# *Collation of Existing Marine Biodegradation Data and its Use in Environmental Risk Assessment*

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## ECETOC TECHNICAL REPORT No. 108


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## SUMMARY

Extrapolation is a tool used to account for uncertainty in environmental risk assessments and PBT/vPvB assessments (EC, 2003; ECHA, 2008a,b,c). Within environmental effects assessments extrapolation or uncertainty factors are applied to acute and/or sub-lethal ecotoxicological studies in order to calculate a predicted no-effect concentration (PNEC). Within environmental exposure assessment (i.e. the determination of predicted environmental concentrations; PECs), preference is given to higher tiered degradation test data that have measured rates of abiotic or biotic degradation. However, in reality very little higher tiered 'simulation' test data exists. Therefore default rate constants are applied to the pass/fail outcome of relatively simple screening studies such as the tests to determine Ready Biodegradability (OECD 301 series and 310). The guidance contained within the Technical Guidance Document (EC, 2003) and Registration, Evaluation and Authorisation of Chemicals (REACH; ECHA, 2008c) for environment risk assessment and PBT/vPvB assessments applies default rate constants for almost all environmental compartments based on the outcome of biodegradation screening tests. This includes extrapolating from the outcome of biodegradation in freshwater screening studies to default half-lives for removal in the marine environment.

In recent years, increased emphasis has been placed on protecting the marine environment (EC, 2003). This recognised that there were specific concerns that: (i) hazardous substances may accumulate in parts of the marine environment and result in unpredictable and irreversible long-term effects; and (ii) remote areas of the oceans should remain untouched by hazardous substances and that pristine environments should be protected. The default half-lives used in exposure assessment and persistency assessments assume that the degradation potential in the estuarine environment is equivalent to that of the freshwater environment. However, the degradation potential within the marine environment is considered to be between three and four times slower than the freshwater and estuarine environments. This task force has reviewed the publicly available standard and non-standard test data and prepared the ECETOC Marine Biodegradation Kinetics database (EMBK) which consists of >800 data. These data have been used to review the scientific basis of the current TGD (EC, 2003) and REACH (ECHA, 2008c) default values and whether they need to be refined.

Quality control and assurance criteria have been established for the identification of suitable non-standard biodegradation test data. These included information with respect to: the identity and purity of the test substance, the origin and pretreatment of the *inoculum*, the analyte being measured, and the way in which the rate or extent of degradation was being described. In total, 806 kinetic data, mainly from marine environments, together with the corresponding information on test design and conditions, *inoculum* source and sampling conditions were collated. The 26 most data rich substances (507 datasets) where subjected to further statistical investigations.

There were a number of ways that degradation rates were expressed (e.g. turn-over rate, $T_{50}$; $DT_{50}$, half-life, etc.). Where possible these rates were converted to half-lives. These data demonstrated that there was not a single rate of degradation for any given substance but that a distribution of rates existed. This supported the distribution concept described by ECETOC in its technical review of environmental persistence (ECETOC, 2003).

Based on the data reviewed, extrapolation between the aquatic systems was feasible for readily biodegradable as chemicals that had been shown to degrade in the freshwater environment had also been shown to degradation the marine environment and *vice versa*. Statistical analysis of these data concluded that there was a scientific basis to propose some revision of the current default values assigned to readily biodegradable chemicals that are described in the TGD (EC, 2003) and REACH (ECHA, 2008c). The TF proposes that the current default values in the TGD (EC, 2003) and REACH (ECHA, 2008c) could be revised in light of the published data. Default values of 5 days for the freshwater environment, 15 days for the estuarine environment and 11 days for the marine environment may be more appropriate. However, these proposals are based on a limited dataset and more robust recommendations require further empirical work especially for the estuarine and marine environments.

In making these proposals the TF has applied the upper 95% confidence limit of the median half-life rather than using the overall median half life value (for all chemicals) or the lower 95$^{th}$ percentile confidence limit. By taking this approach the TF considers that the precautionary nature of exposure assessment has not been undermined. At present insufficient data exist to draw similar conclusions for chemicals substances that are considered to be inherently or not readily biodegradable. More research is required to fill these data gaps and a number of research recommendations have been identified.

# 1. INTRODUCTION

Degradation is a major process that can result in the loss or transformation of a chemical substance in the environment. Consequently, it is a major factor that determines the long-term fate and persistence of a chemical in the environment and the subsequent risk of long-term adverse effects that the chemical may pose to wildlife.

Degradation processes can be abiotic or biotic. Abiotic or non-biological degradation can occur by physico-chemical processes such as hydrolysis, oxidation and photolysis. Removal due to biotic or biological degradation is commonly known as biodegradation and is usually catalysed by the activity of microorganisms. Biodegradation can occur in the presence of oxygen (aerobic biodegradation) or in the absence of oxygen (anaerobic biodegradation). Biodegradation is often preceded by the terms primary or ultimate. Primary biodegradation describes the initial transformation of a chemical by microorganisms to another organic chemical, a transformation or degradation product; ultimate biodegradation or mineralisation describes the degradation pathway leading to inorganic end-products (such as carbon dioxide) and biomass (ECETOC, 2003).

Information on the degradability (both biotic and abiotic) of chemicals is used in: (i) hazard assessments (e.g. for classification, packaging and labelling) to identify rapidly degradable chemicals, (ii) environmental risk assessments (for chemical safety assessment) and (iii) persistency assessments (for PBT/vPvB assessment; EC, 2006). Hazard and persistency assessments, or risk in general, are largely based on data obtained in standardised screening tests for ready biodegradability and hydrolysis.

As a result of the importance of environmental persistence and the technical limitations associated with existing biodegradation screening tests several task forces, expert groups and workshops have been organised to identify possible improvements. The key points identified in each of these have been summarised in Appendix A.

In 2003 risk assessment in the EU was extended to include the marine environment. This recognised that there are additional concerns for the risk assessment of the marine environment, which may not be adequately addressed by the methodologies used for the inland environmental risk assessment (EC, 2003). The specific concerns were: (a) that hazardous substances may accumulate in parts of the marine environment with unpredictable long-term effects and that the accumulation would be difficult to reverse; and (b) that remote areas of the oceans should remain untouched by hazardous substances resulting from human activity, and that the intrinsic value of pristine environments should be protected. Of these additional concerns (a) was seen as the main concern as it was characterised by a spatial and temporal scale not covered by the inland risk assessment approach (EC, 2003).

The EU Technical Guidance Document (TGD) (EC, 2003) and REACH (ECHA, 2008c) preferred higher tiered degradation data in order to conduct a marine risk assessment, including an assessment of persistence. However, as with freshwater risk assessments, when only results from marine or freshwater biodegradation screening tests are available, it is recommended to use the default mineralisation half-lives for the pelagic compartment. In assigning these default half-lives for degradation the TGD (EC, 2003) and REACH (ECHA, 2008c) assumed that:

- The degradation potential in the estuarine environment is equivalent to that of the freshwater environment;
- the degradation potential within the marine environment is considered to be between three and four times slower than the freshwater and estuarine environments;
- chemicals with a half-life > 60 days for marine water, or > 180 days for marine sediment, are persistent.

Consequently, assessing the risk that chemicals pose to the marine environment is becoming increasingly important. However, for exposure and persistency assessments very little marine data exist and rates of biodegradation in the marine environment are extrapolated from ready biodegradation screening studies conducted in artificial mineral media. ECETOC (ECETOC, 2003) concluded that the scientific basis of the default rate constants, as described in the TGD (EC, 2003) and REACH (ECHA, 2008c), for extrapolating between freshwater, estuarine and marine environments should be investigated. The TGD (EC, 2003) and REACH (ECHA, 2008c) also recognised that default rate constants were based on a limited dataset and that "the half-lives for the marine environments are provisional recommendations, which should be reconsidered, when sufficient data for degradation of different substances in screening tests and simulation tests have been evaluated." The purpose of this task force is to determine whether sufficient data now exist to improve the scientific basis of these default values.

In order to achieve this, and identify possible future research needs, it was necessary to review all standard and non-standard biodegradation data for the marine environment. To address these points, an ECETOC Task Force was commissioned with the following Terms of Reference:

- Develop selection criteria to identify good quality non-standard biodegradation data;
- collect, review and build a database of marine and freshwater biodegradation rates for organic chemicals;
- compare freshwater and marine data to assess whether there is a scientific basis for extrapolation between the two types of environmental compartments;
- where possible compare published rates with TGD default values;
- make an overall assessment, highlight data gaps and identify research opportunities.

# 2. MODUS OPERANDI

## *2.1 Scope*

The primary focus for the TF was to prepare a high quality biodegradation kinetics database (see Chapter 4 for details on quality criteria) for marine environments. Laboratory, mesocosm and field study data were recorded for estuarine, coastal and open sea environments. In general all experimental data from open source literature and, where available, industry in-house data were identified and evaluated. Where sufficient marine data were identified for a substance the literature search was extended to find corresponding biodegradation data for fresh water and sediments in order to provide a means of comparison between marine and fresh water environments for a representative number of substances. No attempt was made to make the database a comprehensive or exhaustive collection of biodegradation rates in the fresh water compartments.

## *2.2 Literature*

The kinetic data for the ECETOC Marine Biodegradation Kinetic (EMBK) database were extracted from a literature search covering the 16 years of research between 1990 and 2006. Literature was reviewed and data collected in accordance with the criteria described in section 2.3. Applying the criteria to the 82 papers reviewed resulted in 69 papers being suitable for inclusion in the database.

The following journals were screened:

Applied Environmental Microbiology;
Applied Microbiology and Biotechnology;
Aquatic Toxicology;
Archives Microbiology;
Biodegradation;
Chemosphere;
Ecotoxicology and Environmental Safety;
Environmental Microbiology;
Environmental Pollution;
Environmental Science and Technology;
Environmental Toxicology and Chemistry;
Extremophiles;
FEMS;
Freshwater Biology;
Industrial Microbiology and Biochemistry;
International Biodeterioration and Biodegradation;
International Microbiology;
Journal of Applied Microbiology;
Journal of Bacteriology;
Journal of Marine Biotechnology;
Marine Biology;
Marine Chemistry;
Marine Ecology;
Marine Environmental Research;
Marine Pollution Bulletin;
Microbial Ecology;
Organic Geochemistry;
Progress in Polymer Science of the Total Environment;
Spill Science and Technology Bulletin;
Tenside;
Water Research;
Water Science and Technology.

Where the search was carried out electronically the following terms and key words were used to ensure consistency:

| | | |
|---|---|---|
| - Transformation<br>- Biodegradation<br>- * Degradability<br>- * Degradation | and | - Marine<br>- Coastal<br>- Estuarine. |

* Truncation of unknown prefix.

## *2.3 Criteria for selection of data*

### Data Pool

In compiling the database the following criteria were applied for the selection of data during the first phase:

- The marine environment;
- single substances tested;
- biodegradation rates were given;
- study was not carried out under anaerobic conditions.

Three classifications of paper were identified:

| | |
|---|---|
| A1 | Meets all the criteria above - all A1 papers were subjected to detailed evaluation/review. |
| A2 | Meets some of the above criteria but needs working on to derive rates - all A2 papers were kept aside for more consideration / work in the second phase of the work (i.e. after the Task Force had completed a gap identification exercise). |
| Rejected* | Not considered to contain any rate data that can be used by the Task Force. |

*Main reasons for rejection were:

- Degradation without determination of the substance concentration and/ or test duration;
- results restricted to just the percentage biodegradation or substance concentration at the start and end of the test duration only;
- the test substance was applied as a component of a complex mixture (e.g. n-alkane in a gasoline preparation).

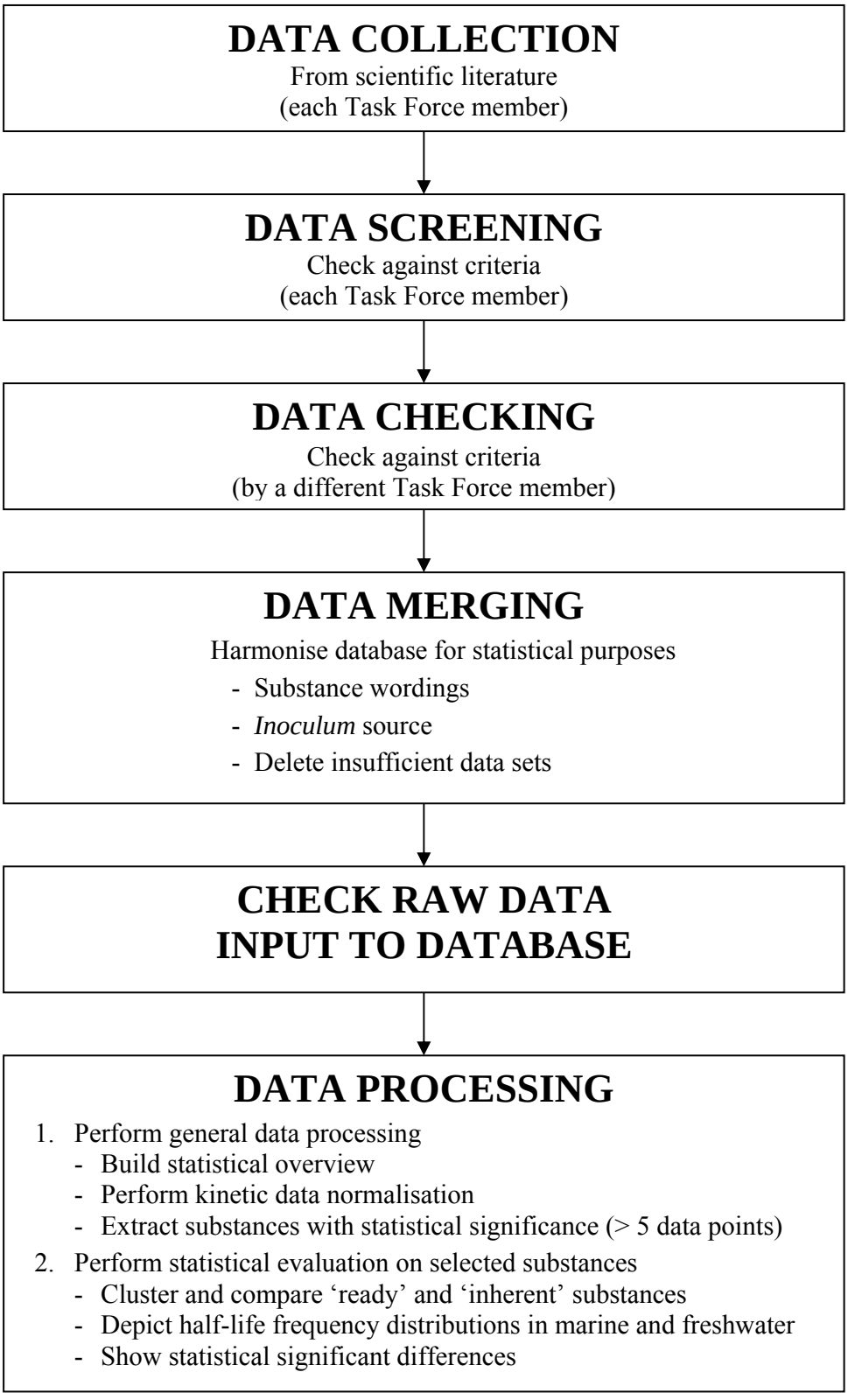
In addition to the journal publications, a significant amount of data was taken from 'Aerobic biodegradation of organic chemicals in environmental media: A summary of field and laboratory studies' (SRC, 1999). This aerobic biodegradation rate constant database includes rate constant information from soil, surface water, sediment, as well as aquifer environments. In this study, Syracuse Research Corporation reviewed the available aerobic biodegradation literature for 25 common organic chemicals (including benzene, toluene, ethyl benzene and xylene (BTEX), chlorinated aliphatic compounds, phenolic compounds, and polychlorinated aromatic hydrocarbons) and identified biodegradation rate constants from these studies. Because this database consisted of reviewed literature and provided sufficient kinetic information, the information has been considered A1. Note that this information also included literature published before 1990. All literature citations can be found in the 'Database Bibliography' section of this report.

The product of the first phase was a dataset of marine biodegradation half-lives for a number of organic chemicals. If there were more than five data for any given chemical in the marine environment then, in the second phase, a literature search was conducted to find data for the freshwater environment.

### *2.4 Data collection and evaluation*

A diagrammatic overview of the approach used for the data collection and evaluation is shown in Figure 1. Data were collected from 69 publications. All data were crosschecked and classified (see section 2.3) by a second reviewer in the TF. Each data entry was assigned a single record number in the database. This number is used to identify lines of original input data throughout the processing.

***Figure 1: Procedure of data collection and evaluation***



## *2.5 General information on database fields*

The database was populated with information relating to the following fields:

### 2.5.1 Record number

This number was used to identify lines of input data.

### 2.5.2 Chemical name

The name of the chemical.

### 2.5.3 Environmental media

Indicates whether the origin of the *inoculum* is natural or artificial. Pure cultures are considered to be artificial. Supplemented / amended refers to nutrient addition including buffering.

### 2.5.4 *Inoculum* source

Three environmental media were recognised: fresh water and/or sediment, estuarine water and/or sediment or marine water or sediment. The database distinguishes marine data between coastal and open sea (limited use for statistical analysis).

### 2.5.5 Location details and characterisation of *inoculum*

This includes information about the site location and type of site (e.g. spill site, industrial location, pristine site or landfill) and sampling temperature. Information about whether or not the *inoculum* was aged (or acclimated) (e.g. number of days), or whether biomass had been increased (e.g. pre-concentrated by centrifugation or filtration).

### 2.5.6 Mode of delivery

Information is given on whether co-solvents, emulsifiers, sonication, etc., or immobilisation were used to introduce the test substance into the text.

#### 2.5.7 Test guideline

The standard guideline number is given (e.g. OECD 301A-F, etc.) where appropriate.

#### 2.5.8 Test design

Details are given about the type of study (e.g. field or laboratory static or shake test, microcosm, *in situ* microcosm), the sampling protocol, and method of analysis, the test duration, the number of data points pH, temperature, dissolved oxygen concentrations, redox conditions. This includes information on whether the test media was supplemented with additional nutrients or buffered and whether the test substance was present as sole carbon source or in the presence of others. The biodegradation endpoint is recorded as either parent compound removal (i.e. primary biodegradation), $CO_2$ release, $O_2$ uptake (ultimate biodegradation) or DOC removal. All kinetic data are included. There may be one or more kinetics expressions given (e.g. $DT_{50}$, half-life).

#### 2.5.9 Substance concentration

The test substance concentration is recorded in mg/l or mg/kg unless other stated in the database.

#### 2.5.10 Reference

The references are indicated as one or two names followed by the year of publication. One name indicates one author, two names two authors and one name followed by 'ea' is the first name of a multi-author publication.

### *2.6 Normalisation of kinetic data (calculation of half-lives)*

The comparison of various kinetic data drawn from biodegradation studies is only possible when the data have been normalised to a single kinetic description i.e. converted into half-lives. First order degradation rate constants, and activities (zero order) were therefore transformed into half-lives according to equations described in Table 1. In many cases the test duration of the published studies was far shorter than the half-life or turnover rate reported. These data should be treated with caution as they exclude the potential for natural adaptation or enrichment of low abundance members of the microbial *inoculum*. In some publications the published half-life is ten times greater than the duration of the test (see supporting database). Ideally the ratio of test duration to reported half-life should not exceed 1:2. In these short-term investigations microbial growth can be excluded. Turnover times (residence times) used in some papers to record

degradation rates were transformed into the half-lives by multiplying the turnover time by 0.69. Reported $DT_{50}$ values and half-live were assumed equal. Interpretation of kinetics in experiments in which growth occurs is more difficult and not straightforward. The half-lives may be highly variable because of varying biomass concentrations (X). The background of biodegradation kinetics is summarised in Appendix B.

***Table 1: Overview of the most important biodegradation kinetics***

| Reaction order | Equation | Half-life | |
|---|---|---|---|
| Zero | $dS/dt = -k$ | $t_{0.5} = S_0/2\ k$ | No growth or steady state |
| First | $dS/dt = -kS$ | $t_{0.5} = \ln 2/k$ | No growth or steady state |
| Second | $dS/dt = k\ \{S\ X\}$ | $t_{0.5}$ variable | Growth of microorganisms |

## *2.7 Conclusions*

The purpose for which the studies have been performed is wide ranging and because there was no regulatory driver behind the study, the results were often expressed in a way that could not be readily translated into a half-life. Whilst this is understandable, it would be of some value if scientists working on the fate and degradation of chemicals could be encouraged to either express the data in a more uniform way or include the appropriate data in the publication such that the data could be used directly in single substance environmental exposure assessments.

The number of publications containing high quality marine biodegradation data was limited. This in turn limited the amount of data that could be obtained for any given substance (with one or two notable exceptions e.g. naphthalene and phenol). The interpretation of the available information was complicated because of the many variable parameters of non-standard test conditions (e.g. different incubation temperatures, different substrate concentrations, test duration, number of samples and data points, etc.). Interestingly, the initial test substance concentration was not reported in a number of the studies and consequently half-lives could not be calculated. The test duration varied widely, this is important where the rate (or expression) of degradation has been calculated by extrapolation, in particular the TF rejected half-lives estimated to be between one year and infinity based on studies of a few hours to a few days.

A number of different endpoints have been used to measure biodegradation. For example, many studies measure biodegradation through the disappearance of parent material, which only generates primary biodegradation kinetics. In other studies the uptake of $O_2$ or production of $CO_2$ are used as endpoints, which leads to ultimate biodegradation kinetics.

# 3. POTENTIAL REGULATORY UTILITY OF BIODEGRADATION STUDIES

Data on the biodegradability of chemicals are required in a number of regulatory frameworks. These include: (i) hazard assessments (e.g. for classification, packaging and labelling) to identify rapidly degradable chemicals, (ii) environmental risk assessments (for chemical safety assessment) and (iii) persistency assessments (for PBT/vPvB assessment). These data are usually generated to highly prescribed international guidelines (OECD, ISO, etc.) and in compliance with Good Laboratory Practice (GLP). However, there also exists a wealth of biodegradation data that are not generated in GLP compliant standardised tests. The use of degradation data in regulation including both standard and non-standard biodegradation studies is discussed in this chapter.

## *3.1 Hazard assessment (e.g. for use in classification, packaging and labelling)*

Environmental hazard classification requires information on aquatic toxicity, degradation and bioaccumulation. In the current EU classification system (Council Directive 67/548/EEC) and in the "Globally Harmonized System of classification and labelling of chemicals (GHS)" (United Nations, 2003), the determination of the appropriate risk phrases or hazard statements is often based on an integration of this information.

Under the degradation part of the EU and GHS classification criteria two aspects need to be evaluated:

*Current EU system:*

- Whether "the substance is not readily degradable".
- Whether "additional scientific evidence concerning degradation" is available, i.e. whether there is "a proven potential to degrade rapidly in the environment".

*GHS:*

- Whether there is a "lack of rapid degradability".
- Whether there is "other evidence of lack of rapid degradation".

For the purposes of decisions on classification and testing strategies, the two terms 'not readily degradable' and 'lack of rapid degradation' may be considered as synonymous. The definition of ready (or rapid) degradability covers both biotic and abiotic degradation, the latter principally meaning hydrolysis. Data on either or both biotic or abiotic degradation would be sufficient to make a decision on rapid degradation.

Within GHS, rapid degradation in the aquatic environment may be demonstrated by other data than just the standard methods for assessing ready biodegradation and hydrolysis. Scientific evidence demonstrating that the substance is degraded in the aquatic environment to a level of > 70% within a 28-day period is required. If first-order kinetics is assumed, which is reasonable

at the low substance concentrations prevailing in most aquatic environments, the degradation rate will be relatively constant for the 28-day period. Thus the degradation requirement will be fulfilled with an average degradation rate constant, $k > 0.043$ $day^{-1}$ which corresponds to a degradation half-life of 16 days.

### *3.2 Risk assessment (for chemical safety assessment)*

Transport and transformation ('fate') describe the distribution of a substance in the environment, or in organisms, and its changes with time (in concentration, chemical form, etc.), thus including both biotic and abiotic transformation or degradation processes (EC, 2003; ECHA, 2008c). Consequently, degradation data are important when establishing predicted environmental concentrations (PEC) within environmental exposure assessments.

Whilst it is preferred to have degradation data from studies under conditions that attempt to simulate the conditions in various environmental compartments, it has to be recognised that the majority of data that exist are derived from relatively simple screening studies (e.g. ready biodegradation tests). Consequently, degradation rates and half-lives have to be estimated from these screening test data. When only results from marine or freshwater biodegradation screening tests are available, it is recommended to use the default half-lives for the pelagic compartment as specified in Table 2 (EC, 2003; ECHA, 2008c).

