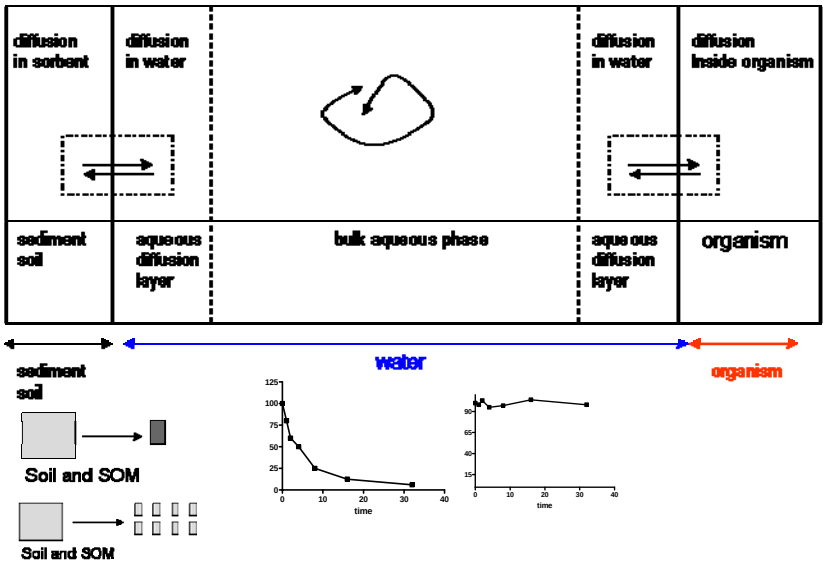
APPENDIX E: USE OF ANALYTICAL TOOLS AND KINETIC MODELS TO CHARACTERISE BIOAVAILABILITY AND BOUND RESIDUES

Joop L. M. Hermens
Institute for Risk Assessment Sciences, Utrecht University, the Netherlands

The exact meaning of the terms bioavailability, bioaccessiblity and bound residue is not straightforward. It is obvious, however, that total concentrations in soil often do not represent the concentrations of organic contaminants that are available for uptake into organisms. The freely dissolved concentration is a first step in better defining the exposure because the dose inside the organism, the cell membrane, or at the target is directly related to the freely dissolved concentration. Several analytical tools for measuring bioavailability have been developed during the last decades. The presentation will focus on measurement techniques for bioavailability, and more specifically of freely dissolved concentrations. Emphasis will be given to measurements with solid phase micro-extraction (SPME) as a simple sampling method.

Although for most of the time the concept of bioavailability, as well as measurement techniques, is based on the assumption of an equilibrium situation, it is clear that this assumption is certainly not always valid. The presentation will discuss the topic of bioavailability from a more dynamic point of view. These more dynamic aspects may also be important in the discussion of bound residues.

Bioavailability is a dynamic process: Some concluding remarks



- A matrix may change in time (quantity and/or quality).
- A change in matrix may affect bioavailability and bioaccessibility.
- The concentration in a compartment (including organisms) depends on rates of exchange and degradation processes.
- Dynamic (diffusion based) models will lead to a better understanding of the concentration in a compartment and subsequent potential risks.
- Dynamic models for simulation of worst case scenarios.
- Need for information of kinetic parameters for exchange between phases, degradation rates, diffusion coefficients, etc.
- A simple (single point) chemical extraction technique cannot describe this complex process.
- Dynamic modelling of fate of pesticides in soil (including leaching) has been done.

APPENDIX F: IMPLICATIONS OF BOUND RESIDUES FOR THE ASSESSMENT OF EFFECTS

Dave R. Mount
U.S. Environmental Protection Agency, Office of Research and Development, USA

Assessment principles used in evaluating chemical risks in water and sediment are used as a backdrop for considering the potential effects of bound residues. In broad terms, toxicological potency of chemicals in the environment is often indexed to chemical activity, which can be thought of as the 'chemical pressure' exerted by the environment onto the organism. Frequently, this chemical pressure is much lower than the total chemical concentration in the environment, and is correlated with specific fractions, such as the freely dissolved concentration in water. This approach has been effectively used to assess / predict chemical toxicity in complex matrices, such as sediment. For non-polar organic chemicals which distribute primarily by diffusion, the approach has proven fairly robust. For other chemicals, such as metals, there are many complicating factors, including temporal changes in chemical speciation and uncertainty regarding the implications of dietary and waterborne exposure. Examples from experimental work are presented to frame questions relevant to assessment of bound residues.

