

Persistence of Chemicals in the Environment

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Persistence of Chemicals in the Environment	
Persistence of Chemicals in the Environment	
CONTENT	
SUMMARY	1
	-
1. INTRODUCTION	7
2. DEFINITIONS OF PERSISTENCE AND PERSISTENCE CRITERIA	10
2.1 Persistence	10
2.2 Persistence criteria in EU	12
2.2.1 EC Notification of New Substances (NONS) and Existing	14
Substances Regulations (ESR)	
2.2.2 OSPAR	17
2.2.3 Overview of persistence criteria 2.3 Conclusions	18
2.3 Conclusions	21
3. FACTORS AFFECTING PERSISTENCE: LABORATORY AND FIELD CONSIDERATIONS	23
3.1 Introduction	23
3.2 Persistence and ready biodegradability	23
3.3 The half-life concept	24
3.4 Microbial diversity, growth and adaptation	26
3.4.1 Diversity	26
3.4.2 Microbial Growth	28
3.5 Cometabolism	37
3.6 Temperature	38
3.7 Oxygen content 3.8 Bioavailability	40 41
3.9 Metabolism by higher organisms	44
3.10 Conclusions	46
	10
4. EXTRAPOLATION FROM LABORATORY AND FIELD DATA	49
4.1 Extrapolation from standard tests	49
4.2 Defaults and standard ready test data	51
4.2.1 Aqueous data	52
4.2.2 Terrestrial data	65
4.3 Discussion and conclusions	67
5. IMPROVEMENTS TO EXISTING BIODEGRADABILITY TEST METHODS	69
5.1 Source and density of inoculum	71
5.2 Test chemical concentration	80
5.3 Test temperature	80
5.4 Duration of test, required number of replicates and the frequency of sampling	81
5.5 Conclusions	81

6. ABIOTIC DEGRADATION PROCESSES	83
6.1 Hydrolysis	84
6.1.1 Kinetics	85
6.1.2 Measurement of hydrolysis rate	85
6.1.3 Factors influencing hydrolysis	86
6.1.4 Estimates of hydrolytic rates	88
6.1.5 Prediction and extrapolation of hydrolysis rates	89
6.2 Photolysis	89
6.2.1 General theory	90
6.2.2 Measurement of direct photolysis rate	92
6.2.3 Estimation of direct photolytic rates	92
6.2.4 Factors influencing photolysis	96
6.2.5 Prediction of photolysis, uncertainties and variation	99
6.3 Indirect photooxidation or chemical oxidation	99
6.3.1 General theory	100
6.3.2 Estimation of total loss	103
6.4 Conclusions	104
7. MODELLING	106
	100
7.1 Introduction	106
7.2 Review of use of modelling to estimate persistence	107
7.2.1 Elements of multi-media fate models	107
7.2.2 Available multi-media models	116
7.2.3 Model Output	119
7.3 Conclusions	122
8. A TEST-BASED STRATEGY TO IDENTIFY PERSISTENCE	E CLASSES 124
FROM DEGRADATION MEASUREMENTS	
8.1 T ¹ / ₂ Distribution ranges	127
8.2 Strategy for predicting persistence of substances in the	environment 128
8.3 Conclusions	135
9. CONCLUSIONS AND RECOMMENDATIONS	107
9. CONCLUSIONS AND RECOMMENDATIONS	136
GLOSSARY	140
	110
APPENDIX A: SUMMARY OF METHODS FOR DETERMIN DEGRADATION RATES AND BIODEGRADABILITY	NING 143
DEGRADATION RATES AND DIODEGRADADILITT	
APPENDIX B: DEGRADATION RATE DISTRIBUTIONS	165
BIBLIOGRAPHY	169
MEMBERS OF THE TASK FORCE	189
	107
MEMBERS OF THE SCIENTIFIC COMMITTEE	190

SUMMARY

The ability of certain chemicals substances to persist (P) in the environment is an issue of global concern that requires careful consideration in environmental risk assessment. This is especially true when this ability is coupled with bioaccumulative (B) and toxicological (T) properties, i.e. when it is classed as a PBT substance.

Assessing the persistence of chemical substances in the environment is not straightforward. Persistence cannot be directly measured; it can only be inferred from the continued presence of a substance in the environment or the lack of observed degradative data in the laboratory.