***Table 2: Default first order rate constants and half-lives for removal in freshwater***

| Test result | Rate constant k ($d^{-1}$) | Half-life (d) |
|---|---|---|
| Readily degradable | $4.7 \times 10^{-2}$ | 15 |
| Readily degradable, but failing 10-day window | $1.4 \times 10^{-2}$ | 50 |
| Inherently degradable | $4.7 \times 10^{-3}$ | 150 |
| Not biodegradable | 0 | $\infty$ |

In 2003 the environmental risk assessment process was extended to include the marine environment. This recognised that there are additional concerns associated with the marine environment, which may not be adequately addressed by the methodologies used for the inland environmental risk assessment (EC, 2003; ECHA, 2008c). Specifically:

a. The concern that hazardous substances may accumulate in parts of the marine environment and that:
   - the effects of such accumulation are unpredictable in the long-term;
   - such accumulation would be practically difficult to reverse;
b. the concern that remote areas of the oceans should remain untouched by hazardous substances resulting from human activity, and that the intrinsic value of pristine environments should be protected.

Of these additional concerns (a) was seen as the main concern as it was characterised by a spatial and temporal scale not covered by the current environmental risk assessment approach (EC, 2003; ECHA, 2008c).

When conducting a risk assessment for the marine environment, preference is again given to higher tiered degradation data (e.g. marine OECD 308 water-sediment transformation study). However, when only results from marine or freshwater biodegradation screening tests are available, it is recommended to use the default mineralisation half-lives for the pelagic compartment as specified in Table 3. This assumes that the degradation potential in the estuarine environment is equivalent to that of the freshwater environment. However, the degradation potential within the marine environment is considered to be between three and four times slower than the freshwater and estuarine environments. This is based on:

- A review of existing data (ECETOC, 1991) that showed that biodegradation in estuaries was approximately a factor of four lower than in freshwater environments for a variety of substances: Linear alkylbenzene sulphonates, linear alcohol ethoxylates, m-cresol, chlorobenzenes, p-nitrophenol glutamate, hexadecane, and methylparathion. However, for substances known to be very rapidly biodegradable (such as sodium acetate, sodium benzoate and sodium dodecylsulphate), the rates were similar in estuarine and freshwater environments.
- An assumption that the conditions favouring microbial activity in the marine environment are less favourable (e.g. low microbial density, low concentrations of chemicals, reduced adaptation potential and lower average temperatures).

However, these assumptions were based on a limited dataset and the EU TGD and REACH text states, “The half-lives for the marine environments are provisional recommendations, which should be reconsidered, when sufficient data for degradation of different substances in screening tests and simulation tests have been evaluated.” One of the main aims of this Technical Report is to examine whether sufficient data exist to substantiate or refine these default half-life values.

***Table 3: Default first order rate constants and half-lives for removal in surface waters (EC, 2003; ECHA, 2008c)***

| | **Freshwater[1]** | **Estuaries[4]** | **Other marine environments[5]** |
|---|---|---|---|
| Degradation in marine screening test | n.a. | 15 | 50 |
| Readily degradable[2] | 15 | 15 | 50 |
| Readily degradable, but failing 10-day window | 50 | 50 | 150 |
| Inherently degradable[3] | 150 | 150 | 150 |
| Persistent | ∞ | ∞ | ∞ |

[1] Half-lives from Table 3.

[2] Pass level >70% DOC removal or > 60% ThOD in 28 days. Not applicable for freshwater.

[3] A half-life of 150 days may be used only for those inherently degradable substances that are quickly mineralised in the MITI II or the Zahn Wellens Test.

[4] Also including shallow marine water closest to the coastline.

[5] The half-lives mentioned under this heading are normally to be used in the regional assessment (coastal model).

In the risk assessment process, there may be a need to refine the currently used default value for readily biodegradable chemicals. For some chemicals the circumstance may arise whereby a readily biodegradable chemical could have an adverse risk profile i.e. PEC/PNEC > 1. In such cases, further refinement of the default rate constant applied to the outcome of the ready test may be required. This may include further examination of the curve for biodegradation to determine whether the removal rate is significantly greater than the default values within the TGD.

## *3.3 Persistence assessments (for PBT assessment)*

The most topical aspect of degradation assessments at present is related to environmental persistence. The ability of certain chemicals to persist in the environment is an issue of worldwide concern that requires careful consideration in environmental risk assessment. However, assessing the environmental persistence of chemical substances is not straightforward. Persistence cannot be measured directly; it can only be inferred from the continued presence of a substance in the environment or the lack of observed degradation data in the environment (ECETOC, 2003).

Criteria for persistence have been proposed by a number of organisations, and international bodies such as OECD, ECETOC, EC, US-EPA, CEPA (see Table 4) and national bodies (e.g. BSI, DIN, ASTM, AFNOR, NEN, etc.) have all developed 'Standard' methods for measuring a chemical's degradability. The Existing Substances and Notification of New Substances Regulations and the revised version of the TGD (EC, 2003) also address persistence and there are a number of other initiatives within the EU aimed at regulation of persistent,

bioaccumulating and toxic (PBT) substances, for example the Water Framework Directive (EC, 2000) and the PBT Management Strategy.

The range of half-lives presented in Table 4 demonstrates a marked inconsistency in persistency criteria at the international level. Environmental half-lives for the freshwater habitat range from as little as 40 days (EC, 2003; ECHA, 2008c) to 182 days (CEPA). Given the magnitude of these variations in half-lives there may be merit in harmonising the approach for assigning environmental persistence. In addition, the scientific basis behind these national and international persistence criteria is not transparent; the basis for these half-lives needs to be disclosed and subject to broader scientific scrutiny.

***Table 4: International and national persistence criteria***

| UNEP | UNECE | CEPA | EU TGD - P (EC, 2003) | EU TGD - vP (EC, 2003) | US EPA PBT Profiler | US EPA TSCA New chemicals programme[1] | US EPA TSCA New chemicals programme[2] | EU REACH P (ECHA, 2008a) | EU REACH vP (ECHA, 2008a) |
|---|---|---|---|---|---|---|---|---|---|
| Water<br>t½ > 60 d | Water<br>t½ > 60 d | Water<br>t½ > 182 d | Fresh water<br>t½ > 40 d | FW or MW<br>t½ > 60 d | Water<br>t½ > 60 d | Aquatic environment<br>t½ > 60 d | Aquatic environment<br>t½ > 180 d | FW<br>t½ > 40 d | FW or MW<br>t½ > 60 d |
| | | | Marine water<br>t½ > 60 d | | | | | Marine water<br>t½ > 60 d | |
| Sediment<br>t½ > 180 d | Sediment<br>t½ > 180 d | Sediment<br>t½ > 365 d | FW sediment<br>t½ > 120 d | FW or MW sediment<br>t½ > 180 d | Sediment<br>t½ > 60 d | | | FW sediment<br>t½ > 120 d | FW or MW sediment<br>t½ > 180 d |
| | | | MW sediment<br>t½ > 180 d | | | | | MW sediment<br>t½ > 180 d | |
| Soil<br>t½ > 180 d | Soil<br>t½ > 180 d | Soil<br>t½ > 182 d | | | Soil<br>t½ > 60 d | | | Soil<br>t½ > 120 d | Soil<br>t½ > 180 d |
| Or sufficient concerns | Or sufficient concerns | | | | | | | | |

[1] TSCA New Chemicals Programme – Moderate Action Level

[2] TSCA New Chemicals Programme – High Action Level

FW – Fresh water

MW – Marine water

Recently the European Commission proposed a new approach to chemicals management, known as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) (EC, 2006). This new legislation will implement an authorisation system for the use of all chemicals designated to be of 'very high concern' (EC, 2007). Those are defined as substances being carcinogenic, mutagenic or toxic to reproduction (CMR), those that fulfil the PBT or vPvB criteria or those deemed to be of 'equivalent concern' (e.g. endocrine disrupters) (see Table 5 for the definitive and screening P criteria under REACH; ECHA, 2008a). In contrast to the EU TGD, the PBT assessment within REACH applies not only to the marine environment but is intended for use in all environmental compartments.

***Table 5: EU REACH Criteria for definitive and screening assignments of environmental persistence (EC, 2003; ECHA, 2008a)***

| Type of data | Criterion | Definitive assignment | Screening assignment [1] |
|---|---|---|---|
| $DT_{50}$ marine water | > 60 d | VP | - |
| $DT_{50}$ freshwater [2] | > 40 d | P [3] | - |
| | > 60 d | VP | - |
| $DT_{50}$ marine sediment | > 180 d | VP | - |
| $DT_{50}$ freshwater sediment [2] | > 120 d | P [3] | - |
| | > 180 d | VP | - |
| Readily biodegradable [4] | Yes | Not P | - |
| | No | - | P or vP |
| Inherently degradable | Yes | Not P [5] | - |
| | No | - | P or vP |
| QSAR | | - | P or vP |

[1] These screening methods give an 'open-ended' categorisation of the substance as either being potentially P or vP, which cannot easily be related to a half-life for biodegradation.

[2] Data for estuaries should also be considered in this category.

[3] Half-life data in freshwater and freshwater sediment can be overruled by data obtained under marine conditions.

[4] Regardless of whether the 10-d window criterion is fulfilled.

[5] This only applies to cases where the specific criteria as mentioned in Section 4.4.3.3 of the EU TGD (EC, 2003) are fulfilled.

As described above, biodegradation rates or half-lives would ideally be determined from laboratory-based degradation tests 'simulating' the biodegradation in water, aquatic sediment and soil. However, the majority of hazard and exposure assessments are based on data obtained in standardised screening tests (e.g. tests for ready biodegradability and hydrolysis). Other types of test data that may be considered in an assessment of the potential environmental hazard or risk include sewage treatment plant (STP) simulation data, inherent biodegradability, anaerobic

biodegradability, biodegradability in seawater and abiotic transformation. However, the use of sewage treatment simulation data in persistency assessments is at present restricted to use within a weight of evidence assessment (ECHA, 2008b).

For substances where a range of degradation data is available, a 'weight of evidence' approach is required. When more than one simulation test result is available, a suitable half-life should be selected taking into account the realism, relevance, quality and documentation of the studies in relation to environmental conditions (ECHA, 2008b). When more than one screening test result is available, positive test results should be considered valid, irrespective of negative results, when the scientific quality is good and the test conditions are well documented, i.e. guideline criteria are fulfilled, including the use of non-adapted *inoculum* (ECHA, 2008b). Negative results in screening tests are frequently observed due to the toxic or inhibitory effects of the test substance. Whereas simulation tests that use a low concentration of the test substance may give a more realistic estimate of the degradation in the environment and reduce the potential for inhibition. By using all available degradability test data in this way, it is possible to establish a comprehensive assessment of the degradability of the substance.

Once all the relevant information has been gathered in relation to the requirements of REACH (ECHA, 2008b), it needs to be determined whether sufficient information exists to draw conclusions for each of the three regulatory endpoints: Hazard assessment (e.g. for classification and labelling), risk assessment (for chemical safety assessment) and persistency assessments (for PBT/vPvB assessment). If insufficient information exists then the data gaps for each regulatory endpoint need to be identified together with a summary of any remaining uncertainty. For substances at tonnages that require simulation data, the most appropriate environmental compartments to support both P/vP assessment and chemical safety assessment should be identified (ECHA, 2008a). For PEC assessments, additional consideration will be needed to determine whether or not inherent biodegradation test data (OECD 302 series, OECD, 1981; 1992c; 2009) or sewage treatment simulation test data are required to refine the $PEC_{local}$ and $PEC_{regional}$.

### *3.4 Standardised tests*

Standard test methods in use for assessing biodegradability of chemicals are described in national and international guidelines (e.g. OECD, ISO). A comprehensive review of the standard biodegradation tests was conducted by ECETOC (ECETOC, 2003) and this has been included within the current REACH guidance documents addressing the information requirement for degradation (ECHA, 2008b). The OECD methodology provides a tiered framework for assessing biodegradation. The first tier known as the 'ready biodegradability' tests, are based on stringent, fairly unrealistic screening tests that are relatively short term in duration (typically 28 days).

Modifications and enhancements, including situations under which the test duration can be extended, to the ready biodegradability test were identified in the RIP 3.3.2 EWG 9 (see Appendix A; ECHA, 2008b).

The ready tests are batch experiments, utilising relatively low numbers of microorganisms, and test substance concentrations in the range of 2 to 100 mg/L. High concentrations are required due to analytical limits of detection, so as to enable an accurate assessment of biodegradation (i.e. to distinguish 'measured' versus 'background' respiration or removal). Pass levels for ready biodegradability are 70% removal of Dissolved Organic Carbon (DOC) and 60% of the Theoretical Oxygen Demand (ThOD) or Theoretical Carbon Dioxide ($ThCO_2$) production for respirometric methods. A pass in a ready test provides the confidence and assurance that the chemical will be readily biodegraded in the environment (Painter, 1995). Where a sufficient number of data points are generated, the data in these tests are increasingly being used to estimate the kinetics of degradation (e.g. half-lives or second order rate constants). However, the scientific justification for the use of measured kinetic data from screening studies requires further scrutiny.

Test method OECD 306 (OECD, 1992a), which is a variant of ready biodegradability OECD screening tests, is the only standardised test available for assessing biodegradability of individual substances in seawater. The OECD 306 test can be carried out as either a shake flask (DOC-parameter) or closed bottle method ($O_2$-parameter) and the *inoculum* is derived from natural seawater. The test guideline does state that this test does not simulate the marine environment. Only growth-linked biodegradation under aerobic conditions is assessed in the OECD 306 and OECD 301 tests (OECD, 1992b) because the test substance is added as sole carbon and energy source at 'very high' concentrations compared to environmental concentrations. As with the ready biodegradability tests described earlier, a favourable result i.e. $\geq$ 60% $CO_2$ or $\geq$ 70% DOC for biodegradation, strongly indicates that the substance is both easily and ultimately biodegradable. The test duration for the marine biodegradability screening studies are 60 days.

The second tier of tests known as the 'inherent biodegradation' tests allow the 'potential' for biodegradation to be demonstrated (OECD 302 series). However, their use in persistency assessments is somewhat restricted because the *inoculum* is derived from sewage treatment plants and the potential for sorption to sludge solids is quite high (ECHA, 2008a). Like the ready tests, there are specific pass criteria (i.e. 70% DOC removal) and rates of degradation are not normally assessed.

Higher tiered biodegradation tests such as the aerobic and anaerobic transformation in soil test (OECD 307, 2002a) and the aerobic and anaerobic transformation in aquatic sediment systems (OECD 308, 2002b) describe the rate of disappearance usually via a $DT_{50}$ value

(i.e. the time within which the initial concentration of the test substance is reduced by 50%). These higher tiered tests were not designed to assess persistence of general chemicals; they were designed to assess the transformation of pesticides. Typical endpoints being assessed include:

- The measurement of the transformation rate of the test substance in a water-sediment system;
- the measurement of the transformation rate of the test substance in the sediment;
- the measurement of the mineralisation rate of the test substance and/or its transformation products (when $^{14}C$-labelled test substance is used);
- the identification and quantification of transformation products in the water and sediment phases including mass balance (when $^{14}C$-labelled test substance is used);
- the measurement of the distribution of the test substance and its transformation products between the two phases during a period of incubation in the dark (to avoid, for example, algal blooms) at constant temperature.

It is stated that half-lives and dissipation times ($DT_{50}$, $DT_{75}$ and $DT_{90}$ values) may be determined where the data warrant, but should not be extrapolated far past the experimental period.

### *3.5 Describing rates of biodegradation*

Many aspects of environmental regulation assume that biodegradation follows first-order reaction kinetics. The concept of half-life time originates from the description of the physical phenomenon that radioactive isotopes show an exponential decline of the intensity of radiation over time. The application of this concept for biological processes has also become wider used in pharmacology as biological half-lives are frequently used to express the disappearance of a drug from the body (ECETOC, 2003).

The use of the half-life concept for biodegradation in the environment has become familiar in the scope of pesticide residues in soil (ECETOC, 2003). In some cases the concept fits rather well with empirical data; however in many cases first order reaction kinetics do not describe the rate of biodegradation (Appendix B). The application of kinetic expressions to describe biodegradation data has been reviewed in detail by Alexander (1999) and Battersby (1990). The persistence of chemicals is almost always expressed in terms of the biological half-lives (see criteria in Table 5).

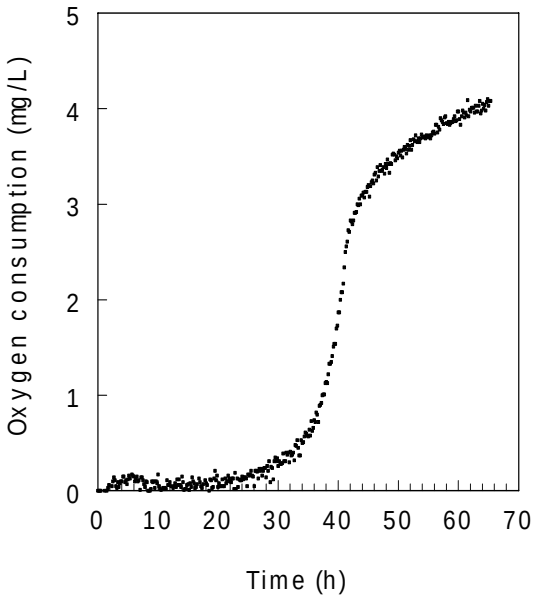
The rate and extent of biodegradation is described in many ways for both standard and non-standard studies. These include: pass/fail approaches using fixed arbitrary criteria, half-lives, $T_{50}$ values, $DT_{50}$ values, percentage removal with time and activity measurements per unit of biomass. The endpoints of biodegradation are also diverse, including parent compound

analysis, and semi-specific endpoints such as carbon dioxide evolution, oxygen demand, dissolved organic carbon removal, removal of chemical oxygen demand and loss of surface active properties (e.g. Methylene Blue Active Substances; MBAS and Bismuth Active Substance; BiAS). Consequently, when collating data from standard and non-standard studies it can be very difficult to directly compare data even for a single chemical. This makes the interpretation of biodegradability data against specific regulatory thresholds (e.g. those for persistency assessments) problematic. Some examples of how different kinetic and pseudo-kinetic criteria are applied to standard biodegradation assays are given below.

In ready biodegradation screening tests pass criteria are defined by the time it takes to reach a certain percentage values for semi-specific endpoints e.g. carbon dioxide evolution, oxygen demand or removal of dissolved organic carbon. The following pass levels of biodegradation, obtained within 28 days, may be regarded as evidence of ready biodegradability: 70% DOC removal (OECD 301A and 301E); 60% theoretical carbon dioxide ($ThCO_2$; OECD 301B); 60% theoretical oxygen demand (ThOD; OECD 301C, 301D and 301F). These pass levels have to be reached in a 10-day window within the duration of the test. The 10-day window begins when the degree of biodegradation has reached 10% DOC removal, ThOD or $ThCO_2$ and must end before or on day 28 of the test. Default rate constants for removal in different environmental media are applied on the pass/fail basis of these tests with and without the 10-day window (see above).

Treating biodegradation in OECD biodegradability tests as a first order process ignores the fact that biodegradation in these tests is ruled by Monod kinetics (i.e. biodegradation is linked to growth of the microbial population; Alexander, 1999). A biodegradation curve from such tests with sufficient data points can (should) therefore not be described with first order kinetics. The curve is probably best described by a logistic equation. The initial part of a biodegradability curve of the OECD 301 and 306 tests are usually logarithmic. In the next phase the biodegradation curve levels off because the concentration of the chemical substance has fallen below the level of the substrate affinity ($k_s$) of the microorganisms, and/or microbial storage material is now being respired. Although these tests were not designed for obtaining values of kinetic constants, the first part of the biodegradation curves can, in some cases, be used to obtain a growth rate by a process of re-iteration to find a kinetic equation that can 'best fit' the curve and describe a rate. To assist this, the OECD tests can be modified to gather more data points allowing a more accurate determination of growth rate (van Ginkel *et al*, 1992). Using a ready biodegradability test with a representative *inoculum* and many data points, a maximum specific growth rate ($\mu_{max}$) of 3 $h^{-1}$ can be calculated for the substance shown in Figure 2.

***Figure 2: Typical sigmoidal biodegradation curve***



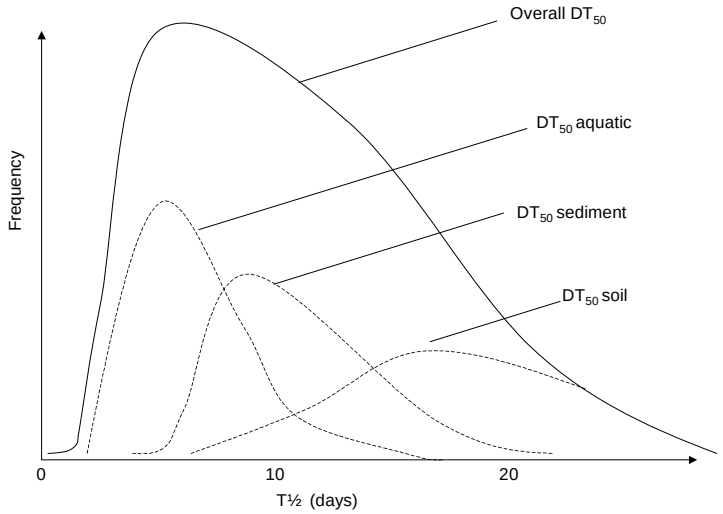

This $\mu_{max}$ corresponds with a half-life (doubling time) of < 0.2 days. This approach rules out the lag period often found in ready biodegradability tests. The lag period is also not considered when determining the $DT_{50}$ in the example given in the OECD 306 guideline (OECD, 1992a). Lag periods in OECD tests usually represent undetectable growth (and degradation of the test substance) due to the 'high' substrate concentrations used and the poor detection limits associated with the semi-specific endpoints used to assess degradation (e.g. DOC removal and oxygen demand). A second pragmatic approach is to measure the time required to reach the point where the curve starts to plateau. The time typically needed to reach the plateau is two half-lives (approximately 2 days in the example shown in Figure 3). These modifications improve determination of half-lives in ecosystems and could reduce the need to perform higher tiered tests.

Higher tiered biodegradation tests such as the aerobic and anaerobic transformation in soil test (OECD 307) and the aerobic and anaerobic transformation in aquatic sediment systems (OECD 308) describe the rate of disappearance usually via a $DT_{50}$ value (i.e. the time within which the initial concentration of the test substance is reduced by 50%). The DT value does not just reflect the rate of biodegradation; it also includes removal due to sorption to the soil organic matter or sediment and any losses due to volatilisation. The kinetics of these higher tiered studies can also be complex and do not always follow first order reaction kinetics.

In conclusion it is clear that the use of a single first order rate constant to describe biodegradation in the environment is overly simplistic. The kinetics occurring within laboratory test and the environment are much more complex and variable. It is not unreasonable to assume that a

number of different types of biodegradation kinetics may be contributing to the overall rate of degradation and a single rate of degradation will not exist. For a given chemical substance, in any environment, a range of degradation rates will exist (see Figure 3; ECETOC, 2003). This concept is illustrated further with the data gathered as part of this review (see Chapter 4). Therefore the concept of biological half-lives or any other single kinetic description is not applicable for characterisation of intrinsic substance properties and not applicable to describe a measurement of biodegradability or persistence. What is required is a kinetic description that reflects the overall distribution of rates in the environment independent of the type(s) of reaction kinetics operating. A concise review of biodegradation kinetics can be found in Appendix B.

***Figure 3: Illustration of the T distribution and range (ECETOC, 2003)***




## *3.6 Guidance on assessing kinetic data from non-standard biodegradation tests*

Environmental microbiology is devoted to the study of microbial conversions (biodegradation) of chemicals in the environment and technosphere (wastewater treatment plant). A large number of methods have been developed to study the biodegradation of chemicals. These methods such as selective enrichments, isolations, continuous and batch cultivation, and assessing activities of whole cell suspensions and enzymes have been conducted since the early 20th century. The incentives for conducting these studies were both applied and fundamental and thus the primary use of the data was not in a regulatory context. Most of these studies were performed in order to understand microbial conversions in ecosystems and biological treatment systems. Examples of environmental microbiology can be found in numerous articles and textbooks on the biodegradation of chemicals in (marine) environments (Alexander, 1999; Harayama *et al*, 1999;

Reineke, 2001; Paul, 2001; Munn, 2004) and the biological treatment of (saline) industrial wastewater (Grady *et al*, 1998; Kargi and Dincer, 1999; Zhao *et al*, 2006). A basic understanding of how microorganisms transform naturally occurring and anthropogenic chemicals that underlie both survival and replication is therefore available. This scientific knowledge is not, as yet, used for regulatory purposes. However, it is forming the basis of training sets for mechanistic QSBRs such as Catabol and Meteor. A significant amount of biodegradation pathway data can be found on the University of Minnesota Biocatalysis/Biodegradation Database[1]. This knowledge may be used to progress the process of assessment of persistence and to ensure more robust classifications of chemicals by balancing information generated for regulatory, applied and fundamental purposes.

Where available, results obtained in standard tests and environmental microbiology studies should be considered as complementary. To this end, all biodegradability data, standard and non-standard, should be collated. When all available information has been evaluated, a judgement of the degradability of a substance should take place by use of a 'weight of evidence' approach. More reliable classifications are basically due to interpreting data supplied in peer-reviewed journals, books, reports of standard tests. Standard tests are carried out under controlled conditions which often do not reflect the complexity experienced in the environment (e.g. standard screening tests). Consequently, they rarely take into account important parameters such as variations, presence of other organic compounds, realistic test substance concentrations, microbial adaptation, redox potential, etc. Consequently negative results obtained in standard tests, especially from screening tests, are not generally appropriate to exclude biodegradation (to assess persistence) of chemicals in the environment.