This abstract does not necessarily represent the views or policy of the US EPA.

Concluding questions

- Is it reasonable to assume that 'bound' chemical is not contributing meaningfully to chemical activity at present?
- Is it plausible that 'bound' chemical could be released under other biological conditions (e.g. the gut)?
- If changing environmental conditions can result in the release of bound chemical, is it likely to happen at a rate that would create risk?

APPENDIX G: SYNDICATE GROUPS

Syndicate 1-A

Moderator: Peter Burauel
Rapporteur: Tom Federle
Marc Geurts
Martin Holt
Caroline Moermond
Jason Snape
Henrik Tyle
David Werner
Tomohiko Yoshida

Syndicate 1-B

Moderator: Eric Verbruggen
Rapporteur: Torben Madsen
Jon Ericson
Thomas Knacker
Robin Oliver
Gary Roberts
Ed Schaefer
Michael Spiteller

Syndicate 2-C

Moderator: Gerrit Schüürmann
Rapporteur: Jörg Klasmeier
Marc Bonnell
Alistair Boxall
Dominic Di Toro
Joop Hermens
Martin Krauss
Jan Renger van de Veen
Mick Whelan

Syndicate 3-D

Moderator: Dave Mount
Rapporteur: John Parsons
Joop de Knecht
Margaret Feehan
Malyka Galay Burgos
Andreas Hoellrigl-Rosta
Reinhard Laenge
Fraser Lewis
Stuart Marshall
Andreas Schäffer
Alex Tait
Elisabeth Thumm

Syndicate 4-E

Moderator: Jon Ericson
Rapporteur: Ed Schaefer
Alistair Boxall
Peter Burauel
Tom Federle
Marc Geurts
Thomas Knacker
Martin Krauss
Torben Madsen
Henrik Tyle
Jan Renger van de Veen

Syndicate 4-F

Moderator: Gary Roberts
Rapporteur: Mick Whelan
Martin Holt
Jörg Klasmeier
Caroline Moermond
Robin Oliver
John Parsons
Jason Snape
Elisabeth Thumm
David Werner
Tomohiko Yoshida

Syndicate 4-G

Moderator: Fraser Lewis
Rapporteur: Dave Mount
Margaret Feehan
Reinhard Laenge
Andreas Schäffer
Gerrit Schüürmann
Michael Spiteller
Eric Verbruggen

Syndicate 4-H

Moderator: Stuart Marshall
Rapporteur: Dominic Di Toro
Mark Bonnell
Joop de Knecht
Malyka Galay Burgos
Joop Hermens
Andreas Hoellrigl-Rosta
Alex Tait

Syndicate 5-I

Moderator: Alistair Boxall
Rapporteur: Stuart Marshall
Joop de Knecht
Jon Ericson
Margaret Feehan
Malyka Galay Burgos
Thomas Knacker
Reinhard Laenge
Caroline Moermond
Robin Oliver
Gerrit Schüürmann
Michael Spiteller
Alex Tait
Henrik Tyle
Jan Renger van de Veen
Eric Verbruggen
Mick Whelan

Syndicate 5-J

Moderator: Joop Hermens
Rapporteur: Mark Bonnell
Peter Burauel
Tom Federle
Marc Geurts
Andreas Hoellrigl-Rosta
Martin Holt
Jörg Klasmeier
Martin Krauss
Fraser Lewis
Torben Madsen
Dave Mount
John Parsons
Gary Roberts
Ed Schaefer
Andreas Schäffer
Elisabeth Thumm
David Werner
Tomohiko Yoshida

APPENDIX H: REQUEST FOR RESEARCH PROPOSALS (RfPs)

Four broad themes emerged over the two-day workshop:

- Impact of environmental changes on NER.
- Need for predictive methods to quantify and characterise NERs.
- Framework for intelligent extraction strategies.
- Ecotoxicity testing for NERs including mixtures and accumulation.