Extrapolation is a major component of any strategy to assess the fate and persistence of chemical substances in the environment. As it is not practical to measure degradation under all environmental conditions, and for all environmental compartments, it is therefore necessary to relate laboratory test results from one or two degradation studies to removal rates in all environmental compartments. Consequently, a number of regulatory bodies have proposed criteria for prioritising the environmental risk associated with a chemical substance based on its PBT properties (EC, 2003; OSPAR, 1998). These regulatory authorities (e.g. the European Commission) describe the persistence of chemicals in terms of single medium half-lives or default half-lives based on the outcome of standard ready and inherent biodegradation tests. While some of these tests are suitable for identifying substances that are non-persistent (i.e. substances that undergo rapid and ultimate biodegradation in all environmental compartments) they should not be used to classify a chemical as 'persistent'.

This study reviews the current approaches for defining, determining, interpreting and inferring the persistence of a substance in the environment. Potential modifications designed to improve the inference of the persistence of chemical substances, based on sound scientific principles, are also identified in this report. The review commences with a comprehensive overview of all the definitions currently used by the relevant organisations worldwide to describe the persistence of a chemical substance. The factors that affect environmental persistence, and the current methodologies used to assess the abiotic and biotic degradation of substances in water, air, soil and sediment, are also discussed in detail. The limitations of these methods are discussed, and modifications identified to improve the assessment of persistence.

The extrapolation of laboratory data to the environment is described in detail. Measured degradation values have been compared with the current default values that are applied based on the outcome of ready and inherent biodegradation tests. Data presented within this ECETOC review demonstrate that the default half-lives assigned against data generated under these test guidelines are too conservative i.e. the default values described in the EU Technical Guidance Documents (TGD) (EC, 2003) overestimate the persistence of a substance in the environment.

The value of multi-media modelling as a tool for targeting degradation testing in relevant environmental compartments has also been considered. As well as providing information on realistic presence in different phases, models can predict overall persistence which can be compared with persistence in individual environmental media. This concept has been reviewed. Finally, the review concludes with a combined test- and model- based strategy which employs a two-stage process to characterise chemical persistence. This strategy maximises the use of existing standard and non-standard degradation data and provides a framework in which data from new, targeted test systems can be used in a pragmatic manner to determine overall persistence. Conclusions and recommendations for further research are also provided.

In the regulatory context, persistence has been defined in terms of environmental half-lives, which in turn have been assigned to standard test results. This notion of half-life is clearly scientifically linked to first order kinetics. In reality degradation mechanisms are often much more complex and cannot be described by a single rate parameter or type of reaction kinetics. Consequently, the use of a single half-life ($t^{1/2}$) to describe the degradation of a chemical in the environment may not be appropriate. Nevertheless, the idea of a half-life to describe persistence is widely used and forms part of many regulatory schemes. For consistency with such schemes we continue to use this term but propose that a range or distribution of half-lives ($T^{1/2}$) replaces the use of a single value. This $T^{1/2}$ distribution is independent of any specific environmental medium and attempts to reflect all spatial and temporal differences in degradation rates and degradation kinetics that may be operating. For mathematical expediency, degradation (biotic and abiotic) is assumed to occur under first order kinetics in multi-media models, with the rates in each medium described using $T^{1/2}$ values.

The T¹/₂ distribution should relate to the degradation of the parent compound whenever possible. Where a stable metabolite or bound residue is identified, persistence should be considered independently as the partitioning behaviour of the metabolite may differ from that of the parent compound. The currently available abiotic test methods are considered suitable for the generation of T¹/₂ values. However, in order to measure biologically mediated transformations of a substance in the environment, biodegradation tests are required. Such tests should maximise environmental realism without compromising biodegradative potential. Only then can an accurate assessment of biodegradation be derived. A number of suggestions for an improved testing approach to biodegradation are proposed.