As described in Chapter 2, and in the master database CD, the information in peer-reviewed journals and books can be highly variable because of the different methods used, e.g. diverse microorganisms (isolates), various ecosystems and treatment systems studied, different ways of expressing biodegradation kinetics. Many of these published non-standard biodegradation studies are of significantly higher scientific quality than many of the standardised screening biodegradation tests. The maximal use of information in peer-reviewed journals therefore requires expert judgement. Screening biodegradability tests like OECD 306 results inform about the following aspects of biodegradation: a) ultimate (complete) biodegradation, b) rate of biodegradation and c) number and occurrence of competent microorganisms present in 'unadapted' ecosystems and biological treatment plants. Ultimate degradation of organic compounds is assumed when > 60% degradation is achieved. Besides reaching the pass level valuable (additional) evidence on ultimate biodegradation may be acquired from catabolic pathways, growth experiments, mass balances of carbon nitrogen, etc. Although the OECD 306 guideline already encourages the derivation of kinetic data (TL, length of lag phase and $t_{50}$; time taken for 50% degradation to occur from the start of the TL) from the test results, rates of

[1] http://umbbd.msi.umn.edu/

biodegradation (half-lives) may also be gathered from pure culture studies (growth rates), biological wastewater treatment, etc. Finally, the number and occurrence of competent microorganisms may be extracted from information on the *inocula* used, their ease of isolation, and the length of the lag period.

A standardised approach of judging the information in peer-reviewed papers, books and reports is not possible due to differences in study design. However, during the compilation of the marine database, several key criteria required for the determination of kinetic behaviour from biodegradation studies were identified as being critical. The remainder of this section will highlight these key quality criteria and illustrate how biodegradation potential can be established with three case studies i.e. naphthalene, methyl bromide and nitrilotriacetic acid.

### 3.6.1 Test substance (identity and purity)

The full chemical name and purity of the test substance should be known. For assessing a half-life, it is imperative that the test substance is a single chemical and not a mixture of chemicals. Information on the composition, possible presence of enantiomers, or homologues is therefore very important. Chemical formula and CAS number are useful but not a prerequisite. The presence of solvents and significant impurities should be quantitatively known. Failure to provide this information may result in an incorrect estimation of biodegradation within the system especially when the biodegradation rates are not determined using specific analyses.

The composition of the test chemical will significantly impact biodegradation itself, and the resulting biodegradation curves. The presence of mixtures of homologues can lead to one or more biodegradation curves which make the interpretation of the kinetics difficult or even impossible. The biodegradation kinetics (lag period, growth rate, yield, etc.) of the individual compounds in the mixture are not necessarily the same. Thus the biodegradation of a mixture in a test using a-specific analyses is the sum of the different individual curves. For example, biodegradation of the enantiomers of EDDS has been reported by Schowanek *et al*, (1997). The EDDS structure has two chiral carbon atoms and has three stereoisomers [R,R], [R,S/S,R] and [S,S]. A biodegradation test programme which included the SCAS, Sturm and soil biodegradation tests was conducted with the isomer mixture 25% [S,S]; 25% [R,R] and 50% [S,R]/[S,R]. To determine the rates and potential of biodegradation for the individual EDDS stereoisomers, three separate SCAS units were acclimatised to a mixture of EDDS. This was followed by a $CO_2$ production Sturm test by taking the *inocula* from the three separate units. The tests showed that mineralisation of [S,S] was rapid, achieving > 90% in 28 days, while [R,R] EDDS degradation was negligible and the mixture did not exceed 35%. This clearly demonstrates that kinetic parameters derived from a test with mixture of enantiomers need to be assessed with some caution.

### 3.6.2 Characterisation of the *inoculum*

The *inoculum* has to be evaluated to ensure that the findings are representative of the 'targeted environment'. The *inoculum* should be characterised and the source clearly identified (activated sludge, sewage, surface water, seawater, soils, or a mixture of these). Information on concentration and history (exposure of the *inoculum* to the test substance) of the *inoculum* would assist in the interpretation of the biodegradability data. This information is also important for assigning a half-life to the test substance in a targeted environment. Ideally, the biomass concentration used in the test should be comparable with the concentrations occurring in the ecosystems. However, the biomass concentrations in standard screening tests (RBT) are between 1-2 orders of magnitude lower than that in environmental waters. In many standard studies, a positive reference compound is used to demonstrate biomass is viable e.g. OECD 309 (OECD, 2004). However, a reference compound is not usually used in non-standard studies or some of the higher tier standard studies e.g. OECD 307 and 308 (OECD, 2002a,b). Knowledge of a correctly operating biological treatment plant, and skilled sampling should ensure the use of viable *inocula* for a biodegradability test. For pure cultures the ecosystem or treatment system where the isolates originated from, and preferably the size of the *inoculum* used during enrichment should be known. Detailed information on pure cultures such as ecological prevalence and potential to degrade other chemicals might enable assessment of half-lives in the environment. This information will usually allow assessment of the potential and the extent of the test substance to biodegrade.

Studies by Thouand *et al*, (1995), have investigated the relationship between biomass concentration and the probability of observing ready biodegradability. Thouand *et al*, (1995) concluded that environmentally relevant biomass concentrations increase the probability for observing biodegradability, and reduce the potential for false negatives.

Work by Davenport *et al*, (2008) and Goodhead *et al*, (2008) used the principles described by Thouand *et al*, (1995; 1996) to investigate the role of *inoculum* source, density and measures of diversity on ready biodegradability outcome using 96-well plate high-throughput enhanced BSTs. These studies supported and extended Thouand's findings, which show that *inoculum* density is an important factor in determining the probability of degradation for a given compound. Davenport *et al*, (2008) and Goodhead *et al*, (2008) demonstrated that the *inocula* concentrations used in current RBTs have a high failure rate and can lead to variable and conflicting results. DNA-based molecular techniques showed that standard OECD *inoculum* preparation methods reduced the detectable diversity and density of the microbial community from an activated sludge sample. Whereas Thouand *et al*, (1995) only investigated one *inoculum* source for activated sludge and an indeterminate number of river sources, Davenport *et al*, (2008) and Goodhead *et al*, (2008) investigated *inocula* prepared from numerous locations from various environmental compartments using the same BST method. The following environmental compartments were used, shown in order of highest assumed diversity (Curtis *et al*, 2002). The number of locations

for each compartment are shown in parentheses; soils (11) > activated sludge (6) > lakes (5) ≡ rivers (20) > coastal waters (5). This resulted in the equivalent of over 400 RBTs, each with 96 replicates. Davenport *et al*, (2008) and Goodhead *et al*, (2008) also developed a novel colorimetric method based on azo-coupling reactions in order to extend the capabilities of the high throughput enhanced BST for the detection of different phenolic compounds (e.g. *para*-chlorophenol).

In contrast to Thouand's work, data from Davenport *et al*, (2008) and Goodhead *et al*, (2008) showed that there was considerable variation in the number of competent degraders from different locations in the same environmental compartment. Activated sludge showed the highest variation and coastal waters showed the least variation. Generally, the *inocula* showed better biodegradation potential for *para*-nitrophenol than *para*-chlorophenol, although for coastal water samples the reverse was true. Importantly, there is a low frequency of biodegradability of the test compounds at *inocula* densities used in standard tests, and even at ambient environmental cell densities for some compartments (e.g. rivers and coastal waters). Molecular analysis indicated that the biodegradation potential for *para*-chlorophenol nearly matched the order of the perceived diversity for each of the environmental compartments.

Improved predictions of the marine biodegradability of linear alkylbenzene sulphonate (LAS) have also been achieved through elevating the biomass level within laboratory-based marine biodegradation tests. Work by Mauffret *et al*, (2007; 2008) pre-colonised glass beads with marine bacteria derived from Sweden (annual temperature range of 1-21°C) and Spain (annual temperature range of 9-25°C). The glass beads were colonised in a flow through system of natural seawater and acclimated to a low concentration of LAS (20 µg $l^{-1}$). For each site temperatures outside the annual field temperature range resulted in lower rates of LAS mineralisation. This was particularly true for studies conducted at 6°C using the Spanish *inoculum* source where less than 10% mineralisation was observed. At 6°C the studies conducted using the Swedish *inoculum* source still exhibited approximately 40% mineralisation.

### 3.6.3 Test system design

The test set-up used to determine biodegradation should take into account the physico-chemical properties of the test compound, such as solubility in water, vapour pressure, sorption characteristics, etc. When the test substance has been tested above its solubility limit, the dissolution rate could also be determined. Removal of the test substance through evaporation might occur in 'open' test systems. Volatile substances should therefore always be examined in closed systems. Septa used to close systems should not adsorb the test material. The test system (i.e. batch, semi continuous or continuous) and the test chemicals used should therefore be sufficiently described, highlighting the possible pitfalls that may need to be considered for assessing biodegradation. An appropriate method for analytical measurements should also

be used. Analyses (not used in standard tests) such as ammonium, nitrate, phosphate and halides are in some cases very interesting alternatives. Rates of biodegradation cannot always be determined at 'high' concentrations, and thus suitable methods are required to determine the biodegradation at 'low' concentrations. For substances capable of sorption onto biomass, material of the test system, etc., a validated method to recover the substance should be available. The use of carbon dioxide formation or oxygen consumption always requires adequate controls.

Research by Ingerslev and Nyholm (2000) showed that increasing the total volume of test medium resulted in decreased lag times (TL) in biodegradability shake flask batch tests conducted with (i) surface water and (ii) synthetic mineral medium inoculated with supernatant from settled activated sludge. Test volumes ranging from 1.8 ml to 100 L were investigated using two chemicals (2,4-dichlorophenoxyacetic acid and p-nitrophenol). Ingerslev and Nyholm (2000) demonstrated that lag times increased with decreasing test volume, even when a single test batch was redistributed into smaller flasks. At small volumes of 10 ml or less, degradation failed randomly i.e. the test gave false negatives. This was attributed to the absence of a sufficient total amount as well as a sufficient concentration of specifically degrading microorganisms or consortia of bacteria at low volumes.

Work by Snape (2005) also found that test volume had a significant impact on the marine biodegradability of naphthalene, phenol, glucose, para-nitrophenol and aniline. Higher levels of variation and an increased occurrence of false negatives were observed between replicate flasks at lower volumes. Snape (2005) demonstrated that the biodegradation assays routinely used to assess ready biodegradability or biodegradation of chemicals in freshwater environments, such as the ISO $CO_2$ headspace test or the OECD 310 test (OECD, 2006), produced high and unacceptable levels of replicate variability when used to conduct marine assessments. Snape (2005) concluded that marine versions of the ready biodegradability protocols such as the ISO $CO_2$ headspace test or the draft OECD 310 test were not suitable for assessing the persistence of chemicals in the marine environment. The variability observed in these studies could not be attributed to nutrient limitation, as nutrient amendment did not reduce replicate variability. Further investigations by Snape (2005) identified that biological variability between the replicate flasks (i.e. whether or not the *inoculum* source contained a competent microorganism that could degrade the test substance) was the cause of the variation. This low working volume restricted the amount of microbial diversity introduced into the test at the start of the experiment.

In the course of this research Snape (2005) reduced the replicate variability and enhanced the robustness of marine biodegradability assessments using three different approaches:

- Increasing the size of the test vessel.

  Increasing the volume of the test media, including the test substance and *inoculum* source, from a working volume of 70 ml to 4 L improved the reproducibility between replicate flasks. Increasing the test volume increased the microbial biodiversity of the *inoculum* providing an increased chance of introducing a competent microorganism without increasing the density of the *inoculum* (number of cells per litre). At volumes of 4 L this variation was removed and replicates were reproducible. A straightforward practical implication of the findings is that the test volume in biodegradability tests can significantly influence the lag time and thus sometimes be decisive for the outcome in biodegradation studies. This suggests that the observations made by Ingerslev and Nyholm (2000) for river water also apply to marine waters; however the levels of variability in marine studies appeared to extend to higher test volumes. Therefore the volume of the biodegradation tests should be considered during the design phase. This is particularly important given business and regulatory pressures to generate small-scale test protocols for environmental assessments that do not entail excessive costs.

- Introducing a static sediment phase into the test system.

  Conducting marine biodegradability studies in a two-phase system with marine water and sediment increased both the probability of biodegradation taking place and the rate of marine biodegradation when compared to a single-phase system with just marine water. The presence of the sediment phase increased the size and diversity of the inoculum. In experiments using aniline and 4-nitrophenol as the test substances biodegradability was consistently observed when static sediment was present, little or no biodegradation was observed for these substances in the absence of static sediment. Comparable results were achieved in freshwater biodegradation test systems with added sediment (Junker et al, 2006).

- Using semi-continuous approaches to assessing marine biodegradation.

  Semi-continuous assessments of marine biodegradability, conducted in the presence and absence of static marine sediment, confirmed the observations from batch studies that the rate of biodegradation was faster in the two-phase system. The semi-continuous studies, operated with a 28-day hydraulic residence time (i.e. 0.5 L of the 1 L test volume was replaced every 14 days), also demonstrated that the degradation rates for glucose, para-nitrophenol and aniline increased with time i.e. natural adaptation or enrichment of competent populations was occurring within this marine biodegradability test within relatively short time periods.

### 3.6.4 Test substance concentration

The concentration of test substance is important as this in turn also determines how the endpoint of biodegradation will be measured. Typically for standard studies this is usually 10mg/L. Although this does not reflect environmental concentrations, historically it is within the limits of analytical detection, particularly for inorganic carbon and dissolved oxygen measurements. However, sophisticated analytical techniques such as LC/MS, do allow for measurements in the 1-2 µg/L range. Below this concentration range (i.e. ng/L) requires the use of $^{14}C$ for accurate analytical measurements. Low concentrations are not a requirement for testing biodegradability in continuous systems.

The standardised ready biodegradation test methods were initially developed to evaluate the biodegradability of test substances which are non-adsorbing and soluble in water to at least 100 mg $l^{-1}$ provided they are non-volatile (Battersby, 2000). Techniques to administer poorly water-soluble chemicals when assessing biodegradability were originally described by the OECD and include:

a. Homogenisation of solid materials in water by the appropriate mean;
b. the use of an emulsifier to give a stable dispersion of the chemical;
c. the use of solvents;
d. the use of solid carriers for solid test substances (OECD, 1992b: Annex III).

(or
1. direct addition;
2. ultrasonic dispersion;
3. adsorption on an inert support;
4. creating a dispersion or emulsion.)

Agitation during the test may be used. The International Standards Organization (ISO) concluded in 1995 that the development of a single method for evaluating the biodegradability of poorly water-soluble organic substances may not be feasible in the immediate future. Consequently, ISO proposed a series of methods where the final selection was based on a judgment of the physico-chemical properties of the test substance (ISO, 1995). The methods described to administer chemicals are important to assess the ready biodegradability of test substances. These methods do not however, enable a sound assessment of the biodegradation kinetics. The use of the approaches advocated by OECD (1992b) and ISO (1995) have been formalised under REACH (ECHA, 2008b). However, when assessing the validity of non-standard studies it must be demonstrated that the carrier did not contribute towards the rate or extent of biodegradation that was measured. Examples demonstrating the use of carriers, solvents and emulsifiers to enhance the bioavailability of chemicals in biodegradation screening tests are described in Appendix C.

### 3.6.5 Test conditions

Experimental findings are only valid in a targeted 'environment' when the conditions of the incubation reflect the conditions in the environment. Conditions considered important are redox potential (the presence of oxygen), pH, temperature and agitation. This information has to be controlled and/or checked throughout the incubation period. The temperature is usually controlled in incubators. The pH may be regulated by using buffers or with a pH meter and controller. Aerobic conditions may be maintained by adequate aeration in, for example, bioreactors, using stirrers or by using low concentrations of biomass and test substance. Temperature and pH are usually maintained within the range 20-35°C, and 6.5-7.5, respectively. Biodegradation data generated at temperatures or pH values outside these ranges are acceptable provided the conditions used are clearly stated and other quality criteria are followed. Rates at various temperatures have been estimated using the Arrhenius law, however this is not recommended since this approach ignores the existence of different groups of microorganisms (psychrophilic, mesophilic).

### 3.6.6 Test duration

Test duration is another important requisite in determining kinetics of biodegradation. This provides an understanding of whether biodegradation of the test substance has occurred over a sufficient time period to enable a half-life to be reported or calculated, i.e. extrapolation to a long half-life from a very short test duration period. Studies that extrapolate from test durations of just a few days to half-lives of greater than a few months should be avoided as they ignore the potential for microbial adaptation.

### 3.6.7 Degradation rate

The way in which biodegradation results are reported is also important in determining kinetics of biodegradation. For example, details of the test substance concentration, a sufficient number of data points and measurement of the extent of removal.

### 3.6.8 Biodegradation endpoints

The endpoint of biodegradation is an important requisite as this provides a direct measure of the fate of the substance. Several endpoints are typically measured to describe the extent of biodegradation. This includes dissolved oxygen (DO), $CO_2$ production, $O_2$ uptake, dissolved organic carbon (DOC), BOD and removal of parent compound. Some of these are more equivocal measures e.g. DOC, as the removal of organic carbon may not solely be due to

biodegradation. Unequivocal measures of biodegradation include $CO_2$ production and $O_2$ uptake which are direct measures of respiratory activity, and much more conclusive of ultimate biodegradation. Where specific chemical analysis has been used to demonstrate primary biodegradation the formation of degradation products and their identity needs careful consideration. It should also be noted that the endpoints are not always directly comparable. ECETOC (2003) demonstrated that the time to meet the pass criteria for $CO_2$ evolution and DOC removal for glucose, aniline, 4-nitrophenol and 4-chloroaniline were not equivalent.

### 3.6.9 Additional information requirements from biodegradation studies

In addition to the essential parameters described above, other useful parameters such as the motivation for conducting the study and biomass concentration [M] can also aid in determining kinetic information. Typical measurements of biomass concentration include total and viable cell counts, fumigation extraction and dried cell weight. The most probable number (MPN) approach can help identify the competent fraction of the biomass. However information on the numbers of bacteria present, combined with the test substance concentration, is useful for determining the type of kinetics occurring.

## *3.7 Case studies assessing non-standard data*

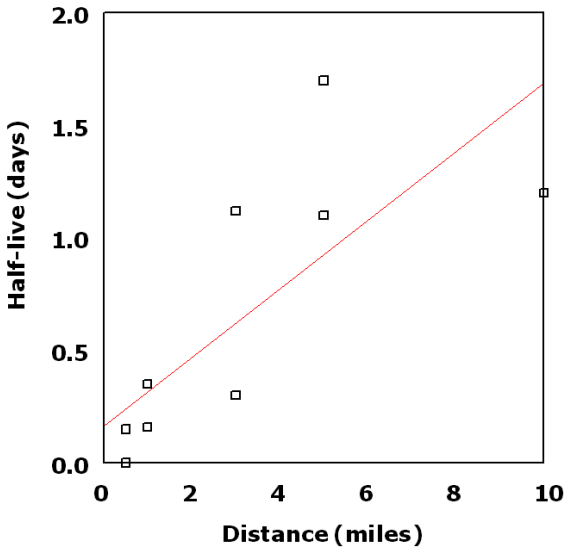
Three case studies are described below that illustrate how non-standard marine biodegradation data demonstrate that chemicals biodegrade in the natural environment through a variety of mechanisms. In the first example growth-linked biodegradation of naphthalene is illustrated, as is the natural enrichment of competent degraders near oil platforms. The second example illustrates that some chemicals e.g. methyl bromide can be degraded through growth-linked degradation and co-metabolism. Finally, the importance of a weight of evidence assessment is demonstrated using NTA to address conflicting data.

### 3.7.1 Example of growth-linked biodegradation and natural adaptation

Demonstration of growth-linked degradation may be illustrated with a study performed in the North Sea (Salzmann, 1982). Biodegradation rates of naphthalene in sediments were measured at various distances from the Beryl oil platform. These degradation rates have been converted into half-lives. It was found that the half-lives were greater with increasing distances from the platform demonstrating that exposure resulted in shorter half-lives (Figure 4). The competent microbial communities had been enriched by exposure to naphthalene near the platform. Mineralisation of naphthalene was demonstrated in this paper whilst complete degradation of

naphthalene has been demonstrated with both pure cultures (Smith, 1994) and in mixed marine microbial communities (Snape, 2005).

***Figure 4: Half-lives (days) of naphthalene versus distance from the Beryl offshore oil platform demonstrating the capacity of microorganisms to utilise this substance for growth in the marine environment***




### 3.7.2 Example of degradation via growth-linked removal and cometabolism

Methyl bromide is an example of an organic compound that can be degraded in the marine environment by growth-linked and co-metabolic activity. The capacity of microorganisms to utilise methyl bromide as carbon and energy source is demonstrated by the 'logarithmic' increase of the production of carbon dioxide by microorganisms in a seawater sample (Goodwin *et al*, 1998). The use of a chloramphenicol control demonstrated that microorganisms are capable of adapting to methyl bromide. The doubling time with methyl bromide of microorganisms present in the sample was estimated to be only 10 hours. In a separate experiment with an enrichment culture, the carbon of methyl bromide was stoichiometrically recovered as carbon dioxide. This formation of carbon dioxide demonstrates that this substance is ultimately biodegradable (Goodwin *et al*, 1998).

Typically in ready biodegradability screening tests, the test chemical is introduced as the sole carbon source at a relatively high concentration compared to the initial biomass concentration. However in the real environment, the test chemical will be one of a complex mixture of chemicals, all of which will influence biodegradation. Primary degradation of organic

compounds is either catalysed by microorganisms growing on the test substance or by microorganisms grown on another substrate (co-metabolism). Co-metabolism is defined as the microbial metabolic activities on chemicals from which microorganisms do not primarily receive energy. Such metabolic activities require the utilisation of existing enzymatic systems. Co-metabolism therefore depends on the fact that certain enzymes are sufficiently non-specific to catalyse degradation of compounds other than their 'normal' substrate. Co-metabolic transformation may be the initial step in the degradation of an organic compound making the substrate more susceptible to further transformation resulting in mineralisation. However, a co-metabolic conversion may also result in formation of a persistent chemical(s). Mono-oxygenase enzymes are known to have wide substrate specificity. For example, toluene-oxidising microorganisms have mono-oxygenases capable of catalysing the initial biodegradation steps of a number of alkanes and alkenes. This co-metabolic transformation can be illustrated by the work of Goodwin *et al,* (2005) who isolated a bacterium capable of growing on toluene. The bacterium did not grow on methyl bromide; however, methyl bromide was oxidised by toluene-grown bacteria. Co-metabolic degradation of methyl bromide has also been demonstrated with marine nitrifying bacteria (Rasche *et al*, 1990). The example of co-metabolism clearly indicates that for certain substances a standardised screening test cannot sufficiently reflect the real environmental situation (i.e. co-metabolism is unlikely to occur in the OECD screening tests). Additional information from non-standardised test can improve the assessment of the environmental behaviour of the substance.

### 3.7.3 Example of weight of evidence approach to assess conflicting data

NTA is another example illustrating the importance of the use of non-standardised test methods in a 'weight of evidence' approach. Whilst the biodegradation of this compound under fresh water conditions has been established comprehensively (Bucheli-Witschel and Egli, 2001) there are conflicting results from studies assessing the biodegradation of NTA in estuarine and marine conditions (Kirk *et al*, 1983; Bourquin and Przybyszewski, 1977; Hales and Ernst, 1979; Bartholomew and Pfaender, 1983; Hunter *et al*, 1986; Palumbo *et al*, 1988). NTA concentrations and salinity had a marked effect on biodegradation. At an NTA concentration of 8 mg/L and salt concentrations of $\geq$ 1.8% no NTA degradation was detected. However, at low NTA concentrations i.e. 0.8 mg/L NTA, degradation was observed at salinities varying from 0.1 to 3.0% (Hunter *et al*, 1986). The data demonstrate that biodegradation of NTA is possible in marine environments although compared with NTA degradation in fresh water at lower rates. NTA biodegradation rates measured in marine environments allow calculation of half-lives in the range from 10 to 30 days.

The biodegradability of NTA has not been demonstrated with OECD 306 tests inoculated with seawater sampled from the coast of the Netherlands (Henkel, 1998; Akzo Nobel, 2003a) although NTA degradation in seawater with a half-life of 10 days has been demonstrated. Reasons for the

negative results might be a 'high' initial test substance concentration (Hales and Ernst, 1991; Hunter *et al*, 1986), or the prescribed low inoculation of the test. In order to address this, increased numbers of microorganisms were introduced in biodegradability tests with a sediment layer in a fed batch system. Part of the seawater spiked with 50 mg/L of NTA in the fed batch system was replaced weekly giving a hydraulic retention time of 10.5 days. After an incubation period of approximately 50 days almost complete removal of NTA was observed. This result shows that NTA can be removed from seawater with a half-life of ≤ 5 days (Akzo Nobel, 2003b) and the microbial community are able to adapt to NTA in a relatively short time. This illustrates the need to evaluate all data, standard and non-standard in 'weight of evidence' approach for the assessment of persistence.

