Assessing Environmental Persistence
6-7 November 2012, Paris

Workshop Report No. 24

Co-sponsored by the CEFIC ECO 11 LRI project,
and co-organised by representatives from ECETOC,
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EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

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Assessing Environmental Persistence

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1. EXECUTIVE SUMMARY

Many chemical regulatory schemes exist around the world that contain hazard-based criteria to identify and prioritise persistent, bioaccumulative and toxic (PBT), or very persistent very bioaccumulative (vPvB) chemicals. These are chemicals that have the potential to persist in the environment, accumulate within the tissue of living organisms and, in the case of chemicals categorised as PBTs, show adverse effects following long-term exposure (ECETOC, 2005).

This two-day workshop, co-sponsored by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) and the CEFIC ECO 11 LRI project, and co-organised by representatives from ECETOC, Industry, the Federal Environment Agency of Germany (UBA) and the Environment Agency (EA) of England and Wales, took place at Les Salons France-Amérique, Paris, France on the 6th and 7th November 2012. This was a follow up to the 2007 "Biodegradation and Persistence" Holmes Chapel workshop co-hosted by ECETOC and the Environment Agency of England and Wales to assess areas of research required to help develop the scientific understanding of factors that affect the persistence of chemicals in the environment. The 45 attendees, from academia, regulatory agencies and industry, discussed the challenges and uncertainty faced with persistency assessments at the screening and confirmatory testing levels.

The primary aims of the 2012 workshop were to:

a) Identify whether / how the programmes initiated as a consequence of the Holmes Chapel Workshop have helped further the understanding of biodegradation / persistence related issues,
b) Identify and prioritise key areas for further future research.

The presentations and discussions clearly indicated that the knowledge and science-base were moving forward within the field of persistence assessment. Significant developments include: the ECETOC and UBA activities to define and characterise extractable and non-extractable residues (NERs) formed in soil and sediment, the CEFIC funded work to understand the importance of biomass concentration and diversity within screening assessments for biodegradability, and the inclusion of more ecological realism and relevance within persistency assessments through the inclusion of light, natural waters and assessing adaptation potential and biodegradation outcome over time.

These scientific advancements at the screening and confirmatory level of persistence assessment were helping to (i) increase the body of data and experience for stakeholders (ii) address some uncertainties in persistence assessment and (iii) identify the key research needs that still need to be addressed to achieve a consensus position. The syndicate sessions identified themes for future research and development including:

- Convening an OECD Expert Working Group to consolidate and update the ready biodegradability tests (RBTs) to reflect (i) the availability of new instrumentation with increased analytical sensitivity (ii) the use of tests with combined analytes (e.g. biochemical oxygen demand (BOD) and carbon dioxide evolution), and (iii) the need to screen for biodegradability in water-sediment systems.
- Assessing the influence that temperature has on rates of biodegradability in aquatic and aquatic-sediment habitats and, consequently, the need for temperature extrapolation.

- Assessing the ecological significance of adaptation and developing appropriate test methods and guidance accordingly.
- Developing and validating models to predict non-extractable residue formation and guidance on how to assess the risks posed by NERs over time.
- Benchmarking the microbiological and kinetic performance of the OECD 314, 308, 309 and enhanced biomass tests using appropriate reference chemicals.
- Developing and validating tools and guidance to predict and assess the formation of transformation products in biodegradation studies.
- Clarifying and resolving the test-based and model-based issues associated with the persistence assessment of complex substances.
- Investigating the value of genetic sequencing procedures in determining differences / similarities in inocula to compare the relevance of laboratory inocula with the field situation.

2. WORKSHOP OVERVIEW

2.1 Introduction

ECETOC has been actively involved in developing the scientific understanding of factors that affect the persistence of chemicals in the environment for over 15 years (see for example ECETOC, 2003, 2005, 2009, 2011). In 2007, ECETOC and the Environment Agency (EA) of England and Wales co-hosted a workshop on "Biodegradation and Persistence" at Holmes Chapel in the United Kingdom. Attendees from academia, regulatory agencies and industry discussed the challenges and uncertainty faced with persistency assessments at the screening and confirmatory testing level. Nine research topics were identified at the 2007 workshop and request for proposals (RfPs) were drafted to fill these research needs. From these, four RfPs were assigned highest priority for action. Significant progress has been made such that they are all at or near completion (see also Appendix 1). These include:

- Development of a validation set of chemicals for biodegradation research (funded by CEFIC/LRI and completed in 2010);
- Addressing the uncertainty associated with bound residues or non-extractable residues formed in higher tiered persistency assessments. This resulted in a subsequent ECETOC Workshop (ECETOC, 2009) and two ECETOC Task Forces which were due to report in 2013 (ECETOC, 2013a,b);
- Development of new persistency screening tools with biodegradation studies using enhanced biomass levels (funded by CEFIC/LRI and due for completion in 2013);
- Measuring biodegradation half-lives and identifying sources of variability and uncertainty (funded by CEFIC/LRI and project started in 2012).

2.2 Workshop objectives

A second persistence workshop was convened to: (i) discuss and evaluate the progress and any scientific advances made as a consequence of the activities initiated following the 2007 Holmes Chapel workshop, (ii) discuss other scientific developments within the field of persistence, and (iii) learn from the chemical industry and environmental regulators' experience of persistency assessments conducted within the initial phases of REACH.

The technical programme will address the following themes through a combination of invited and submitted keynote presentations (using case study measured data where possible) and syndicate sessions:

- Regulatory efforts to harmonise persistency criteria and its evaluation across the EU;
- Validation of biodegradability tests for persistency evaluation and the development of appropriate quality assurance / quality control standards;
- Effective prioritisation of persistence at the screening level including the role of quantitative structure-biodegradability relationship (QSBRs), modified and enhanced (ready) biodegradation tests;
- Biodegradability assessments with 'difficult to test' substances;

- Assessment of non-extractable residues associated with sludge, soils and sediments within higher tiered studies;
- Predication, detection, identification and evaluation of degradation products;
- Microbial adaptation and its relevance to exposure and persistency assessment.

The key objectives of the workshop were to:

a) Identify whether / how the programmes initiated as a consequence of the 2007 workshop have helped further the understanding of biodegradation / persistence related issues;
b) Identify and prioritise key areas for further future research.

2.3 Workshop structure

- Series of 20-minute talks and case studies;
- Syndicate sessions addressing specific questions;
- Plenary feedback;
- Further discussions;
- Conclusions and recommendations;
- Areas for future research.

2.4 Plenary presentations

2.4.1 Introduction and stakeholder perspectives of persistence

The aim of this session was to introduce the aims and objectives of the workshop together with some of the key stakeholder perspectives and challenges faced with assessing the persistence of chemicals in the environment. A series of stakeholder presentations introducing the issues relating to persistence assessment was followed by a panel discussion and two syndicate sessions.

Jason Snape *(AstraZeneca, UK)* provided a brief introduction to the scientific remit of ECETOC and summary of its activities related to environmental persistence. This included Task Force reports describing (i) Refined Approaches for Risk Assessment of PBT/vPvB Chemicals (2011), (ii) The Collation of Existing Marine Biodegradation Data and its Use in Environmental Risk Assessment (2009), (iii) The Risk Assessment of PBT Chemicals (2005), and (iv) The Persistence of Chemicals in the Environment (2003). Jason used the PBT profiler definition of chemical persistence where *"persistence is the ability of a chemical substance to remain in an environment in an unchanged form. The longer a chemical persists, the higher the potential for human or environmental exposure to it. The individual environmental media for which a chemical's persistence is usually measured or estimated are air, water, soil, and sediment."*

Jason outlined the biodegradation testing paradigm together with a brief summary of relative strengths and weaknesses of the ready, inherent and higher tiered biodegradability tests. Jason also highlighted the rationale behind why the modified and enhanced biodegradation screening studies had been included within the REACH technical guidance for exposure and persistency assessments. This rationale was to circumvent the high number of false negatives that could be attributed to data from the ready biodegradation tests by addressing the underlying reasons for chemicals failing to degrade in these studies (e.g. testing above the chemical water solubility, low biomass levels excluding competent degraders and testing at concentrations where the chemical is toxic to the inocula).

Jason described the key outputs from the 2007, ECETOC and the Environment Agency (EA) of England and Wales "Biodegradation and Persistence" Workshop at Holmes Chapel in the United Kingdom. Many of the outputs agreed at this workshop had been taken forward by ECETOC and CEFIC. These included workshops, Task Forces and CEFIC/LRI projects to advance the science associated with non-extractable residues, enhanced biodegradation screening studies and the generation of the OECD 314 technical guideline. Jason indicated that the outputs of these activities, and parallel work by other interested parties, would also be shared over the next two days to (i) identify where there is consensus on best practice for persistency assessment at the screening or confirmatory stage, (ii) identify where we still have areas of uncertainty that can be addressed through research, scientific reviews or further dedicated workshops and (iii) prioritise and agree these research needs.

Eric Verbruggen *(National Institute for Public Health and the Environment (RIVM), the Netherlands)* described the several different regulatory frameworks that exist within Europe to deal with specific groups of chemicals (e.g. industrial chemicals, biocides, pesticides, pharmaceuticals), or which serve a particular purpose (e.g. protection of the marine environment). Several of these regulatory frameworks have their own criteria for dealing with Persistent, Bioaccumulative, and Toxic (PBT) substances or

Persistent Organic Pollutants (POP). Eric focused on criteria for persistence of substances and compared the criteria from different regulatory frameworks. It appears that for persistence, differences in criteria are relatively small. Eric highlighted some examples of aspects of persistence and approaches to persistence assessments that are treated differently in the different regulatory frameworks. Some frameworks do not use their own criteria but refer to criteria from REACH or the TGD (Technical Guidance Document) used in the former new and existing substances legislation. Despite these small differences in criteria among the regulatory frameworks, details in the assessment procedure could cause the final assessment of persistence to deviate substantially among the frameworks. Even when the criteria are the same, the way the information from experimental studies is used may vary greatly. For example, the half-life of a substance could refer to degradation only, or it could be a half-life for dissipation. This reflected the way in which bound residues are regarded, with the extreme cases of bound residues being considered as completely disappeared versus completely persistent. It is also important how the results from field studies are considered in the assessment. Eric also highlighted issues associated with temperature for which the criteria are defined (ambient or room temperature), and if a temperature correction should be applied. It was also indicated that the interpretation and use in how to deal with photolysis and hydrolysis is often not well defined. Eric concluded by making the case for further harmonisation of the persistence assessment between different regulatory frameworks. This harmonisation needs to harmonise not only the criteria on which the persistence assessment is based, but also the guidance documents on the interpretation of the data.

Thomas W. Federle *(Procter & Gamble, USA)* indicated that the ability to accurately determine the potential for an organic chemical to degrade and the rate at which degradation will occur in environmental compartments where it is released and ultimately resides is critical in evaluating its environmental persistence. Moreover, this understanding is essential for accurately estimating environmental exposure when conducting an environmental risk assessment. Historically, ready and inherent biodegradation tests have been the principle regulatory tools utilised for assessing degradability. However, these tests are ineffective for chemicals that are difficult to test due to their physico-chemical properties, are not used as growth substrates by microorganisms or whose degraders are rare in standard test inocula. While some of these limitations are remedied in simulation tests, these tests come with their own unique issues.

Tom surveyed some of the challenges commonly encountered in accurately evaluating the degradation and persistence of organic chemicals. These include challenges that are not only scientific and methodological but also financial and practical. Methodological challenges include dosing difficult to handle substances and having sufficient analytical signal above background to quantitatively measure biodegradation at test concentrations, which are not inhibitory to the microbes or at which mass transfer is not a limitation. Scientific challenges include having an inoculum that is of sufficient size and diversity that rare degraders are present and, in the case of substances that are co-metabolised rather than used as a growth substrate, having a metabolically active microbial community available in the test. The former is complicated by regulatory restrictions on using pre-adapted inocula, which is particularly a problem for chemicals that are new to the world. Tom illustrated the importance of adaptation within the natural environmental and how the biodegradability of a chemical can change over time. This example highlighted that rates of degradation increased over time and the frequency of observing consistent positive biodegradation outcomes also increased through the routine use and discharge of a chemical.

Tom also highlighted that the use of simulation tests come with their own specific challenges, many of which are of a practical nature. These include not only the cost but the difficulty of obtaining high quality and well characterised radiolabelled test materials with the consolidation and contraction that has occurred in the industry during the past few years. Others relate to the complexity of such tests, the difficulty of successfully executing them, uncertainty about the results themselves and even their regulatory acceptance. This latter uncertainty includes whether the results from scientifically sound but non-prescribed tests (e.g. OECD 314) will even be considered by regulatory agencies, potential variability in how individual regulators or agencies will weigh and interpret such tests and how they will consider bound residues in the ultimate assessment. Unfortunately, such uncertainty can translate into reluctance by business managers to proactively fund testing and research that could lead to more definitive understanding on the fate of many chemicals in the environment. Tom concluded with the charge that by identifying the challenges, whether scientific or practical, and the dilemmas that they pose, this workshop can catalyse the development of improved approaches that will ultimately advance our understanding of chemical fate and result in better environmental protection.

Johanna Peltola-Thies *(ECHA, Finland)* described the regulatory process for PBT assessment before and after the implementation of REACH. The PBT working group, under the interim strategy period before REACH, discussed around 120 existing substances and also several new substances and biocides. The assessment conclusion 'not persistent' for existing substances was drawn in equal amounts from biodegradation screening information and other information (e.g. abiotic degradation, information about reactivity). For a large number of the substances, the discussions consisted of considerations on the validity of available data in the light of the physico-chemical (PC) properties and chemical reactivity. In discussion on available experimental degradation studies the relevance of the test conditions for PBT assessment was crucial. Conventions established at that time were incorporated into ECHA's guidance, but for some paths (photodegradation, anaerobic biodegradation, hydrolysis, bound residues) uncertainties still remained in terms of whether and how to use the respective data in the context of PBT assessment. Johanna also highlighted a number of challenges. These included: identifying the compartment of concern, understanding the relative role and importance of aquatic photodegradation, how to present and introduce the test chemical(s), improving our understanding of the complex fate of substances in tests and in the environment, understanding the importance of anaerobic biodegradation and the use of monitoring data from contaminated sites.

Johanna indicated that a year ago about 150+ registered substances were prioritised for further PBT screening assessment by EU Member State experts as an activity beyond the formal REACH processes. The basis for the prioritisation was mainly quantitative structure-activity relationship (QSAR) estimations. Only a small proportion of submitted registrations contained experimentally derived degradation data. Furthermore, only a small number of degradation simulation testing proposals have been submitted with the registrations. These facts, and also considering the registration data quality findings of ECHA, appear to indicate that understanding on the assessment of persistence among the registrants in general may still be different from the perception of the authorities. It seems that the main part of the ongoing PBT assessment work of the Member State experts will cover similar aspects as the PBT assessment work in the past. However, in addition to the issues of uncertainty mentioned above, search and assessment concepts of potentially persistent constituents or impurities present in unknown, variable composition or biological

(UVCB) substances need to be developed or further refined. It has not been possible so far to cover UVCBs in the PBT screening activities in a balanced way.

2.4.2 Screening for environmental persistence

The aim of this session was to identify recent advances in the assessment of biodegradation and persistence using screening test methodology and where further improvements are needed. A series of presentations introducing the issues relating to screening studies was followed by a panel discussion and two syndicate sessions.

Gary Bending *(University of Warwick, UK)* presented research undertaken by his group investigating issues of pragmatism and realism related to the assessment of persistence. The environmental relevance of laboratory screening biodegradation studies is limited because standardised test conditions represent only a small range of environmental complexity and heterogeneity. The challenge is to obtain reproducible test outcomes whilst achieving environmental relevance. There are many factors influencing the catabolic potential of an inoculum including density, diversity, composition of inocula as well as water chemistry and compartment variability.

Gary presented data comparing the biodegradation of p-nitrophenol by different inocula and under different environmental conditions. Microbial biofilms, that are absent from biodegradation screening studies, have several advantages over inocula from water including higher density and active biomass, community interactions, microhabitat heterogeneity, greater genetic variation and exchange, better consistency (longer site history). Seasonal variation was a greater determinant of bacterial community composition than proximity to the outflow of a sewage treatment plant (STP). River water collected from the STP outflow showed more consistent degradation of p-nitrophenol than water collected from upstream or downstream. River biofilms provided similar rates of biodegradation to river water, despite the larger amounts of biomass applied in degradation assays. A lack of degradation of p-nitrophenol in some river water samples was associated with factors controlling bacterial proliferation rather than an absence of catabolic potential (competent organisms).

At environmental concentrations of most xenobiotics (low µg/L) degradation is likely to be via co-metabolism, whereas at higher concentrations typically used in lab studies, biodegradation is likely to be growth-linked. The p-nitrophenol experiments support this with more realistic (low) concentrations undergoing more variable and lower rates of biodegradation. Furthermore, studies also demonstrated that light can also be an important factor, as algal photosynthesis increased pH in non-buffered waters resulting in the reduced growth of degrading organisms. Currently, all biodegradability studies are conducted in the absence of light.

The addition of complexity into test systems may therefore affect the outcome of biodegradation tests in a manner which is hard to predict.

Kees van Ginkel *(Akzo Nobel, the Netherlands)* discussed modified and enhanced ready biodegradation tests. Ready biodegradability tests only detect growth-linked biodegradation because there is only one substrate and this is presented at a high concentration. This, combined with the low inocula density, leads to a

stringent test. However, a pass in a ready test indicates a high probability that the chemical tested will undergo rapid and complete biodegradation in the environment. Modifications and enhancements of the ready biodegradability tests have been designed to reduce the likelihood of false negatives and to overcome several difficulties with chemicals that are poorly water soluble, i.e. are of limited bioavailability, or toxic to the inoculum. For example, the toxicity of quaternary ammonium compounds can be avoided by the addition of silica gel, humic acids or lignosulphonic acids salts. Addition of silicone oil has been shown to reduce the toxicity and volatilisation of a fragrance. Agitation of the test media and addition of surfactants have also been used to successfully increase bioavailability of poorly water soluble substances. The slow release of poorly water soluble chemicals in the environment may limit the rate of biodegradation. Although this may suggest the chemical may be persistent, the risks may be low because the bioavailable fraction can be degraded.

Enhanced (less stringent) biodegradation tests can be achieved by pre-adapting inocula and extending the test duration. This allows competent microorganisms present in low numbers in the environment to multiply, not true for prolonged ready biodegradability test results. At least one competent organism was present at the start of the test, multiplying to numbers which enable detection of biodegradation of test chemicals in screening tests. For example, although N-methylpiperazine is not readily or inherently biodegradable, competent micro-organisms were obtained after acclimatisation to low concentrations of N-methylpiperazine in SCAS (semi-continuous activated sludge) units. Pre-adapted inocula also degraded this chemical in a closed bottle test when grown on glass beads and additional nutrients (glucose) to stimulate growth.

It was concluded that modifications and enhancements have improved the assessment of persistence in the environment. There still is an element of trial and error involved in the assessment of biodegradation with modified tests. Pre-adaptation is useful in assessing potential for inherent biodegradation but it may not indicate how widespread a competent micro-organisms might be in the environment. Running screening tests for a prolonged period (60 days) was considered generally appropriate. Interpretation of the results of modified and enhanced test should be more science based. For instance, in environmental microbiology growth-linked biodegradation is considered superior compared to cometabolic transformations often detected in simulation tests. There was interest in comparing the performance of chemicals in both the new enhanced tests and in standard ready biodegradation tests.

Russell Davenport *(Newcastle University, UK)* presented the outcome of a research project that has provided a better understanding of the factors that influence screening tests. Ready biodegradability tests (RBTs) have been core to the determination of the biodegradation of chemicals in regulatory frameworks for 2-3 decades. There are seven test methods varying in inoculum source and preparation and consequently there are variable probabilities of inclusion of specific degraders in the test system. This leads to a high failure rate and high variability largely due to the use of low inocula concentrations (microbial concentrations in inocula can vary by 4 orders of magnitude within the standardised biodegradation test guidelines). Together with their short duration, this makes them unreliable for persistence assessments. Variation in density of inocula should be reduced.