RfP 1 – Guidance document for extraction schemes

Aim of the research

Development of guidance document for extraction schemes in soils and sediments based on functional groups and provide scientific rational for approaches recommended.

Research deliverables

- Extraction scheme for different kind of compounds with different functional groups.
- Input parameters from predictive methods (which scheme to use and why).
- Consider how to address potential degradation products.
- Publish the final outcome.

Cost and timing

€ 75 k 1-2 years.

RfP 2 – Characterisation of bonding

Aim of the research

Characterisation of the bonds between a compound and an environmental matrix.

Research deliverables

- Generate information on the nature of the binding sites and their impact on bioavailability, include parent compound and metabolites.
- Can the binding site(s) be modified?
- Investigate whether binding is reversible or irreversible.
- Mechanisms of binding due to physical entrapment or chemical binding.
- Effect of changes in matrix (e.g. effect of changes of fertiliser, ploughing, drying / wetting).
- Investigate whether different matrices result in different bonds.
- Soluble bound compounds (e.g. colloids).

Cost and timing

3-4 years.

RfP 3 – Comparison of bioassay bioavailability versus chemical extraction methods

Aim of the research

Review current literature to assess if chemical methods from extraction schemes compare to uptake in organisms.

Research deliverables

- Investigate changes in matrix.
- Include pore water and colloids in pore water.
- Experimental work to compare extraction techniques (described in output from RfP 1).
- Compare soils and sediments and different species.
- Include some ionic compounds.
- Partition based techniques / mimics.
- Gut fluid extraction *in vitro* methods.
- Is information from human gut uptake transferable?

Cost and timing

1-year literature review.
4-year experimental.

RfP 4 – Develop simple and rapid assays to evaluate irreversible binding of chemicals to soil and sediment

Aim of the research

Develop simple and rapid assays to evaluate irreversible binding of chemicals to soil and sediment constituents. Relate binding characteristics to underlying mechanisms.

Research deliverables

- Review studies on compound-matrix interaction leading to bound residues.
- High throughput assays to evaluate different types of irreversible binding to clay minerals, iron / aluminium oxihydroxides, natural organic matter in soil, sediment and sewage sludge.
- Explore influence of boundary conditions (pH, ionic strength, type of ions) on binding and derive appropriate standard conditions for the assays (e.g. high / low pH, presence / absence of divalent cations).
- Validation using chemicals with known binding behaviour.

Cost and timing

€ 450 k over 3 years.
Cooperation with RfP 5 is strongly recommended.

RfP 5 – Develop simple and rapid assays to evaluate irreversible binding of chemicals to soil and sediment

Aim of the research

Develop understanding of major mechanisms of non-covalent and covalent binding to soil and sediment matrices. Develop structural rules for differentiating between high and low extent of non-extractable residues.

Research deliverables

- Review of literature data on bound residues.
- Describe compound classes associated with potential for non-extractable residues.
- Characterise matrix components relevant for residue formation.
- Computerised structural alert model for classifying compounds according to high versus low binding capacity.

Cost and timing

€ 250 k over 2 years.
Cooperation with RfP 4 is strongly recommended.

RfP 6 – How will environmental change affect non-extractable residues of chemicals in soil or sediment

Aim of the research

To better understand the impact of environmental changes (temperature variation, humidity variation, changes in redox conditions in response to flooding) on the availability / release of bound residues.

Research deliverables

- Define plausible critical conditions where environmental change would impact the bioaccessibility / bioavailability of bound residues of chemicals.
- Provide plausible mechanisms for the occurrence of this change.
- Relate results to eventual changes in risk assessment strategies.
- Review all published information.

Cost and timing

€ 300 k over 4 years.

RfP 7 – Will a better understanding of soil organic matter pool dynamics be the key to understanding the non-extractable pool dynamics?

Aim of the research

To better understand the impact of changes in soil management (agriculture for instance) or changes in weather conditions on the soil organic matter pool and how this impacts the non-extractable pool of chemicals.