## *3.8 Conclusions*

Degradation, including biodegradation, is an important process that can reduce the environment burden of a chemical and its long-term persistence in the environment. Consequently, standardised methods exist to assess biodegradability within the regulatory frameworks for Classification and Labelling, Chemical Safety Assessment (exposure assessment) and PBT/vPvB assessments.

Many of these standardised approaches, originally developed to assess biodegradation in freshwater aquatic systems, cannot be directly applied to assess marine biodegradability. Studies by Thouand *et al*, (1995, 1996), Ingerslev and Nyholm (2000), Snape (2005), Mauffret *et al*, (2007; 2008), Goodhead *et al*, (2008) and Davenport *et al*, (2008) have all demonstrated that these standard tests, particularly the ready biodegradation tests, have a high potential to produce conflicting results and false negatives. Therefore new tests are required that can accurately assess rates of degradation. One approach could include the use of increased *inoculum* biomass densities or test designs that allow natural adaptation to occur, as advocated by ECETOC (2003), Snape (2005) and REACH (ECHA, 2008a,b). This would provide: i) more reliable (repeatable) biodegradation tests, ii) more environmental realism (cell abundances and diversities typically encountered in the environment), and iii) increased sensitivity i.e. maintained F:M ratios with increased test chemical mass to observe degradation with standard analyses equivalent to low test compound concentrations.

In addition to standard tests there is a wealth of published non-standard biodegradation data of variable quality. It is essential that the maximum use of these data be made, particularly when conducting a weight of evidence assessment to conclude on environmental persistence. The key criteria that need to be evaluated in such studies are:

- The identity, purity and concentration of the test substance;
- the origin of the *inoculum* source including any pre-treatment;
- the analyte being measured (primary or ultimate degradation);
- the rate and extent of degradation.

Numerous other parameters can also be collated (see the database associated with this technical report) including temperature, pH, *inoculum* concentration, salinity, etc. However, these data are not as critical to make a final conclusion on the value or suitability of the study. The case studies identified within this Chapter and data analysis conducted elsewhere in this report demonstrates that non-standard biodegradation data, that meet these minimum data quality requirements, can be used in regulatory assessments as part of a weight of evidence assessment.

# 4. FRESHWATER TO SALTWATER COMPARISONS

## *4.1 Introduction*

The main aims of these comparisons was to try and establish whether the reported rates of biodegradation of a given chemical in fresh waters are significantly faster, equal to or slower than those measured for the same chemical in salt water environments, whether there is a scientific basis to extrapolate between the two and compare published rates with the default values published in the EU TGD (EC, 2003) and REACH (ECHA, 2008c). The data have also been used to demonstrate the range of half-lives that are found for chemicals in any compartment and to illustrate the value of assigning half-life distributions as advocated by ECETOC (2003), as opposed to a the currently used approach of stipulating a specific half-life based on one or two data points.

The EMBK database contains 806 kinetic datasets, mainly from marine environments, together with the corresponding relevant information on test design and conditions, *inoculum* source and sampling conditions were collected in a raw database. In order to retrieve statistical information from this database, kinetic descriptors were harmonised to a normalised half-live ($D_{1/2}$, see section 2.6.). Data where this normalisation procedure was not possible have been disregarded in any further statistical evaluation. In addition, data retrieved from pure culture studies and from enrichments have not been used for statistic analysing. Finally 650 kinetic data for 125 different substances were processed for statistical comparisons between the environmental compartments (see Figure 5). Of these data 48% were from marine environments, 27% from estuarine, and 25% from freshwater habitats (surface waters and sediments) (see Figure 6).

The data collection exercise focussed initially on the marine environment and is considered by the TF to be reasonably comprehensive. Nevertheless, the number of publications containing high quality marine biodegradation data was limited. This in turn limited the amount of data that could be obtained for any given substance (with one or two notable exceptions e.g. naphthalene and phenol). The interpretation of the available information was complicated because of the many variable parameters of non-standard test conditions (e.g. different incubation temperatures, different substrate concentrations, test duration, number of samples and data points, etc.). The amount of data recorded for the freshwater environment is not, and was not, intended to be comprehensive since it was only collected for comparative purposes for the chemicals for which there was a significant amount of marine data. Very little standard data has been published and a wealth of untapped standard and non-standard test studies are available but not presented in a format from which it is possible to get rates.

***Figure 5: Statistical overview of ECETOC Marine Database (n = 650)***

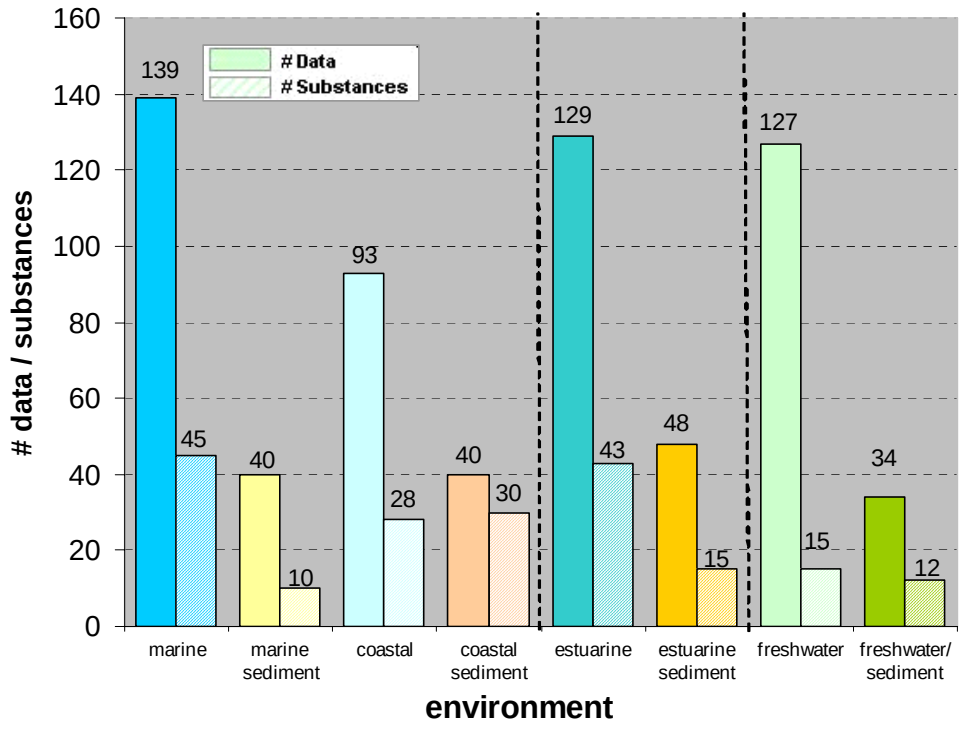
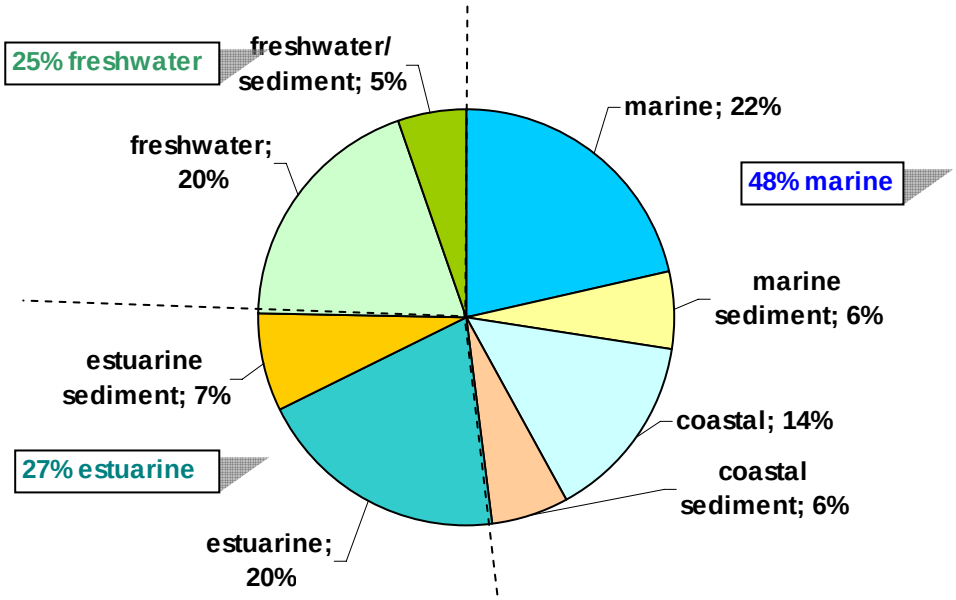



***Figure 6: Distribution of data in ECETOC marine biodegradation kinetic database (EBKD) with respect to environmental compartments***



## *4.2 Data rich substances*

Only studies that can be used to obtain a measure of half-life were subjected to further statistical investigations (Table 6). The 26 most data rich substances (507 data points) were 1,2,4-trichlorobenzene (7), 2-methyl naphthalene (5), amino acids (34), aniline (35), benzene (8), benzo(a)pyrene (26), alcohol ethoxylates (AEO) (25), 4-chloroaniline (21), chlorobenzene (6), fluorene (9), hexadecane (8), linear alkylbenzene sulphonate (LAS) (21), m-cresol (15), methyl bromide (11), methyl parathion (6), naphthalene (96), 4-nitrophenol (12), nonylphenol ethoxylates (NPE) (21), nitriloacetic acid (NTA) (9), phenanthrene (22), phenol (44), pyrene (5), sodium acetate (5), sodium benzoate (13), toluene (29) and tributyltin (14).

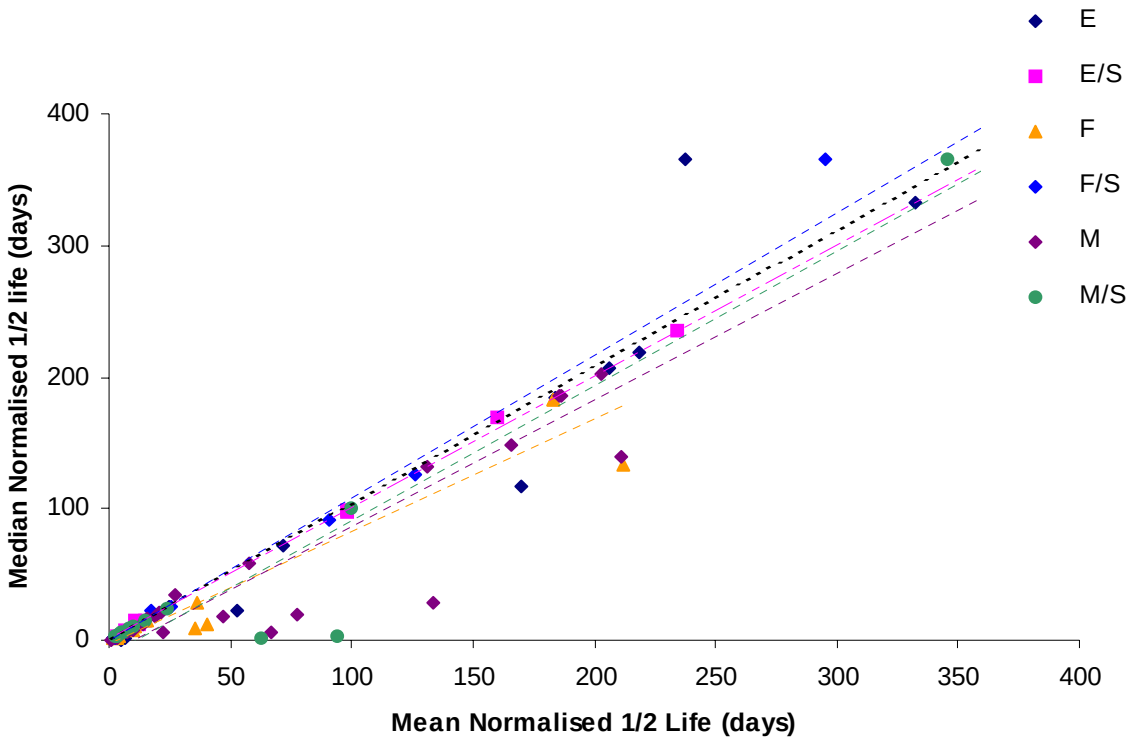
A minimum number of data points are required for each compartment (preferably > 5 data points per substance per compartment) for any given substance to enable comparisons between compartments to be drawn. Therefore the data for the marine compartment were merged with the data for coastal compartment to provide sufficient number of data points for the total marine compartment. Benthic and pelagic data have also been combined for each compartment resulting in the three basic environmental compartments, 'marine', 'estuarine' and 'freshwater'. Seventeen substances had half-lives for all three compartments (representing 450 of the data). Each of the compartments has relatively equal coverage ('marine' = 44%, 'estuarine' = 29% and 'freshwater' = 33%) enabling direct statistical comparisons (cf. section 2.6).

For each chemical, the median and mean of all half-life values in each environmental compartment was determined. A scatter-plot and regression of mean values against median values indicated that overall within each compartment there was an approximate 1:1 relationship between mean values and median values with the exception of some obvious outliers (Figure 7; $r^2$ 0.979). Comparisons of kinetic data for each substance in each of the compartments were carried out using median rather than mean values to prevent outliers biasing the small data sets. In addition to the median value, the maximum and minimum values for each chemical were determined. These values have been plotted to indicate the range of normalised half-life values obtained for each chemical occurring within each *inoculum* source, and are shown in Figures 9 and 10. Substances that showed no degradation were usually reported with very long or infinite half-lives. These long half-lives were attributed to either no degradation being observed or limitations in the test design (e.g. test duration too short). To enable statistical analysis, an upper limit of one year (365 days) was defined as the longest half-life. Limitations of the normalisation methodology are outlined in Appendix B.

***Table 6: Biodegradation kinetic data basis for statistical analysis (number of data in respective environment; F = freshwater; F/S = freshwater-sediment; E = estuarine; E/S = estuarine-sediment; M = marine; M/S = marine-sediment; C = coastal; C/S = coastal sediment)***

| Substance | CAS No | F | F/S | E | E/S | M | M/S | C | C/S | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| 1,2,4-trichlorobenzene | 102-82-1 | 2 | 0 | 2 | 0 | 1 | 0 | 2 | 0 | 7 |
| 2-methyl naphthalene | 91-57-6 | 0 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 5 |
| Amino acids | - | 5 | 0 | 5 | 0 | 19 | 0 | 5 | 0 | 34 |
| Aniline | 62-53-3 | 20 | 0 | 2 | 0 | 12 | 0 | 1 | 0 | 35 |
| Benzene | 71-43-2 | 6 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 8 |
| Benzo(a)pyrene | 50-32-8 | 0 | 8 | 4 | 1 | 0 | 8 | 0 | 5 | 26 |
| AEO | - | 14 | 0 | 7 | 0 | 2 | 0 | 2 | 0 | 25 |
| 4-chloroaniline | 106-47-8 | 10 | 0 | 5 | 0 | 5 | 0 | 1 | 0 | 21 |
| Chlorobenzene | 108-90-7 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 6 |
| Fluorene | 86-73-7 | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 4 | 9 |
| Hexadecane | 544-76-3 | 0 | 2 | 2 | 2 | 0 | 1 | 1 | 0 | 8 |
| LAS | - | 6 | 0 | 4 | 2 | 6 | 0 | 3 | 0 | 21 |
| m-cresol | 108-39-4 | 8 | 0 | 6 | 0 | 0 | 0 | 1 | 0 | 15 |
| Methyl bromide | 74-83-9 | 3 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 11 |
| Methyl parathion | 298-00-0 | 0 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 6 |
| Naphthalene | 91-20-3 | 6 | 9 | 19 | 8 | 0 | 24 | 29 | 1 | 96 |
| 4-nitrophenol | 100-02-7 | 0 | 1 | 1 | 0 | 10 | 0 | 0 | 0 | 12 |
| NPE | - | 2 | 0 | 0 | 18 | 1 | 0 | 0 | 0 | 21 |
| NTA | 139-13-9 | 2 | 0 | 1 | 0 | 3 | 1 | 2 | 0 | 9 |
| Phenanthrene | 85-01-8 | 1 | 2 | 15 | 2 | 0 | 1 | 0 | 1 | 22 |
| Phenol | 108-95-2 | 28 | 2 | 4 | 2 | 0 | 0 | 6 | 2 | 44 |
| Pyrene | 129-00-0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 1 | 5 |
| Sodium acetate | 6131-90-4 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 |
| Sodium benzoate | 532-32-1 | 1 | 0 | 0 | 0 | 10 | 0 | 2 | 0 | 13 |
| Toluene | 108-88-3 | 10 | 0 | 2 | 0 | 0 | 0 | 17 | 0 | 29 |
| Tributyltin | 688-73-3 | 0 | 0 | 11 | 1 | 0 | 1 | 0 | 1 | 14 |
| **Total** | | 126 | 30 | 98 | 43 | 81 | 37 | 77 | 15 | 507 |

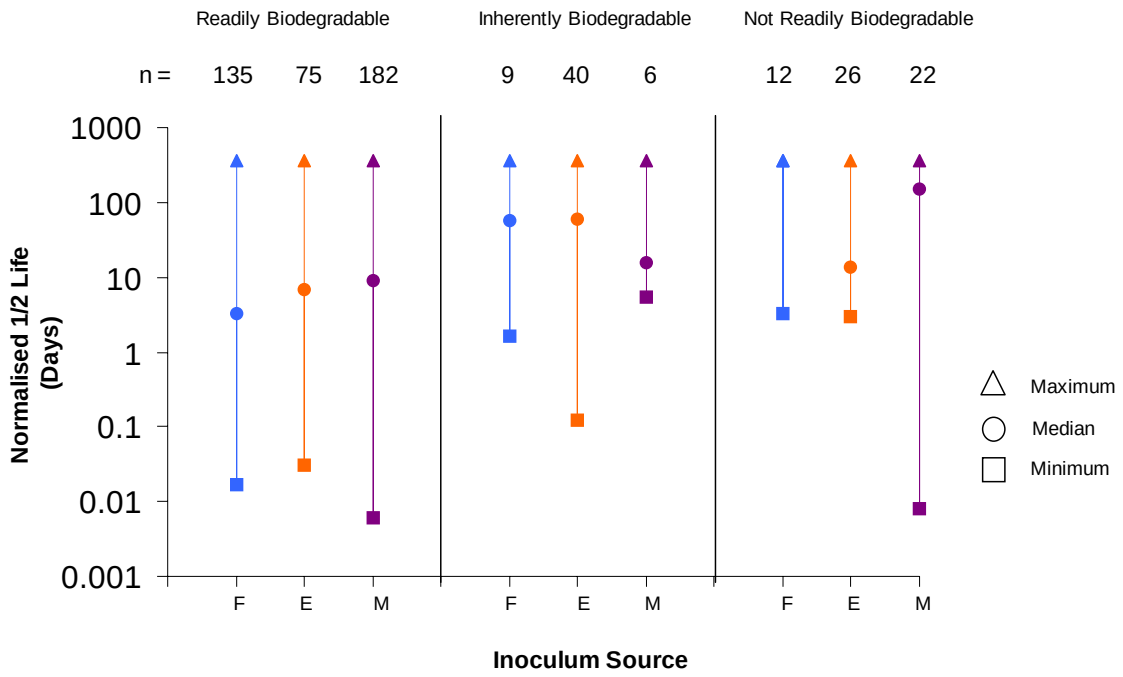
***Figure 7: Scatter plot of mean versus median half-lives (in days)***




The 26 chemicals were categorised as either readily biodegradable (RBT), inherently biodegradable (IBT) or not readily biodegradable (NB). The categorisation was based on results from standard ready tests or 'weight of evidence' (i.e. expert judgement) e.g. aniline was assigned RBT as it is a positive standard for ready biodegradation tests. Note, that for further analysis, the longest half-life assigned was set to 365 days, even when no degradation was observed.

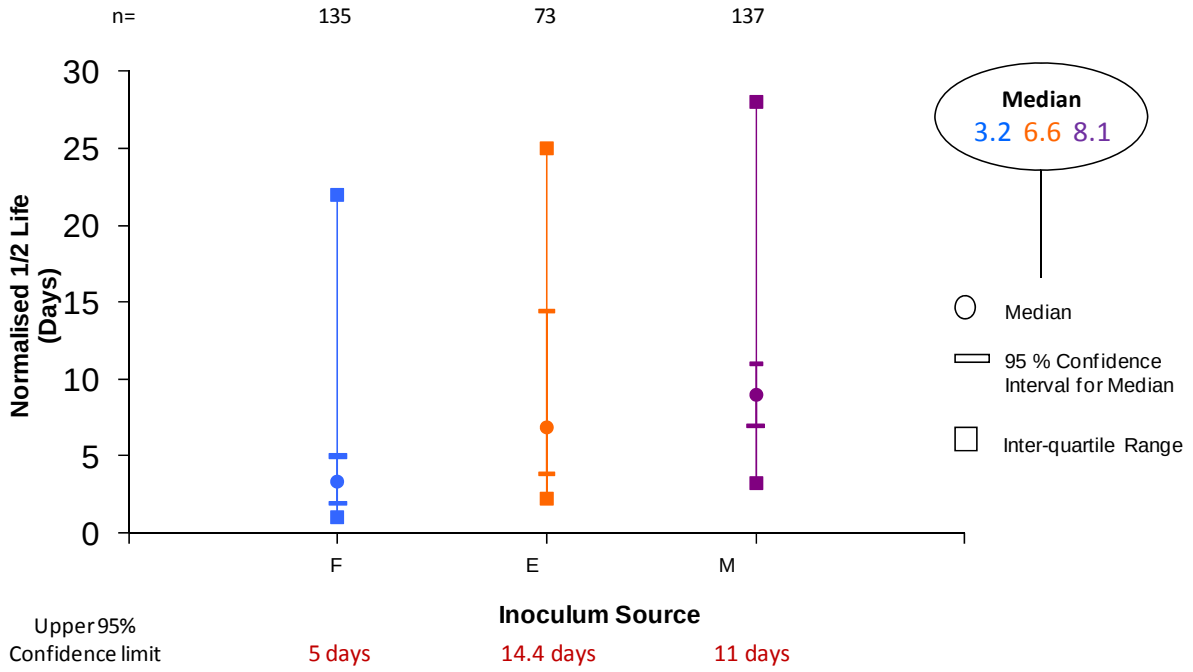
The data in Figure 8 illustrate that chemicals, even those considered readily degradable, can have half-lives that vary over 2-3 orders of magnitude. The median half-lives for the readily biodegradable substances are similar in all three compartments and are lower than those considered not readily biodegradable. The readily biodegradable substances have median half-lives that are lower than the TGD default values, particularly for the marine environment (approximately 10 days versus the TGD default of 50 days). For chemicals considered to be inherently degradable there is some evidence that they can have shorter half-lives (< 100 days in all environmental compartments) than the 150 default values in the TGD. There will always be instances where slow or no degradation is observed. These data support the half-life distribution approach (ECETOC, 2003) and demonstrate that chemicals will have different values in each test.

***Figure 8: Ranges of half-lives in marine (M), estuarine (E) and freshwater (F) compartments for readily, inherently and non-readily biodegradable chemicals***




Normalised half-life values (for all readily biodegradable chemicals) were log-transformed prior to statistical analysis and the data were then assessed for normality. The data were found to be non-normally distributed and were assessed for equality of variances between each compartment using Levene's test (Levene, 1960; Brown and Forsythe, 1974). Variances between the compartments were found to be equal and a one-way Analysis of Variance, followed by a series of Tukey's tests (Zar, 1999) were performed to assess whether any differences existed between the (log-transformed) normalised half-lives. The analysis indicated that freshwater half-lives were significantly faster ($P < 0.05$) than the marine and estuarine half-lives. The freshwater half-lives have a median value of 3.2 days, whereas the estuarine and marine data had median half-lives of 6.6 days and 8.1 days respectively (Figure 9). These median values are significantly faster than the default assigned in the TGD for readily degradable substances (Table 3).

***Figure 9: statistical analysis of chemicals considered to be readily biodegradable to determine inter-quartile ranges and 95$^{th}$ percentile confidence limits***




The TGD default values are considered as provisional and currently result in a substance, which is readily biodegradable being allocated a marine biodegradation half-life of 50 days if the 10-day window is passed and 150 days if the ready pass criteria are achieved outside the 10-day window. Further statistical analysis of the data in order to assign the upper 95% percentile confidence limits for the median half life values (i.e. the median value for each chemical) has been conducted for the chemicals considered to be readily biodegradable, irrespective of the 10-day window (Figure 9). The data presented in Figure 10 excludes the outlying half-life data i.e. the fastest and slowest 25% of half-life values published.