Enhanced screening tests, for use in persistence assessment rather than ready biodegradability, may include increasing inocula to environmentally relevant concentrations. This increases the likelihood that the microbial diversity will be environmentally realistic.

The CEFIC/LRI ECO 11 (*www.cefic-lri.org//ECO11*: Towards rationally designed hazard, risk and persistency assessment: Putting the "bio" back into biodegradability tests) project has been investigating how variations in inocula concentration, community composition and diversity relate to the variation and reliability in screening test outcomes. In addition, the bias and pragmatism of different methods to concentrate cells in inocula for enhanced tests has been assessed. These enhancements were validated using a set of reference chemicals (chosen by CEFIC/LRI ECO 12 project) that represent different rates and extents of biodegradation in the environment. It was found that greater variation occurred between sources (different compartments) of inocula than between different inocula from the same compartment. Enhancement of activated sludge inocula concentrations had a greater effect on reliability than test volume in scaled-up biodegradation tests, but not necessarily for marine inocula which showed particularly high variability in the lag phase. Further investigation is needed to achieve a reliable screening test for the marine compartment. Russell recommended a move to high throughput screening tests using multiple sources of inocula and a probabilistic approach to interpreting the results. This would be aided by better understanding of the influence of genetic diversity on biodegradation. In most cases a 100-fold increase in the concentration of inocula resulted in (i) less variability within and between studies, (ii) shorter and more consistent lag periods, (iii) a higher probability of reaching the pass-criterion for ready biodegradability for chemicals considered as non-persistent, and (iv) no false positives for chemicals considered to be persistent.

Dan Salvito *(RIFM, USA)* introduced the difficulties of testing natural complex substances. Natural Complex Substances (NCS) are materials extracted from plants and used in the preparation of fragrance mixtures for a variety of consumer products. Typically these are classified as UVCBs (Unknown, Variable Composition, or Biologicals), or, minimally, Multi-Component Substances (MCS) for the less chemically complex extracts. Assessment of these materials is required under various regulatory schemes including REACH. While there are methods for considering the ecotoxicity of a mixture using additivity, little has been published on approaches for either environmental fate studies or other assessment methods for NCS. The International Fragrance Association's Environmental Task Force has provided recommended approaches for NCS biodegradation assessment. Dan presented studies using NCS as examples to assess the ready biodegradability of key constituents of these mixtures in order to provide an overall assessment of the biodegradability of the NCS itself. Two approaches were presented. If the mixture contains mostly substances that are structurally similar, then the mixture could be tested. More work is needed to develop this approach. If the mixture contains structurally dissimilar substances then individual constituents should be assessed. In this case the outcome of the constituent assessments can be used in providing a statement about the biodegradability of the NCS. When individual constituents are unavailable to test as single substances, e.g. in the case of sesquiterpenes which are common constituents of fragrance oils, QSBRs can be used to predict biodegradability. These predictions need to be considered carefully as predictions from different versions of the US EPA EPI Suite BIOWIN software are often contradictory and also may not concur with experimental tests.

2.4.3 Experiences with higher tiered assessment of persistence

The aim of this session was to describe experiences in the assessment of biodegradation and persistence using higher tiered tests. A series of presentations addressing issues including (i) experiences with the OECD water-sediment transformation test, (ii) the definition and characterisation of non-extractable residues, (iii) strategies to identify transformation products, (iv) the impact of light on degradation in aquatic-sediment environments and (v) the challenges posed by testing complex or difficult substances. This was followed by a panel discussion and two syndicate sessions.

Jon Ericson (*Pfizer*) described some of the experiences that the pharmaceutical industry has gained with the OECD 308 Sediment-Water Transformation Test (OECD, 2002a) since its introduction into the European Medicines Agency (EMA) environmental risk assessment guidance in 2006 (EMA, 2006). Four pharmaceutical companies (Pfizer, GlaxoSmithKline, AstraZeneca and Novartis) have collated the data for 31 pharmaceutical compounds to determine how the data is used and what can be learnt from the studies. Jon challenged the purpose of the study as it is used under the EMA regulations versus its original design. Within EMA, the OECD 308 test is used to simulate degradation of a down-the-drain chemical such as a pharmaceutical within a river; however the test was originally designed to simulate degradation of plant protection products in shallow ditches exposed via spray drift. The study is also used as a trigger to assess effects on sediment organisms if greater than 10% of the substance is present in the sediment layer at any time point after 14 days.

Jon described and illustrated the OECD 308 test system and summarised the key endpoints that are derived from the study. Some of these endpoints were not always possible to assess for all studies. These included the chemical half-life in sediment and the total ^{14}C half-life for the total system. Failure to measure these half-lives was attributed to the formation of NER. This was true for all groups of pharmaceuticals (neutral, cationic and anionic), although a greater proportion of NER and lower level of transformation products was formed with cationic pharmaceuticals. This NER effect had an impact on the half-life for the total system leading high variability for cationic and neutral pharmaceuticals. A mean parent half-life for the total system of 56 days ±79 days was observed across all classes of the pharmaceuticals tested. Jon reported that there was no correlation between dissipation rate and K_d or K_{oc} within the data set. However, the amount of total parent found in the water and sediment at day 50 and day 100 correlated well to the parent total system half-life. Jon described this observation as a possible case for having a single point screening study rather than a full OECD 308 study.

In summary, Jon concluded that: (i) the high level of NER formed in the studies posed an extraction and interpretation challenge that limits the use of the data in risk assessment unless the NER is considered removed and unavailable, (ii) the data from the OECD 308 are not used to revise the predicted environmental concentration (PEC) for surface waters or sediment and it is unclear how to do this within the EMA guidance should the PEC need revision, (iii) a separate sediment biodegradation test that is relevant to pharmaceutical risk assessments is required, (iv) the total system half-life should be used to refine the PEC within the risk assessment, and (v) the OECD 308 study should not be required at the screening level of a pharmaceutical risk assessment.

Graham Whale (*Shell*) gave a presentation outlining the challenges with undertaking soil biodegradation assessments on a complex hydrocarbon substance. The current recommended method uses the OECD Guideline 307 for testing chemicals: Aerobic - Anaerobic Transformation in Soil (OECD, 2002b) which was originally designed to provide degradation rate data for crop protection products. However, it is apparent that this test is now being undertaken to provide data for other 'chemicals' under the auspices of the EU REACH regulations.

Assessing the fate of complex substances raises many issues and there are scientists who question both the applicability and relevance of the current test guidelines. For example, the current approach recommended by CONCAWE (Conservation of Clean Air and Water in Europe) for complex hydrocarbon substances is to model persistence of the constituent hydrocarbon blocks.

In the case presented by Graham an OECD 307 assessment of a gas to liquid (GTL) fuel was requested by ECHA to determine potential persistent hydrocarbon components of the substance which may warrant further investigation. Consequently, a series of OECD 307 studies were undertaken on GTL fuel and individual alkanes. Difficulties were encountered with these studies that related to analytical constraints, different physico-chemical properties of components and dose rates at which the test can be conducted. All of these factors can complicate interpretation of results and it needs to be recognised that, when using an OECD 307 type soil study to assess the fate of components of complex substances, the objectives will differ to those for 'standard' OECD 307 studies.

A key recommendation was the inclusion of sterile (abiotic) dosed soil systems to assess the losses by abiotic factors (e.g. volatilisation and/or non-extractable residues). By incorporating sterile controls the OECD 307 test has potential to improve the understanding of the fate of components of complex substances like GTL fuel in soil. For example, it has potential to give an indication of the disappearance rate of components and identify recalcitrant components which may warrant further investigation. For the case study presented it appeared that the predicted half-lives of alkanes were conservative and, that no additional bioaccumulation assessments of the components of GTL fuel were warranted (based on the premise that even if some remain in soil they will not be bioavailable to soil organisms because they cannot be extracted using acetone and hexane).

To reiterate the key points although incorporating sterile controls can provide an indication of physical losses, for complex substances like the GTL fuel the OECD 307 test will ultimately determine 'disappearance' of components. This raises the issue that for complex substances it is unlikely to be feasible to calculate biodegradation rates per se using the OECD 307 test, and the data from such studies needs to be put into an appropriate context in an overall risk assessment strategy.

Non-extractable residues

Two presentations by **Charles Eadsforth** *(Shell, UK)* and **Caren Rauert** *(Umweltbundesamt, Germany)* on non-extractable residues (NER) were followed by a lively discussion:

Formation of non-extractable residues is regularly observed in studies on the fate of organic chemicals in soil. NER formation may be interpreted as a specific form of compound persistency – a 'hidden hazard' - or as a detoxification step – a 'safe sink'. Despite the considerable scientific progress made in analysing NER and

identifying their binding types, these insights, which are described in the following section, have not yet been incorporated into the regulatory risk assessment framework.

Following the ECETOC workshop on "Significance of bound residues in environmental risk assessment" in 2009, two Task Forces were set up to (1) understand the relationship between extraction technique and bioavailability and (2) develop interim guidance for the inclusion of non-extractable residues in environmental risk assessment. Charles Eadsforth gave a summary of the aims, objectives and outputs of these two ECETOC Task Force activities. The goal of the first Task Force was to address knowledge gaps in the relationship between bioavailability and extraction technique with regards to bound and non-extractable residues with the ultimate goal being the development of a standard framework for intelligent extraction strategies. A number of residue 'categories' were defined (dissolved, readily desorbed, slowly desorbed, irreversibly sorbed and incorporated) as well as the terms bioavailable and bioaccessible which were aligned with each type of residue within the framework model. It was decided to differentiate residues termed 'reversibly bound' into those 'readily desorbed' and 'slowly desorbed'. This differentiation was based on the solvent strength necessary to extract each type of residue and led to the development of the extraction regime to tie in with the framework model.

To be able to better predict the chemical dynamics once a chemical enters the soil, it is necessary to understand its interaction with the soil matrix. Generally, chemicals which were most strongly associated with the soil (and least bioaccessible) were either covalently bound to the soil, or physically sequestered and trapped in soil pores. Other interactions which were shown to lead to NER or slowly desorbed residues included ionic and ligand exchange. Chemicals were also shown to interact with the soil matrix via Van der Waals forces, hydrophobic partitioning, charge transfer complexes and hydrogen bonds, these interactions are generally thought of as weaker and most likely to lead to desorbable residues. The various interactions studied (and their bond strength ranges) were aligned with the extraction regime and framework model.

One of the major issues of particular concern with regards to environmental risk assessment is the potential for future re-release of NER. For example, there is evidence that physical processes such as freeze-thaw and wet-dry cycling can cause the release of sequestered residues via the breakup of the soil matrix and soil organic matter (SOM). Additionally, chemical and biological processes such as microbial metabolism and pH changes have been found to cause the release of NER. The current literature suggests that the amounts of NER released by such processes do not pose an environmental risk. However, it was agreed that further research is necessary in this area, especially with regards to release caused by physical processes because of the current paucity of studies on this topic.

One of the key components of soil is organic matter and the potential interactions between chemicals and this complex soil constituent are poorly understood and warrant further research. However, some steps have been undertaken to redress this situation and the ECETOC Task Force has developed a framework model and extraction scheme (ECETOC, 2013a). Furthermore, it is expected that research in this area will greatly increase over the coming years as environmental risk assessment of chemicals in soil becomes an increasingly important issue.

In a workshop held at the German Federal Environment Agency (UBA) in June 2010, a slightly different terminology than that used by ECETOC was used to describe NERs. Caren Rauert gave an overview of the key outputs of this UBA Workshop. 'Type 1' NER were defined as those substance molecules that may be

remobilised, possibly over prolonged times, 'Type 2' NER as those that are unlikely to be released in their original structure under environmental conditions. Finally, NER can also be formed via incorporation of single labelled atoms or small fragments from the original substance into biomass. These 'biogenic' NER are no longer structurally related to the original substance. While the formation of Type 2 and biogenic NER can be considered a 'safe sink', Type 1 NER would constitute a 'hidden hazard'. Where no information on their nature is available, NER should in principle be assumed to belong to Type 1 (i.e. worst case scenario), unless experimental data is provided to support categorization as Type 2 NER or as biogenic.

Formation of Type 1 NER should have implications on the environmental risk and hazard assessment. In particular, their potential for substance remobilisation will impact groundwater risk assessment and persistence assessment. Existing trigger values and decision criteria for NER formation were deemed inappropriate for addressing those concerns; hence, a need for developing new criteria was identified.

In a recent publication (Kaestner *et al*, 2013) a classification scheme has been proposed for differentiating type I NER (xenobiotic, sequestered), type II NER (xenobiotic, covalently bound), and type III NER (biogenic), respectively, with decreasing environmental risk in the order I, II, and III NER. The further development of extraction schemes for separating type 1 NER and type 2 NER (and type 3 NER) was suggested, although it seems necessary to distinguish different schemes for different substance classes. Another way forward could be the identification and quantification of the fraction of biogenic residues within the NER (Nowak *et al*, 2013).

Identification of degradation products and their risks

Kathrin Fenner *(EAWAG, Switzerland)* presented strategies to identify degradation products and their risks. At the higher tiers of chemical risk assessment, regulatory guidance typically recommends the performance of simulation-type transformation studies to identify major transformation products (TPs). However, most risk assessment guidelines fall short of providing guidance on how the risk of identified TPs should ultimately be assessed.

In this presentation two possible approaches to identify risk-relevant TPs were presented and contrasted in terms of their advantages and disadvantages. This was based on earlier published work (Escher and Fenner, 2011). The default approach recommended in most regulatory risk assessment frameworks is exposure-driven, i.e. chemical-analytical identification of major TPs followed up by their synthesis and subsequent effect testing. Recent approaches to speed up TP structure identification (see Helbling *et al*, 2010) such as high-resolution mass spectrometry combined with high-throughput data analysis tools were discussed in this context. An effect-driven approach based on toxicity was presented as an alternative, potentially more direct way of identifying toxicologically relevant TPs. In this approach, samples from simulation studies are not only subjected to chemical analysis, but are also analysed with one or more bioassays to follow the development of toxicity over the course of the experiment. Comparison of parent compound concentration and toxicity development over time then indicates whether any toxicologically relevant TPs are formed.

Both of the above-mentioned experimental approaches are laborious and time consuming suggesting that there is a role for models for prioritisation of TPs which warrant further investigation. A model to estimate relative concentrations of pesticide TPs in surface waters was presented and its performance assessed

relative to measured field data. Further, a model for estimating plausible ranges of toxic effects of TPs relative to their parent compounds was discussed. A combination of such models could potentially help to estimate the contribution of TPs to overall environmental risk caused by the release of a given parent compound.

Experiences with higher tier study designs to investigate the fate and behaviour of chemicals in the environment

Robin Oliver *(Syngenta, UK)* presented his experiences with higher tier study designs to investigate the fate and behaviour of chemicals in the environment. Many regulatory risk assessments for chemicals are based on laboratory studies in which the key processes of sorption, hydrolysis, photolysis and microbial degradation are evaluated separately in simple, standardised systems, in accordance with the appropriate guidelines. These studies provide information on the fate and behaviour of the chemical in soil, sludge, sediment and water.

Over recent years Syngenta has developed test systems to investigate the potential significance of degradation resulting from indirect photolysis and metabolism by phototrophic organisms in soil and sediment / water systems. A semi-field aquatic test system has also been developed to enable the determination of the rate and route of degradation, when multiple processes are acting together.

Study results showed that the overall rate of photodegradation in natural waters is a combination of direct and indirect photolysis and, in some cases, light scavenging by constituents of the water can reduce the rate of direct photolysis to a greater extent than is compensated for by indirect photolysis. These findings suggest that this will only be significant for compounds where direct photolysis is very rapid and the overall photodegradation rate in natural waters will still be fast. For those compounds that are not degraded very rapidly by direct photolysis, photodegradation in natural water is likely to be significantly faster than that observed in a sterile buffer.

In his talk, Robin Oliver had described aquatic simulation studies in which algae had been included in an attempt to more realistically mimic environmentally relevant conditions. It was thought that generation of hydroxyl and other oxygen radical species by virtue of the algae's presence might contribute to the increased degradation seen in these studies, although this mechanism was not described in detail.

In conclusion there are studies indicating that under more realistic environmental conditions other factors such as light and presence of algae or soil surface dwelling phototrophs can increased the relevance and realism of higher tiered exposure studies as well as leading to more rapid degradation of chemicals. However, there is currently no clear understanding of how this could be incorporated into the determination of environmental persistence for regulatory risk assessments.

2.4.4 Panel discussions on perspectives of persistence

A discussion panel was held after each of the three main plenary sessions to (i) understand the different stakeholder perspectives of persistence (ii) highlight innovation, best practice and challenges in assigning persistency at the screening stage, and (iii) share experiences in higher tiered assessments of persistence.

Due to the number of common themes that were raised across these discussions, the issues discussed in the three sessions have been collated around a number of key themes. These issues are outlined in more detail below. Many of these issues are explored in more detail and given further consideration in the syndicate sessions described later within this workshop report.

Regulatory Aspects

Some questions highlighted problems associated with variability in the way REACH registrants report persistence and other data, and asked what compliance or checking mechanisms in REACH can be used to deal with this issue. It was also unclear what the proportion of chemical substances that require simulation or higher tier testing at tonnages > 10 tpa under REACH was and the relative weight given to conflicting screening and higher tiered studies. Other questions focused on the different data requirements required under different regulatory frameworks and the potential for a given chemical to be classified differently within different regulatory schemes. Finally, one attendee questioned whether the data contained within the publically available REACH database was sufficient to conclude on PBT properties, and whether the current REACH instruments were fit for purpose. On the question whether data requirements may be insufficient for persistence assessment, ECHA responded that the understanding of the quality of reporting and of the use of data tends to differ a lot between registrants and authorities.

Validation and reference chemicals

The CEFIC-funded ECO 12 report was considered useful. The inclusion of the ECO 12 chemicals in any future method development and guideline development was seen as important. Testing some of these reference chemicals within the OECD 314 studies in order to increase stakeholder confidence in lab-to-field interpretation from these studies was also seen as a priority.

However, it was noted that the reference chemicals from different persistence bins do not provide any mechanistic information to help predict whether chemicals will be degradable / persistent. It was unclear whether the biodegradability of ECO 12 chemicals could be explained by a mechanistic understanding, e.g. of growth, co-metabolism or the biochemical pathways for biodegradation. It was agreed that a more mechanistic approach to identifying an appropriate validation or reference chemical list could assist the development of next generation QSBRs and structure / degradation rule bases. It was noted that the latest version of Biowin is based on empirical data rather than expert knowledge of degradation pathways.

Enhanced screening studies for persistence

It was suggested that the current RBTs were a ‘bacterial lottery’ and there was a critical need to improve understanding on a number of factors including what concentrations of chemicals should be tested and how to obtain an appropriate level of diversity of bacteria in the inoculum used. There was also some consensus that enhancements to existing RBTs were required to improve persistence assessments but there were different views about the extent to which some of these enhancements could be made. Some participants were keen to point out that the fact that biodegradability can occur is very positive and an indication that a substance is unlikely to persist. However, the regulatory concern was that if tests were ‘over enhanced’ these would underestimate persistence in certain environments (i.e. poorly degradable substances easily

passing enhanced RBTs and then being found in environmental monitoring programmes such as those conducted under the auspices of the Water Framework Directive) (EC, 2000).

A participant stated that the discussion was mainly based on deriving a relevant inoculum size sufficient to ensure that it would include relevant degraders from the environment. A member of the panel said that the number of microorganisms is in the order of 10^6 per ml of water, 10^9 per gram of soil (Whitman *et al*. 1998), and 10^9 per ml activated sludge (Goodfellow *et al*, 1996). The issue is finding a better screening test for persistence, since in his opinion the available ready tests are tests of inocula capability, not substance biodegradability.