A number of pre-requisites need to be satisfied for biodegradation observed in the laboratory to be realised in the field: Competent organisms must be present in close proximity to the chemical substance; the competent organisms must be viable and active under the prevailing environmental conditions; the substance must be present in a bioavailable form. The number of factors that influence the biodegradation of a substance in the laboratory and the environment are manifold, and a single factor in isolation to all the others can radically alter biodegradation rates. It is these factors, therefore, that result in the uncertainty in extrapolating laboratory data to the field, and between environmental compartments. Existing biodegradation tests are designed to provide scales of biodegradative potential and their simulation of environmental compartments, other than for the aerobic sewage treatment environment, are minimal. Ready biodegradation test methods were designed as stringent screening tests to identify those substances that would undergo rapid and ultimate degradation in the environment and pose little or no long-term environmental concern. This is a purpose that these tests serve well as they provide a relatively simple and inexpensive technique to identify substances that do not require further information regarding their biodegradability. However, there is no scientific basis to suggest that a fail in a standard ready biodegradation test is an indication that a chemical will be persistent. Identifying and prioritising a substance as persistent on the basis of a failed ready biodegradation test will prevent the effective prioritisation of substances that do pose a long-term concern and may result in unnecessary bioaccumulation and sub-acute ecotoxicological studies. Moreover, a scientifically sound and robust test-based strategy to prioritise substances based on their persistence in the environment may lead to reductions in the level of animal testing if implemented and applied correctly.

Microbes involved in degradation are not uniformly distributed throughout the environment. They have evolved and adapted according to the specific conditions in the different environmental compartments and sub-compartments. These selection pressures mean that they have adapted to the general characteristics specific for that system and this influences their capacity to degrade new compounds to which they may not necessarily have been exposed previously. When assessing degradation in a compartment-specific biodegradation test, it is therefore essential that the inoculum is derived from the medium to which the substance is likely to be released, or partitioned. Some form of a priori assessment is required to ensure that the level of environmental realism and, thus, the level of confidence in the outcome of the tests is enhanced (i.e. an increase the level of certainty in extrapolating from laboratory data to the field). The main areas of improvement were identified as test substance concentration, inoculum density and diversity, issues regarding adaptation of micro-organisms (e.g. use of an adapted inoculum from the appropriate compartment), and temperature. Such tests should also minimise laboratory-based artefacts due to the test geometry and apparatus.

The proposed strategy (see Figure 1) comprises a screening phase and a confirmatory phase and is applicable to both new and existing chemicals. Where degradation data exist, the strategy seeks to maximise the use of these data when assessing the persistence of a chemical substance. A structured testing strategy is also proposed where no, insufficient or inadequate data are available. The strategy incorporates improved tests including the use of pre-exposed inocula and enhanced biomass levels (for marine and freshwater studies). This is a major change to the current approach to the assessment of persistence. In brief, this strategy maximises the use of existing degradation test data, from standard and non-standard test systems, and promotes the generation of new data from novel and emerging test regimes that are targeted to specific environmental compartments (Figure 1).

Four persistency classes, ranging from 'easily degradable' (P4) to 'persistent in the environment' (P1), are proposed. Categorising a substance into one of the four categories is based on the T¹/₂ range that the substance is predicted to fall into. The T¹/₂ is derived from a combination of biodegradation results, the potential for biodegradation to occur and the abiotic half-life. Substances categorised as P4 and P3 are identified in the screening phase and are considered to be of no further concern (non-persistent).

Substances categorised as P2 and P1 should be subjected to more detailed scrutiny in the confirmatory phase. Clearly any such substance released into one medium may transfer to another, the extent of which will depend on the physico-chemical properties of the substance and the environment.

Multi-media Fate and Transport Models may be used to help guide testing and eventual classification. If no degradation data exist and if reliable (Q)SARs are not available then a level I model may be used to give an indication of those compartments to which the substance is most likely to partition. This can then be used to prioritise testing. However, level I models give no information about environmental persistence. If degradation data (or reliable (Q)SARs) are available then higher level models may be used. If mode of entry data exist (the proportion of total emission to each environmental compartment), a level III model should be used to derive (1) the fraction of total steady-state mass which would be expected in each environmental compartment (giving a general indication of the relative importance of each compartment) and (2) the overall half-life of the substance. In the absence of accurate mode of entry data, a level II model may be used to give the same outputs. It should be noted, however, that the output from level II and level III models might differ as a consequence of the (sometimes unrealistic) assumption in level II models of complete equilibrium between phases. Testing priority should be given to those compartments which are expected to contain most of the substance of interest.