The data presented within this report would support a revision of current default values. This TF proposes that based on the limited data available that the default half-life values could be revised. The data reviewed within this report indicate that default half-lives of 5 days for the freshwater environment, 15 days for the estuarine environment and 11 days for the marine environment may be more appropriate (Table 7). In making these proposals the TF has applied the upper 95% confidence limit of the median half-life rather than using the overall median half life value (for all chemicals) or the lower 95th percentile confidence limit. By taking this approach the TF considers that the precautionary nature of exposure assessment has not been undermined. However, the TF does recognise:

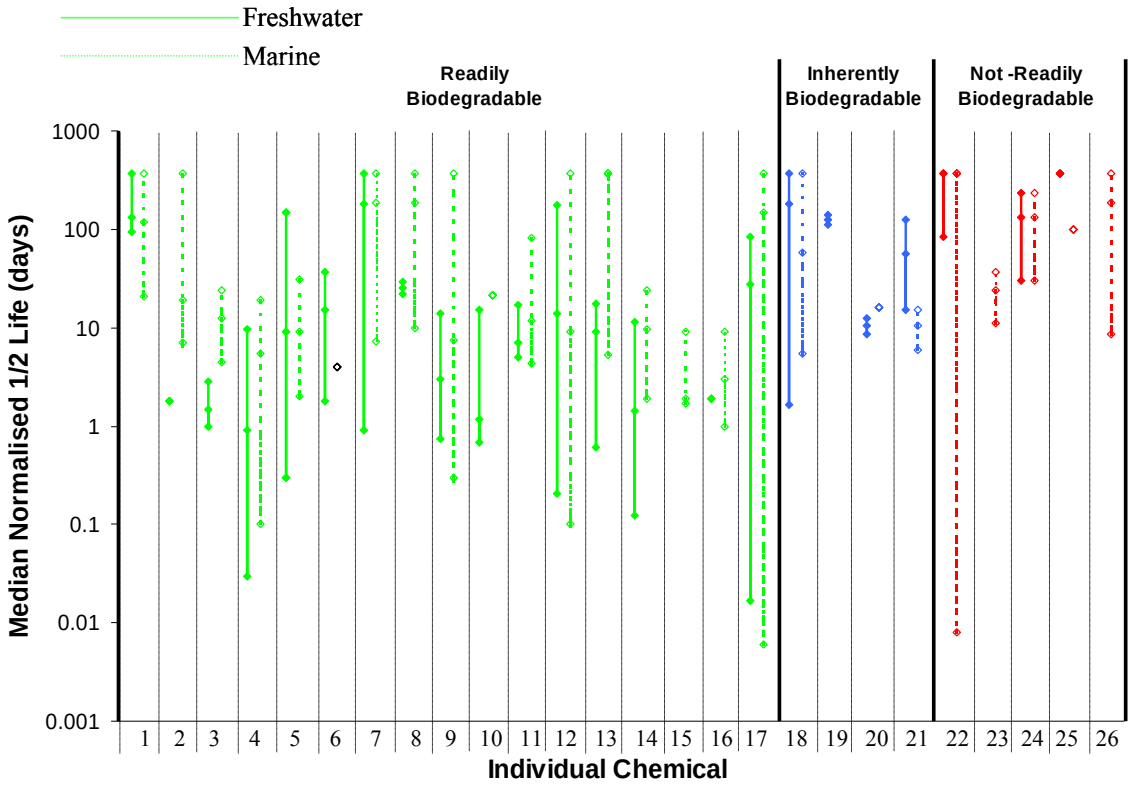
- The proposals are based on a limited dataset and more robust recommendations require further empirical work especially for the estuarine and marine environments.
- Whilst the review of marine and estuarine data is exhaustive, the review of freshwater data is incomplete as it has only been populated with data for chemicals that have existing marine and estuarine data.
- The proposed default half-life value for the estuarine environment is slower than that for the marine environment even though the median half-life value is faster. This reflects the smaller number of chemicals for which estuarine data exist and a broader range or distribution of half-life values for that compartment.

***Table 7: Proposed revision to the TGD and REACH default half-lives***

| Proposed | Freshwater | Estuarine | Other Marine Environments |
|---|---|---|---|
| Degradation in marine screening test | 5 | 15 | Measured rate |
| Readily biodegradable irrespective of 10-day window | 5 | 15 | 11 |
| Inherently biodegradable | 150 | 150 | 150 |
| Persistent | ∞ | ∞ | ∞ |

The data in Figure 10 show the ranges of half-lives for individual chemicals in the marine and freshwater habitat. Readily biodegradable substances can have half-lives above the P criteria and the default rate constants in the TGD (EC, 2003) and REACH (2008c). However, the median half-life is consistently lower than 10 days for both the freshwater and marine compartments. This indicates that the default half-life for the marine environment is overly conservative albeit based on a limited dataset. The data support the distribution approach illustrated in Figure 3 and advocated by ECETOC, 2003. Where data are at the extremes of the distribution (i.e. very short or infinite half-lives) expert judgement is required according to the criteria described in Chapter 3.

***Figure 10: Range of half-lives reported for readily, inherently and non-readily biodegradable chemicals in the freshwater and marine compartments***




Biodegradation was not compartment specific as the degradation of none of the substances was found to be restricted to a single compartment. If a substance is shown to degrade in the freshwater environment evidence also exists to show that it will degrade in marine environments. The evidence indicates that, the extrapolation from freshwater to marine environments is feasible for readily biodegradable substance, but there are insufficient data to conclude whether this can be done for inherently or not readily biodegradable substances. Insufficient data also existed to examine the scientific basis for the default value of 150 days applied to inherently biodegradable chemicals irrespective of the environmental compartment.

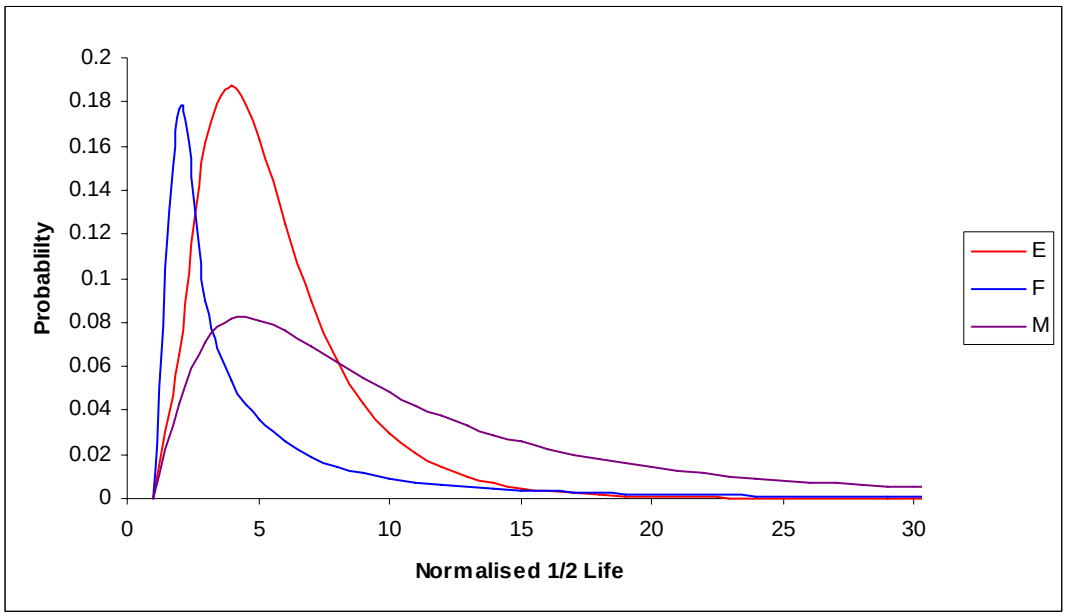
The range and distribution of half-lives for freshwater and saltwater for the most data rich substances (i.e. aniline, AEO, 4-chloroaniline, LAS, m-cresol, naphthalene, phenol and toluene) is further illustrated by the data in Table 8 and is additional supporting evidence for the ECETOC approach to assessing persistence using half-life distributions (ECETOC, 2003). The median half-life values for these relatively data rich substance also indicate that the new default half-lives being proposed by this TF remain conservative.

***Table 8: Reported half-lives for aniline, AEO, 4-chloroaniline, m-cresol, LAS, naphthalene, phenol and toluene in freshwater and saltwater compartments***

| Chemical | Freshwater half -lives | | | *Saltwater half-lives | | |
|---|---|---|---|---|---|---|
| | *No of data* | *Median* | *Range* | *No of data* | *Median* | *Range* |
| **Aniline** | 20 | 9.0 | 0.3 - 150 | 15 | 7 | 1.1 - 31 |
| **AEO** | 14 | 1.45 | 1.0 - 2.8 | 11 | 5.3 | 2 - 24.2 |
| **m-cresol** | 8 | 1.16 | 0.7 - 15 | 7 | 1.9 | 1 - 29 |
| **LAS** | 6 | 2.98 | 0.75 - 14 | 15 | 6.9 | 0.3 - 365+ |
| **Phenol** | 30 | 1.41 | 0.12 - 11.5 | 14 | 7 | 1.93 - 24 |
| **Toluene** | 10 | 28.0 | 0.02 - 83.3 | 19 | 79 | 0.01 - 365+ |
| **Naphthalene** | 15 | 13.9 | 0.2 - 177.7 | 81 | 12.6 | 0.1 - 365+ |
| **4-chloroaniline** | 10 | 133 | 93 - 365+ | 11 | 139 | 21 - 365+ |

*Please note that the saltwater half-lives reported are based on both the marine and estuarine data.

***Figure 11: Distribution of normalised half-lives for phenol in freshwater (F) estuarine (E) and marine (M) compartments***

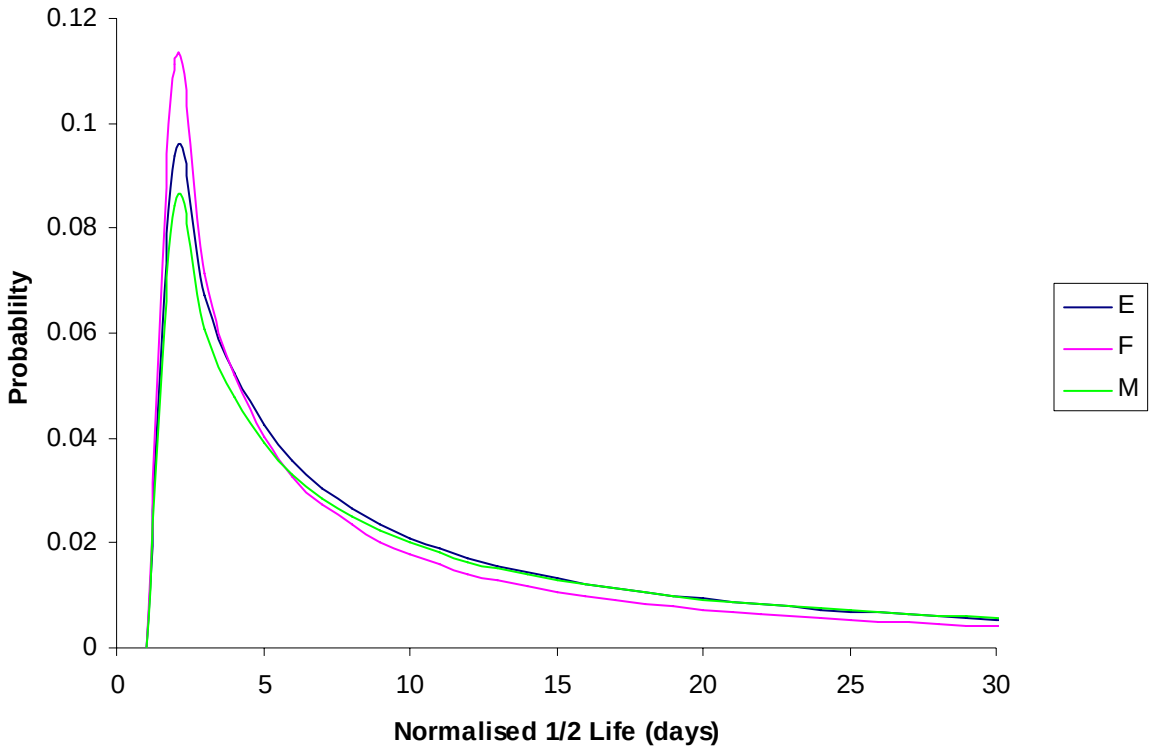



A graphical representation of the data for phenol contained in Table 8 is shown in Figure 11. This illustrates the relatively short half-lives (< 2 days) measured in freshwater, and the slightly longer half-lives (< 10 days) for the marine compartment, as concluded by the analysis of variance. There is a wider distribution of half-lives for the marine environment than the half-life data observed in the freshwater environment. Typically the biodegradability of a substance is

only measured once or twice and therefore it is not possible to know where this rate lies in the overall distribution of half-lives that will exist for the substance. The broader distribution observed in the marine environment leads to greater uncertainty where only a few data exist for a given substance. To increase confidence in where the half-life of a substance lies in the half-life distribution, improvements need to be made to the standardised tests to reduce variability and conflicting results. These are currently exhibited because the low biomass and the use of high substrate concentration can result in toxicity or inhibition. The next generation of tests need to more accurately mimic the environment they are designed to simulate. This is particularly true for the marine environment where low volume ready type laboratory tests have a high false negative failure rate (Snape, 2005).

Finally, the data in figure 12 captures the distribution of half-lives for all the chemicals designated as readily biodegradable. The data in this figure shows that the most frequently observed half-lives are in the range of 2-3 days for all three aquatic systems, with the freshwater system have the highest frequency of observing a shorter half-life. These data again illustrate the importance of the ECETOC (2003) distribution approach to assessing biodegradation data and the conservative nature of the revised half-lives being proposed by this TF.

***Figure 12: Distribution of normalised half-lives for all readily biodegradable chemicals in freshwater (F) estuarine (E) and marine (M) compartments***



## *4.3 Conclusions*

The number of chemicals for which there was a statistically robust dataset was limited and was largely derived from non-standard studies. However it can be concluded that:

1. A wide range of biodegradation half-lives exists for any given chemical in each environmental compartment.
2. The median half-life should be used to conclude on persistency and to determine PECs where there are several data for a chemical.
3. The current default values in the TGD (EC, 2003) and REACH (ECHA, 2008c) could be revised in light of the data presented in this report. Default values of 5 days for the freshwater environment, 15 days for the estuarine environment and 11 days for the marine environment may be more appropriate. However, these proposals are based on a limited dataset and more robust recommendations require further empirical work especially for the estuarine and marine environments.
4. Whilst the review of marine and estuarine data is exhaustive, the review of freshwater data is incomplete as it has only been populated with data for chemicals that have existing marine and estuarine data. Further freshwater data may be available to improve the basis for the TF half-life proposals without the need for additional testing.
5. The proposed default half-life value for the estuarine environment is slower than that for the marine environment even though the median half-life value is faster. This reflects the smaller number of chemicals for which estuarine data exist and a broader range or distribution of half-life values for that compartment.
6. In general, chemicals that have been shown to degrade in the freshwater environment have also been shown to degrade in the marine environment and *vice-versa*.
7. The extrapolation from freshwater to marine is feasible for readily biodegradable substances, but there are no sufficient data to conclude whether this can be done for inherently and not readily biodegradable substances. Insufficient data also existed to examine the scientific basis for the default value of 150 days applied to inherently biodegradable chemicals irrespective of the environmental compartment.

# 5. CONCLUSIONS AND RECOMMENDATIONS

Selection criteria have been used to identify good quality marine and corresponding freshwater biodegradation data. Provided the biodegradation data published in peer reviewed literature satisfy these selection criteria, the TF recommend that they should be considered suitable for use in environmental risk assessment. The key information that needs to be given is:

- The identity, purity and concentration of the test substance;
- the origin of the *inoculum* source including any pre-treatment;
- the analyte being measured (primary or ultimate degradation);
- the rate and extent of degradation.

These selection criteria were used to populate a marine biodegradation database. There are a limited number of published data describing rates of biodegradation in marine environments. The database contains > 800 data for 198 chemicals. Of these chemicals only 26 were considered data rich and were subsequently used to determine whether there was a scientific basis to extrapolate between environmental compartments and to compare actual published rates with the TGD (EC, 2003) and REACH (ECHA, 2008c) default values.

Long half-lives are often quoted having been extrapolated from short-term studies e.g. half-lives of > 100 days being calculated from studies with a duration of < 1 day. The TF suggest that this practice is unsound and that ratio between the reported half-life and the test duration should not exceed 2.

Data were only used from published papers with rates of degradation clearly reported. To increase the dataset, it would be a valuable exercise to extract degradation rates from biodegradation curves published in papers that were collated but not considered within this TF. The database has focused on only obtaining freshwater and estuarine data for chemicals where marine biodegradability data exist. Further work is required to expand the database to include all relevant freshwater and estuarine data.

There are a number of ways of expressing degradation rates (percentage degraded after a specific time, turnover rate, $T_{50}$, $DT_{50}$, half-life, etc.). To overcome this, the data have been normalised wherever possible and converted to a half-life. The data clearly illustrate that there is no single rate of degradation for any given substance. A distribution or range of degradation rates will exist for each environmental compartment. In many cases the biodegradability of a chemical is only assessed once; in such cases it is not known whether the rate of degradation observed is at the higher or lower end of the half-life distribution. The median half-life should be used to conclude on persistency and to determine PECs where there are several data for a chemical.

The current default values in the TGD (EC, 2003) and REACH (ECHA, 2008c) text could be revised in light of the data presented in this report. Default values of 5 days for the freshwater environment, 15 days for the estuarine environment and 11 days for the marine environment may be more appropriate subject to the caveats listed in Chapter 4.

In general, chemicals that have been shown to degrade in the freshwater environment have also been shown to degrade in the marine environment and vice-versa.

The extrapolation from freshwater to marine is feasible for readily biodegradable substance, but there is not sufficient data to conclude whether this can be done for inherently and not readily biodegradable substances.

### *Research needs*

Given the limited data available for analysis, there is clearly potential to increase the knowledge of biodegradation in marine environments. The priority should be to 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 the methods that were being developed were too aggressive. A number of research needs have been identified, these are described below. The findings of this report complement the output and the RfPs generated from the ECETOC Persistence Workshop (ECETOC, 2007; summary in Appendix A).

Research should be carried out to:

- Compare marine and freshwater biodegradability for inherently biodegradable chemicals to demonstrate the importance of adaptation. Insufficient data exist to conclude whether the default half-life of 150 days for chemicals considered to be inherently biodegradable is justified. Work is required to fill this important data to generate freshwater, estuarine and marine biodegradation data for chemicals considered to be inherently biodegradable by the regulatory authorities. Biodegradation data for each habitat should be generated in systems that do and do not allow for adaptation to occur. This could ensure that the number of false negatives observed are minimised, especially for marine assessments (as observed in CEFIC LRi ECO 2a; http://www.cefic/lri.be). Two approaches favouring adaptation have been identified within the REACH guidance (ECHA, 2008b) for enhanced biodegradability and persistency assessments. Research in this area will need to:
  - Identify, with appropriate regulators and industry, inherently biodegradable chemicals to form the basis for experimental phase of work.

- Develop and refine test systems that can evaluation biodegradability in environments continually exposed to chemicals.
- Generate freshwater, estuarine and marine biodegradation data for each of these chemicals in test systems that allow for and do not allow for adaptation to occur.
- Compare the freshwater, estuarine and marine data to assess whether there is a scientific basis for extrapolation between the environmental compartments.
- Compare the measured rates to the REACH (ECHA, 2008c) default values.
- Evaluate the importance / role of having tests that demonstrate natural adaptation as part of a tiered approach to biodegradation / persistence assessments.
- Make an overall assessment, publish results and develop appropriate guidelines and guidance.

Other research that needs careful consideration includes the:

- Development of methods to increase both diversity and density of microbial biomass of *inoculum* for use in screening studies. Understand the impact of the biomass and its density on the data generated. Address density versus volume; include comparison of beads versus other pre-concentration methods.
- Development of a battery of tests to determine natural variability within and between environmental compartments (i.e. determine the shape of biodegradation distributions). 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?
- Increased understanding of degradative processes. In general, standardised screening tests do not encourage all degradative processes e.g. co-metabolic transformations are unlikely to occur in ready biodegradability tests, and consequently a substance may appear to be persistent under standardised test conditions using only one carbon source, whilst in nature the substance may degrade. Thus studies to develop enhanced biodegradability testing to bridge the gap between chemicals passing ready biodegradability tests and substances not supporting growth of microorganisms should be encouraged.

## GLOSSARY

| Term | Definition |
|---|---|
| Abiotic degradation | Degradation mediated through processes other than biodegradation such as hydrolysis, photolysis and interactions with other chemicals. Abiotic degradation studies typically provide a measure of primary degradation. |
| Biodegradation | The biologically mediated degradation or transformation of chemicals usually carried out by microorganisms. |
| Degradation product(s) | The chemicals produced as a result of degradation processes. For aerobic ultimate degradation, or mineralisation, these are carbon dioxide, water and mineral salts. |
| Degradation rate constant | A first order or pseudo first order kinetic rate constant, k ($d^{-1}$), which indicates the rate of the degradation processes. |
| Disappearance Time 50 ($DT_{50}$) | The time within which the initial concentration of the test substance is reduced by 50%. It should be stated whether the DT50 refers to primary degradation or mineralisation (ultimate biodegradation). |
| Disappearance Time 90 ($DT_{90}$) | The time within which the initial concentration of the test substance is reduced by 90%. |
| Fate | Distribution of a chemical in various environmental compartments (e.g. soil or sediment, water, air, biota) as a result of transport, partitioning, transformation, and degradation. |
| Field Data | Representative, standardised measurement, evaluation, and reporting of specified properties of a chemical in the environment, in order to define the current state of the environment, and to establish environmental trends in this respect. |

| Term | Definition |
|---|---|
| Half-life, $t_{1/2}$ | Term used to characterise the rate of a first order reaction. It is the time interval that corresponds to a concentration decrease by a factor 2. The half-life and the degradation rate constant are related by the equation $t_{1/2} = -\ln 2/k$. Half-lives are usually expressed in hours or days and can be assigned to either primary degradation or ultimate biodegradation (mineralisation). |
| Inherent biodegradability tests | Tests inoculated with a high concentration of microorganisms carried out under aerobic conditions in which biodegradation rate and/or extent are measured. The test procedures offer a higher chance of detecting biodegradation compared to tests for ready biodegradability. |
| Mineralisation | The breakdown of a chemical substance or organic matter by microorganisms in the presence of oxygen to carbon dioxide, water, and mineral salts of other elements present. |
| Monitoring | Long-term, standardised measurement, evaluation and reporting of the concentration of specific chemicals to establish the current state of the environment and to establish trends. Surveys and surveillance are both used to achieve this objective. |
| Persistence | A chemical that resists degradation processes and is present in the environment for a long time. Specific criteria have been established in Persistent Organic Pollutant (POP) protocols, in the TGD and in REACH. In the latter persistent (P) and very persistent (vP) refers to chemicals that have degradation half-lives above certain trigger values in surface water, sediment and soil. |
| Predicted environmental concentration (PEC) | The concentration of a chemical in the environment, predicted on the basis of available information on certain of its properties. Its use and discharge patterns and quantities involved. |
| Primary biodegradation | The structural change (transformation) of a chemical substance by microorganisms resulting in the loss of the original chemical identity. |

| Term | Definition |
|---|---|
| Ready biodegradability tests | Stringent screening tests, conducted under aerobic conditions, in which a high concentration of the test substance (in the range of 2 to 100 mg/L) is used and ultimate biodegradation is measured by non-specific parameters like Dissolved Organic Carbon (DOC), Biochemical Oxygen Demand (BOD) and CO2 production. Small amounts of domestic sewage, activated sludge or secondary effluent form the microbial *inoculum* in tests for ready biodegradability. The *inoculum* should not have been pre-adapted to the test substance through previous exposure to either the test substance or structurally related chemicals. A positive result in a test for ready biodegradability can be considered as indicative of rapid and ultimate degradation in most environments including biological STPs. |
| Simulation tests | Aerobic and anaerobic tests that provide data for biodegradation under specified environmentally relevant conditions. These tests attempt to simulate degradation in a specific environment by use of indigenous biomass, media, relevant solids (i.e. soil, sediment, activated sludge or other surfaces) to allow sorption of the chemical, and a typical temperature that represents the particular environment. A low concentration of test substance is used in tests designed to determine the biodegradation rate constant whereas higher concentrations are normally used for identification and quantification of major transformation products for analytical reasons. |
| Ultimate aerobic biodegradation | The breakdown of a chemical by microorganisms in the presence of oxygen resulting in the formation of carbon dioxide and final reduction products like methane, $H_2S$, $N_2$ or $NH_3$, mineral salts and new biomass. |
| Ultimate anaerobic biodegradation | The breakdown of a chemical in absence of oxygen resulting in the formation of carbon dioxide and final reduction products like methane, $H_2S$, $N_2$ or $NH_3$, mineral salts and new biomass. |
| Weight of evidence (WOE) | There is a strong connection between the issue of uncertainty and 'weight of evidence' approach. WOE is a decision making process, often by an expert, to integrate all aspects of the uncertainty (about data quality, model uncertainty, etc.). The weight attached to data point or fact is simply the subjective probability of the data/fact being 'true'. |

## ABBREVIATIONS

| | |
|---|---|
| AE | Alcohol ethoxylates |
| AEO | Linear alcohol ethoxylates |
| BiAS | Bismuth active substance |
| BOD | Biochemical oxygen demand |
| BTEX | Benzene, toluene, ethyl benzene and xylene |
| CMR | Carcinogenic, mutagenic and reprotoxic |
| CSA | Chemical safety assessment |
| DO | Dissolved oxygen |
| DOC | Dissolved organic carbon |
| DT | Disappearance time |
| EDDS | Ethylene diamine disuccinate |
| EMBK | ECETOC marine biodegradation kinetics |
| GHS | Globally harmonised system |
| GLP | Good laboratory practice |
| IBT | Inherently biodegradation test |
| ISO | International Standards Organisation |
| LAS | Linear alkylbenzene sulphonates |
| LRI | Long-range Research Initiative |
| MBAS | Methylene blue active substance |
| NB | Not biodegradable |
| NPE | Nonylphenol ethoxylates |
| NTA | Nitriloacetic acid |
| OECD | Organisation for Economic Co-operation and Development |
| P | Persistence |
| PBT | Persistent, bioaccumulative, toxic |
| PEC | Predicted environmental concentration |
| PEG | Polyethylene glycol |
| PNEC | Predicted no effect concentration |
| POP | Persistent organic pollutant |
| RBT | Ready biodegradation test |
| REACH | Registration, evaluation, authorisation and restriction of chemicals |

| | |
|---|---|
| SETAC | Society of Environmental Toxicology and Chemistry |
| SPE(s) | Soluble sucrose polyester(s) |
| SSD | Species sensitivity distribution |
| STEP | Simulation testing for environmental persistence |
| STP | Sewage treatment plant |
| | |
| TGD | Technical Guidance Document |
| $ThCO_2$ | Theoretical carbon dioxide |
| ThOD | Theoretical oxygen demand |
| TL | Lag times |
| TOC | Total organic carbon |
| | |
| US EPA | United States Environmental Protection Agency |
| vP | very persistent |
| vPvB | very persistent, very bioaccumulative |
| WOE | Weight of evidence |

# APPENDIX A: KEY ISSUES IDENTIFIED TO ASSESS RATES OF BIODEGRADATION IN THE ENVIRONMENT

Assessing rates of biodegradation to use in environmental exposure assessments and environmental persistency assessments has become increasingly important in recent years. As a result of this increased importance and the inherent technical limitations associated with the existing approaches to assessing degradability and persistence several task forces, expert groups and workshops have been organised to identify possible improvements and provide technical guidance. Some of the key issues identified by the work addressed by these activities are summarised in this appendix.