It was also questioned whether the Oslo/Paris Convention (for the Protection of the Marine Environment of the North-East Atlantic) (OSPAR) marine degradation test was a good candidate for targeted field flow fractionation. The response was that it would be; however the variability in marine inocula is high and test durations need to exceed 60 days for many positive reference chemicals. This prompted some discussion about how realistic the biodegradation test durations are with respect to the lack of reference conditions. In response to that, it was indicated that ideally one needs to know the rules that govern inocula behaviour, certainly the relationship between diversity and degradation and the kinetics involved.

It was asked, from a pragmatic point of view, at what point testing should stop and when it is decided that no degradation is going to occur. A member of the panel replied that they would try one of the inherent biodegradation tests (OECD 302 tests) (OECD, 1981a,b; 1992a) for several months, then a closed bottle test and if that does not provide any evidence of primary degradation it would be sufficient to conclude that the chemical is persistent.

A comment was made on the need to see some form of comparison of new enhanced tests with existing ready tests (OECD 301) (OECD, 1992a-h),. It was also mentioned that microbial density and endpoint (e.g. time) need to go hand-in-hand as it becomes a kinetic issue for simulation type tests. The response was that we need to use reference chemicals, with an established range of persistency profiles (such as those identified in ECO 12), against a range of inoculum densities that includes those encountered within the existing ready tests (OECD 301) and (OECD, 310) (OECD, 2006). The work presented by Russell Davenport demonstrated that slight increases in inoculum density reduced variation between studies and increased confidence in the persistency conclusion.

There was also some discussion on the value of microbial profiling which, coupled with information on substance composition, could provide a greater insight to the potential persistence of substances. Currently, there is limited experience with these tools but their value could be enhanced as metagenomic libraries develop and grow and more is known about the catabolic potential of microbial communities.

Use of higher tiered tests in persistence assessments

Regulatory participants indicated that they would not accept the use of wastewater treatment plant (WWTP) simulation studies to simulate degradation in the environment and were clear that the ECHA and Member States have rejected the OECD 314 and OECD 303 tests. Their justification is that:

- the inoculum is derived from WWTPs and not the environment;

- there are concerns that not all chemicals, including down the drain chemicals, pass through WWTPs prior to entering the natural environment;
- there is a large variability in the WWTP removal efficiency for micropollutants depending amongst others on the treatment technology, operation mode and weather conditions;
- the duration of the OECD 314B study (OECD, 2008b), which at 28 days, is substantially longer than typical hydraulic sludge retention times in full scale WWTPs and this could lead to overly favourable assessment of degradation in the OECD 314B.

The arguments against accepting the OECD 314 test were challenged by some participants who re-emphasised that there are studies showing that the vast majority of 'down the drain' chemicals pass through a WWTP before being discharged into the environment and that experience with the OECD 314B indicates that most of the degradation takes place over the first few days, i.e. within the hydraulic and sludge retention time of most WWTPs. It was emphasised that the additional duration of the OECD 314B was to enable slowly degrading intermediates to be identified. From a scientific perspective it was pointed out that WWTPs contain natural assemblages of bacteria and other biota that are also present in the receiving waters. As such the tests assess whether substances will have potential to be degraded or will persist in the environment.

Furthermore, it was questioned why the regulators would accept the OECD 301 tests, that also contain inocula derived from sewage, when this was even more unrealistic than the OECD 314B test (which uses more relevant test concentrations, no nutrient solutions, etc.). The response was that a lot more was known about the OECD 301 test and it had more stakeholder confidence. In support of the OECD 314B test, participants indicated that the study is data- and knowledge- rich providing information on elimination pathway, transformation products and degradation kinetics. It was clear that there was a divergent view between industry proponents and regulators. This could potentially be resolved by generating more data on a reference set of chemicals. One other option that was identified within these discussions was that the persistency criteria applied to the OECD 302B (OECD, 1992a) inherent biodegradation test for the length of the lag and log phase could be applied to the OECD 314B.

There was a general concern that the higher tier tests required to support product registrations (e.g. the OECD 308 and 309) (OECD, 2002a, 2004) were expensive to undertake considering the lack of validation and consensus on the value of the data they provide. The discussion then moved on to the OECD 309 test which is being requested by regulatory agencies although there was a consensus that this test had not been subject to extensive validation and there were different levels of relative stakeholder experience of this study versus alternative test systems such as the OECD 314D and 314E (OECD, 2008c,d).

Overall persistence (P_{OV})

The question why overall persistence (P_{OV}) was not used in P assessment was asked. P_{ov} describes the average lifetime of a chemical in the environment. It therefore encapsulates the net effect of the reactivity of a chemical in individual media (usually in terms of single-media half-lives), residence time, partitioning behaviour and the distribution of emissions (ECETOC, 2003). A response was made that P_{OV} depends very much on use profile (not just partitioning behaviour) so if use pattern changes, P_{OV} might change too. However, it was widely accepted that P_{OV} was useful in identifying the most appropriate, or relevant environmental matrix or compartments, to generate the environmental half-life data. This approach is

advocated by ECETOC in its persistence report (ECETOC, 2003). Since the overall persistence is the weighted average of the residence time in all media, it may sometimes be greater than any of the individual medium-specific half-lives for some chemicals (depending on their transport properties). There is an ongoing debate in the scientific community on the best method for using the overall persistence. At this point in time, however, there is no agreement on the criteria that should be applied to the overall persistence.

Degradability versus bioavailability

A question was asked about the relationship between bioavailability and persistence i.e. when is a bound residue available and when is it not? Specific examples (e.g. alkyl benzene sulfonates) identified by the participants considered the impact of sorption and partitioning on biodegradation kinetics e.g. how to deal with an adsorptive readily degradable chemical that is slowly released from sediment / soil, such that half-lives may be longer than P/vP criteria and low level exposure could occur. This was followed by a discussion on how bioavailability affects persistence assessment, for instance sorptive chemicals which are slowly released.

This raised the issue about PBT risk assessment because the substance may be categorised as persistent but risks may be mitigated if it is released slowly and once released it is then biodegraded. As a result of these discussions, several participants highlighted the need for a more holistic approach that considers bioavailability (to microorganisms and higher organisms), bioaccumulation and toxicity, rather than focusing attention on a single property such as persistence or environmental half-life. One participant highlighted that the re-suspension of particulates may increase bioavailability of adsorbed chemical materials and the need to account for this phenomenon within test designs.

A member of the group questioned the presenter Charles Eadsforth on the subject of bioavailability and bioaccessibility of NERs. He said that an ISO standard addressing extraction is available, and that this was not covered in his talk. However, this ISO standard was developed with neutral non-polar chemicals in mind and may not be applicable to all classes of chemicals. A specific example highlighted that some antibiotics behave in a different way to agrochemical products in that they tend to stick in the soil but there is evidence that these are mineralised further once in the soil (a figure of 20% or more being degraded within 100 days). It was noted in the discussions that instantaneous formation of large percentages of NER, as seen with various pharmaceuticals, seems to be a different mechanism than slow steady NER formation (Müller *et al*, 2013).

Given the difficulties in characterising UVCBs, it was suggested that an overall dissipation half-life is a practical measure of the persistence of a UVCB and can be related from laboratory to the field. However, some argue this is an unsatisfactory measure for assessing risks since the ultimate fate of the components are unknown. Furthermore, it was pointed out that there was a legal requirement under REACH to provide information on biodegradation rates. A risk assessment will need to distinguish between loss processes such as leaching, volatilisation or formation of NERs. There are significant concerns with NERs because with respect to legal interpretation this is a ‘black box’ under REACH Annex 13.

Further discussions on standardised extraction schemes to characterise NER continued. Many thought this would be very difficult given the diversity of chemicals and variety of matrices found in the environment. It was suggested that using all the available information, including functional groups present in the test substance, knowledge of binding mechanisms, knowledge of the types of sorbents used and other available

information could support the development of 'extraction models' useful in defining the best extraction approach for any given chemical. This was identified as a topic for further research.

The use of sterile controls and different extraction procedures may help to differentiate between biodegradation and physical losses and assess potential availability of NERs. This was considered essential for assessing complex substances although some practitioners had experienced problems maintaining the sterility of soils and sediments.

Transformation products

There was discussion on the importance of a 'thinking phase' before initiating any higher tier studies. Basically this was to look at possible degradation pathways before starting the study, write down SMILES of potential transformation products and run prediction models to gain an insight on their physico-chemical properties. It should then be possible to optimise extraction scheme and analytical methodology as well as gaining information on predicted toxicity and log K_{ow} of the transformation products. There was consensus that there should be increased use of *in silico* screening to help focus and adapt the experimental strategy. This was particularly relevant to pharmaceuticals where there should be a lot of potentially relevant information prior to the need to conduct higher tier testing.

Temperature correction

Where studies were conducted at 20°C, one participant indicated that temperature should be normalised using the Q_{10} value of 2.8 to account for a temperature difference of 10°C (EFSA Journal, 2007). However, it was pointed out that the Arrhenius equation temperature correction is not always applicable to biotic transformation as different environmental species have different temperature optima and that the situation seems to be substance and inoculum-specific. It was also pointed out that some bacteria in the inoculum would die if the experimental incubation temperature exceeded their inherent temperature range. Work to date appears to indicate that some degraders perform better at low temperatures e.g. some microbes have temperature ranges between -15 and 10°C (which are classified as *psychrophiles*) and could be excluded from higher tiered studies at 20°C. Whilst no agreement could be made about routinely normalizing studies with respect to temperature, it was agreed that more realism may be provided by testing at environmentally relevant temperatures or the temperature at which the inocula were collected from the environment. One participant disagreed because if the tests were not made at standardised temperatures like 20°C the outcome of the studies will not be comparable. However, it was also noted that as different inocula are used for different studies then they will never be comparable and truly standardised.

Biodegradation kinetics

In response to the presentation from Kees van Ginkel, one participant asked a question about how relevant laboratory observations showing that growth-linked biodegradation can occur are to environmental conditions where chemical concentrations are too low to support microbial growth or that the relative abundance of the competent microbial population may be too low to measure removal. The presenter, Kees van Ginkel, responded that in his studies he added some glucose into test systems not to promote cometabolism but to stimulate inocula growth (including competent degraders). This provides some realism where continuous nutrient input is occurring in natural habitats. In combination with similar substances, levels of degradation can increase in the environment through growth-linked degradation, not always

through cometabolism. Another response suggested that we need to bear in mind the complexity of the community where some microorganisms may be cometabolising in the environment while others grow as they degrade substances.

Several of the talks had referred to the importance of photodegradation (either direct or indirect) in aquatic persistence testing, with one talk describing modified simulation studies that included a defined photoperiod that resulted in shorter dissipation rates. In his talk, Robin Oliver had described aquatic simulation studies in which algae had been included in an attempt to more realistically mimic environmentally relevant conditions. It was thought that generation of hydroxyl and other oxygen radical species by virtue of algal presence might contribute to the increased degradation seen in these studies. A discussion focused on the relevance for the environment of photodegradation in aquatic environments; where suspended matter, depth and shading will all decrease the intensity of light available for photolysis. A comment was raised that the issue of photodegradation was irrelevant for groundwater. In the discussions a recent report (Jiménez and van de Meent, 2011) on the relevance of direct and indirect photolysis in aquatic environments by RIVM was referred to, in which the conclusion had been drawn that photolysis was unlikely to contribute to rates of degradation compared to other abiotic and biotic mechanisms. However, some other members of the workshop disagreed with this view, citing that primary productivity by algae and cyanobacteria in oceans was global and occurs at depths of up to 200m. No consensus was reached with respect to the relevance of light in persistence assessments in aquatic environments.

2.5 Syndicate discussions

Four parallel syndicate sessions were held in each of the two afternoon sessions to discuss specific challenges associated with degradation and persistence assessments. The Organising Committee identified a number of key questions for members of the four syndicate groups to address in each afternoon session. The four syndicates discussed questions under the following themes: (i) the challenges with the persistence assessment of difficult to test substances, (ii) improved screening approaches for persistence assessment, (iii) interpretation of higher tiered studies, and (iv) enhanced realism within persistence assessment. A plenary was held at the end of each of the syndicate sessions. Each rapporteur of the four syndicate groups provided feedback to all participants; an opportunity to clarify any of the points that were raised and for open discussion was also provided.

Syndicate 1: Challenges with the persistence assessment of difficult-to-test substances

Moderator: Graham Whale
Rapporteur: Daniel Merckel

Rolf-Alexander Düring
Charles Eadsforth *
Malyka Galay Burgos
Bruno Hubesch
Anu Kapanen
Georg Kreutzer
Jacques l'Haridon *
Marie-Chantal Huet *
Laurence Libelo *
Dan Salvito
Markus Seyfried
Kees van Ginkel

* Participated in Syndicate session 1B.

1. *When should higher tiered tests on complex substances be waived?*
2. *If degradation data is required, under what circumstances can this be provided on the basis of predicted data for representative molecules?*
3. *From an analytical perspective, what should be measured and how?*
4. *Can endpoints be based on fate of whole substance as well as components?*
5. *How should complex substances be dosed into the test bearing in mind potential differences in physico-chemical properties of components?*
6. *Transformation products and non-extractable residues – do they matter?*

Syndicate session report

The syndicate began with a tour de table on what participants understood by 'difficult-to-test substances'. The responses can be grouped under two headings (with potential crossover): (i) substances with difficulties mainly around their characterisation and analysis, and (ii) substances whose properties mean that practical aspects of testing are difficult. Examples given were petroleum-like products (UVCB examples, i.e. not deliberate mixtures, with components with various physico-chemical properties), natural complex substances (with compositions that can vary each year according to different natural product crops), single substances that are poorly soluble and/or volatile, amphiphilics, and other substances for which analysis / characterisation is difficult (e.g. substances subject to major interferences from background contamination).

1. When should higher tiered tests on complex substances be waived?

The group thought that no general conclusion was possible on situations where higher tier testing could be waived; it is dependent on specific examples. For complex substances, use of read across approaches between components (interpolation and extrapolation) were thought to be important in cases where ready biodegradability could be used as the basis for waiving higher tier testing when ready biodegradability test (RBT) data were available on the substance itself or major components of the substance. The group recognised that in such cases residue analysis was important to check if some components were not degrading in a substance that 'passed' a ready test (ideally specific analysis for parent disappearance and metabolite formation – i.e. more detail than secondary measures of biodegradation like CO_2 production and O_2 uptake). This would especially be the case for more poorly characterised materials where structural similarity of components was less certain. One recommendation was a non-target approach to look for both non-degrading components and metabolites. This would require high-resolution mass spectroscopy (MS) combined with ambient ionisation techniques.

There was also some discussion on the role of microbial profiling to give an indication of the presence and growth of different types of degraders to provide weight of evidence but the general consensus was this was not sufficiently developed to be reliable. Another consideration was that increase in biomass could be a useful measure but there was no consensus on the value for complex substances, the concern being that this does not give any information on substances that do not degrade.

2. If degradation data is required, under what circumstances can this be provided on the basis of predicted data for representative molecules? From an analytical perspective, what should be measured and how?

A recurring theme in the group was the need to characterise substances (especially complex ones) as far as possible to allow robust conclusions to be drawn for regulatory purposes. Weight of evidence approaches are important, but the ability to identify the majority or nearly all constituents in a substance was thought essential to drawn meaningful conclusions using read across approaches. The same is true of predictive (QSAR) approaches where modelling is dependent on representative molecular structures (and associated physico-chemical properties). This discussion included approaches like the hydrocarbon block, and the dangers of splitting complex substances into too many sub-groups – delineation of physico-chemical properties and knowing where to place the thresholds. It was recognised that although future developments in analytical techniques may allow more straightforward detection and characterisation of the constituents and degradants within multi-component substances, analysis remains one of the major challenges and is extremely resource-intensive.

The idea to use available REACH registration data to train existing biodegradation QSAR models further was also mooted.

One of the concerns discussed was that experience suggests the ECHA take a precautionary approach regarding read across of data between endpoints. If a similar attitude prevails with read across to components of complex substances this could become a significant issue. An alternative perspective is that Annex 13 allows more freedom than before and the critical point is to build up a robust case. The key point regarding persistence assessments here is how can the reliability and suitability of component data (either measured or predicted) be demonstrated such that it gains regulatory acceptance.

4. Can endpoints be based on fate of whole substance as well as components?

The group discussed the question at what point one stops attempting to identify a complex substance's constituents and focuses on testing the 'whole' substance. Again it was felt no general rules could be elaborated, that a 'case-by-case' approach would be needed. The experience of one group member was that a 'constituent approach' can be successful for many higher volume (more economically important) products, but that for some substances they were forced to assess the complex substance itself as the number of constituents and structures and properties of the constituents diverged and became unmanageable in a constituent approach. It seemed probable that lower volume (less economically important) substances would be likely to undergo whole substance assessment on cost grounds.

This led onto a discussion on the use of chemical profiling to follow (ready) biodegradation as a pragmatic means to give more information than the usual secondary measures of ready biodegradation, and as a way to inform the hazard assessment (mainly PBT) for the parent substance and degradation products. Ideas put forward included: following a complex substance's average molecular weight change (i.e. decline) over the course of a test using MS; from a hazard assessment point of view, upon completion of an RBT running a log K_{ow} test (e.g. HPLC or shake flask) on the residue and comparing the log K_{ow} against that of the parent substance to inform on the degradation product's / residue's potential for bioaccumulation (i.e. does the resulting mixture's log K_{ow} fall below the threshold for PBT concern, or, if present, can components in the 'boundary of concern' be identified and put forward for further testing?); and use of surrogate testing e.g. change in toxicity over time for discharges from SCAS type systems. It was noted there will be exceptions to the supposition that toxicity of degradants will be lower than the parent (e.g. alkyl phenol ethoxylate degradation).

There was a comment made that the issues encountered with assessing complex substances were in many ways analogous to those encountered in whole effluent assessments (WEAs) developed to assess whether PBT substances were present in effluent discharges. As such, the approach could follow / adapt some of the guidance which has been developed by OSPAR in their practical guide for WEA (OSPAR, 2007). This was considered relevant because the OSPAR WEA approach had considered schemes for not just assessing the persistency of effluent components but the persistence of potentially bioaccumulating and toxic substances. For example, solid-phase micro extraction (SPME) run before and after degradation assessments can give information on the persistence of potentially bioaccumulating substances.

5. How should complex substances be dosed into the test bearing in mind potential differences in physico-chemical properties of components?

One of the problems with the whole substance approach was that this could contain components with considerably different properties which could not only cause problems with the way that the test was set up but also how the results should be interpreted. For example, petroleum products can contain components which are volatile whilst also containing very poorly soluble components. This leads to problems that some components will be lost via volatilisation before others have had any chance to reach equilibrium within the soil / sediment. There was also debate on how poorly water soluble substances should be dosed. From an ecotoxicity perspective the use of solvents for preparation of test media may affect the expected toxicity. Therefore it is likely that similar issues will apply to degradation tests (i.e. solvents may affect the availability of the substance to competent degraders).

6. Transformation products and non-extractable residues – do they matter?

The idea of specifically investigating a modified OECD 301C ("modified MITI I test"), in which degradants are characterised, with conditions more suited to biodegradation (lower substance concentration, more viable inoculum, larger test vessels) was put forward.

Following on from the idea to screen for changes in hydrophobicity following a ready test, one of the group members described a project that is being planned between the Research Institute for Fragrance Materials (RIFM) and University of Stockholm (Michael McLachlan). A wastewater treatment simulation study will be followed by the McLachlan 'better BCF' approach (Adolfsson-Erici *et al*, 2012) for selected (6 – 10) fragrance chemicals, followed by 'simple' natural products if successful, thereby linking the assessment of P and B (for degradants / persistent parent constituents). It was suggested that ECETOC could be involved in this project, as it is very relevant for other types of complex substance. Links / co-funding and the possibility of a CEFIC/LRI RfP could be explored.