Half lives for media in which a realistic presence (>5%) is expected should be considered within the confirmatory stage. Once the environmental compartment(s) of interest have been identified an appropriate test protocol should be employed in order to maximise information regarding the fate and behaviour of the substance in that compartment. The prediction of the overall persistence (P_{ov}) or overall half-life (T_{2ov}) produced by level II and level III MFTMs can also potentially be used within the evaluation system, although the threshold criteria have yet to receive regulatory acceptance. We suggest that a reasonable screening threshold for potential persistence using MFTMs would be $T_{2ov}^{1/2} > 60$ days, which corresponds with the individual half-life thresholds adopted by may regulatory authorities for water, soil and sediment (e.g. US-EPA). It is substances that are classified as P1 and P2 after the confirmatory phase that should be prioritised for B and T assessments.

	lity of Degradation in the Environment Classification
ys Very Hig	gh Non-persistent
lays High	Non-persistent
0 days Uncerta	in Cause for concern
ays Low	Persistent
	,

Table 1: Summary of definitions P1 -> P4

*Assigned P2 or P1 at the Screening Stage, this classification may be revised at the Confirmatory Stage

The report concludes with recommendations for future work to improve the accuracy and practicality of methodologies to assess persistence with a particular focus on laboratory and field measurements. Models should be developed further and applied to key chemicals to develop a benchmarking for overall persistence.

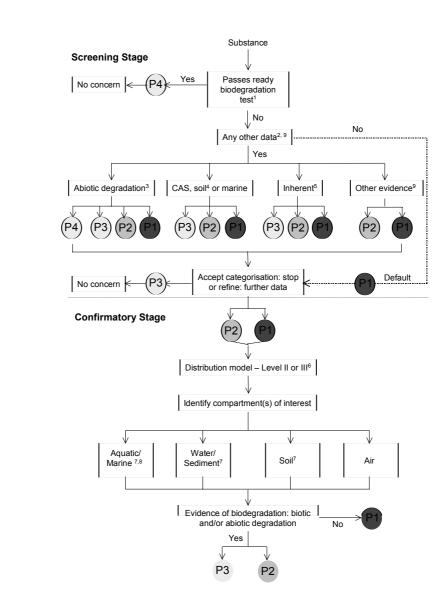


Figure 1: A test-based strategy to determine the persistence of a chemical substance in the environment

- ¹ Includes phytodegradation, although current database is limited, phytotransformations in soil and water environments should be considered.
- ² QSAR predicts fail go to Confirmatory Stage
- ³ Abiotic degradation testing can be applied at all stages, the usefulness will depend on the specific substance and the distribution between the environmental compartments. Abiotic degradation may be combined with degradation to assess persistency.
- ⁴ T¹/₂ for soil is measured directly in laboratory soil die-away tests or in field studies.
- ⁵ Passing an inherent or simulation test has been defined in the specific OECD guidelines.
- ⁶ If emission data is available
- ⁷ Option to expose to 0, 7, 14, 21 and 28d for continuously released substances
- ⁸ Enhanced biomass studies for batch marine and river water studies
- ⁹ Monitoring data

1. INTRODUCTION

Greater environmental awareness by today's society has led governments, via scientific evidence, to recognise the potential threat of chemicals to human health and to the environment. Concerns have been mounting over the past four decades, as scientific evidence has shown that certain chemicals, organics as well as metals, have caused adverse effects to both human health and the environment. A number of these chemicals, such as DDT, dioxins and furans, polychlorinated biphenyls (PCBs) and some polynuclear aromatic hydrocarbons (PAHs) are characterised by their persistence (P), bioaccumulation potential (B) and toxicity (T) (collectively referred to as 'PBT' properties) and their ability to be transported for long distances from their points of emission. A chemical exhibiting all these properties is termed a persistent organic pollutant (POP). Governments, the scientific community, and industry are now acting to ensure that the release of existing substances, that have PBT properties, and which could be harmful to human health and the environment, are controlled and that new chemicals with such properties are not placed on to the market. As a consequence, a robust regime is required to identify chemicals with PBT properties.