## *SETAC Biodegradation Kinetics Workshop (1996)*

The 1996 workshop (SETAC, 1996), held in Port Sunlight, UK, was an outgrowth of a previous SETAC Workshop "Applications of Multimedia Models for Regulatory Decision Making" and the EU expert meeting on "Biodegradability". The workshop recognised that several regulatory agencies were introducing persistency categories outside the context of the classical risk assessment paradigm i.e. there was a move to regulatory action based on hazard and not risk.

The main objectives for the workshop were to: i) review the possibility of using kinetic rate data form existing standard biodegradability tests and apply these rates to environmental compartments; ii) identify modifications to standard biodegradability tests that would enable the generation of kinetic information; and iii) identify research to improve the understanding of biodegradation.

The workshop recommendations included the following:

- Conducting a review of all available biodegradation test data, in the literature and in the archives of business and government. The review should aim to elucidate any relationships between the biodegradability and structure, and between biodegradability in screening tests and the environment.
- Allowing periods of pre-adaptation prior to biodegradability assessments for new chemicals with continuous discharge patterns.
- Improved understanding of the factors producing variation in screening and laboratory simulations e.g. species profiles to characterise the microbial communities.

### *SETAC Workshop on Persistence and Long-Range Transport (1998)*

To foster the development of a sound scientific foundation for the evaluation of POPs and PBTs, SETAC convened a workshop to discuss the evaluation of persistence and long-range transport. The working group addressing persistence in soil, water, sediment concluded that:

- The persistence of a substance in soil, water and sediment is determined by the rates of removal by physical, biological and chemical processes. Microbial degradation is the principal biotic degradation process. Important abiotic degradation processes are hydrolysis, direct/indirect photolysis, and oxidation / reduction reactions.
- Standardised test methods exist for most but not all degradation processes. Some tests yield rate constants or half-lives but in all cases, these values are most relevant to the experimental conditions.
- A wide variety of factors can influence degradation rates in the environment. A particularly important factor that influences biodegradation rate is the number of competent microorganisms.

### *CEFIC LRI Scientific Workshop "Degradation of Chemicals in the Environment and Prediction of Persistence" (2001)*

A workshop was organised by ECETOC on behalf of the Cefic Long-Range Research Initiative (LRI) Persistence Project investigating the persistence of chemicals in the marine and terrestrial environment. The workshop brought together the leading international experts in environmental fate and exposure assessment from government, academia and industry to debate the scientific issues associated with the persistence of chemicals in the environment. The objective of the workshop was to promote the scientific understanding of the issues that determine a chemical substances persistence in the environment and promote areas of research that are required to improve the scientific basis of future legislation. The focus of the workshop was: (i) to describe the key factors governing degradation of chemicals in the terrestrial and marine environments; (ii) to assess criteria for selecting model test chemicals and methodology for testing chemicals for assessing degradation; (iii) to identify when and why predicted (from the laboratory) and measured (in the environment) rates of degradation converge/diverge; and (iv) to propose a testing strategy for predicting persistence and expected rates of degradation of compounds that are not persistent.

Key conclusions from the scientific workshop included that (Evans and Nyholm, 2001):

- A persistent substance is one that is resistant to chemical and biological degradation and transformation processes (under both aerobic and anaerobic conditions) for a sufficient time.
- Persistence alone should not be an issue. Persistence must be associated with toxicity, bioaccumulation, human health, and the conservation of precious assets, etc.
- The half-life concept is useful in practice but is not necessarily the best description of biodegradation. First order kinetics may provide an approximate description of degradation, but is not always adequate to describe biodegradation in the environment.
- Estimates of biodegradation from currently available freshwater data on biodegradability of chemicals that pass the OECD 'ready' biodegradability test may not be unreasonable. However, the differences between the three environmental compartments may be too great to expect that kinetic data, or rates and extent of biodegradation, can be extrapolated from one compartment to another. Modification of the freshwater tests may provide kinetic data for the freshwater environment, but extrapolation from the current and future modifications of these tests to the other compartments is unlikely to be scientifically sound.
- QSBRs should only be used as a guide to expected persistence and that they do not provide a valid alternative to measured data, particularly with regard to biodegradation.
- The minimum data needed for reliable models to predict fate of chemicals in the environment are: The octanol/water coefficient, $K_{ow}$; the Henry's law constant, H; degradation rates or half-lives for each environmental compartment; vapour pressure and

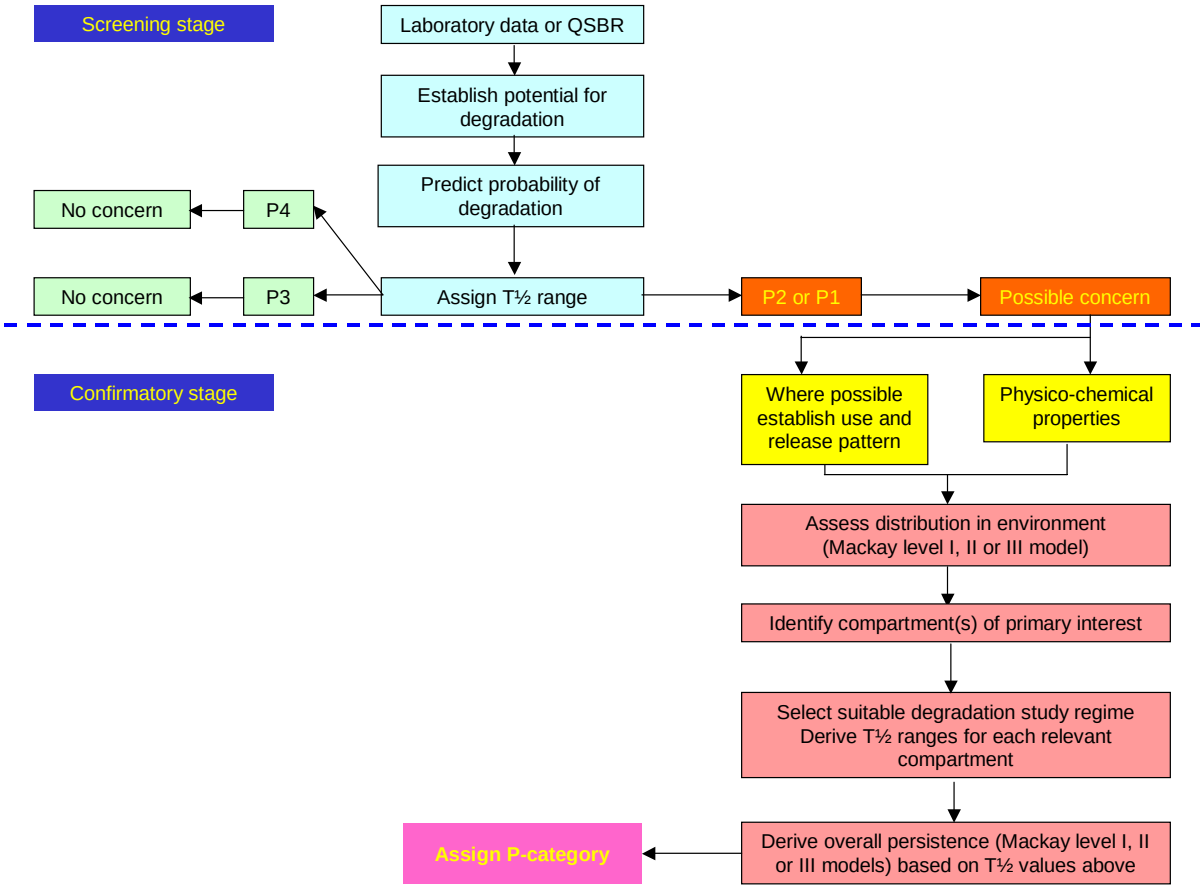
solubility; the volumes of air and/or water and/or soil; and the quantity of organic matter in the receiving compartment.

- In order to predict persistence of a chemical in any environmental compartment with a reasonable degree of confidence, a better understanding of biodegradation and test methods that provide realistic measurements of rates of biodegradation in the environment are necessary.
- A long-term testing strategy must strive to produce tests that simulate conditions in each of the environmental compartments and provide realistic data on the rate and extent of biodegradation. However, in the short-term the strategy must be to provide improved data that can be used within the current regulations.

### *ECETOC Persistence Task Force (2003)*

In 2003 ECETOC reviewed the persistence of chemicals in the environment and concluded by advocating a two-phase strategy to identify substances that persist in the environment (see Figure A1). The strategy comprised a screening phase and a confirmatory phase that was equally applicable to both new and existing chemical substances. Where standard and non-standard degradation test data existed, the strategy aimed to promote the maximum use of these data. In the absence of data, the strategy aimed to focus testing on the most relevant environmental compartment(s).

***Figure A1: ECETOC strategy for assigning environmental persistence***




ECETOC proposed four persistence categories, ranging from 'easily degradable' (P4) to 'persistent in the environment' (P1). A specific range of half-life values defined each category. These categories were derived from a combination of biodegradation test results, the potential for biodegradation to occur, and the abiotic half-life. Substances categorised as P4 and P3 in the

screening phase were considered to be of no further concern; i.e. not persistent. Whereas, substances categorised as P2 and P1 would require more detailed scrutiny in the confirmatory phase.

For the confirmatory stage, the strategy advocates the use of multimedia fate and transport modelling to prioritise the environmental compartments that are expected to contain most of the substance of interest, for testing purposes. Compartments considered relevant are those for which a 'realistic presence' of the substance (> 5% by mass) is forecast by modelling. Once the environmental compartment(s) of interest have been identified, an appropriate test protocol should be employed to generate the most useful information on fate of the substance in that compartment. Substances that were classified as P1 and P2 after the confirmatory phase should then be prioritised relative to bioaccumulation potential and toxicity, following the PBT paradigm.

In addition to the testing strategy proposed, ECETOC made a series of other conclusions and recommendations. These included that:

- Whilst the ready biodegradation screening tests can be used to identify chemicals that will undergo rapid biodegradation in the environment, they are not suitable for identifying chemicals that are likely to persist in the environment. Consequently, they are not an effective screen for assessing persistence or prioritising chemicals based on their degradability.
- The rate of degradation in the environment is not restricted to first order kinetics and cannot be described using a single first order rate constant. TA range of degradation rates will exist for any chemical that will reflect the conditions in the receiving environment. The fact that the biodegradability of a chemical is typically only assessed once makes it impossible to know at what point the rate appears on the distribution of rates for that chemical i.e. is it at the faster or slower end of the distribution?
- New laboratory tests are required to assess biodegradation at the screening stage. Such tests need to ensure environmental relevance with respect to the microbial diversity in the *inoculum* source and that the potential for adaptation through routine exposure is not excluded.
- The criteria used to assess biodegradation and persistence (e.g. carbon dioxide evolution, oxygen demand, removal of dissolved organic carbon and parent compound analysis) are currently treated uniformly. However, these endpoints are not equivalent and cannot be compared with each other.
- The default rates for biodegradation assigned to the outcome of screening studies e.g. ready biodegradation tests appear to be very cautious based on values that have been published. Further evaluation of the scientific basis of these default rates is required.

### *Simulation testing environment persistence workshop (2004)*

The STEP workshop (STEP, 2004) was organised with two objectives: i) to develop and/or refine strategies that will facilitate the use of biodegradation simulation tests in both persistence identification and risk assessment; and ii) to propose where necessary, new guidelines or additions to existing guidelines in order to optimise the data emerging from simulation tests for use in persistence identification and risk assessment. The workshop conclusions included:

- The selection of the appropriate (sub-)compartment should be based on the intrinsic properties of the substance determining its environmental fate (water solubility, log $K_{ow}$, $K_p$, Henry´s laws constant, etc.). Multimedia risk assessment models (such as the MacKay level III model contained in EUSES) may be used to assess whether atmospheric transport of the substance in the gaseous phase plays a role in the environmental distribution of the substance. Should atmospheric transport be likely, then, it is necessary to estimate the fraction reaching open-ocean (pristine) environments via airborne long-range transport.

- When significant atmospheric transport can be ruled out as a distribution process, then the relevant compartment to be investigated is that exposed via the water phase, i.e. receiving waters such as rivers, lakes, estuaries, the coastal zone, and/or their respective sediments. The surface water environmental compartment receiving the bulk of the input volume of a chemical should be focussed upon. This requires an adequate knowledge of production, supply use, and discharge of the chemical, i.e. the dominant emission pattern to surface waters. Four aquatic sub-compartments were recognised:
  - River-water;
  - estuarine water;
  - near-shore, coastal zone[2];
  - open ocean.

- Environmental samples to be brought to the laboratory for the purpose of simulation testing should be selected in such a way that they are suited to the purpose of testing and will depend inter alia on the emission scenario and the properties of the chemical. It is acceptable to select average ambient conditions, bearing in mind that these can vary depending for example on season, weather, hydrographic conditions (such as coastal water depth), etc. The test guidelines discussed below contain guidance on collecting samples.

- The test substance concentration selected for the simulation test should be chosen from representative and environmentally realistic concentrations relevant to the compartment, bearing in mind the water solubility of the substance and the analytical limits of quantification.

[2] Offshore conditions, more representative of the continental shelf are covered under the OSPAR regulations.

A number of potential improvements to the biodegradation screening tests were identified at the Simulation Testing for Environmental Persistence workshop held in 2004 (e.g. extended test duration for poorly soluble substances and lower test substance concentration) (STEP, 2004). This workshop also concluded that a number of enhancements for increasing the reliability of the 'ready' test had been identified but that further guidance needed to be developed. The draft OECD 314 guideline (OECD, 2008) was developed as a result of this workshop.

### *REACH Implementation Project (RIP) guidance*

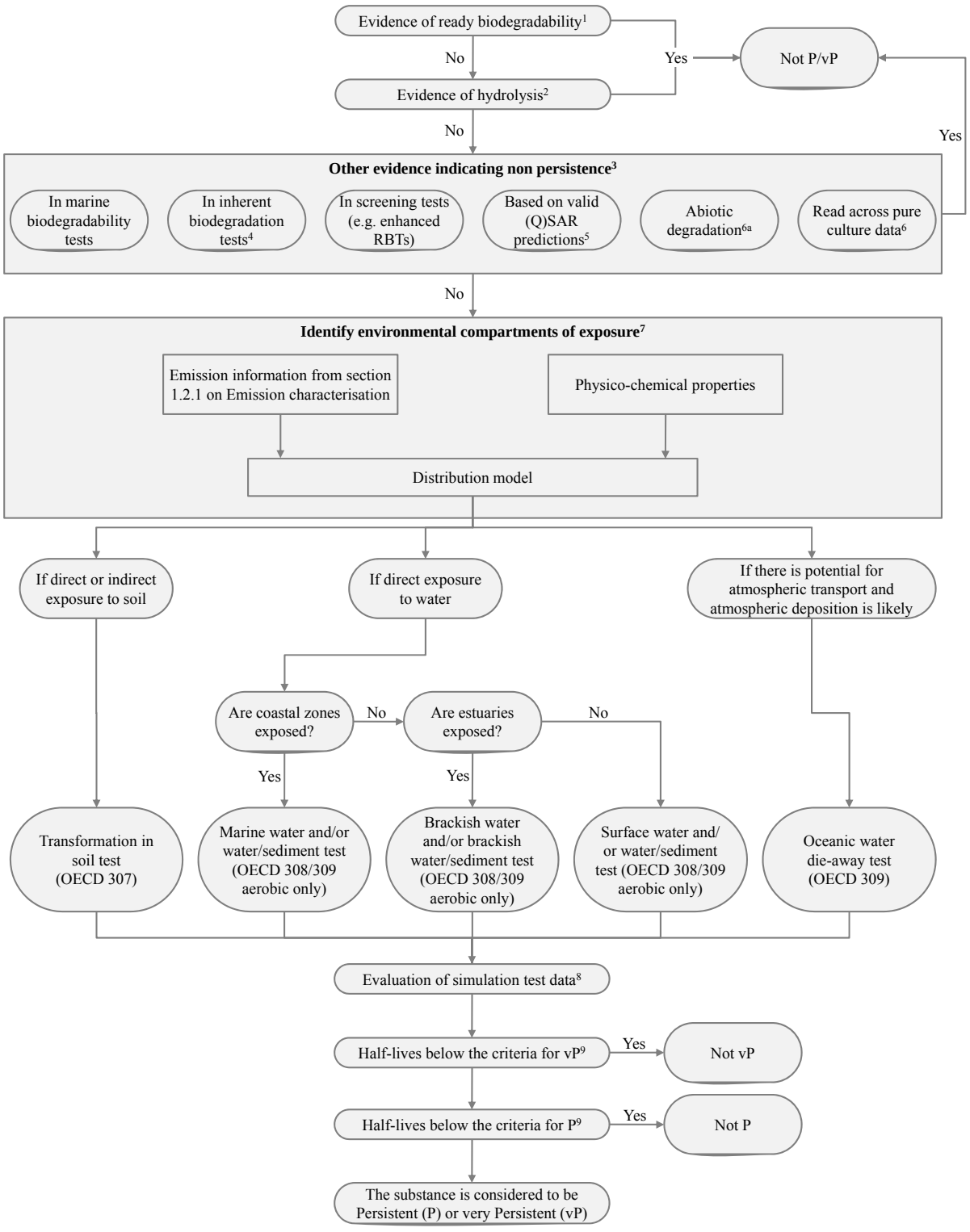
In order to implement REACH, Technical Guidance Document (TGDs) have been prepared in the RIPs with the purpose of helping industry and regulatory authorities fulfil their legislative responsibilities. As discussed above chemicals with PBT properties require authorisation under REACH. The strategy for the assessment of P (persistence), which is described in the TGDs, aims to guide the user through evaluation of existing information. This may lead to classification of a substance as either persistent or not persistent, or indicate the need for further testing (Figure A2).

The discussions that led to the development of the TGDs identified a need for new types of screening tests that can be used to assess whether or not a substance fulfils the P criteria. These new methods build on the principles of the OECD 301 series of tests for ready biodegradability and may be used for a simple low-cost testing of persistence that may lead to conclusions of 'persistent' or 'not persistent' before confirmatory testing by use of simulation biodegradation tests is triggered. Two of the new types of screening tests deserve mentioning:

#### *Modified Ready Biodegradability Tests*

Two modifications to the standard OECD 301 tests for ready biodegradability have been identified. These consider biodegradability testing at low-test substance concentrations and assessing the biodegradation of poorly water-soluble substances. Provided that all other conditions in the ready biodegradability tests are fulfilled, these tests are regarded as ready biodegradability tests and the results can be used directly in classification, environmental risk assessment and persistency assessments. Modified ready biodegradability tests using a lower test substance concentration are relevant, when the test substance is known or expected to exert toxicity to the microbial *inoculum*. Strategies to apply poorly water-soluble substances and determine their biodegradability are described in the TGD (RIP 3.3-2 final draft).

***Figure A2. RIP strategy for the evaluation of persistence (ECHA, 2008a)***



**Explanatory Notes to the Flowchart (quoted from ECHA, 2008a, Chapter R. 11)**

[1] ***Evidence of ready biodegradation –*** If the substance is readily biodegradable, or if the criteria for ready biodegradability are fulfilled with exception of the 10-day window, there is no reason to perform further biodegradation tests for the PBT/vPvB assessment. The conclusion is that the substance is not fulfilling the criteria for Persistence (P) (see Sections R.7.9.4 and R.7.9.5).

[2] ***Evidence of hydrolysis –*** If significant and substantial abiotic degradation has been confirmed and the hydrolysis transformation products have been assessed and concluded not to be PBT/vPvBs, no further testing of degradation is required for the PBT/vPvB assessment. The half-lives obtained in an hydrolysis test have to be compared to persistence criteria of Annex XIII (i.e. a substance fulfils the P(vP) criterion if $T_{1/2}$ > 40 (60) days). Careful consideration will need to be given to the formation of stable degradation products with PBT/vPvB properties. An attempt should be made to identify at least degradation products of >10% of the concentration of the parent substance (see Sections R.7.9.4 and R.7.9.5).

[3] ***Other evidence indicating non persistence –*** if the substance is confirmed to degrade in other biodegradation screening tests than the tests for ready biodegradability, the results may be used to indicate that the substance will not persist in the environment. For example, a result of more than 60% ultimate biodegradability (ThOD, $CO_2$ evolution) or 70% ultimate biodegradability (DOC removal) obtained during 28 days in an enhanced ready biodegradability test may be used to indicate that the criteria for P are not fulfilled (see Sections R.7.9.4 and R.7.9.5). This is also applicable to standardised marine biodegradability tests (OECD TG 306, Marine $CO_2$ Evolution test, Marine BODIS test, and the Marine $CO_2$ Headspace test).

Before concluding under consideration of Explanatory Notes 3 - 6a that a substance is "not P" or "not vP", it should be carefully examined if there exists conflicting evidence from monitoring data (see Note 9 for more information).

[4] ***Assessment of inherent biodegradation test data –*** Results of specified tests of inherent biodegradability, i.e. only Zahn-Wellens test (OECD TG 302B) or MITI II test (OECD TG 302C) may be used to confirm that the substance is *not* fulfilling the criteria for P provided that certain additional conditions are fulfilled. In the Zahn-Wellens test, a level of 70% mineralization (DOC removal) must be reached within 7 days, the log phase should be no longer than 3 days, and the percentage removal in the test before degradation occurs should be below 15% (pre-adaptation of the *inoculum* is not allowed). In the MITI II test, a level of 70% mineralisation ($O_2$ uptake) must be reached within 14 days, and the log phase should be no longer than 3 days (pre-adaptation of the *inoculum* is not allowed). If test results are available showing that a substance is not inherently biodegradable under the mentioned conditions this is a clear indication that the substance will not biodegrade in the marine environment and, hence, shall be regarded as persistent.

[5] ***Use of (Q)SAR (both QSARs and SARs) estimates*** – Such estimates may be used for preliminary identification of substances with a potential for persistency (see Section R.11.1.3.1). The combined results of the three freely available estimation models BIOWIN 2,6 and 3 in the EPI suite (US EPA 2000) may be used as follows:

- Non-linear model prediction (BIOWIN 2): Does not biodegrade fast (probability <0.5) and ultimate biodegradation timeframe prediction (BIOWIN 3): ≥ months (value <2.2), **or**
- MITI non-linear model prediction (BIOWIN 6): Does not biodegrade fast (probability <0.5) and ultimate biodegradation timeframe prediction (BIOWIN 3): ≥ months (value <2.2).

When the QSAR predictions using these models are reliable and the estimation results clearly indicate that the substance is not persistent, further information will normally not be required for the PBT and vPvB assessment, and it may be considered as not fulfilling the criteria for P. This implies that borderline cases should be carefully examined, e.g. when the estimate of the ultimate degradation time gives a result in the range 2.2 to 2.7 (see Sections R.7.9.4 and R.7.9.5). Note however that in any case all other existing and reliable QSAR predictions, read across and test data information should be considered for deriving a conclusion regarding the persistency status of the substance (cf. the other boxes regarding the various types of other potentially available information).