Research deliverables

- Review all published information.
- Define methods to distinguish between recalcitrant and mobile pools of organic matter and methods to define different fractions of organic carbon.
- Define critical changes in soil management or weather condition which affect the soil organic matter pool.
- Evaluate systematically how changes in the soil organic matter affect the non-extractable pool of chemicals.
- Using adequate tracer techniques such as radioactive and stable isotopes, define what portion of the non-extractable pool of chemicals becomes part of the soil organic matter pool, what portion is metabolised completely to CO_2 and what portion is parent compound / metabolite.
- Verify laboratory results in outdoor lysimeter experiments.
- Relate results to eventual changes in risk assessment strategies.

Cost and timing

€ 500 k over 4 years.

RfP 8 – How do changes in land use affect the non-extractable pool of chemicals in soils?

Aim of the research

To better understand how changes in land use (such as minimal tillage, fertilisation, increased chemical application, crops or afforestation / deforestation) affect the non-extractable pool of chemicals in soil.

Research deliverables

- Review all published information.
- For one plausible and relevant change in land use (for example change from tillage to non-tillage agriculture and increased use of agricultural chemicals, or removal of flood defence to allow temporary flooding of land), investigate systematically how the non-extractable pool of chemicals will be affected, and under what conditions the bioavailability / bioaccessibility of the non-extractable pool of chemicals in soil will change.

Cost and timing

€ 300 k over 3 years.

RfP 9 – Development of methods for testing the ecotoxicity of NER as formed in OECD 307/308 tests and similar procedures

Aim of the research

Methods are needed to determine the potential ecotoxicity of NER, as an input to regulatory risk assessment. Ecotoxicity of NER will be evaluated by comparing ecotoxicity in tests with no NER formation (parent chemical only), with NER only (ER has been removed from the test matrix); and both ER and NER. Assessment of ecotoxicity in the context of measured / predicted freely dissolved chemical concentration will be used to both compare potencies of different exposures, and to determine whether the freely dissolved paradigm is suitable for evaluating potential effects of NER. Because the regulatory concern over NER stems from NER formation in tests like OECD 307/308 (OECD, 2002b,a), test procedure development should provide for NER formation in a similar manner.

Research deliverables

- Develop methods to prepare substrates with NER in ways consistent with OECD 307/308 or similar procedures.
- Evaluate the relative ecotoxicity of NER:
  - in matrices that contain only NER (i.e. extractable residues that have been removed);
    - develop methods to remove extractable residues (ER);
  - in matrices that contain both ER and NER.
- Conduct reference exposures of parent compound in substrates with minimal sorption / NER formation.
- Select soil and sediment toxicity test methods believed to capture exposure to NER and provide justifications for their selection. Emphasise long-term exposure and ingestion of substrate.
- For both approaches, evaluate the adequacy of the freely dissolved chemical paradigm for predicting the potency of the exposures (i.e. does exposure to NER create effects that cannot be accounted for by the measured and/or predicted freely dissolved concentration in interstitial water?).
- Use chemicals known / suspected to have different mechanisms of NER formation.
- Use range of soils / sediments with different amount of NER formation.
- Propose / develop quality / performance criteria for newly developed procedures.

APPENDIX I: LIST OF PARTICIPANTS

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APPENDIX J: WORKSHOP PROGRAMME

Wednesday 14th October 2009 – morning

08.30 – 09.00	Registration and coffee	
09.00 – 09.10	Welcome and Introduction	Chair: Gary Roberts AstraZeneca

Session 1:

HIGHER TIER BIODEGRADATION TESTING METHODOLOGY AND DATA INTERPRETATION

09.10 - 09.35	Consideration of bound residues in regulatory environmental risk assessment – Problems and open issues	Andreas Hoellrigl-Rosta UBA
09.35 - 10.00	Availability of organic contaminant residues in soil	Peter Burauel, Forschungszentrum Jülich on behalf of Kirk Semple, Lancaster University
10.00 - 10.25	Experiences with bound residues in environmental fate studies – Plant protection products	Robin Oliver Syngenta
10.25 - 10.50	*Coffee*	
10.50 - 11.15	Experiences with bound residues in OECD 308 tests – Human pharmaceuticals	Jon Ericson PhRMA

Session 2:

DEPLETION, MODELLING AND EFFECTS ASSESSMENT OF BOUND RESIDUES

11.15 - 11.40	Use of analytical tools and kinetic models to characterise bioavailability and bound residues	Joop Hermens Utrecht University
11.40 - 12.05	Implications of bound residues for the assessment of effects	Dave Mount US EPA
12.05 - 12.15	Introduction to syndicate sessions	
12.15 - 13.15	*Lunch*	