The most difficult property to define is persistence. Persistence cannot be measured directly, only inferred from measurements of degradation; furthermore, there is a commonly held belief that persistence is always a negative attribute. Consequently, a regulatory trend is growing to categorise the term 'persistence' in the same way that 'bioaccumulation' has been categorised, i.e. as a key environmental property *per se*, thus ignoring any link to transport, toxicity or environmental conditions. Whilst environmental studies may offer the most realistic measurement of degradation potential and rates, such studies are site-specific, and subject to influence from numerous environmental parameters, which can make interpretation of data very difficult. Criteria for persistence have been proposed by a number of organisations (e.g. OSPAR, UNEP, EC, Environment Canada). These criteria are generally based on degradation half-lives for substances in the environment, even though the degradation reaction mechanisms may not necessarily follow first order kinetics.

The majority of experimental data used to predict persistence is derived from standard biotic and abiotic laboratory tests for soils, sediments and water (fresh and marine). Organisations such as OECD, ECETOC, US-EPA, ISO and national bodies (e.g. BSI, DIN, ASTM, AFNOR, NEN,) have developed standard methods for measuring a chemical's degradability. Whilst abiotic tests measure primary degradation, biodegradation tests typically measure or infer ultimate degradation.

These 'standard' tests are carried out under controlled laboratory conditions that often do not reflect the complexity of those in the environment. Moreover, such tests were not designed to determine kinetic rate constants or half-lives. Consequently, they rarely take into account or record complete information on important parameters, such as variations in soil/sediment or water properties (e.g. for soil/sediment: organic carbon content, clay fraction, water content, ageing, acclimation, redox potential; and for water: organic carbon content, chemical composition, temperature, microbial content, acclimation). This makes it difficult to extrapolate data derived from laboratory tests to that actually experienced in the various environmental compartments.

Fate and degradation rates can be estimated using computer programmes. With the development of more powerful computers and sophisticated software, it is now possible to predict the persistence of certain chemicals using models. These models may be divided into two different categories:

- 1. Quantitative Structure Activity Relationships ((Q)SARs), which provide estimations of biotic and abiotic chemical processes;
- 2. Partitioning models, based on fugacity or multi-compartment models, which are used to assess the overall persistence of a compound.

The use of models offers a more convenient way of assessing a substance's persistence than laboratory or field studies, and this approach is often used as a first stage screening process. The main disadvantage with models is that their level of sophistication is directly proportional to the amount and quality of input data required, and hence the accuracy of their predictions can be poor.

An ECETOC Task Force (TF) was commissioned with the following Terms of Reference:

- Review the definitions (e.g. EU TGD, UNEP, OSPAR, Environment Canada, Swedish Chemical Policy) of persistence of natural and synthetic chemicals in the environment, and examine the criteria on which the definitions are based. Include rate(s) of degradation/dissipation, and loss to sinks that remove carbon from the carbon cycle;
- propose a scientifically sound definition of persistence;
- discuss the validity of the half-life concept for biotic and abiotic degradation and the extrapolation process to persistence in a real multi-compartment environment versus a single environmental compartment. All degradation and dissipation processes should be accounted for, together with the effects of environmental conditions on persistence;

- review and analyse critically existing biotic and abiotic tests of degradation and default values and their uses in regulatory context (e.g. EU TGD, UNEP, OSPAR, Environment Canada, Swedish Chemical Policy). Particular reference should be made to the way in which extrapolations are derived from laboratory tests in defining/predicting persistence in the environment. The factors which may influence the validity of the extrapolation process (e.g. temperature, concentration, adaptation, microbial population changes, biotransformation, etc.) should be fully explored. The strengths, limitations and weaknesses of the current approaches should be illustrated with examples using laboratory and field data. A comparison of default/trigger values with actual laboratory and field data should be carried out;
- discuss possible improvements of existing test methods to better predict persistence in order to contribute to appropriate science based regulatory action;
- propose an improved strategy for predicting environmental persistence (global or single compartment) based on better scientific knowledge/relevance and/or novel testing methodology to address the needs of regulators and other stakeholders;
- review the validity of existing multi-compartment models as tools to predict persistence globally.

2. DEFINITIONS OF PERSISTENCE AND PERSISTENCE CRITERIA

This section explores the definition of persistence with respect to chemical substances in the environment, and identifies the persistence criteria that are currently proposed for regulatory use.