Another idea that came out of the group was for a project entitled "Methods to characterise the poorly defined fraction of complex materials and assess through a screening approach P, B and T endpoints of these complex materials". This would involve literature searching and potentially a subsequent ring test of complex mixtures.

Syndicate 2: Improved screening approaches for persistence assessment

Moderator: Jason Snape
Rapporteur: Russell Davenport

François Brillet
Juha Einola
Tom Fisher
Marie-Chantal Huet
Ulrich Jöhncke
Thomas Junker
Anu Kapanen *
Georg Kreutzer *
Laurence Libelo
Kathleen McDonough
Markus Seyfried *
Eric Verbruggen

* Participated in Syndicate session 2B.

1. *Is there value in current RBTs in P assessment?*
2. *What are the views on extending the duration from 28 to 60 day tests (impact of test duration)?*
3. *What are the opinions on modified tests (how confident are we in the current modified approaches)?*
4. *Do OECD 314 tests have value in persistence assessments?*
5. *How to incorporate pre-adaptation in screening-level persistence assessments?*
6. *How confident are we in enhanced approaches?*
7. *Can we get more out of inherent biodegradation studies (e.g. MITI II)?*
8. *How can we reliably assess marine persistence?*
9. *What are useful endpoints for persistence assessment (primary versus ultimate)?*

The order of the questions provided at the workshop was changed and further questions were posed. The questions above reflect those used during the break-out session.

1. Is there value in current RBTs in P assessment?

It was widely agreed that there is still a role for ready biodegradation tests (RBTs) in persistence assessment. This role is limited to the initial screening assessment stage where there is a high degree of confidence that chemicals that pass the RBT will be non-persistent in the environment.

Currently, there are seven RBTs (OECD 301A-F and OECD 310) (OECD, 1992c-h;2006) assessing ready biodegradability using a variety of semi-specific endpoints such as Biochemical Oxygen Demand (BOD), Dissolved Organic Carbon (DOC) removal and carbon dioxide evolution. It was recognised that there was significant room to consolidate these RBTs. In reality, the most frequently used RBTs are the OECD 301B, D

and F and the OECD 310. The OECD 301 series of tests that rely on DOC removal (OECD 301 A and E) are not widely used as they are not suitable for adsorbing, volatile or poorly water soluble test substances. Based on some experience of enhanced biodegradation screening under REACH where the test durations had been extended to 60 days there was some concern that the fixed 28 day test duration of the RBT was missing the degradation phase for some chemicals with extended lag periods.

Finally, some technical innovations were also discussed. These included: (i) the availability of new instrumentation that increased analytical sensitivity (ii) the use of tests with combined analytes (e.g. BOD and carbon dioxide evolution), and (iii) the introduction of a test to screen for biodegradability in water-sediment systems (Junker *et al*, 2010).

2. What are the views on extending the duration from 28 to 60 day tests?

The issue of prolonging test duration is already written into REACH technical guidance and in principle it was agreed that this was a good idea but not following any pre-adaptation approaches. It was also highlighted that in some cases lag periods extended to beyond 60 days and so a case could be made to extend the test duration further; this is particularly true for marine biodegradability assessments using OECD 306 and 309 tests (OECD, 1992i;2004a) (see below). The use of these data in persistency assessments would be subject to expert judgement based on the nature of the biodegradation curves and the behaviour of any reference compounds.

3. What are opinions on modified tests?

It is important to recognise the nature of chemicals being tested. It was felt that the current REACH guidance allowing modified tests to assess chemicals with (i) poor water solubility and/or (ii) toxicity overcame some of the limitations associated with the RBTs. These were accepted as a good idea and should be allowed.

4. Do OECD 314 tests have value in P assessment?

There was a difference of opinion between regulators and practitioners with respect to the use of OECD 314 tests, which comprise five separate tests simulating the fate of down-the-drain chemicals in wastewater treatment plants and surface waters. The greatest concern was associated with the use of the OECD 314B within persistence assessments due to its high level of activated sludge solids. One participant highlighted that the OECD 314B was a more up-to-date version of the OCED 302B Zahn-Wellens test and that the test should be able to be used in persistence assessments if the strict criteria applied to the OECD 302B test were applied to the OECD 314B test. Some regulatory stakeholders appear to have more confidence with the OECD 309 Surface Water Transformation test. However, it was recognised that there are few data for both the OECD 309 and 314 tests at present. The OECD 314 series are better described than OECD 309 tests, and it was pointed out that the OECD 309 test also allows the inclusion of more solids and provides an option for some pre-exposure. It was felt that the OECD 314D and E tests may have value in P assessment, but some of the modification in Annex of 309 may be preferable e.g. pre-adaptation. It was agreed that more data was required for both studies to increase the confidence in the studies with regulators and practitioners.

5. How to incorporate pre-adaptation in screening-level P assessments?

There was a good discussion about the advantages and limitations / concerns of pre-adaptation. Most of these discussions focused on (i) concerns over ecological significance of adaptation, (ii) the timescales required for adaptation to occur and (iii) its relevance and importance for different habitats.

The various ways in which adaption or pre-exposure could take place were discussed and it was agreed that there was a need to (i) review and define adaptation (ii) describe what is happening in extended lag periods, (iii) identify relevant experimental adaptation regimes, (iv) validate these approaches with appropriate benchmark chemicals and, (iv) define appropriate pass criteria (e.g. pass / fail or half-lives).

6. How confident are we in enhanced approaches in P assessment?

Following on from the presentations earlier in the day, there was general all round support for the use of (i) extended test durations and (ii) enhanced inocula concentrations but particularly when used in conjunction with a reference validation set of chemicals.

It was felt that progress in this area was such that a large ring test for enhanced tests in comparison with other current screening tests and with a reference set of validation chemicals would be necessary. It was also felt that more validation around adaptation methods was required.

7. Can we get more out of the current inherent biodegradation studies?

Inherent biodegradation studies were designed to establish whether the potential for degradation existed (OECD, 2006). It was agreed within the syndicate that the failure to observe any biodegradation within an Inherent biodegradation study could be used as evidence of environmental persistence. However, there was some concern that where inherent biodegradability studies provide evidence of degradation potential the knowledge generated has limited use under REACH within a persistence assessment. A major limiting factor is associated with the short lag and log period criteria defined within REACH that would not be assessed within the sampling regime of a standard OECD 302B study. The OECD 302A (modified Semi-Continuous Activated Sludge Test; SCAS) cannot be used within persistence and exposure assessments due to concerns over its infinite sludge retention time and strong adaptation potential. However, subtle changes to the 302A and 302B studies addressing test compound concentrations, test durations, sample intervals and the inclusion of a sludge retention time within the SCAS could improve the relevance and reliability of these studies. In part the more recent OECD 314B is a more rigorous and widely applicable test than the OECD 302B test that addresses many of these refinements and can meet the specific criteria laid out under REACH (see question 4 above). It was also recognised that the enhanced biodegradation screening tests, with increased inocula levels, might also provide a more robust alternative to the OECD 302B and OECD 302C tests.

8. How can we reliably assess marine persistence?

Marine persistence is usually assessed by the OECD 306 and/or 309 test guidelines (OECD, 1992i; 2004a). Both these studies have a test duration that ends at day 60. Marine studies presented on Day 1 clearly demonstrated that lag periods for chemicals known to be non-persistent can exceed these test durations and result in a false persistency assignment. In many cases positive reference chemicals (e.g. aniline) can fail

marine OECD 309 tests, and lag periods of > 70-80 days can be observed before the onset of rapid degradation even with enhanced marine inocula at 100X cell concentrations. Data also presented on Day 1 also indicated that the greatest sources of variation in inocula were also associated with microbes derived from marine sources (Davenport *et al*, presentation). The current level of experience and confidence with marine biodegradability assessments remains limited and further research work is required. One option suggested included the use of marine water-sediment systems or a semi-continuous test regime with low level adaptation as described within the Annexes of the OECD 309 test guideline.

9. What are useful endpoints for persistence assessment (primary versus ultimate)?

Whilst persistence assessment is focused predominantly on the parent compound it was widely recognised that the mineralisation endpoint (CO_2 and BOD) can be more robust as it accounts for losses that can be attributed to both parent compound and degradation products. The group did recognise that (i) the mineralisation pass criteria for RBTs should not be assigned to higher-tiered biodegradation studies, (ii) the rates and extents of degradation observed differ for each endpoint (e.g. parent removal, DOC removal, carbon dioxide evolution), and (iii) the levels of degradation observed change with substance and inocula concentrations.

The group also recognised that mineralisation data would not always be available for higher tiered tests. A radiolabel was required and this is not always feasible (e.g. complex mixtures and some chemical moieties). In such circumstances many higher tiered tests will only provide evidence of removal or dissipation and not necessarily biodegradation. However, it was agreed that the more endpoints that you could assess the higher the level of confidence in the study. It was also agreed that every effort should be made to assess degradation or transformation products formed at > 10% of the applied parent or ^{14}C material (e.g. OECD, 2002b). Within REACH an obligation exists to look at transformation products formed at >0.1% of the applied parent or ^{14}C material. However, this poses some significant practical issues as most higher tiered biodegradation studies can only resolve 2% of the applied radioactivity due to (i) the specific activity associated with the radiolabelled test material and (ii) dosing near an environmentally relevant concentration.

Recommendations

- The OECD should consider convening an Expert Working Group to consolidate and update the RBTs to reflect (i) the availability of new instrumentation with increased analytical sensitivity (ii) the use of tests with combined analytes (e.g. BOD and carbon dioxide evolution), and (iii) the need to screen for biodegradability in water-sediment systems.
- A laboratory-based study to compare the performance of the OECD 309 and relevant OECD 314 tests, using appropriate benchmark chemicals with known biodegradability / persistency is required to increase industrial and regulatory stakeholder confidence in these studies. There are a growing number of OECD 309 studies being performed for the AIR process of plant protection products. Up to now there is only limited confidence in this test especially with respect to the biomass variation during the experiment. It was also recommended that the enhanced biomass studies could be included as part of this exercise. It was also recommended that this exercise includes a ring test for enhanced biomass studies to compare their performance against other current screening tests with a reference set of validation chemicals.

- Research is conducted to demonstrate the ecological significance of adaptation and get a better understanding of adaptation processes, the probability of these to occur under environmental conditions, including the mechanisms of adaptation and suitable test systems to allow provision for these mechanisms to occur. This approach should also develop appropriate marine studies with a reference set of validation chemicals.
- Test durations should be made more flexible and be allowed to extend to beyond 60 days as marine biodegradation studies often have lengthy lag periods before the onset of degradation.
- It is important to recognise the physico-chemical properties of chemicals being tested (e.g. solubility and volatility) and the limitations of each biodegradation test system. It was recommended that the current REACH guidance allowing modified tests, to assess chemicals with (i) poor water solubility and/or (ii) toxicity, should be allowed as they overcame some of the limitations associated with the existing RBTs.

Syndicate 3: Interpretation of higher tiered studies

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1. *Non-extractable residue (NER) characterisation and interpretation.*
2. *DT_{50} versus $DegT_{50}$.*
3. *Impact of temperature and normalisation of results.*
4. *Transformation products (TP) identity and need for Environmental Risk Assessment (ERA) risk.*
5. *Endpoints for persistence assessment (primary versus ultimate).*
6. *Dealing with half-life differences between soils and sediments.*

1. NER characterisation and interpretation

It was noted early in the syndicate discussions that it would be helpful to have updates, or reports from the two ECETOC Task Forces (TF) (ECETOC, 2013a,b) heading up discussions on the characterisation and assessment of non-extractable residues. It was suggested that a review of these TF activities on the ECETOC web page would be good follow-up for interested parties. In light of this, the following represents the discussion of this syndicate realising that many of the issues may have already been discussed and/or may already have recommendations in place. It was also noted that NER will likely be part of other syndicate discussions at this workshop.

In general, a review of terms was helpful to ensure everyone has the same understanding. Non-extractable residue (NER) has always been an operational definition, defined by the steps taken to extract the residues from the various environmental matrices without destroying the matrix itself or altering the nature of the residue in question. Anything remaining is considered NER.

NER is no more and no less than the fraction that cannot be extracted under the analytical constraints of the method used. This does not include any information on processes or binding mechanisms that may be responsible for the observed 'loss of extractability' (compared to the desired 100% yield).

The term bound residue (BR) has sometimes been used synonymously to NER, but this is obviously not useful. The term **bound** is an indication that this fraction should be considered to only encompass molecules that are irreversibly bound to the matrix. Therefore, BR constitute a sub-fraction of NER (BR ≤ NER), which could be clarified by defining BR as covalently or irreversibly bound residues (or incorporated into biomass, see below). In this case, BR formation would be an ultimate loss process for the substance in question and contribute to its relief in terms of persistence.

This is not justified, however, for NER. The reason for this statement is two-fold:

a) Since the NER fraction depends on the method used; a substance could be described as an NER based on the use of an inappropriate extraction procedure. Consequently, the methods of extraction require careful scrutiny and judgement.
b) It has been unequivocally demonstrated for some compounds (atrazine, sulfadiazine) that there is the possibility of long-term remobilisation of the parent compound from solid matrices (sediment, soil) on a time scale of years although the extractability of the compounds was low on shorter time scales (high amounts of NER). Obviously, there is a fraction that is not bound strong enough to ensure its ultimate 'disappearance' from the system. This sub-fraction of NER should be considered at least in long-term risk assessment.

One opinion was that what may have been characterised as 'remobilisation' of parent may be due to a change in redox conditions from aerobic to anaerobic. Anaerobic processes may be responsible for reappearance of the parent (or an active metabolite) that has been either immobilised or biotransformed by aerobic processes - anaerobic conditions do exist in 'aerobic' water-sediment test systems. The group felt it is necessary to generally understand remobilisation in order to define to what extent it may be an issue in risk assessment. There is also a need to define suitable (case-specific?) metrics for this risk.

In recent months, UBA has introduced other terms (type 1 and type 2 NER) that result in a similar distinction of NER: Type 1 NER is the fraction that may be remobilised as parent substance (i.e. sulfadiazine) or active metabolite due to environmental events and may thus be considered as a hidden hazard. Type 2 is the NER fraction that is incorporated as parent molecule and/or metabolite into humic substances by covalent binding and thus will not be remobilised. Some syndicate participants felt that the Type 2 NER was the same as what has been previously defined as 'Bound Residue'. Yet another group additionally introduced type 3 NER, separating those that are (truly degraded and) incorporated into biomass as biogenic residues, e.g. amino acids, phospholipids, etc. (no environmental risk). The further development of extraction schemes for separating type 1 NER and type 2 NER (and type 3 NER) was suggested, although it seems necessary to distinguish different schemes for different substance classes. Another way forward could be the identification and quantification of the fraction of biogenic residues within the NER, which is what Andreas Schäffer *et al* are working on. (e.g. Kaestner *et al*, 2013)

It was remarked that instantaneous formation of large percentages of NER, as seen with various pharmaceuticals, seems to be a different mechanism than slow steady NER formation observed for other types of compound (Müller *et al*, 2013).

The formation of NER leads to the additional problem that allows for a wider scope of interpretation. As discussed above, a significant emphasis has focused on the interpretation of NER in terms of persistence

assessment and it is still under debate i.e. should NER be regarded as degraded or as potential reservoir that has to be excluded from the persistence evaluation. Regardless of which interpretation is agreed upon, it is necessary to distinguish the different processes and pathways. In water-sediment studies (OECD 308) where estimating a total system half-life is achievable, the derivation of valid $DegT_{50}$ values for the separate water and sediment compartments is often difficult, due to competing processes of the test substance dissipating from the water phase into the sediment, and the test substance forming NER. Separate $DegT_{50}$ for the water and the sediment phase can often not be estimated because the models used parameter estimation and include too many unknown parameters and the degrees of freedom becomes too high. By using an inverse modelling approach transformation of the chemical and formation of NER can be mathematically separated. It may be possible to differentiate between the fraction of chemical that is non-extractable, but may be remobilised (equivalent to type I NER, entrapped NER, as proposed by UBA and Kästner *et al*, 2013) and the fraction that is permanently removed (equivalent to type 2 NER, bound residues), if sufficient data were available. Such an inverse modelling approach has been applied to data from degradation tests in soil for a number of pesticides (Matthies *et al*, 2008; Loos *et al*, 2012) resulting in half-lives for total dissipation (DT_{50}) and degradation only ($DegT_{50}$).

How should additional information on NER, such as differentiation between NER and BR, be used? What implication could that have on risk assessment? Several examples of bioassays were discussed to assess the potential ecotoxicity of NER. Such approaches are needed to better understand the potential risk posed by NER and provide additional tools for risk assessors to enable more definite action with respect to having no risk, or having a risk that requires further follow-up. The concept of a 'soup' test has been recommended by the ECETOC TF on assessing NERs where sediment ecotoxicity data is developed in real sediments where NERs are present as well as potential transformation products (ECETOC, 2013b). Such a test would help identify where no risk exists, and/or identify where further follow-up is needed either in terms of the bound residue or potential TPs that may have formed. Also discussed is the assessment of NER found in manure, where soil germination and growth studies are conducted in soil amended with manure.

Further discussions on standardised extraction schemes to characterise NER continued. Many thought this would be very difficult given the diversity of chemicals and variety of matrices found in the environment. It was suggested that using all the available information, including functional groups present in the test substance, knowledge of binding mechanisms, knowledge of the types of sorbents used and other available information could support the development of 'extraction models' useful in defining the best extraction approach for any given chemical. This was identified as a topic for further research.

2. DT_{50} versus $DegT_{50}$

The syndicate discussed the current definitions of DT_{50} and $DegT_{50}$. Typically, DT_{50} and $DegT_{50}$ are determined from the remaining concentrations of extractable substance in the test system. DT_{50} is usually defined as the time for 50% of the substance to dissipate (i.e. by degradation or other loss processes such as sorption, leaching, volatilisation, etc.) as determined by observation or extrapolation of substance disappearance, whereas $DegT_{50}$ is considered as the time for 50% degradation of the substance. There are cases where $DegT_{50}$ values are difficult to derive, i.e. for field study data, if appropriate abiotic controls are not possible or lacking, or experimental systems where several compartments are involved, e.g. for OECD 308 studies. While estimating a total system half-life is readily achievable in water-sediment studies (OECD 308), the derivation

of valid $DegT_{50}$ values for the separate water and sediment compartments is often difficult, due to competing processes of the test substance dissipating from the water phase into the sediment, and the test substance forming NER.

The scope of this procedure is the mathematical derivation of characteristic numbers to be used in persistence assessment or multimedia fate modelling. Thereby, it has to be regarded that multimedia fate models implicitly assume first-order kinetics of all loss processes and that regulatory frameworks use half-lives in environmental compartments as persistence criteria. Therefore, it is inevitably necessary to ensure that the derived characteristic "half-lives" fulfil the basic assumption of representing first-order processes.

As described above, by definition DT_{50} does not necessarily obey first-order kinetics, but constitutes the observed time needed to decrease the concentration of a chemical by 50% within an environmental system or compartment – be it in the field or in a controlled experiment. Such DT_{50} values depend on the initial concentration which can be seen from the fact that even within one dataset multiple DT_{50} may occur (often the loss of the first 50% is faster than the subsequent loss of another 50% of the remains). This is due to the fact that observed DT_{50} values are the result of several different overlying processes such as degradation, phase transfer, adsorption/desorption or binding to humic matter. Even though the single processes are of first-order, this does not hold for the observed decrease of the chemicals' concentration. This is especially true for one of the standard outputs of the OECD 308 test - the total system DT_{50}. Therefore, observed DT_{50} values, which have not been clearly shown to describe a first-order process, are inappropriate as input parameters for multimedia fate models and at least questionable to be used as half-life equivalent in persistence assessment.