[6] ***Use of pure culture data*** – The data derived from studies with pure culture cannot on its own be used within persistency assessment, however these types of data should be considered as part of the weight of evidence approach.

[6a] ***Use of other abiotic data –*** Data derived from this studies (e.g. photodegradation, oxidation, reduction) cannot on their own be used within persistency assessment, but in a weight of evidence approach.

[7] ***Identification of the environmental compartment of exposure*** for simulation testing (see Section R.11.1.3.1).

[8] ***Evaluation of simulation test data –*** In order to evaluate the outcome of the simulation test, the following information is required:

a. Test conditions
b. First order, pseudo-first order rate constant, degradation half-life or DT50
c. Length of the lag phase
d. Fraction of mineralised label, and, if specific analyses are used, the final level of primary degradation
e. Mass balance during and at the end of the study
f. Identification and concentration of major transformation products, where appropriate
g. An indication of the level of bound residues
h. A proposed pathway of transformation, where appropriate
i. Rate of elimination (e.g. for risk assessment purposes)

[9] ***Evaluation versus the P and vP criteria*** (Section R.11.1.2)

Before concluding finally that a substance is "not P" or "not vP" it should be carefully examined if there exists conflicting evidence from monitoring data either from national monitoring programmes of Member States or internationally acknowledged organisations such as e.g. OSPAR or the Danube Convention. This could include, for example, findings of significant concentrations of the substance under consideration in remote and pristine environments such as the arctic sea or Alpine lakes. Also, significant concentrations of the substance in higher levels of the food chain in unpolluted areas may indicate high persistence (beside a potential to bioaccumulate). If such evidence indicates that the substance may be persistent, further investigations are required.

*Enhanced Biodegradability Screening Tests*

Several potential enhancements to the ready biodegradability tests have been identified with the aim to assist in assessments of persistence (RIP 3.3-2 final draft). Results obtained by use of these methods are not applicable for classification and labelling. Test approaches in enhanced biodegradability screening tests could include:

- **Increased test duration:** The test duration for poorly soluble substances and substances with extended lag phases is important. Where biodegradation is still occurring in a ready biodegradability test weekly determinations could be continued up to day 60.
- **Testing in larger vessels:** Conducting biodegradation tests using larger volumes of environmental sample increases the total number of microorganisms introduced into the test, and the number of different types, without changing the density of microorganisms introduced. This will increase the probability of introducing a competent degrading microorganism into the test vessel.
- **Increasing the biomass concentration:** This approach recognises that when conducting biodegradability tests with less than one litre of an environmental water sample it will not reflect the total number and types of microorganisms that a substance will encounter in the environment. A suitable procedure could be to concentrate the microorganisms of a larger water volume (e.g. by use of filtering or centrifugation) and re-suspend the microbial *inoculum* in the liquid test volume.
- **Low-level pre-adaptation test systems:** Adaptation of environmental microorganisms that can degrade particular substances is a natural phenomenon. Low-level pre-adaptation tests could include conducting applying a sample from a completed ready biodegradability test to inoculate a subsequent ready biodegradability test. This may reduce the lag period preceding the onset of biodegradation.
- **Semi-continuous biodegradability tests:** Semi-continuous test systems help maintain the diversity, viability and nutrient status of the biodegradability tests whilst allowing the potential for adaptation to be determined over time (such as in the semi-static version of OECD 309).

### *ECETOC Persistence Workshop (2007)*

ECETOC and the Environment Agency for England and Wales hosted a workshop (ECETOC, 2007) to identify the key areas of research that were needed improve persistency assessments conducted under REACH. Eight possible research projects, having the support of industrial, regulatory and academic research scientists, were identified. These projects have been submitted to the CEFIC LRI planning group for consideration. The ECETOC workshop conclusions included:

- That the current ready tests were not designed to and were not very good at differentiating persistent chemicals because they vary widely in their test conditions and contain many historical differences which lead to very variable data. Nevertheless, more information could be extracted from ready tests e.g. shape of the degradation curve and it may also be opportune to consider systematic standardisation of the ready tests (e.g. substrate concentration, *inoculum* size, etc.).

- There was a broad consensus that an enhanced tier of biodegradation screening studies is required to aid in the prioritisation of PBT and vP/vB assessments. Enhancements discussed included extending the test duration, increasing the test volume, enhancing the biomass levels and allowing for acclimation. Whilst extension of the test duration and conducting studies using higher test volumes posed little concern to regulatory members of the workshop, it was felt that some validation and standardisation were needed with respect to working with higher biomass levels and acclimated *inocula*. It was envisaged that such enhanced tests could contribute to a weight of evidence approach to decide if a chemical is persistent.

- The group also decided that, to prevent confusion over the terms acclimation and adaptation, the terminology "deliberate pre-exposure of the *inoculum* to the test chemical" should be used to describe adapted or acclimated *inocula*. Some participants recommended the use of highly adapted systems as a positive screen for persistence i.e. chemicals that could not degrade in such systems can be assumed to persist. The workshop concluded that adaptation should be taken into account in any assessment of the persistence of a chemical (pre-exposure should be environmentally realistic concentrations).

- There was a general consensus that there are no soil, sediment or water biodegradation studies that accurately simulate biodegradation in the 'natural' environment. There are higher tiered biodegradation studies (e.g. OECD 307, 308 and 309) that use environmental media that can describe degradation under conditions that have a greater environmental relevance than the ready biodegradation test. However, many of these studies do pose considerable problems associated with their interpretation.

Problems identified included:

- Selection of the most appropriate compartment for testing.
- How to deal with bound residues.
- How to discriminate between degradation half-lives for persistency assessments and rates of dissipation from water or sediment.
- How to correct for temperature differences and is it really necessary.

# APPENDIX B: BIODEGRADATION KINETICS; GROWTH AND ACTIVITIES

In order to be able to predict more precise concentrations of chemicals in the environment, it is important to know the kinetic constants of degradation of chemicals in the various environmental compartments. Numerous mathematical models for biodegradation kinetics have therefore been developed. These biodegradation kinetics have been described in detail elsewhere (Alexander, 1999; Battersby, 1990; ECETOC, 1991). In this report kinetic data for biodegradation in marine ecosystems have been compiled. It is evident that the mathematical expression of biodegradation kinetics is, as expected, not uniform. For the assessment of persistence, primarily first order kinetics, often recorded as half-lives, have attracted almost all the attention. However, many environmental scientists do prefer to express biodegradation rates in units such as grams substance per litre seawater per day or mmol substance per kilogram sediment per day. Kinetic data expressed in these units will be referred to as activities. To understand the various biodegradation data, observed biodegradation should be viewed as either growth or non-growth (Alexander, 1999; Simkins and Alexander, 1984). Non-growth biodegradation assumes an instantaneous reaction between the organic compound and the microorganisms in an ecosystem. Inherent to non-growth biodegradation is the assumption that the number of microorganisms is constant. The second group of models assumes growth of microorganisms and consequently varying numbers of microorganisms and activities.

## *B.1 Growth*

In the presence of organic compounds, which act as growth substrates, microorganisms multiply. Upon addition of growth substrates to microbial cells, a period without growth is frequently observed (the so-called 'lag period') during which adaptation of microbial cells occurs by for instance synthesis of enzymes. Next, a phase of exponential growth occurs until the growth of microorganisms is limited by the availability of the substrate. During the exponential phase, microorganisms grow at their maximum rate and cell numbers increase exponentially resulting in increasingly faster activities (rates of conversion). The exponential phase is followed by a stationary phase. During the stationary phase microorganisms usually continue metabolising. A lag period, an exponential growth phase and a stationary can be recognised in most biodegradation tests including the test used to assess biodegradation in seawater (e.g. OECD 306) and in microbial enrichment cultures.

The rate at which an organic compound (carbon and energy source) is consumed is usually related to the rate of microbial growth. In growing cultures, the concentration of biomass increases according to a first order reaction, that is, the biomass changes (dX/dt) are proportional to the biomass present. The specific growth rate $\mu$ and the doubling time are used to quantify growth:

$dX/dt = \mu \cdot X$ and $td = \ln 2/\mu$.

It is well accepted that the Monod equation accurately describes the specific growth rate of microorganisms (Monod, 1949). The specific growth rate is a function of the limiting substrate concentration:

$$\mu = \mu_{max} \cdot S / K_s + S$$

where $\mu$ is the growth rate of the microorganism, S is the substrate concentration, $\mu_{max}$ is the maximum growth rate of the microorganism, and $K_s$ is defined as the substrate concentration at which $\mu = 0.5\mu_{max}$. The unit of this rate constant is expressed as for instance $days^{-1}$. The Monod equation is best used when the microbial population is low in relation to the substrate concentration and time-scales larger than a few hours. These ratios and time-scales are very common under the conditions prevailing in most biodegradability tests including OECD 301, 306, 310 and enrichment cultures.

The rate of biodegradation of a chemical acting as growth substrate is a function of the number of competent microorganisms and the concentration of the chemical. The substrate degradation (Monod-with-growth) expressed mathematically in a differential form can be best employed:

$$- dS/dt = u_{max} \cdot S(S_0 + X_0 - S) / K_s + S$$

where $S_0$ is the initial substrate concentration, and $X_0$ is the biomass concentration at $t = 0$. This equation includes simplifying assumptions of a constant relationship between cell numbers and substrate utilisation (constant cell yield), and no formation of inhibitory substances. This equation is an example of second order biodegradation kinetics describing non-steady state conditions with varying numbers of microorganisms (Table 1). Monod does not deal with lag periods, and stationary phases during growth in batch culturing. Sissons *et al* (1986) extended the Monod model to account for the lag phase, acceleration, deceleration and endogenous phases of growth.

The time-scale of kinetics linked to growth is in the order of days to weeks. During this period, the competent microorganisms grow exponentially, resulting in accelerating biodegradation rates. As a consequence biodegradation rates in the environment differ considerably as found in numerous studies. For instance, Saltzman (1982) reported that the biodegradation rates increased upon exposure.

Growth-linked biodegradation of chemicals is superior compared to fortuitous degradation in the environment (Alexander, 1999). Mediated by the exposure of chemicals supporting growth, adaptation of the microbial community will eventually take place. This leads to faster

degradation. The feature of adaptation of microbial populations in the environment is a key issue in the interpretation of biodegradability data.

### *B.2 No microbial growth*

In contrast to the OECD screening test and other enrichment cultures, minor changes of the biomass concentrations are expected under the conditions prevailing in marine waters. Despite generally lower biomass concentrations in seawater compared to freshwater, the ratio of microorganisms to substance concentration is probably high. This is due to very low substance concentrations generally observed in the marine environment. Under these conditions no or only a slight increase of the number of microorganisms utilising organic substances as carbon and energy source is expected (not true for marine and estuarine sediments). Organic substances, which are only degraded by co-metabolism, do not bring about growth and as a consequence, there is no increase in the number of competent microorganisms. Co-metabolic conversions of an organic compound are catalysed by microorganisms grown on analogous compounds or a compound inducing catabolic enzymes with broad substrate specificity. An example of co-metabolism in the marine environment is given in Chapter 3.

When the biodegradation rates are measured in environmental samples during periods of less than a few hours, appreciable growth of microorganisms is not expected. When growth does not occur in the environment and/or biodegradation assays, biodegradation kinetics may be represented by the classic Michaelis-Menten equation for enzyme kinetics. This equation assumes that the degradation rate by microorganisms and not the microbial population is increasing in relation to increasing substrate concentrations:

$$- dS/dt = v = V_{max} \,.\, S / K_m + S$$

where v is the degradation rate, $V_{max}$ is the maximum degradation rate, and $K_m$ is the Michaelis-Menten constant ($K_s$ in the Monod equation). The unit of this rate constant, expressed as for instance µg substrate $l^{-1}$ seawater $day^{-1}$, or mg substrate $mg^{-1}$ biomass $day^{-1}$.

A zero-order rate constant is used when the substrate concentration is much higher than the $K_m$. In this case the rate of biodegradation is independent of substrate concentration. The rate of a zero-order reaction is linear (a constant amount of the substrate is lost per unit of time) and is represented by the following equation:

$$- dS/dt = V_{max}.$$

First-order rate constants should be used when the substrate concentration is low ($S << K_m$). Under this condition both the substrate concentration and the rate of degradation decrease in proportion with each other. Thus, unlike zero-order kinetics, the rate of biodegradation in a first order reaction is dependent on the substrate concentration and is described by the following equation:

$$- dS/dt = V_{max}/K_m \, . \, S = k \, . \, S$$

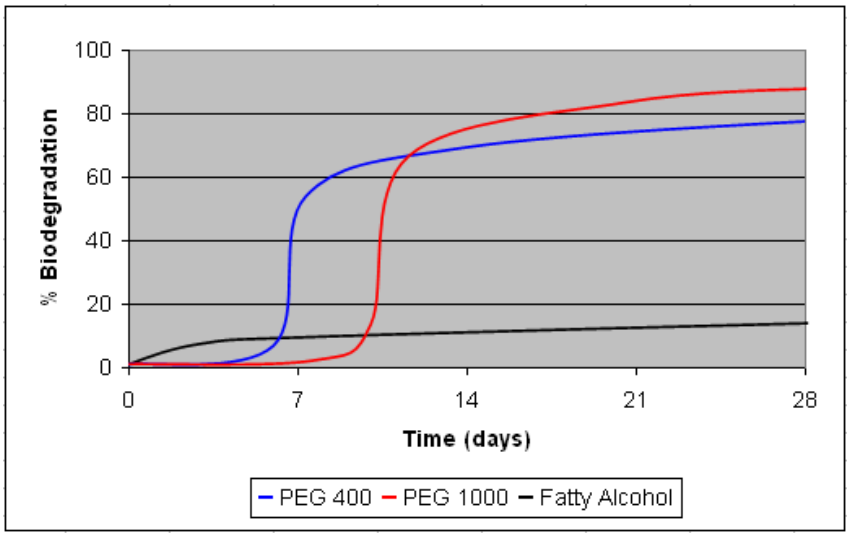
where $V_{max}/K_m$ is the first order rate constant.

# APPENDIX C: FACTORS AFFECTING KINETICS OF BIODEGRADATION

## *C.1 Composition*

Surfactants are typically comprised of a hydrophilic moiety and a hydrophobic (water repelling) moiety. Anionic surfactants comprise a negatively charged hydrophilic moiety. Cationic surfactants comprise a positively charged hydrophilic moiety. Amphoteric surfactants consist of both charges and non-ionic surfactants contain no charge. (van Ginkel, 1996). Thus for complete biodegradation to occur, both the hydrophobic and hydrophilic moieties need to biodegrade which requires a series of different biochemical reactions by a consortia of at least two microorganisms. The metabolism of non-ionic surfactants e.g. alcohol ethoxylates (AEs) typically comprises three different mechanisms of biodegradation. These are: a) central cleavage of the hydrophobic alkyl chain and the hydrophilic moieties, b) oxidation of the alkyl chain, and c) hydrophilic cleavage. Central cleavage of the parent molecule to the corresponding alcohol and polyethylene glycol chain (PEG), as well as the oxidative degradation of the alkyl chain moiety leaving PEG, are the primary biodegradation mechanisms. In either case, the first steps of AE biodegradation will result in fast removal of the alkyl moiety and the formation of an $EO_n$ residue which is biodegraded subsequently (Richterich and Steber, 2001). In many cases a biphasic course of degradation is seen to represent the kinetics of the alcohol and PEG moieties. This is shown in Figure C1 below. These examples demonstrate that deriving biodegradation kinetics from OECD 301 and 306 tests with surfactants should be interpreted with caution because these substances are degraded by consortia of microorganisms. To allow for multiple, subsequent degradation and in order to show potential degradation of the mixture a higher incubation time may be applicable.

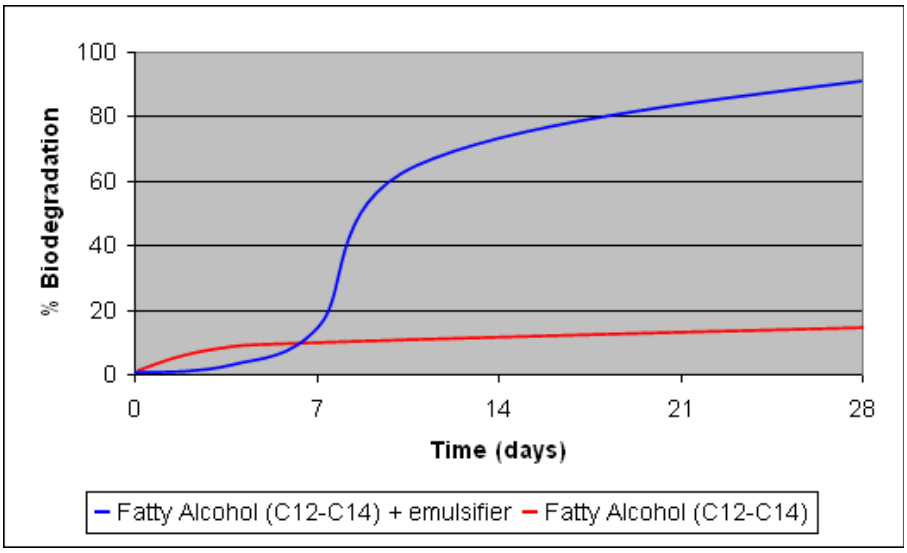
***Figure C1: Biodegradation kinetics showing the kinetics of fatty alcohol and PEG moieties of alcohol ethoxylates (Richterich and Steber, 2001)***



## *C.2 Bioavailability*

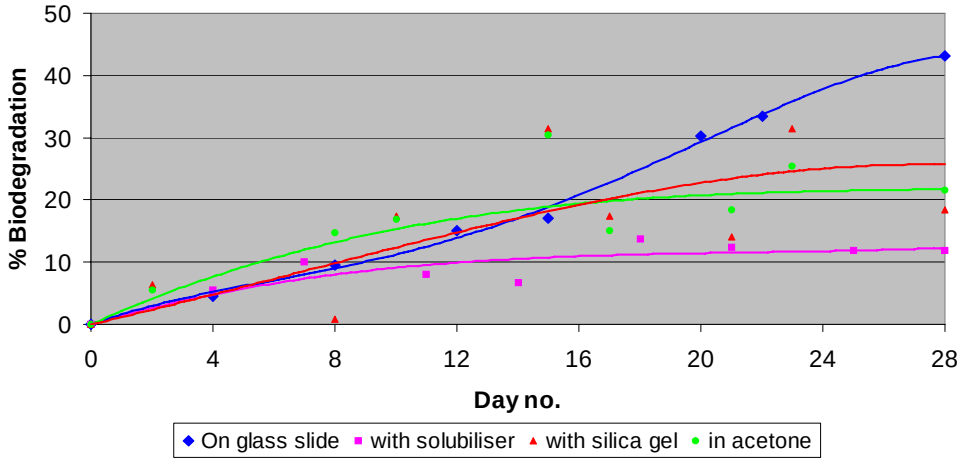
Fatty alcohols also provide a good example of how the aqueous solubility of the test chemical strongly influences the degradation kinetics in a ready biodegradation test (Richterich and Steber, 2001). The degradation kinetics of a poorly soluble C12-14 fatty alcohol in the Sapromat system (OECD 301F respirometry) are almost linear and increased very slowly when the material was tested alone (Figure C2). In contrast, a typical biodegradation curve reaching the 60% pass level within a few days was obtained when a poorly biodegradable emulsifier was used.

***Figure C2: Biodegradation kinetics of poorly soluble fatty alcohols with and without emulsifier***




Another example showing the influence of a poorly soluble chemical on biodegradation kinetics is given by the highly insoluble sucrose polyesters (SPEs). Biodegradation of SPEs was investigated in studies conducted by Melling, 2004. The poor bioavailability of (SPEs) demonstrates differing extents of kinetic behaviour that can be seen when SPEs are solubilised using the techniques as described by ISO, 1995. SPEs were introduced into the test system with acetone, silica gel, a solubiliser and using a glass slide (Figure C3). It can be seen that SPEs cannot be classed as being readily biodegradable; however the biodegradation behaviour with respect to the shape of the curves is distinctly different due to the varying bioavailability of SPE obtained with each of the techniques. This is most evident when SPE is introduced into the test system using a glass slide, compared to the use of solvent and silica gel techniques. This also demonstrates that the 28-day test duration is not necessarily applicable for poorly soluble chemicals, and an extended time period would be more appropriate to accommodate chemical bioavailability.

***Figure C3: Biodegradation kinetics of poorly soluble sucrose polyesters (SPEs) using techniques as described by ISO, 1995 (Melling, 2004)***




Another example is given by the solubility of fatty amine derivatives that decreases with increasing alkyl chains. Also, due to the hydrophobic nature of the alkyl chain and the positive charge, many fatty amine derivatives partition to sludge particles and the glass wall of the test vessel, and this is well illustrated by fatty amine derivatives. Adsorption and poor water solubility of these surfactants is expected to affect biodegradation kinetics because slow desorption and dissolution rates of test substances present at high concentrations may limit the biodegradation in ready biodegradability tests. The biodegradation curves of substances with a poor water solubility and limited bioavailability show a linear curve instead of the anticipated S-shaped curve. Also, under environmental conditions fatty amine derivatives will be present in the environment at very low concentrations.

## *C.3 Variability between screening tests*

Historically, one of the most commonly used biodegradation test methods was the Sturm test for $CO_2$ production (Hales, 1996). In this test biodegradation is determined by monitoring the production of $CO_2$ which is produced in the test medium and then transferred by carrier gas into an alkali trap. This transfer of $CO_2$ from the medium to the traps can take some time and may result in misinterpretations. The headspace $CO_2$ method developed by Birch and Fletcher, (1991) allows for the direct measurement of $CO_2$ in solution and the headspace. Direct measurements with the respirometer are also possible due to the low water solubility of oxygen. This results in different and faster biodegradation curves being obtained which are likely to be a more realistic representation of biodegradation. The studies by Birch and Fletcher, (1991) have shown the differences in biodegradation that can exist between these test systems and this is illustrated with sodium benzoate and LAS (see Figures C4 and C5).

***Figure C4: Biodegradation of sodium benzoate in headspace and Sturm test***

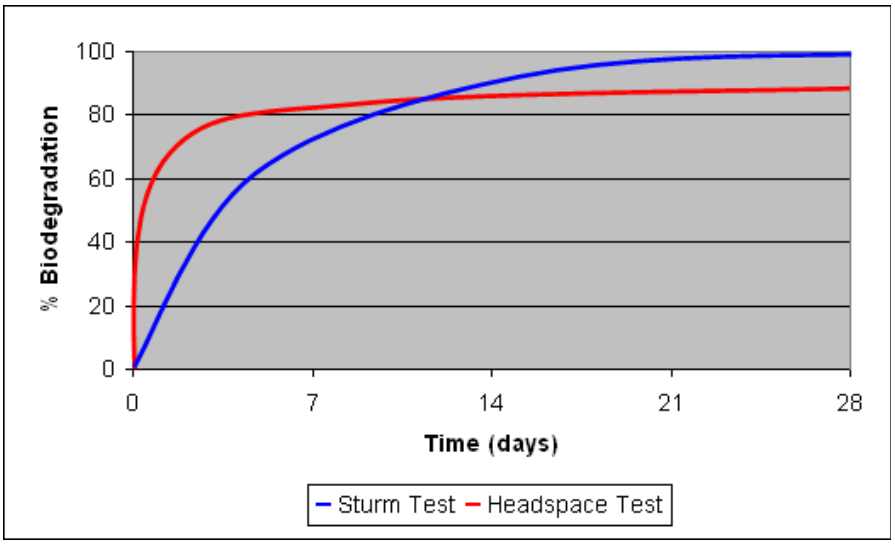
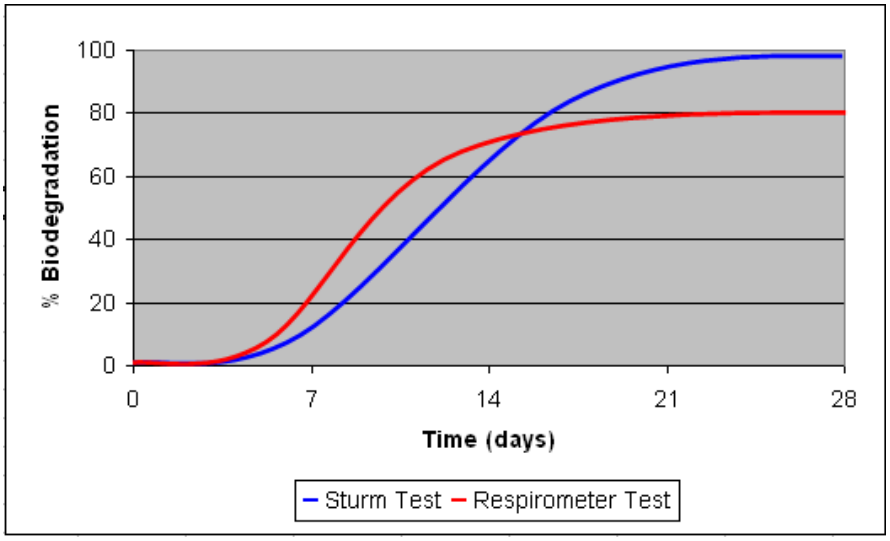



***Figure C5: Biodegradation of LAS in Sturm and respirometer test***




The data sets can be fitted by treating the data as first order with a lag. In this way, the Sturm data gives the same first order rate constant for benzoate and LAS i.e. 0.15 $d^{-1}$. However, different first order rate constants are obtained with the Headspace method, i.e. 0.2 $d^{-1}$ for LAS and 0.5 $d^{-1}$ for benzoate. This illustrates how the Sturm test data underestimates biodegradation rates and does not differentiate between the two materials. It is only possible to fit the ready biodegradation test data to 'first order with a lag' model because of the experimental design, however those respirometric methods which can provide large numbers of data points, provide a more realistic shape of the biodegradation curve as shown below with sodium benzoate and LAS.