2.1 Persistence

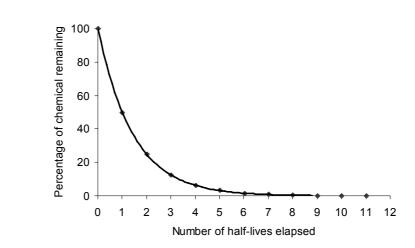
Persistence can be defined as 'the ability to continue steadfastly or obstinately despite opposition' or 'to continue without interruption' (Chambers Dictionary, 1999). The first of these two definitions is the most relevant for chemical substances in the environment since their presence is 'opposed' by abiotic and biotic degradation processes.

The International Council of Chemical Associations (ICCA) (ICCA, 2001) describes persistence as the ability of a chemical to stay unchanged in the environment for a long time, such that it can result in concerns for the environment because:

- Emissions or discharges of the chemical into the environment are only removed slowly, so the amount in the environment can gradually build up to a level that can cause problems.
- The chemical can remain in the environment long enough to be transported long distances from the point of emission or discharge possibly to more sensitive regions.
- Slow removal from the environment means that, even when emissions or discharges are reduced or stopped, environmental concentrations of the chemical will take a long time to fall back to background or non-effect levels.

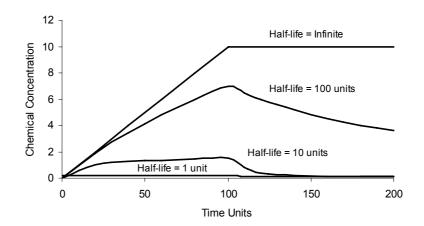
ICCA (2001) also observed that the persistence of a chemical is usually expressed in terms of an environmental half-life, i.e. the time it takes for half the initial amount of chemical to be removed from the environment. The data in Figure 2 show the disappearance of a chemical with time according to first order kinetics. After five half-lives, the amount of the chemical remaining is small, for example, a chemical with a half-life of six days in water will be reduced to only 3% of its initial concentration after a month, while a chemical with a half-life of 70 days, will take a year to reduce in concentration to only 3% of its initial value. The degradation curve shown in Figure 2 is based on the assumption that degradation obeys first order kinetics and that there is no lag phase (or adaptation period) prior to the start of degradation.





The influence of half-life on residence time in the environment is illustrated in Figure 3 (ICCA, 2001). This demonstrates how the environmental concentrations of substances with different half-lives change as a fixed amount of the substance is continually released into the environment. The maximum environmental concentration reached for each substance depends on its half-life. Substances with short half-lives (1 - 10 time units) soon reach a balance between emission and removal at a characteristic (steady-state) environmental concentration. For substances with short half-lives once emissions cease, after 100 time units, the environmental concentration diminishes towards background levels. However, for substances with long half-lives, the environmental concentration decreases at a much slower rate. Half-life can therefore provide a useful measure of persistence since it provides a quantitative way of assessing the length of time a substance may remain in the environment after release, and where it is likely to accumulate.

Figure 3: Influence of half-life on residence time in the environment (emissions cease after 100 time units)



The following considerations need to be taken into account in using half-life data to assess persistence in the environment:

- What is the appropriate method to provide the half-life value ?
- For which environmental compartments are half-lives needed ?
- What is the overall half-life in the environment ?
- Is there an adaptation period and, if so, how long is it?
- Do first order kinetics describe the degradation ?
- Is there a better alternative ?

Persistence will be dependent on the release pattern of the substance and its subsequent distribution between the different environmental compartments. Its distribution will reflect both the physico-chemical properties of the substance and the environmental compartment. This complexity is another reason why it is difficult to correlate the results from standard laboratory degradation tests with observations made in the environment. Some information may be provided by a combination of modelling and monitoring data. These models are discussed in Section 7 of this report.

2.2 Persistence criteria in EC

This section describes the approach and criteria used by EC regulators to assess whether or not a substance is considered to be sufficiently persistent in the environment to present a problem.

Within the EC, there are a number of initiatives that regulate PBT compounds, for example the Water Framework Directive (EC, 2000a) and the PBT Management Strategy. The Existing Substances Regulation (ESR), the Notification of New Substances Regulations (NONS, 1993) and the revision of the Technical Guidance Document (TGD) (EC, 2003) are highly relevant, these will be considered below.