To circumvent this problem, model approaches that consider the individual first-order processes can be used to estimate rate constants which then can be transformed to real half-lives. However, this is often difficult because of too few data points for reliable estimation of the many parameters (low degrees of freedom). Nevertheless, inverse modelling could be applied to results from standard tests such as OECD 307 or 308 and allows for estimation of first-order rate constants (and therefore true half-lives). Unfortunately, the terms half-life and DT_{50} are sometimes used interchangeably. DT_{50} is then defined as the true half-life as a result of all first-order loss processes (including NER formation) and $DegT_{50}$ being the true half-life of all first-order degradation processes. Deviations from first order kinetics must be addressed, and a possible lag phase should be described. Biphasic kinetics may be due to sorption and desorption processes. A discussion of these deviations and an overall description of the important processes that describe biotransformation in test systems may be found in FOCUS (2006).

It should be noted that there are studies where DT_{50} and $DegT_{50}$ have been defined differently than above (Matthies *et al*, 2008; Loos *et al*, 2012). Where DT_{50} in those studies is defined as the time to 50% disappearance due to NER and metabolite formation, $DegT_{50}$ is defined as the time to 50% metabolite formation only. Both studies show that DT_{50} and $DegT_{50}$ values typically do not differ by more than a factor of 2 for the limited number of chemicals studied. However, at present there is no clear simple relationship between $DegT_{50}$ and DT_{50}, since the relative contribution of NER formation to DT_{50} is compound-specific and not (yet) predictable.

More guidance is needed on persistence assessment with data from multiple tests, as it is unclear how the natural variability of $DegT_{50}$ data should be taken into consideration when comparing against a single trigger

value. As a first approach, with multiple (valid) data, the range of $DegT_{50}$ values should be considered along with all available information on soil types and any other sources of variability. Generally, if a log-normal distribution is given, it was suggested that the geometric mean should be used for comparison against the trigger value, but a weight of evidence approach should be used, as suggested in the revised annex XIII of REACH (EC, 2011).

3. Impact of temperature and normalisation of results

The syndicate thought it was logical to use the same reference temperature both for studies and for trigger values, e.g. 12°C for fresh water and soil, and 9°C for marine water, or 20°C alternatively as what is typically used in standard lab persistence tests. However, a need is seen nonetheless to discuss the relevance of temperature normalisation, as opposed to the uncertainty of single $DegT_{50}$ values. Normalised $DegT_{50}$ values may not reflect reality, as this remains to be proven (see research needs). For temperature normalisation, the Q_{10} factor of 2.2 used in plant protection products (PPP) assessment (FOCUS, 2000), or the new Q_{10} of 2.58 as developed by the European Food Safety Authority (EFSA) (EFSA, 2007) which is based on a literature review could be used, or the Arrhenius equation as suggested in REACH. It was discussed that this normalisation may have originated from its use in chemical degradation processes such as hydrolysis where the overall rate is both temperature and pH dependant. Over time, it has been applied to biotic processes as well, though whether it is correct to apply simple temperature correction factors to biological data is still debated today. On the other hand, the diversity of inocula may have a bigger influence on degradation rates than normalisation from different temperature environments. An evaluation from Helbling *et al* (2012) on degradation tests in 10 different sewage treatment plants resulted in a difference of 4 orders of magnitude in half-lives. So here, it seems vital to understand the different processes first.

Benchmarking with reference chemicals was also suggested by the syndicate as an alternative to temperature normalisation, though not necessarily a new concept. Many fate tests (OECD 308, 314, 303) do not require reference chemicals as part of the study protocol. This leaves a challenge of interpreting study results especially when samples for any given study may have unique seasonal and geographical characteristics that may influence study outcomes in a positive (greater transformation rate) or negative way (slower transformation rate). Including such reference chemicals will help normalise study outcomes when such diversity in samples or sample conditions exist and facilitate the use of the data in determining the regulatory outcome. This may be especially important for borderline persistence / non-persistence cases where the estimated half-life approximates the criteria used between one regulatory outcome and the next more persistent one (e.g. non-persistent, persistent or very persistent).

For persistence assessment, all available information should be assessed, using expert judgement such that a more holistic review may be made. This information may include but not be limited to physico-chemical properties (solubility, log D, log P, vapour pressure), metabolism data, other fate data such as hydrolysis, photolysis and sorption data; route(s) of introduction into the environment and volume of substance applied or introduced per a specific period. Each piece of information is considered together with the other data, e.g. data on hydrolysis and sorption: rapid sorption to sediment may prevent the substance from hydrolysing.

4. Transformation products identity and need for ERA risk

The syndicate group had a discussion on whether the persistence of transformation products was a relevant issue in persistence assessment. It is not included in all regulatory frameworks, but it was agreed that generally transformation products tend to be more polar, i.e. more water soluble and less toxic, but it is important not to miss those that deviate from that rule, such as DDT and DDE, and to understand the underlying processes (see research needs). So there is a need to understand when transformation products should be identified, under what circumstances, and for what endpoints (environmental or human safety) they are relevant in the risk assessment (Escher and Fenner, 2011). It was also noted, for example, that more polar transformation products are more mobile and thus may pose a risk to ground water. As such, identification would be necessary, e.g. for estimation of an acceptable daily intake if drinking water was a concern.

In terms of better defining the issue, more examples of when transformation products are a concern would be helpful, especially when defining / developing action criteria for additional testing. And, as risk reduction measures differ between commodity groups, different information may be needed. Regulatory agencies are always looking for information on transformation products.

There were different viewpoints on how to determine what may be relevant transformation products. Some suggested degradation studies at 30°C to 'screen' for potential products, while others preferred simulation studies conducted at environmentally realistic temperatures. While one approach may allow for a quick turnaround time, the other identifies those more closely linked to standard OECD methods.

Depending on the test sampling intervals, test duration and the test temperature, it was noted that it may be possible to miss the formation of some transformation products. Discussion in the group asked whether these transient transformation products are a real risk, as they are transient in nature, or are the ones that tend to accumulate over the study period more of a concern. While accumulating TPs were generally agreed as a potential concern, no consensus was reached over the concern of transient TPs.

For determining transformation rates, especially when there is more than one subsequent transformation product, more sampling points than what is typically required (6) may be needed to appropriately determine the kinetics for each of the transformation products. It is sometimes very challenging to determine how many extra samples could be needed and maintained for such, while not impacting the overall cost of the study. It would be helpful if one could rapidly screen for TPs prior to the study such that this could be determined ahead of time.

5. Endpoints for persistence assessment (primary versus ultimate)

The syndicate discussed that using primary biodegradation endpoints such as biotransformation is useful and needed in the persistence assessment as well as the risk assessment. But there was general consensus that it was not a major issue. In general, information about both endpoints is always needed.

Several technical points were discussed in how fate studies are conducted with respect to the need for more positive controls to confirm the viability of soil / sediment microbial community; and sterile controls to

assess abiotic transformation. It was noted however, for abiotic controls, that it is difficult to achieve 100% sterile samples even with the combination of different techniques (chemical and physical) such as is done with activated sludge. Although there are other very effective alternatives to sterilisation, such as γ-irradiation, it is also a technique that is not easily available to everyone. Most sterilisation techniques offer a 99% kill at best; therefore with a starting population of 10 billion bacteria a 99% kill would still result in there being 100 million viable bacteria remaining. No consensus was established as to whether sterile controls would be useful and significant regulatory questions could be raised about the validity of a study if sterility could not be maintained in an abiotic control.

A discussion on the use of the data from the OECD 314 test for classification purposes was also held. Although it was argued that the OECD 314 may be useful for risk assessment purposes (refinement of the PEC), the authorities participating in the discussion felt it could not be used for PBT assessment as it informs about degradation in the technosphere (STP or STP-influenced receiving stream) and not the natural environment. It was noted by industry that there have been some correlations established (non-published data) between what has been observed in STP degradation to the mixing zone, receiving waters and subsequent surface waters and also that more work is needed in this area. There is a need to bring forth what is known about the bacteria used in this STP test, including but not limited to the profile of micro-organisms present, occurrence of similar micro-organisms or enzyme systems in the natural environment, transformation half-lives of reference compounds and formation of subsequent TPs such that we can determine whether this is a potential model for the natural environment (surface waters) or not. Though the current regulatory position seems to be that such data can't be used in classification schemes; it is also fair to say that without further research and disclosure of what is learned there is no means of furthering the debate or of advancing the science. This was identified as an area of research during the reporting of the syndicate in the session overview. One participant highlighted that the study may be able to have some immediate use in persistence assessments if the OECD 314 test was interpreted using the strict criteria applied under REACH to the OECD 302B test.

6. Dealing with half-life differences between soils and sediments

How does the fate in one compartment impact the other; after all the compartments are interconnected? The syndicate did not add much to this question other than to say that it seems the overall exposure is quite often driven by the compartment with the slowest half-life and that in general this needs to be approached on a case by case analysis. Further discussion on this topic is also found under the DT_{50} vs. $DegT_{50}$ question. In general, this review requires all the information that is available including route of introduction into the environment and rate of introduction into the environment in order to provide the best assessment possible.

Syndicate 4: Enhanced realism within persistence assessment

Moderator: Stuart Marshall
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1. *Importance of adaptation.*
2. *Field relevance of lab data.*
3. *Can the OECD 314 be used to assess the persistence of down the drain chemicals?*
4. *Impact and significance of light on degradation studies.*
5. *Impact of temperature and normalisation of results.*
6. *Batch versus continuous methods to assess degradation.*
7. *Interpretation of BR / NER from persistence perspective; why should we worry about bound residues?*
8. *Assessment of Transformation products.*

1. Importance of adaptation

The importance of considering adaptation in biodegradation assessments was seen largely as a problem for aquatic systems, mostly as a result of high variability in dynamics and heterogeneity in time and space. It was felt that in soils, inocula were larger and more diverse, so that laboratory tests using soil provided closer fit to field degradation rates. It was recognised that there are currently no specific protocols or recommendations available to test for adaptation, although there are a range of published studies which could provide guidance. Studies including an adaptation phase (e.g. Annex V of the OECD 309) would need to provide environmental degradation rates rather than providing a pass/fail assessment of the inherent potential of a chemical to degrade under non-relevant conditions. In other words, adaptation should not lead to over-estimation of the extent or rate of degradation in the environment. There was discussion about the Japanese MITI (Ministry of International Trade and Industry) test and whether a modified test of this nature could prove useful. Guidance would be required about the appropriate duration of tests, which may even require year-long timescales. Rather than assessment against persistence criteria, any such test could be interpreted in terms of pass – i.e. adaptation can occur – or fail, in which case higher tier tests would

need to be conducted. Alternatively, discussions centred on the need for benchmarking against reference chemicals to allow calibration of test results in terms of persistence. Bins 2-4 proposed in the ECO 12 report (Comber and Holt, 2010) could provide a starting set of chemicals to allow this, although there is a need to increase the number of reference chemicals available.

2. Field relevance of lab data

The extent to which the results of laboratory tests need to be relevant to the field situation was discussed. It was suggested that screening tests do not necessarily need to be field relevant if they are sufficiently conservative with enough benchmarking. Generally, it was considered that ideally screening tests should be indicators of field dissipation rates for the appropriate scenario. OECD 307/308 were considered to have reasonable relevance to field rates for which they were originally designed, and for intermediate tests, such as the OECD 314, it was considered to be important that the test reflects the appropriate emission scenario and thereby processes occurring in the environment. The group felt that more confidence and data were needed for the OECD 309 to determine its relevance. With appropriate calibration they could potentially work well in terms of predicting rates, although it was considered that currently evidence is limited. There was discussion that in order for tests to have field relevance then all microbial processes which could act in specific environmental compartments should be considered within tests. In particular, following Robin Oliver's talk, the importance of considering degradation by phototrophs, in addition to bacterial pathways, was considered. Soil testing in particular proves problematic, considering sieved soil alters the biology of the system.

3. Can the OECD 314 be used to assess the persistence of down the drain chemicals?

Scientifically it was considered that the test is robust, it may be a good predictor for the degradation of down the drain chemicals in wastewater treatment plants or in the immediate vicinity of effluents, and it was considered that there were no problems with the test which make it unsuitable for use in a regulatory setting. It was considered that if a substance is persistent in the OECD 314, then it will be also persistent in the aquatic environment. However, discussions within Syndicate 3 described above were more cautious about the use of this study in persistence assessments without any further investigation.

4. Impact and significance of light on degradation studies

It was considered that the relevance of including of light in tests depends on the testing tier; in particular it could be useful in higher tier tests in which greater environmental realism is valued. Additionally, it will depend on the specific protection goal and scenario. It was felt that the registrant should have the option of including light. It should be possible to develop high throughput screening approaches which would work for many compounds, providing the potential for assessment of direct and indirect photolysis, and degradation by phototrophs, and allowing integration of all key processes which are important in specific environmental compartments.

5. Impact of temperature and normalisation of results

There was limited discussion of this topic. It was considered that the need for normalisation of degradation data for temperature was scenario specific. There was considered to be some evidence that temperature

adjustment should be made on the basis of Q_{10}. However, generally it was felt that there was currently insufficient knowledge about the extent to which temperature correction would make a difference to the test outcome.

6. Batch versus continuous methods to assess degradation

The longevity of inoculum in batch tests was considered. There appears to be good evidence that inoculum viability declines very quickly. Therefore, it is important to add the test chemical as soon as possible. In WWTPs a typical hydraulic residence time (HRT) is less than 24 hours. The 28 day duration of OECD 314 tests for this scenario is to assess the dynamics of intermediates and many sample points are clustered to assess removal during both the HRT and sludge residence times. The only continuous test is the OECD 303A test. However the frequent need for radioactively labelled chemical and manpower needed to run the units, makes this test expensive. It was considered that from a regulator's point of view, there would be concern about extrapolating data from this test to the environment under direct discharge conditions, i.e. WWTP simulation tests would not be acceptable for assessing persistence *per se*. Generally it was considered that if a chemical fails to degrade in an OECD 314 test it will not degrade in a WWTP and it will be persistent in aquatic systems and possibly will accumulate in the environment.

7. Interpretation of BR / NER from persistence perspective; why should we worry about bound residues?

It was felt that there was still a need to clarify definitions of bound residues, and the need to integrate chemical and biological assessment of bioavailability. There was consensus that on a scientific basis type II and type III non-extractable residues (see Syndicate 3) represent a negligible risk. No likely scenarios have been outlined which could result in concern over release. It was felt that should a bound chemical or metabolite subsequently become available by slow desorption, risk would be covered by the original assessment. Although there could be an issue regarding differences between acute and chronic toxicity there was felt to be no evidence this could be an issue. However it was recognised that the regulatory position was at odds with this view. A key gap was considered to be understanding and prediction of covalent binding and desorption processes.

8. Assessment of transformation products

It was felt that considering the parent and transformation product together in testing schemes was the best approach. Where there was a shift in toxic mode of action between parent and transformation product this could be a problem, although formation of intermediates more toxic than the parent was considered to be rare. There were discussions about a range of scenarios in which transformation products could prove problematic, such as when polar parent compounds degrade to non-polar transformation products which have potential for bioaccumulation, when transformation products are more mobile than parents, posing a risk of leaching or contamination of raw drinking water, and when the parent and metabolite have different water solubilities and therefore pose risks to different compartments. It was considered that although tools are available to predict the properties of transformation products, such as QSAR, there is insufficient guidance on regulatory requirements, particularly in REACH and the accuracy and confidence of predictions for more complex molecules is limited.

2.6 Research proposals

Following the syndicate and plenary discussions, the research topics worth consideration for further work and funding are listed in Table 1.

Table 1: Research topics and proposals for how to address

Research topic	Description	Action
Effect of temperature	Review the impact that temperature has on biodegradability in aquatic and aquatic-sediment habitats. Does conducting higher tiered biodegradation studies at 12°C and 20°C result in different degradation kinetics for identical sediments? Does an increase in temperature (by 10°C) from the point of sampling to incubation impact the microbial biodiversity and biodegradation potential of the test? Is normalisation to a specific temperature scientifically justified for biodegradability assessments?	Draft RfP – A literature review is needed to investigate whether laboratory conditions are representative of environmental conditions. The review, and (possible published paper), would recommend how to account for the influence of temperature when assessing the environmental relevance of laboratory derived data. For RfP see Appendix 5. ECETOC workshop report 2007.
Bound Residues	Identify and develop models to predict NER and Transformation Product formation. Chemistry of the molecule to target appropriate mechanisms. Develop a systematic approach aided by modelling for developing appropriate extraction strategies for soil and sediments. Assess the bioavailability of NER to appropriate soil organisms to determine whether risks reduced by aging. Clarify Type 1 (entrapped parent and/or metabolites), Type 2 NER and Type 3 NER (biogenic residues) for subsequent regulatory use / action.	Draft RfP.
Comparison of OECD 314 tests, OECD 308/309 tests and enhanced biomass studies using benchmark chemicals	This research project would use the LRI ECO 12 reference chemicals (possibly with additional reference chemicals) to assess the relative performance of each method. A key element of the experimental design would address standardisation of the sourcing and handling of inocula prior to testing. In addition to direct comparison of the different methods, the use of reference / benchmark chemicals would provide potential to assess the persistence of test chemicals by comparison with established benchmarks, and thereby, overcoming some of the problems arising from intra / inter test variation.	Draft RfP

Research topic	Description	Action
Laboratory to field extrapolation	The aim of this work would be to assess the predictive value of laboratory enhanced / modified screening tests by comparing with field data. A key question to address would be whether such laboratory tests are sufficiently complex to include the major processes.	Task Force
Transformation products	The aim of this work would be to develop tools and guidance to: Identify types of transformations resulting in greater toxicity; i.e. more non-polar; change in pKa resulting in greater uptake; or non-target receptor with greater toxicity. Are these predictable? What models may be helpful in identifying these? Match commodity products with mechanism of greater toxicity observed. Characterise the extent of increase in toxicity; is this a concern from a hazard or risk assessment perspective? Characterise mobility of persistent TPs for protection of groundwater / source water for drinking water production.	No action to date
STP model	Investigate whether (or not) the technosphere (STP) model is representative of the natural environment; and if so, how that may be used for each regulatory framework (e.g. classification and persistence assessment). Define limiting parameters for such. Such work may include documenting the occurrence of micro-organisms / enzymes systems in both environments, comparative metabolism for reference materials, mitigating factors for each, etc.	Draft RfP
Complex substances	The aim of this work would be to clarify the problems with undertaking persistence assessment of complex substances. Provide an overview of potential approaches identifying strengths and weaknesses of predicted versus whole substance assessments (i.e. *in silico* versus higher tier tests). Investigate the practicality of applying WEA PBT approaches to the assessment of UVCBs. Monitor and report key findings / relevance of RIFM project.	Task Force

Research topic	Description	Action
Demonstrating the ecological significance of adaptation	Pre-exposure of an inoculum to the test chemical may result in more environmentally realistic responses in biodegradation tests. Systematically evaluate various sources of inocula, various test material concentrations, times for pre-exposure in simple batch, intermittently fed batch and continuously fed chemostat-type exposure systems on the biodegradation of representative non-persistent and known persistent chemicals in screening tests (e.g. OECD 301). Different pre-exposure protocols for inocula to test the biodegradation of problematic chemicals in screening biodegradation tests are needed with the goal of defining guidance on the proper use of pre-exposed inocula for persistence assessments.	Initially a workshop to review approaches (see TF's list) and agree the research needed. Implementation of the research would be aimed at achieving agreement of the 'approved' processes. For RfP see Appendix 9. ECETOC Workshop Report 2007.
Microbial characterisation of inocula	This research project would investigate the value of genetic sequencing procedures in determining differences / similarities in inocula to compare the relevance of laboratory inocula compared to the field situation; to cross compare different communities (e.g. compartment, geographical region) and assess whether there is commonality in terms of diversity and composition; to investigate and predict generic processes which drive transformation e.g. co-metabolism; to apply microbial ecology theory to interpret and understand test outcomes; to improve and refine systems. This research project would investigate the value of genetic sequencing procedures in determining differences in microbial communities during the course of higher tier tests. Information could be obtained from any higher tiered ring testing associated with any of the above projects. The greatest interest may be in looking at differences in microbial communities in higher tier assessments of UVCBs	Draft RfP
Understanding the impact of low substrate concentration and cometabolism on biodegradation test data	The relationship between biological processes occurring in high concentration biodegradation tests and those at low concentrations including cometabolism needs to be understood. There is data to suggest that it is not practical to extrapolate from laboratory studies done at high concentrations to environmental conditions. If this is the case, it probably needs to be understood why and what should or could be done to overcome these concerns.	Review literature conduct laboratory studies at <10 µg/l and compare to studies conducted at higher concentrations. For RfP see Appendix 6. ECETOC Workshop Report 2007.