Wednesday 14th October 2009 - afternoon

Session 3: Syndicates

13.15 - 15.15 Syndicate groups addressing questions related to presentations

Syndicate 1 - Exposure Assessment of Bound Residues

A Moderator: Peter Burauel Rapporteur: Tom Federle
B Moderator: Eric Verbruggen Rapporteur: Torben Madsen

- How should bound residues be defined / characterised?
- What extraction methods are considered suitable / unsuitable?
- How relevant are they to the conditions experienced in the environment?
- Are all bound residues equivalent in exposure as determined by these characterisation and extraction methods?
- How suitable are current biodegradation tests for the assessment of bound residues and persistence?
- Gaps and research needs associated with the above.

Syndicate 2 - Depletion Mechanisms and Modelling of Bound Residues

C Moderator: Gerrit Schüürmann Rapporteur: Jörg Klasmeier

- Scientific understanding of the mechanisms of binding that lead to bound and non-extractable residues (NER):
  - Key literature and/or authors.
  - Identify key mechanism for bound and non-extractable residues, covalent binding, partitioning, hydrogen, ionic, etc.
  - Role of functional groups (NH_2, -NH, -N-, COOH, other).
- Evaluation of models that may predict:
  - The extent and nature (mechanism) of bound residues based on structure, physico-chemical properties, or other.
  - Reliability or accuracy of such models; if not, what other weight of evidence is needed before assigning such mechanism?
  - The microbial biotransformation products (MBP) of test materials that are subsequently incurred in the binding process and contribute to the non-extractable residues.
  - Reliability or accuracy of such models; if not, what other weight of evidence is needed before assigning such structures?
- Gaps and research needs associated with the above.

Syndicate 3 - Effects Assessment of Bound Residues

D Moderator: Dave Mount Rapporteur: John Parsons

- Current scientific understanding - what are the key influences on bioavailability?
- How suitable are current ecotoxicity methods for assessing effects of bound residues in soil, freshwater and marine sediments?
- What improvements / modifications are needed?
- How can total residue / bioavailable concentration be related to observed effects?
- What are suitable tests that account for different exposure routes (ingesters versus non-ingesters)?
- What soil / sediment species should be included in testing strategy and how should different trophic levels be addressed?
- Are there any *in vitro* / *in situ* tests that can be used to assess bioavailability of soil / sediment residues and therefore preclude effects testing?
- What research is needed to answer these questions?

Wednesday 14th October 2009 – afternoon, cont'd

15.15 - 15.30 *Tea*

15.30 - 16.30 Feedback from Syndicate groups

16.30 - 17.00 Plenary review - Identification of possible research projects

17.00 - 18.00 Poster session

Presented by	***Authors***	***Poster title***
Peter Burauel	N. D. Jablonowski S. Köppchen A. Schäffer P. Burauel	Sorption and distribution of aged atrazine residues in the drainage system of an outdoor lysimeter experiment
Peter Burauel	N. D. Jablonowski S. Köppchen D. Hofmann A. Schäffer P. Burauel	Characterization of ^{14}C-labeled atrazine residues after 22 years of aging under outdoor conditions
Peter Burauel	N. D. Jablonowski S. Köppchen A. Schäffer P. Burauel	Environmental long-term persistence of ^{14}C-labeled atrazine and its residues
Peter Burauel	A. E. Berns H. Philipp H.-D. Narres F. Schnitzler E. Klumpp H. Lewandowski	^{15}N-CPMAS-NMR spectra and quantum chemical calculations of sulfadiazine and its reaction with soil components
Peter Burauel	J. Modler N. D. Jablonowski P. Burauel	Bioaccessibility of naturally aged ^{14}C-atrazine residues in an agriculturally used soil and its different soil particle size fractions
Gary Roberts	G. Roberts P. McCormack R. Oliver H. Noble T. Garcia de Oteyza Felderman	Standard protocol for the extraction of pharmaceuticals from soil, sediment and biosolids
Michael Spiteller	M. Lamshöft P. Sukul M. Spiteller	Determination of bound residues of fluoroquinolones as influence by solvent extraction
David Werner	D. Werner	Adding activated carbon to polluted sediments to bind residues of persistent organic compounds