The Water Framework Directive aims to improve the way in which the quality of the aquatic environment is characterised. The Combined Monitoring-based and Modellingbased Priority Setting Scheme (COMMPS) has been used to prioritise chemical parameters, leading to a ranking of exposure based on monitoring and model predicted data. Persistence was one of the criteria that formed part of the algorithm used for ranking substances in the modelling part of the approach. COMMPS has resulted in the selection and listing of a number of priority hazardous substances which must be addressed under the Water Framework Directive. The EC is also taking a major initiative on PBT and 'very persistent, very bioaccumulative' substances (vPvB) and has proposed an interim strategy for dealing with such substances (EC, 2001). The objective of this strategy is to develop a coherent approach for identification and management of PBT and vPvB substances in the EC. The strategy document reviewed current international approaches on PBT management (*viz* those in the USA, Sweden, Canada, the Netherlands and UNEP and OSPAR) and the way in which current EC chemical policy manages new and existing substances with these properties. The review identified a number of problems with the existing EC approach, namely that:

- The process is cumbersome, resource intensive and slow;
- the lack of data about intrinsic properties and environmental concentrations of most existing substances impedes identification of PBT and vPvB substances;
- estimation of overall released quantities is slow and difficult;
- tracing the sources of persistent accumulating substances is often not possible.

As a consequence, three steps are being taken which are of particular relevance for the current review:

- Development of PBT and vPvB testing strategies;
- identification of potential PBT or vPvB substances using screening data and screening estimation techniques ((Q)SARs) for substances for which relevant data are missing;
- verification of PBT or vPvB properties by additional testing.

Proposed criteria for identification of PBT and vPvB substances

The EC review concluded that although there is no clear-cut scientific answer to which substances should be regarded as a PBT or vPvB substance, there is some scientific consensus, particularly on what constitute adverse PBT properties. The criteria proposed are summarised in Table 2.

	PBT - Criteria	vPvB - Criteria
P	Half-life >60 days in marine water or >40 days in freshwater or half-life >180 days in marine sediment or >120 days in freshwater sediment	Half-life >60 days in marine or freshwater or half-life >180 days in marine or freshwater sediment
В	BCF > 2000	BCF > 5000
T	Chronic NOEC < 0.01 mg l ⁻¹ or CMR or other evidence of long-term mammalian toxicity (e.g. endocrine disrupting effects)	Not applicable

Table 2: EC criteria for PBT and vPvB substances

The testing strategy for the persistence, P, criterion appears to be almost identical to that proposed by OSPAR (see below) with the following exceptions:

Experimental data on persistence: Substances with a half-life in marine water > 60 days or a half-life in marine sediment > 180 days are considered to be persistent. If marine data are not available then freshwater half-life data can be used, in which case, a criterion of > 40 days for water, and > 120 days for freshwater sediment, implies persistence. This is because degradation in marine waters is expected to be slower.

2.2.1 EC Notification of New Substances (NONS) and Existing Substances Regulations (ESR)

Environmental risk assessments of new and existing substances is required by these regulations and comprehensive guidance for these is provided in the EU TGD (EC, 2003). The 1st edition of the TGD (EC, 1996) makes no specific reference to persistence, but refers to 'transformation processes of a substance in the environment and in organisms as affecting the fate of a substance'. It states that 'since measured data on degradation processes for different compartments are usually not available, they must be extrapolated from standardised laboratory tests'. Rate constants for abiotic (hydrolysis and photolysis in water and photochemical reactions in the atmosphere) and for biotic (biodegradation in sewage treatment works, surface water, sediment and soil) processes are derived using data from standard laboratory tests. These should preferably be conducted using standardised (e.g. OECD) test procedures performed according to good laboratory practice (GLP). Substances which fail an inherent biodegradability test are considered to be persistent in the environment, and are allocated a first order rate constant of zero. The problem with adopting such an approach is that laboratory test systems do not reflect adequately environmental conditions and the data provided are not suitable for assessing a chemical's persistence. The interpretation of test result data in the TGD (EC, 1996) is shown in Tables 3 and 4.

Test result	Soil half-life (days)		
	Kp soil ≤ 100	Kp soil >100 ≤ 1000	Kp soil >1000 ≤ 10000
Ready biodegradable	30	300	3000
Ready but failing 10-day window	90	900	9000
Inherently biodegradable	300	3000	30000
Not biodegradable	Infinite	Infinite	Infinite

Table 3: Recommended mineralisation half-lives (days) for use in terrestrial riskassessment when only screening data are available

The interpretation for soil is dependent on the sorption properties of the chemical, since degradation is assumed to take place only in soil porewater and adsorbed chemical is considered to be unavailable.