3. CONCLUSIONS AND RECOMMENDATIONS

Although there was insufficient time at the workshop to rank the recommendations, in the process of writing this report the authors have sought an indication of the priority for the work from the workshop participants. The following areas were considered to be of very high priority and should be recommended for further action:

Difficult substances and complex mixtures

It was clear that there were significant challenges in developing appropriate techniques to assess the persistence of complex substances. It was also clear that the higher tiered tests were developed for single substances and need to be tailored for complex substances. Approaches could be based on assessing the predicted properties of components, an assessment of the whole substance or a combination of both. Specific recommendations were as follows:

- Develop a non-target approach to look for both non-degrading components and metabolites. This would require advanced analytical procedures and composition would need to be linked to improved predictive models.
- Assess the potential role of microbial profiling to give an indication of the presence and growth of different types of degraders to provide weight of evidence that degradation of the substance (and relevant constituents) was occurring.
- Use chemical profiling to follow (ready) biodegradation as a pragmatic means to give more information than the usual secondary measures of ready biodegradation e.g. complex substance's average molecular weight change (i.e. decline) over the course of a test using MS.
- Consider using an approach analogous to that recommended by OSPAR for whole effluent assessment (WEA) to not just assess the persistency of certain components but the persistence of potentially bioaccumulating and toxic substances. For example, solid-phase micro extraction (SPME) ran before and after degradation assessments can give information on the persistence of potentially bioaccumulating substances.
- Use sterile controls to help differentiate between physical and biodegradation losses.
- Monitor and assess value of RIFM research project on "Methods to improve the environmental assessment of poorly defined components of complex mixtures" and if appropriate provide to a wider spectrum of complex substances.

Screening level assessment of persistence

It was clear that the current OECD biodegradation screening tests have some limitations associated with the identification and relative prioritisation of chemicals that persist in the environment. There were also concerns expressed (i) about the extent to which many of these standardised tests had been subject to any rigorous validation and ring testing or inter-laboratory comparisons with a range of suitable test chemicals, and (ii) some of the standardised studies are being used in regulatory assessments in a manner for which

they were not designed or intended (e.g. the use of the OECD 308 study being used as a river simulation for down the drain chemicals). Specific recommendations were as follow:

- The OECD should consider convening an Expert Working Group to consolidate and update the RBTs to reflect (i) the availability of new instrumentation with increased analytical sensitivity (ii) the use of tests with combined analytes (e.g. BOD and carbon dioxide evolution), and (iii) the need to screen for biodegradability in water-sediment systems.
- A laboratory-based study to compare the performance of, and identify improvements to, the OECD 309 and relevant OECD 314 tests, using appropriate benchmark chemicals with known biodegradability / persistency is required to increase industrial and regulatory stakeholder confidence in these studies. It was also recommended that the enhanced biomass studies could be included as part of this exercise.
- Research is conducted to demonstrate the ecological significance of adaptation and validate an appropriate experimental approach for its inclusion within persistency assessments. This approach should also develop appropriate marine studies with a reference set of validation chemicals.
- A ring test for enhanced biomass studies to compare their performance against other current screening tests with a reference set of validation chemicals.
- Test durations should be made more flexible and be allowed to extend to beyond 60 days as marine biodegradation studies often have lengthy lag periods before the onset of degradation.
- It was recommended that the current REACH guidance allowing modified tests, to assess chemicals with (i) poor water solubility and/or (ii) toxicity, should be allowed as they overcame some of the limitations associated with the RBTs.

Interpretation of higher tiered studies

Whilst higher tiered biodegradation studies attempt to increase the realism and relevance of persistence assessment, the increased complexity of the test system poses several additional challenges and uncertainties associated with the interpretation of the test data. Specific recommendations were as follows:

- Clarification of terms and definitions associated with chemical-sediment and chemical-soil interactions (e.g. NER and bound residues, type 1-3 NER).
- Improved understanding of remobilisation of test substance or transformation products from NER and the resulting environmental risk.
- Guidance on the use of $DegT_{50}$ values, not DT_{50} values for comparison against the persistence triggers and the role that inverse modelling and other model-based approaches can have in elucidating removal mechanisms.
- Development of guidance for concluding on a persistence assessment where multiple data exist.
- Improved understanding of the impact of temperature on degradation rates as opposed to variability of biomass and other possible influences (see research needs).
- Improved identification of transformation products and associated risks.

- Publish data on correlations between the phylogeny of STP microorganisms and those present in natural environmental waters.

Enhanced realism within persistence assessment

It is recognised that screening and higher tiered persistence assessments lack realism and often introduce laboratory artefacts. These tests are quite often conducted only once so the relevance of the rate of degradation measured and the inherent variability around that measured rate is unknown. Tests are also of very limited duration compared to the environmental exposure of the chemical and the persistence assessment is made using a small number of environmental microbes with no history of pre-exposure and limited or no time to adapt to the test chemical during the study period. Specific recommendations were as follows:

- OECD 314 tests involving activated sludge at high concentrations of biomass are considered valuable in assessing the environmental fate and behaviour for the purposes of risk assessment only. However, the tests would not be accepted (by regulatory agencies) as methods for assessing chemical persistence, even for chemicals discharged to sewer, because sewage treatment may not always be applied to all point source and diffuse discharges. The assessment of chemical transformation products is an important aspect of risk assessment. In most cases assessment of the fate of parent and transformation products can be made in a single study. Data from the OECD 314 activated sludge die-away studies could be used as part of a weight of evidence assessment at the screening stage for persistence if the criteria for the inherent biodegradation in the Zahn-Wellens test (OECD 302B) are fulfilled.
- A set of chemicals with defined persistence criteria, e.g. LRI ECO 12 chemicals, should be established for use in degradation studies to provide internal benchmarks. This is particularly important for aquatic studies where inocula tend to be more variable compared to those in soil systems. This would need to involve considering additional chemicals to the current reference set to include a wider set of chemical structures and properties. There would need to be consideration of the availability of ^{14}C labelled compounds when considering chemicals to include.
- It was considered that key reference chemicals should be assessed in multiple labs, focussing on the complete set of OECD 314 tests (with the exception of the anaerobic test). Ideally OECD 307/308/ 309 should also be included to cover all environmental compartments and to adhere to environmentally relevant conditions (e.g. temperature, suspended particle concentration, etc.). Practically there would need to be a focus around key chemicals and tests. An output of the project would be to improve guidance documents or to revisit persistence cut-off criteria e.g. to consider whether persistence assessment is better done as a relative measure compared to appropriate established benchmarks. It was considered important to standardise inocula collection / handling etc. to minimise variability in the tests, before the testing phase. There would need to be agreed criteria for inoculum quality to allow comparison and reproducibility.
- It was felt that there was a need for a Task Force or further workshop to consider the relevance of laboratory data for the field situation. The aim would be to compile existing data and compare the fit of laboratory enhanced / modified screening tests (not ready or simulation tests) and field data. There may be data available from key sectors e.g. the pesticide industry; there may be potential to use blind data. Some data will be publicly available e.g. FOCUS reports. Once data has been considered an expert

workshop should be held. The findings could be used to determine the need to introduce further complexity into laboratory tests, such as light associated processes.

- Inclusion of an adaptation phase offers potential for relevant assessments of chemical biodegradation but test designs must be interpreted with care or superseded by simulation tests inoculated with adapted biomass. Test designs should take account of the rapid loss of inoculum activity in batch systems. Other modifications to laboratory studies can enhance field relevance, e.g. including phototrophic organisms when relevant.
- Microbial characterisation of inocula using next generation sequencing procedures, which are becoming cheap and accessible. There was debate about how to take this forward- a literature review was suggested although the use of next metagenome and metatranscriptome analysis could be suitably advanced now for application of techniques. Techniques could have application, for instance, to investigate adaptation processes; to identify differences and similarities of inocula and its consequences for test outcome; to compare the relevance of laboratory inocula compared to the field situation; to cross compare different communities (e.g. compartment, geographical region) and assess whether there is commonality in terms of diversity and composition; to investigate and predict generic processes which drive transformation e.g. cometabolism; to apply microbial ecology theory to interpret and understand test outcomes; and to improve and refine systems. There would need to be initial validation of next generation sequencing methods.
- Further work is needed to develop knowledge and predictive capability of covalent binding of chemicals in the environment.

ABBREVIATIONS

AIR	Annex 1 renewal project
BCF	Bioconcentration factor
BOD	Biochemical oxygen demand
BR	Bound residue
CAS	Continuous activated sludge
CEFIC	The European Chemical Industry Council
CONCAWE	Conservation of Clean Air and Water in Europe
CPPs	Crop protection products
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
$DegT_{50}$	Degradation time for 50% of a compound
DOC	Dissolved organic carbon
DT_{50}	Dissipation time for 50% of a compound
EA	Environment Agency
EAWAG	Swiss Federal Institute of Aquatic Science and Technology
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC)
ECHA	European Chemicals Agency
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
EMA	Environmental Medicines Agency
ERA	Environmental risk assessment
EU	European Union
GCMS	Gas chromatography coupled mass spectrometry
GCxGC	Comprehensive two-dimensional gas chromatography
GLP	Good laboratory practice
GTL	Gas to liquid
HPLC	High performance liquid chromatography
HRT	Hydraulic residence time
ISO	International Organisation for Standardisation
K_{ow}	Octanol-water partition coefficient
LCMS	Liquid chromatography coupled mass spectrometry

LOEC	Lowest observed effect concentration
LRI	Long-range research initiative
MCS	Multi-component substances
MITI	Ministry of International Trade and. Industry, Japan
MS	Mass spectrometry
NCS	Natural complex substances
NER	Non-extractable residues
NMR	Nuclear magnetic resonance spectrometry
NOEC	No observed effect concentration
OECD	Organisation for Economic Co-operation and Development
OSPAR	Oslo/Paris Convention (for the Protection of the Marine Environment of the North-East Atlantic)
P/vP	Persistent / very persistent
PBT	Persistent, bioaccumulative and toxic
PC	Physico-chemical
PCR	Polymerase chain reaction
PEC	Predicted environmental concentration
pH	Potential of hydrogen
pKa	The negative logarithm of the dissociation constant
PNEC	Predicted no effect concentration
POP	Persistent organic pollutants
P_{OV}	Overall persistence
PPP	Plant protection products
Q_{10}	Temperature coefficient
QSAR	Quantitative structure-activity relationship
QSBR	Quantitative structure-biodegradability relationship
RBT	Ready biodegradability test
REACH	Registration, Evaluation, Authorisation and Restriction of Chemical Substances
RfP	Request for proposal
RIFM	Research Institute for Fragrance Materials
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Health and Environment)
SCAS	Semi-continuous activated sludge
SMILES	Simplified molecular input line entry specification
SOM	Soil organic matter

SPME	Solid-phase micro extraction
SSD	Species sensitivity distributions
STP	Sewage treatment plant
TF	Task force
TGD	Technical guidance document
TP	Transformation product
tpa	Tonnes *per annum*
UBA	Umweltbundesamt (Federal Environment Agency)
UV	Ultraviolet
UVCB	Unknown, variable composition, or biological
vPvB	Very persistent, very bioaccumulative
WEA	Whole effluent assessment
WWTP	Wastewater treatment plant

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APPENDIX A: RESEARCH TOPICS IDENTIFIED AT THE 2007 WORKSHOP AND PROGRESS TO DATE

Research topic	Description	Progress since 2007
Validation – test chemicals and test methods	Draw up a list of chemicals, with clearly agreed properties and an agreed persistency category. This list would cover chemicals that biodegraded rapidly as well as those that were very slow to biodegrade. The purpose of this reference set would be to establish a set of chemicals that further research could address, and help allay fears that methods that were too aggressive were not being developed. The workshop attendees rated this as a very important exercise, that together with ring tests of any protocols eventually developed, would further the regulatory acceptance of the methods.	CEFIC/LRI Project ECO 12: Validation Chemicals for Assessing Biodegradation Tests Start date: December 2008 End date: December 2009 This project developed a list of reference chemicals covering a range of environmental persistence and non-persistence. This reference set is now available for use to validate modifications to existing biodegradation test methods and to develop new test methods. The validation set should also help address concerns that some of the modifications or new methods could result in tests becoming too powerful or overly protective. The aim of the research was to establish such a list of chemicals, with an agreed (by regulators and industry) set of properties and characterised set of degradation behaviour. The reference set of chemicals will be relevant to projects addressing: - Compartment persistence / biotransformation - Bioavailability and bound residues - Determination of test method variability, e.g. via ring testing - Field monitoring / exposure model validation
Bioavailability and bound residues	Definition is needed to clarify 'bound chemicals', when bound chemicals can be bioavailable and when they are practically unavailable. The problem starts with their identification, proceeding to the impact on the interpretation of data when bound residues are identified within a study. This would link the use of extraction solvents with what fraction is bioavailable and, therefore, has potential for toxicity to micro-organisms and higher organisms. The research should be based initially on a review of the literature to investigate what bound residues can be degraded and if so at what rate; Is it the same for all types of compounds (organic, inorganic, neutral, ionic, etc.)? What are the mechanisms of binding and what types of analytical methods are needed to identify such mechanism? What are the appropriate extraction methods to fractionate available residues while maintaining compound integrity? What is the effect of desorption on bioavailability? Are there parameters that could be used for normalisation?	ECETOC workshop held in 2009 (ECETOC, 2009) ECETOC Task Force on extraction techniques – Started in 2011 and due to report in 2013 (ECETOC, 2013a) ECETOC Task Force on risk assessment of NER – Started in 2011 and due to report in 2013 (ECETOC, 2013b) UBA workshop held in 2010

Research topic	Description	Progress since 2007
Temperature	Investigate whether rates of biodegradation determined at laboratory temperatures can be predictive of rates under a wider range of environmental conditions. For example, if conducting a test at 20 degrees, is extrapolation to other temperatures using the Arrhenius equation appropriate or does the testing need to be done at different temperatures? Is it important to conduct the tests at the environmental temperatures where the inoculum was collected to maintain the microbial community? Is it possible to extrapolate from one test temperature to a range of temperatures in the environment, or is it necessary to generate data at two or more temperatures?	No activities initiated to date
Understanding the impact of low substrate concentration and cometabolism on biodegradation test data	The relationship between biological processes occurring in high concentration biodegradation tests and those at low concentrations including cometabolism needs to be understood.	No activities initiated to date
Addressing the F:M, microbial biodiversity and density	Develop methods to increase both diversity and density of microbial biomass of inoculum for use in screening studies so that the likelihood of false negatives is reduced. This will require an understanding of the impact of the biomass and its density on the data generated. Address density versus volume and pre-concentration methods such as colonisation of glass beads. The aim would be to develop a strategy that can be used to assess chemicals, in fresh and marine waters, without having to conduct the full suite of approaches developed in this research. It was also considered whether there was a need to conduct research on new devices to simplify the test procedure (screening test with easy to use and disposable platform). In this case, a platform would be proposed to allow O_2, CO_2 and cell biomass measurement for each flask to build a balance of the biodegradation. The platform would use disposable flasks with a sensor embedded into the bottom.	Cefic/LRI project ECO 11: Towards rationally designed hazard, risk and persistency assessment: Putting the 'bio' back into biodegradability tests Start date: January 2009 End date: December 2013 The objectives of this project are as follows: **Objective 1a.** Determine the extent and variation in microbial diversity of environmental sources used in biodegradation studies i.e. activated sludge, fresh-, estuarine- and marine-waters. **Objective 1b.** Examine the relationship between microbial diversity, cell density and the probability of biodegradation occurring for chemicals that have a range of environmental persistency. **Objective 2.** Compare different approaches to pre-concentrate biomass and their influence on microbial community composition. These include sandwich filtration; centrifugation; tangential flow filtration; and biofilm development through the colonisation of glass beads. **Objective 3a.** Investigate the relationship between the test volume used in the biodegradation study, the microbial diversity introduced into the test, and the probability of observing degradation at a fixed initial test chemical concentration (i.e. study the impact of varied F:M).

Research topic	Description	Progress since 2007
		Objective 3b. Investigate the effect of proportionally increasing the inoculum density with initial test chemical concentration (i.e. fixed F:M) on biodegradation rates. **Objective 4.** Validate the enhanced biodegradation screening test using the CEFIC/LRI ECO 12 reference chemicals for persistency, and publish the procedure in appropriate journals and submit the final protocol to the OECD or ISO test guidelines programme. Discussions about enhanced marine persistence assessments held between ECETOC and the regulators for offshore chemicals based at CEFAS (August 2013) and OSPAR (October 2013) to share the latest scientific developments.
Investigating pre-exposure	Pre-exposure of an inoculum to the test chemical may result in more environmentally realistic responses in biodegradation tests. Systematically evaluate various sources of inocula, various test material concentrations, times for pre-exposure in simple batch, intermittently fed batch and continuously fed chemostat-type exposure systems on the biodegradation of representative non-persistent and known persistent chemicals in screening tests (e.g. 301). Different pre-exposure protocols for inocula to test the biodegradation of problematic chemicals in screening biodegradation tests are needed with the goal of defining guidance on the proper use of pre-exposed inocula for persistence assessments.	No activities initiated to date
Measuring half-lives and understanding the principle causes of variability	Develop a battery of tests to determine natural variability within and between environmental compartments. If such data become available can they be used to support a probability approach (analogous to the use of SSDs for deriving PNECs) to assessing half-life/persistence? Differences between inocula should be investigated to determine if higher biodiversity means greater catabolic potential. Research on factors influencing half-lives such as the F:M ratio may help. Consider the regulatory issue of how to select a single value from the distribution of values for regulatory decisions. Existing literature on inter-media half-life extrapolation based on key parameters is available. A review of this would be a starting point.	Cefic/LRI LRI-ECO 18-Eawag: Improved strategy to assess chemical persistence at the water-sediment interface Start date: January 2012 End date: September 2014 This project will investigate two hypotheses that should (i) help to better understand the value and information content of the existing OECD 308 protocol, and (ii) help to develop an improved test strategy for assessing persistence in sediment and surface water in a consistent and robust manner.

Research topic	Description	Progress since 2007
New analytical tools for biodegradation assessments	Provide information on the various techniques currently available and their applicability to both screening level and 'simulation' studies. Although there is a growing body of knowledge on appropriate techniques to support single substances such as pesticides and pharmaceutical products, there needs to be clearer guidance on analytical techniques for other molecules and complex substances in particular. In addition to assessing the latest capabilities of ^{14}C or tritiated hydrogen techniques (and other types of radiolabelling), the work would include a review of potential new alternative methods, e.g. use of microfibres to concentrate substances in soil and sediment samples, Fourrier Transform Infra-Red and nuclear magnetic resonance spectrometry (NMR), improved liquid chromatography coupled mass spectrometry (LCMS)/ gas chromatography coupled mass spectrometry (GCMS), advanced comprehensive two-dimensional gas chromatography (GCxGC). This would lead to recommendations on whether these could be practical and feasible to apply to biodegradation assessments of chemicals at low concentrations in a range of media.	No activities initiated to date.