Thursday 15th October 2009

08.30 – 08.45	Review of day 1	Chair: Jon Ericson Pfizer
08.45 - 09.00	Introduction to day 2	Jon Ericson

Session 4: Syndicates

ENVIRONMENTAL RISK ASSESSMENT OF NER AND DEVELOPMENT OF A NER TESTING STRATEGY

9.00 - 10.15 Syndicate 4: PECs and PNECs

E	*Moderator: Jon Ericson*	*Rapporteur: Ed Schaefer*
F	*Moderator: Gary Roberts*	*Rapporteur: Mick Whelan*
G	*Moderator: Fraser Lewis*	*Rapporteur: Dave Mount*
H	*Moderator: Stuart Marshall*	*Rapporteur: Dominic Di Toro*

- When would bound residues be considered a risk? When would bound residues not be considered a risk?
- At what point in the risk assessment would one consider / evaluate bound residues?
- What additional testing (fate and effects) would be appropriate?
- What considerations are important in the risk assessment?
    - Is it important to understand the mechanism of binding – would this affect the way the hazard / risk is assessed?
    - Should bound residues be considered part of the overall depletion rate?
    - How should different rates of dissipation / degradation from a study with different sediments be used in risk / hazard assessment?
    - How should discharge / release pattern and rate be compared to the binding capacity of the receiving matrix (overloading binding capacity)?
    - What specie(s) is best to assess the potential effects of NER?

10.15 - 10.35 Feedback from syndicates

Session 5:

DISCUSSION AND PRIORITISATION OF RESEARCH PROPOSALS

13.30 - 15.45 Drafting of outline RfPs – Identify key players and other research parameters, e.g. budget, time etc

15.45 - 16.00 Summing-up and close of workshop

APPENDIX K: ORGANISING COMMITTEE

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ECETOC WORKSHOP REPORTS

No.	Title
No. 1	Workshop on Availability, Interpretation and Use of Environmental Monitoring Data. 20-21 March 2003, Brussels
No. 2	Strategy Report on Challenges, Opportunities and Research Needs Arising from the Definition, Assessment and Management of Ecological Quality Status as Required by the EU Water Framework Directive Based on the Workshop EQS and WFD versus PNEC and REACh - Are They Doing the Job? 27-28 November 2003, Budapest
No. 3	Workshop on Use of Human Data in Risk Assessment. 23-24 February 2004, Cardiff
No. 4	Influence of Maternal Toxicity in Studies on Developmental Toxicity. 2 March 2004, Berlin
No. 5	Workshop on Alternative Testing Approaches in Environmental Risk Assessment. 7-9 July 2004, Crécy-la-Chapelle
No. 6	Workshop on Chemical Pollution, Respiratory Allergy and Asthma. 16-17 June 2005, Leuven
No. 7	Workshop on Testing Strategies to Establish the Safety of Nanomaterials. 7-8 November 2005, Barcelona
No. 8	Workshop on Societal Aspects of Nanotechnology. 9 November 2005, Barcelona
No. 9	Workshop on the Refinement of Mutagenicity / Genotoxicity Testing. 23-24 April 2007, Malta
No. 10	Workshop on Biodegradation and Persistence. 26-27 June 2007, Holmes Chapel
No. 11	Workshop on the Application of 'Omics in Toxicology and Ecotoxicology: Case Studies and Risk Assessment. 6-7 December 2007, Malaga
No. 12	Workshop on Triggering and Waiving Criteria for the Extended One-Generation Reproduction Toxicity Study. 14-15 April 2008, Barza d'Ispra
No. 13	Counting the Costs and Benefits of Chemical Controls: Role of Environmental Risk Assessment in Socio-Economic Analysis. 4 June 2008, Brussels
No. 14	Use of Markers for Improved Retrospective Exposure Assessment in Epidemiology Studies. 24-25 June 2008, Brussels
No. 15	Workshop on the Probabilistic Approaches for Marine Hazard Assessment. 18-19 June 2008, Oslo
No. 16	Workshop: Guidance on Interpreting Endocrine Disrupting Effects. 29-30 June 2009, Barcelona

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