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# ECETOC PUBLISHED REPORTS

## *Monographs*

No. Title

No. 1 Good Laboratory Practice (Published October 1979)
No. 2 A Contribution to Strategy for Identification and Control of Occupational Carcinogens (Published September 1980)
No. 3 Risk Assessment of Occupational Chemical Carcinogens (Published May 1985)
No. 4 Hepatocarcinogenesis in Laboratory Rodents: Relevance for Man (Published October 1982)
No. 5 Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology) (Published December 1983)
No. 6 Acute Toxicity Tests, $LD_{50}$ ($LC_{50}$) Determinations and Alternatives (Published May 1985)
No. 7 Recommendations for the Harmonisation of International Guidelines for Toxicity Studies (Published December 1985)
No. 8 Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment (Summary) (Published June 1986)
No. 9 Assessment of Mutagenicity of Industrial and Plant Protection Chemicals (Published June 1987)
No. 10 Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man (Published August 1987)
No. 11 Eye Irritation Testing (Published June 1988)
No. 12 Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity) (Published November 1989)
No. 13 DNA and Protein Adducts: Evaluation of their Use in Exposure Monitoring and Risk Assessment (Published October 1989)
No. 14 Skin Sensitisation Testing (Published March 1990)
No. 15 Skin Irritation (Published July 1990)
No. 16 Early Indicators of Non-Genotoxic Carcinogenesis (Published June 1991)
No. 17 Hepatic Peroxisome Proliferation (Published May 1992)
No. 18 Evaluation of the Neurotoxic Potential of Chemicals (Published September 1992)
No. 19 Respiratory Allergy (Published August 1993)
No. 20 Percutaneous Absorption (Published August 1993)
No. 21 Immunotoxicity: Hazard Identification and Risk Characterisation (Published September 1994)
No. 22 Evaluation of Chemicals for Oculotoxicity (Published November 1994)
No. 23 Receptor Mediated Mechanisms in Chemical Carcinogenesis (Published December 1995)
No. 24 Risk Assessment for Carcinogens (Published July 1996)
No. 25 Practical Concepts for Dose Selection in Chronic Toxicity and Carcinogenicity Studies in Rodents (Published February 1996)
No. 26 Aquatic Toxicity Testing of Sparingly Soluble Volatile and Unstable Substances (Published September 1996)
No. 27 Aneuploidy (Published August 1997)
No. 28 Dose-response and threshold-mediated mechanisms in mutagenesis - Mutation Research Special Issue (Published January 2000)
No. 29 Skin Sensitisation Testing for the Purpose of Hazard Identification and Risk Assessment (Published September 2000)
No. 30 Genetic Susceptibility to Environmental Toxicants (Published October 2001)
Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, Volume 482, Issues 1-2, Pages 1-115
www.sciencedirect.com/science/journal/00275107
No. 31 Guidance on Evaluation of Reproductive Toxicity Data (Published February 2002)
No. 32 Use of Human Data in Hazard Classification for Irritation and Sensitisation (Published July 2002)

No. 33 Application of Physiological - Toxicokinetic Modelling to Health Hazard Assessment of Chemical Substances (Published February 2003)
Toxicology Letters, Volume 138, Issues 1-2
www.sciencedirect.com/science/journal/03784274

No. 34 Toxicogenomics in Genetic Toxicology and Hazard Determination (Published August 2005)
Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, Volume 575, Issues 1-2
www.sciencedirect.com/science/journal/00275107

No. 35 Biomarkers and molecular epidemiology (Published August 2006)
Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, Volume 600, Issues 1-2
www.sciencedirect.com/science/journal/00275107

No. 36 Environmental Genotoxins in Children and Adults (Published August 2006)
Mutation Research/Genetic Toxicology and Environmental Mutagenesis, Volume 608, Issue 2
www.sciencedirect.com/science/journal/13835718

No. 37 Biomarkers in Children and Adults (Published July 2007)
Toxicology Letters, Volume 172, Nos. 1-2
www.sciencedirect.com/science/journal/03784274

No. 38 Toxicity of Engineered Nanomaterials (published May 2009)
Toxicology Letters, Volume 186, Issue 3
http://www.sciencedirect.com/science/journal/03784274

## *Technical Reports*

No. Title

No. 1 Assessment of Data on the Effects of Formaldehyde on Humans (Published January 1979) (Updated by TR No. 6)
No. 2 The Mutagenic and Carcinogenic Potential of Formaldehyde (Published May 1981)
No. 3 Assessment of Test Methods for Photodegradation of Chemicals in the Environment (Published August 1981)
No. 4 The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man (Published June 1982) (Updated by TR No. 17)
No. 5 Toxicity of Ethylene Oxide and its Relevance to Man (Published September 1982)
No. 6 Formaldehyde Toxicology: An Up-Dating of ECETOC Technical Reports 1 and 2 (Published September 1982)
No. 7 Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere (Published September 1983)
No. 8 Biodegradation Testing: An Assessment of the Present Status (Published November 1983)
No. 9 Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients (Published December 1983)
No. 10 Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits (Published February 1984)
No. 11 Ethylene Oxide Toxicology and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 5 (Published March 1984)
No. 12 The Phototransformation of Chemicals in Water: Results of a Ring-Test (Published June 1984)
No. 13 The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on the Environment (Published March 1984)
No. 14 The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on Human Health (Published March 1984)
No. 15 The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values (Published June 1984)
No. 16 A Review of Recent Literature on the Toxicology of Benzene (Published December 1984)
No. 17 The Toxicology of Glycol Ethers and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 4) (Published April 1985) (Updated by TR No. 64)
No. 18 Harmonisation of Ready Biodegradability Tests (Published April 1985)
No. 19 An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment (Published May 1985)
No. 20 Biodegradation Tests for Poorly-Soluble Compounds (Published February 1986)
No. 21 Guide to the Classification of Carcinogens, Mutagens, and Teratogens under the 6th Amendment (Published February 1986)
No. 22 Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity (Published January 1987)
No. 23 Evaluation of the Toxicity of Substances to be Assessed for Biodegradability (Published November 1986)
No. 24 The EEC 6th Amendment: Prolonged Fish Toxicity Tests (Published October 1986)
No. 25 Evaluation of Fish Tainting (Published January 1987)
No. 26 The Assessment of Carcinogenic Hazard for Human Beings exposed to Methylene Chloride (Published January 1987)
No. 27 Nitrate and Drinking Water (Published January 1988)
No. 28 Evaluation of Anaerobic Biodegradation (Published June 1988)
No. 29 Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico-Chemical Properties, Tonnage and Use Patterns (Published June 1988)
No. 30 Existing Chemicals: Literature Reviews and Evaluations (Fifth Edition) (No longer available) (Published May 1994)
No. 31 The Mutagenicity and Carcinogenicity of Vinyl Chloride: A Historical Review and Assessment (Published July 1988)
No. 32 Methylene Chloride (Dichloromethane): Human Risk Assessment Using Experimental Animal Data (Published May 1988)

No. 33 Nickel and Nickel Compounds: Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis (Published February 1989)
No. 34 Methylene Chloride (Dichloromethane): An Overview of Experimental Work Investigating Species Differences in Carcinogenicity and their Relevance to Man (Published March 1989)
No. 35 Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments (Published January 1990)
No. 36 Biomonitoring of Industrial Effluents (Published April 1990)
No. 37 Tetrachlorethylene: Assessment of Human Carcinogenic Hazard (Published May 1990)
No. 38 A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens (Published July 1990)
No. 39 Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea (Published July 1990)
No. 40 Hazard Assessment of Chemical Contaminants in Soil (Published April 1992)
No. 41 Human Exposure to N-Nitrosamines, their Effects and a Risk Assessment for N-Nitrosodiethanolamine in Personal Care Products (Published August 1990)
No. 42 Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products (Published February 1991)
No. 43 Emergency Exposure Indices for Industrial Chemicals (Published March 1991)
No. 44 Biodegradation Kinetics (Published September 1991)
No. 45 Nickel, Cobalt and Chromium in Consumer Products: Allergic Contact Dermatitis (Published March 1992)
No. 46 EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals (Published May 1992)
No. 47 EC 7th Amendment "Toxic to Reproduction": Guidance on Classification (Published August 1992)
No. 48 Eye Irritation: Reference Chemicals Data Bank (Second Edition) (Published June 1998)
No. 49 Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration (Published December 1992)
No. 50 Estimating Environmental Concentrations of Chemicals using Fate and Exposure Models (Published November 1992)
No. 51 Environmental Hazard Assessment of Substances (Published January 1993)
No. 52 Styrene Toxicology Investigation on the Potential for Carcinogenicity (Published August 1992)
No. 53 DHTDMAC: Aquatic and Terrestrial Hazard Assessment (CAS No. 61789-80-8) (Published February 1993)
No. 54 Assessment of the Biodegradation of Chemicals in the Marine Environment (Published August 1993)
No. 55 Pulmonary Toxicity of Polyalkylene Glycols (Published December 1997)
No. 56 Aquatic Toxicity Data Evaluation (Published December 1993)
No. 57 Polypropylene Production and Colorectal Cancer (Published February 1994)
No. 58 Assessment of Non-Occupational Exposure to Chemicals (Published May 1994)
No. 59 Testing for Worker Protection (Published April 1994)
No. 60 Trichloroethylene: Assessment of Human Carcinogenic Hazard (Published May 1994)
No. 61 Environmental Exposure Assessment (Published September 1994)
No. 62 Ammonia Emissions to Air in Western Europe (Published July 1994)
No. 63 Reproductive and General Toxicology of some Inorganic Borates and Risk Assessment for Human Beings (Published February 1995)
No. 64 The Toxicology of Glycol Ethers and its Relevance to Man (Published August 1995)
No. 65 Formaldehyde and Human Cancer Risks (Published May 1995)
No. 66 Skin Irritation and Corrosion: Reference Chemicals Data Bank (Published March 1995)
No. 67 The Role of Bioaccumulation in Environmental Risk Assessment: The Aquatic Environment and Related Food Webs (Published October 1995)
No. 68 Assessment Factors in Human Health Risk Assessment (Published August 1995) (Updated by TR No. 86)
No. 69 Toxicology of Man-Made Organic Fibres (Published April 1996)
No. 70 Chronic Neurotoxicity of Solvents (Published February 1996)
No. 71 Inventory of Critical Reviews on Chemicals (Only available to ECETOC members) (Published August 1996)

No. 72 Methyl *tert*-Butyl Ether (MTBE) Health Risk Characterisation (Published June 1997)
No. 73 The Value of Aquatic Model Ecosystem Studies in Ecotoxicology (Published December 1997)
No. 74 QSARs in the Assessment of the Environmental Fate and Effects of Chemicals (Published June 1998)
No. 75 Organophosphorus Pesticides and Long-term Effects on the Nervous System (Published December 1998)
No. 76 Monitoring and Modelling of Industrial Organic Chemicals, with Particular Reference to Aquatic Risk Assessment (Published January 1999)
No. 77 Skin and Respiratory Sensitisers: Reference Chemicals Data Bank (Published August 1999)
No. 78 Skin Sensitisation Testing: Methodological Considerations (Published December 1999)
No. 79 Exposure Factors Sourcebook for European Populations (with Focus on UK Data) (Published June 2001)
No. 80 Aquatic Toxicity of Mixtures (Published July 2001)
No. 81 Human Acute Intoxication from Monochloroacetic Acid: Proposals for Therapy (Published November 2001)
No. 82 Risk Assessment in Marine Environments (Published December 2001)
No. 83 The Use of T25 Estimates and Alternative Methods in the Regulatory Risk Assessment of Non-threshold Carcinogens in the European Union (Published December 2002)
No. 84 Scientific Principles for Soil Hazard Assessment of Substances (Published July 2002)
No. 85 Recognition of, and Differentiation between, Adverse and Non-adverse Effects in Toxicology Studies (Published December 2002)
No. 86 Derivation of Assessment Factors for Human Health Risk Assessment (Published February 2003)
No. 87 Contact Sensitisation: Classification According to Potency (Published April 2003)
No. 88 Environmental Risk Assessment of Difficult Substances (Published June 2003)
No. 89 (Q)SARS: Evaluation of the Commercially Available Software for Human Health and Environmental Endpoints with Respect to Chemical Management Applications (Published September 2003)
No. 90 Persistence of Chemicals in the Environment (Published October 2003)
No. 91 Aquatic Hazard Assessment II (Published November 2003)
No. 92 Soil and Sediment Risk Assessment (Published December 2004)
No. 93 Targeted Risk Assessment (Published December 2004)
No. 94 Whole Effluent Assessment (Published December 2004)
No. 95 The Toxicology of Glycol Ethers and its Relevance to Man (Fourth Edition) Volume I and Volume II Substance Profiles (Published February 2005)
No. 96 Trends in Children's Health and the Role of Chemicals: State of the Science Review (Published June 2005)
No. 97 Alternative Testing Approaches in Environmental Safety Assessment (Published December 2005)
No. 98 Risk Assessment of PBT Chemicals (Published December 2005)
No. 99 Toxicological Modes of Action: Relevance for Human Risk Assessment (Published July 2006)
No. 100 Contribution to the Methodology for the Development of Acute Exposure Threshold Levels in Case of Accidental Chemical Release (Published July 2006)
No. 101 Guidance for Setting Occupational Exposure Limits: Emphasis on Data-Poor Substances (Published October 2006)
No. 102 Intelligent Testing Strategies in Ecotoxicology: Mode of Action Approach for Specifically Acting Chemicals (Published December 2007)
No. 103 Toxicity of Possible Impurities and By-products in Fluorocarbon Products (Published December 2008)
No. 104 Framework for the Integration of Human and Animal Data in Chemical Risk Assessment (Published January 2009)
No. 105 Evaluation of Cardiac Sensitisation Test Methods (Published October 2009)
No. 106 Guidance on Identifying Endocrine Disrupting Effects (Published June 2009)
No. 107 Addendum to ECETOC Targeted Risk assessment report No. 93 (Published December 2009)

## *Joint Assessment of Commodity Chemicals (JACC) Reports*

| No. | Title |
|---|---|
| No. 1 | Melamine (Published February 1983) |
| No. 2 | 1,4-Dioxane (Published February 1983) |
| No. 3 | Methyl Ethyl Ketone (Published February 1983) |
| No. 4 | Methylene Chloride (Published January 1984) |
| No. 5 | Vinylidene Chloride (Published August 1985) |
| No. 6 | Xylenes (Published June 1986) |
| No. 7 | Ethylbenzene (Published August 1986) |
| No. 8 | Methyl Isobutyl Ketone (Published May 1987) |
| No. 9 | Chlorodifluoromethane (Published October 1989) |
| No. 10 | Isophorone (Published September 1989) |
| No. 11 | 1,2-Dichloro-1,1-difluoroethane (HFA-132b) (Published May 1990) |
| No. 12 | 1-Chloro-1,2,2,2-tetrafluoroethane (HFA-124) (Published May 1990) (Updated by JACC No. 25) |
| No. 13 | 1,1-Dichloro-2,2,2-trifluoroethane (HFA-123) (Published May 1990) (Updated by JACC No. 33) |
| No. 14 | 1-Chloro-2,2,2-trifluoromethane (HFA-133a) (Published August 1990) |
| No. 15 | 1-Fluoro 1,1-dichloroethane (HFA-141) (Published August 1990) (Updated by JACC No. 29) |
| No. 16 | Dichlorofluoromethane (HCFC-21) (Published August 1990) |
| No. 17 | 1-Chloro-1,1-difluoroethane (HFA-142b) (Published August 1990) |
| No. 18 | Vinyl Acetate (Published February 1991) |
| No. 19 | Dicyclopentadiene (CAS: 77-73-6) (Published July 1991) |
| No. 20 | Tris-/Bis-/Mono-(2 ethylhexyl) phosphate (Published May 1992) |
| No. 21 | Tris-(2-butoxyethyl)-phosphate (CAS:78-51-3) (Published March 1992) |
| No. 22 | Hydrogen Peroxide (CAS: 7722-84-1) (Published January 1993) |
| No. 23 | Polycarboxylate Polymers as Used in Detergents (Published November 1993) |
| No. 24 | Pentafluoroethane (HFC-125) (CAS: 354-33-6) (Published May 1994) |
| No. 25 | 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0) (Second Edition) (Published July 1994) (Updated by JACC 46) |
| No. 26 | Linear Polydimethylsiloxanes (CAS No. 63148-62-9) (Published September 1994) |
| No. 27 | *n*-Butyl Acrylate (CAS No. 141-32-2) (Published August 1994) |
| No. 28 | Ethyl Acrylate (CAS No. 140-88-5) (Published September 1994) |
| No. 29 | 1,1-Dichloro-1-fluoroethane (HCFC-141b) (CAS No. 1717-00-6) (Published December 1994) |
| No. 30 | Methyl Methacrylate (CAS No. 80-62-6) (Published February 1995) |
| No. 31 | 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2) (Published February 1995) (Updated by JACC No. 50) |
| No. 32 | Difluoromethane (HFC-32) (CAS No. 75-10-5) (Published May 1995) (Updated by JACC No. 54) |
| No. 33 | 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) (CAS No. 306-83-2) (Published February 1996) (Updated by JACC No. 47) |
| No. 34 | Acrylic Acid (CAS No. 79-10-7) (Published September 1995) |
| No. 35 | Methacrylic Acid (CAS No. 79-41-4) (Published May 1996) |
| No. 36 | *n*-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9) (Published December 1996) |
| No. 37 | Methyl Acrylate (CAS No. 96-33-3) (Published September 1998) |
| No. 38 | Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3) (Published June 1999) |
| No. 39 | Tetrachloroethylene (CAS No. 127-18-4) (Published December 1999) |
| No. 40 | Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions (Published January 2001) |

No. 41 *n*-Butanol (CAS No. 71-36-3) (Published March 2004)

No. 42 Tetrafluoroethylene (CAS No. 116-14-3) (Published December 2003)

No. 43 *sec*-Butanol (CAS No. 78-92-2) (Published December 2004)

No. 44 1, 1, 1, 3, 3-Pentafluoropropane (HFC-245fa) (Published June 2004)

No. 45 1, 1-Difluoroethane (HFC-152a) (CAS No. 75-37-6) (Published September 2004)

No. 46 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) CAS No. 2837-89-0 (Third Edition) (Published November 2004)

No. 47 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) CAS No. 306-83-2 (Third Edition) (Published May 2005)

No. 48 Hexafluoropropylene (HFP) CAS No. 116-15-4 (Published September 2005)

No. 49 Vinylidene Fluoride CAS No. 75-38-7 (Published November 2005)

No. 50 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2) (Second Edition) (Published January 2006)

No. 51 Synthetic Amorphous Silica (CAS No. 7631-86-9) (Published September 2006)

No. 52 Trifluoroethane (HFC-143a) CAS No. 420-46-2 (Published October 2006)

No. 53 Cyanides of Hydrogen, Sodium and Potassium, and Acetone Cyanohydrin (CAS No. 74-90-8, 143-33-9,151-50-8 and 75-86-5) (Published September 2007)

No. 54 Difluoromethane (HFC-32) CAS No. 75-10-5 (Second Edition) (Published June 2008)

## *Special Reports*

| No. | Title |
|---|---|
| No. 8 | HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances (Published October 1994) |
| No. 9 | Styrene Criteria Document (Published June 1995) |
| No. 10 | Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1) (Published July 1996) |
| No. 11 | Ecotoxicology of some Inorganic Borates (Published March 1997) |
| No. 12 | 1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0) (Published January 1997) |
| No. 13 | Occupational Exposure Limits for Hydrocarbon Solvents (Published August 1997) |
| No. 14 | *n*-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document (Published May 1998) |
| No. 15 | Examination of a Proposed Skin Notation Strategy (Published September 1998) |
| No. 16 | GREAT-ER User Manual (Published March 1999) |
| No. 17 | Risk Assessment Report for Existing Substances Methyl *tertiary*-Butyl Ether (Published December 2003) |

## *Documents*

| No. | Title |
|---|---|
| No. 32 | Environmental Oestrogens: Male Reproduction and Reproductive Development (Published January 1996) |
| No. 33 | Environmental Oestrogens: A Compendium of Test Methods (Published July 1996) |
| No. 34 | The Challenge Posed by Endocrine-disrupting Chemicals (Published February 1996) |
| No. 35 | Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances (Published May 1997) |
| No. 36 | Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals (Published August 1997) |
| No. 37 | EC Classification of Eye Irritancy (Published December 1997) |
| No. 38 | Wildlife and Endocrine Disrupters: Requirements for Hazard Identification (Published January 1998) |
| No. 39 | Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach (Published January 1999) |
| No. 40 | Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene (Published October 2000) |
| No. 41 | Persistent Organic Pollutants (POPs) Response to UNEP/INC/CEG-I Annex 1 (Published January 2000) |
| No. 42 | Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction (Published April 2001) |
| No. 43 | Contact Sensitisation: Classification According to Potency. A Commentary (Published July 2003) |
| No. 44 | Guidance for the Interpretation of Biomonitoring Data (Published November 2005) |
| No. 45 | Triggering and Waiving Criteria for the Extended One-Generation Reproduction Toxicity Study (Published March 2008) |
| No. 46 | Potency Values from the Local Lymph Node Assay: Application to Classification, Labelling and Risk Assessment (Published December 2008) |

## *Workshop Reports*

| No. | Title |
|---|---|
| No. 1 | Workshop on Availability, Interpretation and Use of Environmental Monitoring Data. 20-21 March 2003, Brussels (Published December 2003) |
| No. 2 | Strategy Report on Challenges, Opportunities and Research needs arising from the Definition, Assessment and Management of Ecological Quality Status as required by the EU Water Framework Directive based on the workshop EQS and WFD versus PNEC and REACH - are they doing the job? 27-28 November 2003, Budapest (Published March 2004) |
| No. 3 | Workshop on the Use of Human Data in Risk Assessment. 23-24 February 2004, Cardiff (Published November 2004) |
| No. 4 | Influence of Maternal Toxicity in Studies on Developmental Toxicity. 2 March 2004, Berlin (Published October 2004) |
| No. 5 | Workshop on Alternative Testing Approaches in Environmental Risk Assessment. 7-9 July 2004, Paris (Published December 2004) |
| No. 6 | Workshop on Chemical Pollution, Respiratory Allergy and Asthma. 16-17 June 2005, Leuven (Published December 2005) |
| No. 7 | Workshop on Testing Strategies to Establish the Safety of Nanomaterials. 7-8 November 2005, Barcelona (Published August 2006) |
| No. 8 | Workshop on Societal Aspects of Nanotechnology. 7-8 November 2005, Barcelona (Published October 2006) |
| No. 9 | Workshop on the Refinement of Mutagenicity/Genotoxicity Testing. 23-24 April 2007, Malta (Published September 2007) |
| No. 10 | Workshop on Biodegradation and Persistence. 26-27 June 2007, Holmes Chapel (Published September 2007) |
| No. 11 | Workshop on the Application of 'Omics in Toxicology and Ecotoxicology: Case Studies and Risk Assessment. 6-7 December 2007, Malaga (Published July 2008) |
| No. 12 | Workshop on Triggering and Waiving Criteria for the Extended One-Generation Reproduction Toxicity Study. 14-15 April 2008, Barza d'Ispra (Published August 2008) |
| No. 13 | Counting the Costs and Benefits of Chemical Controls: Role of Environmental Risk Assessment in Socio-Economic Analysis 4 June 2008, Brussels (Published September 2008) |
| No. 14 | Use of Markers for Improved Retrospective Exposure Assessment in Epidemiology Studies. 24-25 June 2008, Brussels (Published February 2009) |
| No. 15 | The Probabilistic Approaches for Marine Hazard Assessment. 18-19 June 2008, Oslo (Published June 2009) |
| No. 16 | Guidance on interpreting endocrine disrupting effects. 29-30 June 2009, Barcelona (Published October 2009) |
| No. 17 | Significance of Bound Residues in Environmental Risk Assessment. 14-15 October 2009, Brussels (Published December 2009) |
| No. 18 | The Enhancement of the Scientific Process and Transparency of Observational Epidemiology Studies. 24-25 September 2009, London (Published December 2009) |

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