APPENDIX B: PRESENTATIONS

B1. Introduction and stakeholder perspectives of persistence

B1.1 Welcome, introduction and summary of activities since the ECETOC / Environment Agency persistence workshop (2007)

Jason Snape
Brixham Environmental Laboratory, AstraZeneca, Devon, UK

In 2007, ECETOC and the Environment Agency (EA) of England and Wales co-hosted a workshop on "Biodegradation and Persistence" at Holmes Chapel in the United Kingdom. Attendees, from academia, regulatory agencies and industry discussed the challenges and uncertainty faced with persistency assessments at the screening and confirmatory testing level. This presentation summarised the key conclusions and recommendations made during the 2007 workshop and summarised some of the activities that have been progressed since. It also highlighted where new issues or areas of uncertainty have come to light that were then discussed over the next two days. The presentation ended by setting the scene for the next two days of the workshop and providing the challenge for the workshop attendees. In setting the scene, it was emphasised that the output of the workshop would be disseminated as a workshop report and ultimately summarised in a journal publication to ensure that any critical research needs be disseminated.

B1.2 Regulatory overview of persistence assessment within EU

Eric Verbruggen
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In Europe, several different regulatory frameworks exist which deal with specific groups of chemicals (e.g. industrial chemicals, biocides, pesticides, pharmaceuticals), or which serve a particular purpose (e.g. protection of the marine environment). Several of these regulatory frameworks have their own criteria for dealing with Persistent, Bioaccumulative, and Toxic (PBT) substances or Persistent Organic Pollutants (POP). This study focused on criteria for persistence of substances. Criteria from different regulatory frameworks were compared. It appears that for persistence, differences in criteria are relatively small. Some frameworks do not use their own criteria but refer to criteria from REACH or the TGD used in the former new and existing substances legislation. Despite these small differences in criteria among the regulatory frameworks, details in the assessment procedure could cause the final assessment of persistence to deviate substantially among the frameworks. Even when the criteria are the same, the way the information from experimental studies is used may vary greatly. For example, the half-life of a substance could refer to degradation only, or it could be a half-life for dissipation. In this respect, it is also important how the results from field studies are considered in the assessment. Another aspect is the temperature for which the criteria are defined (ambient or room temperature), and if a temperature correction should be applied. Differences can also be caused by the way in which bound residues are regarded, with the extreme cases of bound residues being completely disappeared versus completely persistent. Further, how to deal with photolysis and hydrolysis is often not well defined. These are just some examples of aspects that are treated differently in the different regulatory frameworks. For further harmonisation of the persistence assessment between different regulatory frameworks, it is therefore necessary to harmonise not only the criteria on which the persistence assessment is based, but also the guidance documents on the interpretation of the data.

B1.3 Challenges with assessing degradation and persistence

Thomas W. Federle
Procter & Gamble, USA

The ability to accurately determine the potential for an organic chemical to degrade and the rate at which degradation will occur in environmental compartments where it is released and ultimately resides is critical in evaluating its environmental persistence. Moreover, this understanding is essential for accurately estimating environmental exposure when conducting an environmental risk assessment. Historically, ready and inherent biodegradation tests have been the principle regulatory tools utilised for assessing degradability. However, these tests are ineffective for chemicals that are difficult to test due to their physico-chemical properties, are not used as growth substrates by microorganisms or whose degraders are rare in standard test inocula. While some of these limitations are remedied in simulation tests, these tests come with their own unique issues.

The presentation surveyed some of the challenges commonly encountered in accurately evaluating the degradation and persistence of organic chemicals. These include challenges that are not only scientific and methodological but also financial and practical. Methodological challenges include dosing difficult to handle substances and having sufficient analytical signal above background to quantitatively measure biodegradation at test concentrations, which are not inhibitory to the microbes or at which mass transfer is not a limitation. Scientific challenges include having an inoculum that is of sufficient size and diversity that rare degraders are present and in the case of substances that are co-metabolised rather than used as a growth substrate having a metabolically active microbial community available in the test. The former is complicated by regulatory restrictions on using pre-adapted inocula, which is particularly a problem for chemicals that are new to the world. A consistent scientific challenge relates to having a ratio of test chemical to microbial biomass that is reflective of actual exposure and *in situ* conditions in the environment.

The use of simulation tests come with their own specific challenges, many of which are of a practical nature. These include not only the cost but the difficulty of obtaining high quality and well characterised radiolabelled test materials with the consolidation and contraction that has occurred in the industry during the past few years. Others relate to the complexity of such tests, the difficulty of successfully executing them, uncertainty about the results themselves and even their regulatory acceptance. This latter uncertainty includes whether the results from scientifically sound but non-prescribed tests (e.g. OECD 314) will even be considered by regulatory agencies, potential variability in how individual regulators or agencies will weigh and interpret such tests and how they will consider bound residues in the ultimate assessment. Unfortunately, such uncertainty can translate into reluctance by business managers to proactively fund testing and research that could lead to more definitive understanding on the fate of many chemicals in the environment. The hope is that by identifying the challenges, whether scientific or practical, and the dilemmas that they pose, this workshop can catalyse the development of improved approaches that will ultimately advance our understanding of chemical fate and result in better environmental protection.

B1.4 Current issues and challenges faced on the PBT working group with respect to persistence

Johanna Peltola-Thies
ECHA, Finland

The PBT working group discussed under the interim strategy period before REACH around 120 existing substances and also several new substances and biocides. The assessment conclusion 'not persistent' for existing substances was drawn in equal amounts from biodegradation screening information and other information (e.g. abiotic degradation, information about reactivity). For a large number of the substances, the discussions consisted of considerations on the validity of available data in the light of the physico-chemical (PC)-properties and chemical reactivity. In discussion on available experimental degradation studies the relevance of the test conditions for PBT assessment was crucial. Conventions established at that time were incorporated into ECHA's guidance, but for some paths (photodegradation, anaerobic biodegradation, hydrolysis, bound residues) uncertainties still remained in terms of whether and how to use the respective data in the context of PBT assessment.

Experience gained with substances like endosulfan shows that persistence in one compartment may still be significant although testing information of a specific path such as hydrolysis would primarily indicate rapid degradation.

A year ago about 150+ registered substances were prioritised for further PBT screening assessment by EU Member State experts as an activity beyond the formal REACH processes. The basis for the prioritisation was mainly QSAR estimations due to lack of experimental data. It seems that the main part of the ongoing PBT assessment work of the EU Member State experts will cover similar aspects as the PBT assessment work in the past. This screening and assessment work carried out by the EU Member State experts and ECHA is, among others, discussed in ECHA's PBT Expert Group, which had its first meeting in February 2012. The group is an informal scientific expert group. The work is planned to be extended in future to cover unknown, variable composition or biological (UVCB) substances. It has not been possible so far to cover UVCBs in the PBT screening activities in a balanced way.

The first amendment of REACH Annex XIII, which the registrants must comply with by March 19, 2013, extended registrant's obligations regarding the PBT assessment. Although CoRAP (Community Rolling Action Plan under REACH) remains the most powerful tool for requiring more information for the PBT assessment, the role of dossier compliance check may become more important than it is today as one of the tools for clarifying the PBT concern of a substance. The amendment of REACH Annex XIII also introduced into the legal text the scientific principles of the PBT assessment which were developed and applied by the former PBT WG. Nevertheless, a long list of scientific issues remains to be elaborated by ECHA's PBT Expert Group for the further development of the PBT assessment. Following issues can be mentioned as examples:

- *identifying the compartment of concern, - role of aquatic photodegradation,*
- *influence of the form of test item,*
- *improving understanding of the fate of substances (e.g. how to identify very persistent substances which form very slowly PBT substances),*

- *sediment issues,*
- *role of anaerobic biodegradation,*
- *use of monitoring data from contaminated sites*

The substances so far identified as PBT/vPvB by ECHA's Member State Committee in accordance with REACH Article 59 cover different types of substances which therefore have been assessed for persistence with different approaches. Finally, when looking at the substances identified as PBT/vPvB previously and under REACH, it seems that during the assessment of persistence field monitoring data has played an important role as supporting information.

B2. Screening for Environmental Persistence

B2.1 Assessing environmental persistence: balancing pragmatism with realism

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Standardised laboratory degradation tests provide data which is used to determine chemical behaviour and risk in the environment. The advantage of laboratory tests is that they are conducted with defined environmental substrates under standardised conditions, which generally makes them reproducible, and ensures that test results are readily understood in terms of their regulatory significance. However, use of laboratory test systems inevitably results in loss of complexity, and the extrapolation of laboratory test results to the natural environment can be problematic. For most chemicals, biodegradation represents the major route for dissipation in the environment. A variety of factors which affect pollutant bioavailability and microbial community diversity and functioning differ between natural systems and the laboratory, and could affect biodegradation rates. These include chemical concentration, scale, light / dark cycles, redox and temperature variation and interactions between the water column and sediment. Furthermore there can be great variability in the physico-chemical and biological properties of materials within and between environmental compartments (e.g. environmental heterogeneity) which could affect test outcome. The results of work which investigated the effect of adding complexity and greater environmental realism to degradation screening tests was presented. River biofilms generated on glass slides were found to provide greater inoculum density than unconcentrated river water while preserving diversity. It was shown that bacterial diversity in biofilms and river water showed seasonal variation, and that this was a greater determinant of bacterial community composition than proximity to the outflow of a sewage treatment plant (STP). River water collected from the STP outflow showed more consistent degradation of p-nitrophenol than water collected from upstream and downstream of the STP. River biofilms provided similar rates of biodegradation to river water, despite the larger amounts of biomass applied in degradation assays. In a number of river water samples, biodegradation of p-nitrophenol did not occur. This could not be attributed to reduced biomass or bacterial diversity in these samples. Furthermore quantitative PCR showed that these samples contained genes in the biodegradative pathway of p-nitrophenol, indicating that factors controlling bacterial proliferation, rather than absence of catabolic potential was responsible for the lack of biodegradation. Introduction of natural light to river water biodegradation tests with p-nitrophenol resulted in the inhibition of biodegradation. This was shown to result from growth of algae, which increased pH, preventing growth of degraders. Similarly use of p-nitrophenol concentrations at levels approaching those found in the environment resulted in reduced biodegradation rates, and at the lowest concentrations, variable results between replicates, including the introduction of test failures. The addition of complexity into test systems may therefore affect the outcome of biodegradation tests in a manner which is hard to predict.

B2.2 Modified and enhanced biodegradability testing

Kees van Ginkel
Akzo Nobel, the Netherlands

Ready biodegradability tests only detect growth-linked biodegradation whereas simulation tests also measure cometabolic transformations. Growth-linked biodegradation is superior compared to cometabolic degradation. Ready biodegradability test results should therefore be treated with priority when assessing the biodegradation potential. Modifications and enhancements of the ready biodegradability tests have been designed to improve biodegradability assessments with the methodology of ready biodegradability tests.

This presentation focused on methods of modified and enhanced biodegradability testing. A justifiable outcome of ready biodegradability tests with poorly water soluble and toxic substances requires methods preventing inhibitory effects due to high initial concentrations and/or limited bioavailability. A few examples were discussed. Decrease of the concentration of quaternary ammonium salts in the water phase to a non-toxic level can be achieved through the addition of silica gel, humic and lignosulphonic acids. Silicone oil was introduced into the test vessels when testing with a fragrance to prevent toxic effect and loss of the volatile test substance. Introducing agitation of the test media and lower initial test substance concentrations resulted in a ready biodegradability results with dialkylamines. Biodegradation of poorly water soluble substances though an increase of the bioavailability with surfactants has also been demonstrated.

Competent microorganisms present at low numbers in the environment are often not detected due to the low inoculum size of ready biodegradability tests. This may be solved through acclimatisation at low test substance concentrations. N-methylpiperazine is not biodegradable in ready biodegradability tests nor is it in most inherent biodegradability tests. However, a prolonged Closed Bottle test result indicated that microorganisms capable of utilising N-methylpiperazine as growth substrate do exist. Inocula for Closed Bottle tests with increased number of competent micro-organisms were obtained through acclimatisation of activated sludge and micro-organisms present in river water at low concentrations (1 µg/L to 1 mg/L). The modifications and enhancements improved the assessments of the biodegradation potential with the methodology of ready biodegradability tests. Microbial growth results by definition in adaptation of microbial communities and increased degradation rates in (eco)systems. Detection of growth-linked biodegradation with modified and enhanced tests should, therefore be more appreciated than simulation test results. The inability to detect growth-linked biodegradation with standard ready biodegradability tests are often caused by the high test substance concentrations not occurring in the environment and small inoculum sizes. Enhancements like extending the duration of ready biodegradability tests and low level adaptation do improve detection of growth-linked biodegradation. Detection of growth-linked biodegradation with modified and enhanced tests should also be more appreciated than simulation test results.

The talk concluded that ‘the assessment of biodegradation should become more science based’.

B2.3 Towards rationally designed hazard, risk and persistency assessment: putting the "Bio" back into biodegradation testing

Russell Davenport [1], Andrew Goodhead [1], Timothy Martin [1,2], Jason Snape [2], Jon Ericson [3], Torben Madsen [4]
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Mitigating the risks that manufactured chemicals pose to the environment and human health is a major global concern and one of the greatest challenges for the 21st Century. Regulatory emphasis has recently shifted to identifying and prioritising chemicals that are persistent, liable to bioaccumulate and are toxic (PBT e.g. REACH). Chemicals with these properties have previously been shown to be those most harmful to human health and the environment. Biodegradation is one of the most important but poorly understood fate processes that determines persistence. It is often measured experimentally by observing the degradation of a chemical substance in the presence of a bacterial inoculum. In reality it should be acknowledged that Ready Biodegradability Tests (RBTs) are notoriously variable. For example, microbial concentrations in inocula can vary by 4 orders of magnitude.

RBTs have been the central foundation for understanding the biodegradation of chemicals in regulatory frameworks for hazard and environmental risk assessments for 2-3 decades. They are highly prescribed, standardised and conservative regulatory tests that measure the relative biodegradability of chemicals (e.g. OECD 301 tests). RBTs rely on the probabilistic inclusion of specific degraders in the test system, but have a high failure rate and are highly variable largely due to the use of low inocula concentrations. Together with their short duration, this makes them unsuitable for persistence assessments.

REACH guidance which advocates the introduction of a new tier of enhanced tests to enable efficient and effective identification of persistent chemicals (ECHA, 2008). Reliable extrapolation from any small-scale systems to predict local and regional environmental impacts depends on incorporating environmental realism into test systems, which includes the nature of the microbial populations present. Enhancements may therefore include increasing inocula to environmentally-equivalent concentrations, and thereby the microbial diversity, to levels likely to be met by a given chemical in the environment.

Our CEFIC/LRI ECO 11 project has been investigating how variations in inocula concentration, community composition, and diversity, relate to biodegradation variation and reliability. In addition, the bias and pragmatism of different methods to concentrate cells in inocula for enhanced tests has been assessed. It was found that enhancement of activated sludge inocula concentrations had a greater effect on reliability than test volume in scaled-up biodegradation tests carried out using GLP (Good Laboratory Practice), but not necessarily for marine inocula. Finally, these enhancements using a set of reference chemicals chosen by CEFIC/LRI ECO 12 project for this purpose were validated.

B2.4 Challenges in the assessment of natural complex substances

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Natural Complex Substances (NCS) are materials extracted from plants and used in the preparation of fragrance mixtures for a variety of consumer products. Typically these are classified as UVCBs (Unknown, Variable Composition, or Biologicals), or, minimally, Multi-Component Substances (MCS) for the less chemically complex extracts. Assessment of these materials is required under various regulatory schemes including REACH. While there are methods for considering the ecotoxicity of a mixture using additivity, little has been published on approaches for either environmental fate studies or other assessment methods for NCS. The International Fragrance Association's Environmental Task Force has provided recommended approaches for NCS biodegradation assessment. Presented here are some recent studies using NCS as examples to assess the ready biodegradability of key constituents of these mixtures in order to provide an overall assessment of the biodegradability of the NCS itself. Particularly challenging was the assessment of sesquiterpene compounds; many of which are not used individually within fragrance preparation and, therefore, data are not readily available for constituent assessment.

B3. Experiences with higher tiered assessment of persistence

B3.1 Experiences with the OECD TG 308 with human pharmaceuticals

Jon Ericson
Pfizer, USA

The OECD 308 water-sediment transformation test has been routinely conducted in Phase II Tier A testing of the environmental risk assessment (ERA) for human pharmaceutical marketing authorisation applications in Europe since finalisation of Environmental Medicines Agency (EMA) ERA guidance in June 2006.

An overview of 31 OECD 308 studies conducted by 4 companies with a focus on how pharmaceuticals behave in these water-sediment systems was presented. The mean parent total system half-life for the 31 pharmaceuticals was 56 days ± 79 days. The formation of non-extractable residues (NER) was considerable, averaging 44 ± 25%, with cationic substances averaging 51 ± 27% of the applied radioactivity, neutral substances averaging 32 ± 13% and anionic substances averaging 31 ± 23%. In general there was an inverse relationship to the amount of non-extractable residue and the amount of total transformation products observed at study termination. On the sixteen test materials with OECD 218 (OECD, 2004b)sediment toxicity data, ten reported a LOEC (Lowest Observed Effect Concentration) as the highest concentration tested (range of 1 to 150 mg/kg) and six reported a NOEC (No Observed Effect Concentration), mean value of 98 mg/kg (range 5 to 400 mg/kg). NER challenge extractions at study termination showed no more than 5 to 10% of the dosed ^{14}C-residues recovered during this procedure. This was consistent with the entire data set, though the approaches varied to include some or combinations of the following procedures: series of solvent extractions (polar to non-polar), adjustment of pH, soxhlet extraction and/or use of EDTA (Ethylenediaminetetraacetic acid). A review of whether a simplified one point analysis could reasonably estimate the parent total system half-life showed that the total amount of parent remaining in the water and sediment extracts at day 50 or day 100 correlated fairly well with total system half-life; correlation coefficient r^2 for day 50 and day 100 was 0.83 and 0.93 respectively. This relationship in particular was observed once the aqueous dissipation phase was completed, day 50 for the data set studied. This suggested that there may be some potential for an abbreviated / water-sediment screening study. An approach to water-sediment screening was also presented using voriconazole as a case study. The goal was to develop a short term method (1 week or less) that could screen for potential transformation products typically observed in an OCED 308 study. Such a screen would be helpful in: 1) optimising analytical conditions for the OECD 308; 2) generating transformation products for MS/NMR identification procedures should there be a need; and 3) investigating conditions that may better represent water-sediment conditions found in a STP release environment. Initial design focused on a stirred or agitated reactor using sediment generally following OECD 309 collection procedure with solids levels at 0.1 to 1 g/L, much less than what is seen in the 308 study. Sediment K_d values for test substance was used to target conditions that result in approximately 50-75% of the test substance dissolved in solution. Temperature of 20°C and 30°C were investigated to assess how an elevated temperature would potentially enhance the kinetics without impacting the viability of sediment micro-organisms. Results from the preliminary study showed similar transformation products of the screen when compared to what was observed in the OECD 308. As voriconazole has a low sediment K_d value of 9.7 for an high organic content sediment, it was not anticipated that lowering the solids level would

improve the availability by much. Comparison of the rate of disappearance of voriconazole and the rate of appearance for its hydroxylated transformation product in the OECD 308 and screen showed very similar results. Raising the test conditions to 30°C approximately doubled the rate of disappearance of voriconazole and appearance of the transformation product. Further work is planned to test a substances at a higher K_d boundary condition, and by investigating other approaches to enhance transformation rates by using P450 inducers and/or other co-factors.

Recommendations from this 4 company collaboration included: 1) the need to develop a more relevant water-sediment transformation test reflecting the conditions of the STP discharge scenario more representative of human pharmaceuticals; 2) potential use of a one point estimate of parent total system half-life in the EMA ERA screening phase of testing; 3) use of the parent total system biotransformation half-life in revising predicted environmental concentration (PEC) in ERA; 4) need to investigate approaches to water-sediment screening and 5) routinely conduct sediment toxicity testing in Phase II Tier A given the extent of sediment binding generally observed with pharmaceuticals.

B3.2 Application of the OECD 307 study to assess the persistence of gas to liquid (GTL) fuel

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The main study currently used to assess the biodegradation of chemicals in soil is the OECD Guideline 307 for testing chemicals: Aerobic - Anaerobic Transformation in Soil (OECD, 2002b). This test was originally designed to provide degradation rate data for crop protection products but this is now being undertaken to provide data for other 'chemicals' under the EU Reach regulations. Many do not believe the current guidelines are suitable to assess the fate of complex substances. For example, the current approach recommended by CONCAWE (Conservation of Clean Air and Water in Europe) for complex hydrocarbon substances is to model persistence of the constituent hydrocarbon blocks. However, as part of the registration of a new substance the European Chemicals Agency (ECHA) have stipulated to the registrants that an OECD 307 study should be undertaken to determine potential persistent hydrocarbon components of a gas to liquid (GTL) fuel.

A series of OECD 307 studies were undertaken on GTL fuel which consists predominantly of branched and linear aliphatic hydrocarbons having carbon numbers in the range of C8 to C26. In an initial study the GTL fuel was applied to a single soil and although it was feasible to undertake an OECD 307 study the test was unable to assess whether losses were due to biodegradation alone. In a second study the GTL fuel was once again added to a single soil. However, in addition three individual n-alkanes (dodecane, hexadecane and eicosane) and a C15 iso-alkane were applied separately to a single soil type to monitor their respective degradation rates. The n-alkanes were added to the soil at a concentration comparable to their respective concentrations in the GTL fuel. An important addition to the second series of studies was that sterile (abiotic) soil systems were also treated with GTL fuel, the individual n-alkanes and the iso-alkane to assess the losses by abiotic factors (e.g. volatilisation and/or non-extractable residues).

By incorporating sterile controls the OECD 307 test has potential to improve the understanding of the fate of components of complex substances like GTL fuel in soil. In particular, it has potential to identify recalcitrant components which may warrant further investigation. However, analytical constraints, different physic-chemical properties of components and dose rates at which the test can be conducted will differ significantly to those of individual substances and all of these factors will complicate interpretation of results. Furthermore, it should be recognised that when using an OECD 307 type soil study to assess the fate of components of complex substances the objectives will differ to those for 'standard' OECD 307 studies.

The results of the current studies indicate that, although sterile controls can provide an indication of physical losses, the OECD 307 test will ultimately determine 'disappearance' as opposed to biodegradation of components of a complex substance like GTL fuel. The results of the studies indicate that predicted half-lives are conservative and, that no additional bioaccumulation assessments of the components of GTL fuel are warranted based on the premise that even if some remain in soil they will not be bioavailable to soil organisms because they cannot be extracted using acetone and hexane.

Once again the issue regarding persistence was related to another key factor with the lack of persistent components negating the requirement for further bioconcentration studies.

B3.3 Hidden hazard or safe sink? Approaches to consider non-extractable residues in the regulatory assessment of chemicals [1]

Caren Rauert, Andreas Höllrigl-Rosta, Elisabeth Thumm
Umweltbundesamt, Germany

Formation of non-extractable residues (NER) is regularly observed in studies on the fate of organic chemicals in soil. NER formation may be interpreted as a specific form of compound persistency ('hidden hazard') or as a detoxification step ('safe sink'). Despite the considerable scientific progress made in analysing NER and identifying their binding types, these insights have not yet been utilised in regulatory risk assessment.

In a 2010 workshop held at the German Federal Environment Agency (UBA), it was agreed that three main types of NER should be considered in regulatory schemes: Fixation of substance molecules by physical entrapment in the soil matrix can be reversed under certain environmental conditions. Those 'Type 1' NER must be considered as a reservoir for remobilisation of a chemical over prolonged times. In contrast, formation of strong chemical bonds between substance molecules and soil matrix will produce 'Type 2' NER, which are unlikely to be released in their original structure under environmental conditions. Finally, NER can also be formed via incorporation of single labelled atoms or small fragments from the original substance into biomass. These 'biogenic' NER are no longer structurally related to the original substance. While the formation of Type 2 and biogenic NER can be considered a 'safe sink', Type 1 NER would constitute a 'hidden hazard'.

A generic extraction scheme was proposed for residue analysis in the standard studies on the fate of organic chemicals in soil. Specific methods are required to determine the amount of biogenic NER. Extraction with non-destructive methods allows conclusions to be drawn on the availability of residue fractions.

To differentiate between Type 1 and Type 2 NERs, a set of destructive extraction methods differing in strength is available, which may be complemented with sophisticated spectroscopic techniques. Where no information on their nature is available, NER should in principle be assumed to belong to Type 1 (i.e. worst case scenario).

Formation of Type 1 NER will have different implications on the environmental risk and hazard assessment. In particular, their potential for substance remobilisation will significantly impact groundwater risk assessment and persistence assessment. Existing trigger values and decision criteria for NER formation were deemed inappropriate for addressing those concerns; hence, a need for developing new criteria was identified.

[1] A precursor of this talk was held by Andreas Höllrigl-Rosta at SETAC in Milan 2011.

B3.4 Understanding the relationship between extraction technique and bioavailability / bioaccessibility

Charles Eadsforth
Shell, UK

Following the ECETOC workshop on "Significance of bound residues in environmental risk assessment" in 2009, two Task Forces were set up to (1) understand the relationship between extraction technique and bioavailability and (2) develop interim guidance for the inclusion of non-extractable residues in environmental risk assessment. The goal of the first Task Force was to address knowledge gaps in the relationship between bioavailability and extraction technique with regards to bound and non-extractable residues with the ultimate goal being the development of a standard framework for intelligent extraction strategies. A number of residue 'categories' were defined (dissolved, readily desorbed, slowly desorbed, irreversibly sorbed and incorporated) as well as the terms bioavailable and bioaccessible which were aligned with each type of residue within the framework model. It was decided to differentiate residues termed 'reversibly bound' into those 'readily desorbed' and 'slowly desorbed'. This differentiation was based on the solvent strength necessary to extract each type of residue and led to the development of the extraction regime to tie in with the framework model.

A proposed extraction strategy has been based on extraction and quantitation of the dissolved and readily desorbed fraction (for the bioavailable residue) and in addition the slowly desorbed fraction (for the bioaccessible fraction). A selection of appropriate extraction solvents and parameters, which will not result in destruction of the organic matrix, is proposed. When this extraction framework is applied using a considered and rational methodology, it will provide a conservative evaluation of bioaccessible residues.

An important consideration in predicting the behaviour of chemicals entering the soil is understanding their interaction with the soil matrix. To be able to better predict the chemical dynamics once a chemical enters the soil, it is necessary to understand the processes which govern these interactions. Generally, chemicals which were most strongly associated with the soil (and least bioaccessible) were either covalently bound to the soil, or physically sequestered and trapped in soil pores. Other interactions which were shown to lead to NER or slowly desorbed residues included ionic and ligand exchange. Chemicals were also shown to interact with the soil matrix via Van der Waals forces, hydrophobic partitioning, charge transfer complexes and hydrogen bonds, these interactions are generally thought of as weaker and most likely to lead to desorbable residues. The various interactions studied (and their bond strength ranges) were aligned with the extraction regime and framework model.

One of the major issues of particular concern with regards to environmental risk assessment is the future re-release of NER. It was found that physical processes such as freeze-thaw and wet-dry cycling can cause the release of sequestered residues via the breakup of the soil matrix and soil organic matter (SOM). Additionally, chemical and biological processes such as microorganism metabolism and pH changes have been found to cause the release of NER. The current literature suggests that the amounts of NER released do not pose an environmental risk, however, it was identified that further research is necessary in this area, especially with regards to release caused by physical processes, on which very few studies exist.

In conclusion, the issue of non-extractable residue formation and release is a very complex one. The interaction of chemicals released to the environment with the soil is reliant on a number of factors, not least of all the nature of the soil. Soil organic matter is a key component of soil, this complex soil constituent and the potential interactions it may have with chemicals is not very well understood and needs further research. However, this Task Force has developed a framework model and extraction scheme (ECETOC, 2013a). It is expected that work in this area of research will greatly increase over the coming years as environmental risk assessment becomes an increasingly important issue.

B3.5 Strategies to identify degradation products and their risks

Kathrin Fenner
EAWAG, Swiss Federal Institute of Aquatic Science and Technology, Switzerland

At the higher tiers of chemical risk assessment, regulatory guidance typically recommends the performance of simulation-type transformation studies to identify major transformation products (TPs). However, most risk assessment guidelines fall short of providing guidance on how the risk of identified TPs should ultimately be assessed.

In this presentation two possible approaches to identify risk-relevant TPs were presented and contrasted in terms of their advantages and disadvantages. This was based on earlier published work (Escher and Fenner, 2011). The default approach recommended in most regulatory risk assessment frameworks is exposure-driven, i.e. chemical-analytical identification of major TPs followed up by their synthesis and subsequent effect testing. Recent approaches to speed up TP structure identification (see Helbling *et al*, 2010) such as high-resolution mass spectrometry combined with high-throughput data analysis tools were discussed in this context. An effect-driven approach based on toxicity to the bacteria *Escherichia coli* was presented as an alternative, potentially more direct way of identifying toxicologically relevant TPs. In this approach, samples from simulation studies are not only subjected to chemical analysis, but are also analysed with one or more bioassays to follow the development of toxicity over the course of the experiment. Comparison of parent compound concentration and toxicity development over time then indicates whether any toxicologically relevant TPs are formed.

Both of the above-mentioned experimental approaches are quite labour- and time-intensive suggesting that there is a role of models for prioritisation of TPs for further investigation. A model to estimate relative concentrations of pesticide (trichlosane) TPs in surface waters was presented and its performance assessed relative to measured field data. Further, a model for estimating plausible ranges of toxic effects of TPs relative to their parent compounds was discussed. A combination of such models could potentially help to estimate the contribution of TPs to overall environmental risk caused by the release of a given parent compound.

B3.6 Experiences with higher tier study designs to investigate the fate and behaviour of chemicals in the environment

Robin Oliver and Laurence Hand
Syngenta, UK

Many regulatory risk assessments for chemicals are based on laboratory studies in which the key processes of sorption, hydrolysis, photolysis and microbial degradation are evaluated separately in simple, standardised systems, in accordance with the appropriate guidelines. These studies provide information on the fate and behaviour of the chemical in soil, sludge, sediment and water.

Hydrolysis studies are conducted under sterile conditions in the dark, to exclude microbial and photolytic degradation, respectively. Photolysis studies are also conducted under sterile conditions and at a pH at which hydrolysis is known to be minimal. The most complex laboratory studies are those designed to determine microbial degradation in soil, sludge or sediment / water systems. These are conducted in small vessels that are incubated in the dark under either aerobic or anaerobic conditions. Potential loss mechanisms in these studies include sorption to the solid phase, hydrolysis, and microbial degradation. However, because these studies are conducted in the dark, the contributions of photolysis and metabolism by phototrophic organisms (algae and macrophytes) are excluded. In the regulatory scheme for crop protection products (CPPs) degradation studies under field conditions are a requirement for some compounds. Historically such studies have provided an indication of how different processes combine to influence the rate of degradation of chemicals. However in most field study designs the chemical is applied to recently cultivated, bare soil (which is often not representative of the use conditions), thus excluding the potential contribution of surface dwelling phototrophs and microbes in the rhizosphere. Recent changes in Europe in the guidance for such studies have increased their artificial nature by indicating that degradation by surface processes should be excluded. There are, therefore, no studies in the regulatory suite that provide a realistic integration all of the potential degradative processes in either the terrestrial or aquatic compartments. It is questionable how well the potential persistence of chemicals can be assessed without such studies, as adequately reconstructing potentially complex interactions from processes studied in isolation is extremely difficult.

Over recent years Syngenta has developed test systems to investigate the potential significance of degradation resulting from indirect photolysis and metabolism by phototrophic organisms in soil and sediment / water systems. A semi-field aquatic test system has also been developed to enable the determination of the rate and route of degradation, when multiple processes are acting together.

Indirect photolysis was studied using sixteen waters from corn growing regions of the United States. Six test compounds were selected; two that did not degrade significantly by direct photolysis, two that were degraded slowly by direct photolysis and two that degraded quickly by direct photolysis. The compounds that did not degrade or showed moderate degradation by direct photolysis were degraded significantly faster in all of the natural waters tested. For the two compounds for which degradation by direct photolysis is rapid, one was degraded faster in all of the natural waters tested while the other was degraded more quickly in some natural waters and more slowly in others (but degradation was still rapid in all natural waters). The overall rate of photodegradation in natural waters is a combination of direct and indirect

photolysis and, in some cases, light scavenging by constituents of the water can reduce the rate of direct photolysis to a greater extent than is compensated for by indirect photolysis. These findings suggest that this will only be significant for compounds where direct photolysis is very rapid and the overall photodegradation rate in natural waters will still be fast. For those compounds that are not degraded very rapidly by direct photolysis, photodegradation in natural water is likely to be significantly faster than that observed in sterile buffer.

Degradation in aquatic systems by algae and a representative macrophyte (*Elodea sp.*) was investigated by incubating natural sediment and overlying water under light from fluorescent bulbs (the absence of UV wavelengths means that the contribution of photolysis would be negligible), with or without the macrophyte present. The incubation system enabled any radiolabelled volatile components evolved to be trapped ensuring that a mass balance for the test system could be obtained. For all five compounds tested, degradation in the presence of aquatic plants was significantly faster than in the standard water / sediment system and much closer to the rate obtained in the semi-field study.

The role of soil surface dwelling phototrophs has also been investigated using both sieved soil and intact soil cores incubated under fluorescent lamps (as for the aquatic system). In sieved soil systems the presence of soil phototrophs enhanced the rate of degradation for most but not all of the chemicals tested. The use of intact soil cores further enhanced the degradation rate of the two chemicals tested to date (compared to the rate in the same soil sieved).

These findings suggest that degradative processes that are not currently included in regulatory testing schemes are likely to play a significant part in the degradation of chemicals in the environment. To ensure that assessments of the persistence of chemicals is not biased by a failure to take account of all relevant mechanisms of degradation and their integration, registrants should have the option to include all of these in the estimation of persistence. This can be done cost effectively by the adoption of a tiered testing approach which provides the option to include degradation by all of these processes and, where the degree of complexity requires it, integrated test systems such as semi-field aquatic studies and uncovered field studies.

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APPENDIX D: WORKSHOP PROGRAMME

Tuesday 6 November 2012

Session 1: Introduction and stakeholder perspectives of persistence (Chair: Jason Snape)

09.30 – 10.00	Registration	
10.00 – 10.25	**Welcome, introduction and summary of activities since the ECETOC / Environment Agency Holmes Chapel Persistence Workshop (2007)**	**Jason Snape** AstraZeneca, UK
10.25 – 10.50	**Regulatory overview of persistence assessment within EU**	**Eric Verbruggen** RIVM, The Netherlands
10.50 – 11.15	**Challenges with assessing degradation and persistence**	**Tom Federle** Procter & Gamble, USA
11.15 – 11.40	**Current issues and challenges faced on the PBT Working Group with respect to persistence**	**Johanna Peltola-Thies** ECHA, Finland
11.40 – 12.15	**Panel Discussion**	**Jason Snape** AstraZeneca
12.30 – 13.30	Lunch	

Session 2: Screening for Environmental Persistence (Chair: Stuart Marshall)

13.30 – 13.50	**Assessing environmental persistence: balancing pragmatism with realism**	**Gary Bending** University of Warwick, UK
13.50 – 14.10	**Modified and enhanced biodegradability testing**	**Kees van Ginkel** Akzo Nobel, The Netherlands
14.10 – 14.30	**Towards rationally designed hazard, risk and persistency assessment: Putting the "bio" back into biodegradation testing**	**Russell Davenport** Newcastle University, UK
14.30 – 14.50	**Challenges in the assessment of natural complex substances**	**Dan Salvito** RIFM, USA
14.50 – 15.30	**Panel Discussion**	**Stuart Marshall** Unilever, UK
15.30 – 15.45	Coffee	

Syndicate Session 1

15.45 – 17.15 **Breakout Group Sessions**

Syndicate 1a – Challenges with the persistence assessment of difficult to test substances
Moderator: Graham Whale, Shell, UK
Rapporteur: Andreas Schäffer, RWTH, Germany

Syndicate 2a – Improved screening approaches for persistence assessment
Moderator: Jason Snape, AstraZeneca, UK
Rapporteur: Russell Davenport, Newcastle University, UK

Syndicate 3a – Interpretation of higher tiered studies
Moderator: Caren Rauert, Umweltbundesamt, Germany
Rapporteur: Jon Ericson, Pfizer, USA

Syndicate 4a – Enhanced realism within persistence assessment
Moderator: Stuart Marshall, Unilever, UK
Rapporteur: Gary Bending, University of Warwick, UK

17.15 – 18.15 **Plenary** — **Kathrin Fenner**, EAWAG, Switzerland
Feedback from Syndicate rapporteurs

18.15 – 18.45 **Discussion** — **Kathrin Fenner**, EAWAG, Switzerland

20.00 – 22.00 Dinner

Wednesday 7 November 2012

Session 3: Experiences with higher tiered assessment of persistence (Chair: Daniel Merckel)

08.30 – 08.50	**Experiences with the OECD TG 308 with human pharmaceuticals**	**Jon Ericson** Pfizer, USA
08.50 – 09.10	**Application of the OECD 307 study to assess the persistence of Gas to Liquid (GTL) Fuel**	**Graham Whale** Shell, UK
09.10 – 09.30	**Hidden hazard or safe sink? Approaches to consider non-extractable residues in the regulatory assessment of chemicals**	**Caren Rauert** Umweltbundesamt,Germany
09.30 – 09.50	**Understanding the relationship between extraction technique and bioavailability**	**Charles Eadsforth** Shell, UK
09.50 – 10.10	**Strategies to identify degradation products and their risks**	**Kathrin Fenner** EAWAG, Switzerland
10.10 – 10.30	**Experiences with higher tier study designs to investigate the fate and behaviour of chemicals in the environment**	**Robin Oliver** Syngenta, UK
10.30 – 11.15	**Panel Discussion**	**Daniel Merckel** Environment Agency, UK
11.15 – 11.45	Coffee	

Syndicate Session 2

11.45 – 13.00 **Breakout Group Sessions**

Syndicate 1b – Challenges with the persistence assessment of difficult to test substances
Moderator: Andreas Schäffer, RWTH, Germany
Rapporteur: Daniel Merckel, Environment Agency, UK

Syndicate 2b – Improved screening approaches for persistence assessment
Moderator: Russell Davenport, Newcastle University, UK
Rapporteur: Jason Snape, AstraZeneca, UK

Syndicate 3b – Interpretation of higher tiered studies
Moderator: Jon Ericson, Pfizer, USA
Rapporteur: Caren Rauert, Umweltbundesamt, Germany

Syndicate 4b – Enhanced realism within persistence assessment
Moderator: Gary Bending, University of Warwick, UK
Rapporteur: Stuart Marshall, Unilever, UK

13.00 – 14.00 Lunch

14.00 – 15.00 **Return to Syndicates**

15.00 – 16.00 **Plenary** — **Jorg Klasmeier**, University of Osnabrück, Germany
Feedback from Syndicate rapporteurs

16.00 – 16.30 **Discussion** — **Jorg Klasmeier**, University of Osnabrück, Germany

Close of Workshop

APPENDIX E: ORGANISING COMMITTEE

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Established in 1978, ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) is Europe's leading industry association for developing and promoting top quality science in human and environmental risk assessment of chemicals. Members include the main companies with interests in the manufacture and use of chemicals, biomaterials and pharmaceuticals, and organisations active in these fields. ECETOC is the scientific forum where member company experts meet and co-operate with government and academic scientists, to evaluate and assess the available data, identify gaps in knowledge and recommend research, and publish critical reviews on the ecotoxicology and toxicology of chemicals, biomaterials and pharmaceuticals.