Test result	Aquatic half-life (days)			
	Freshwater	Estuaries	Other marine environments	
Ready biodegradable	15	15	50	
Ready but failing 10-day window	50	50	1 <i>5</i> 0	
Inherently biodegradable	150	150	Infinite	
Not biodegradable	Infinite	Infinite	Infinite	

Table 4: Recommended half-lives (days) for biodegradation in water based on results of screening tests on biodegradability

The half-lives in Table 4 are provisional recommendations, and in the TGD, it is proposed that they should be reconsidered when sufficient data for degradation of different substances in screening and simulation tests have been evaluated. The basis for the recommendation is the assumption that, in general, the degradation of xenobiotics in freshwater and estuaries can be described by similar degradation rates, whereas degradation rates are considered to be lower in other marine environments more distant from the coastline. Adoption of this proposal would lead to the situation that a substance that passed a ready-biodegradability test, but failed the 10-day window, would be considered to be persistent in the marine environment.

It should be noted that the TGD has been revised recently (EC, 2003) and now incorporates specific PBT criteria for marine risk assessment. These are based on the EC interim strategy for dealing with such substances (EC, 2001). As part of the TGD review, it is proposed that the PBT and vPvB criteria in Table 2 be incorporated into the environmental risk assessment procedures for new and existing chemicals.

The revised TGD (EC, 2003) indicates that for most substances, the available data will not allow a definitive answer to the question of whether or not a substance should be considered under the PBT-assessment. The TGD therefore proposes the use of screening data to identify whether the substance has a potential to possess PBT properties. The testing strategies proposed are based largely on standard screening data but should be investigated accordingly. In deciding which information is requested (on P, B or T), care should be taken to avoid animal testing wherever possible. This implies that when further information is needed for several properties of a substance, the assessment should be focussed first on clarifying the potential for persistence. When it is clear that the Pcriterion is fulfilled a stepwise approach should be followed to evaluate next the B-criterion, and finally toxicity testing to clarify the T-criterion.

The TGD recommends that the assessment of the (potential for) persistency in the marine environment should be based in principle on actual half-life data, determined under marine environmental conditions. Depending on whether a substance has a half-life less or greater than the cut-off criterion, it is decided if a substance meets the P-criterion.

When these key data are not available, other types of information on the degradability of a substance can be used to decide if further testing is needed to assess the potential for persistence. In this approach three different levels of information are defined according to their perceived relevance to the criteria:

- Experimental data on persistence in the marine environment;
- other experimental data;
- data from biodegradation estimation models.

The type of information that is relevant within these levels, and the relevant cut-off values, are described in the revised TGD (EC, 2003). However while the TGD looks to focus on marine data, of which there are few, the use of other data is confined almost entirely to standard ready and inherent biodegradability test data. Table 5 summarises how the TGD proposes to use biodegradability data to assign persistence. The revised TGD (EC, 2003) also indicates that marine biodegradability data should override freshwater biodegradability data extrapolated to the marine environment.

Type of data	Criterion	Definitive	Screening
		assignment	assignment ¹
DT ₅₀	>60 d	vP*	-
DT ₅₀ freshwater ²	>40 d	P ³	-
	>60 d	vР	-
DT ₅₀ marine sediment	>180 d	vР	-
DT ₅₀ freshwater sediment ²	>120 d	P ³	-
	>180 d	vР	-
Readily biodegradable ⁴	Yes	Not P	-
	No	-	P or vP
Inherently degradable	Yes	Not P ⁵	-
	No	-	P or vP
QSAR	Non-linear model prediction <0.5 or MITI non-linear model	-	P or vP
	prediction <0.5 and ultimate		
	biodegradation timeframe		
	prediction <2.2		

Table 5: Overview of P-assignment for different types of biodegradation data

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 TGD (EC, 2003) are fulfilled.

- vP = Very Persitent

2.2.2 OSPAR

In 1998 the OSPAR Ministerial Meeting agreed on an OSPAR Strategy with regard to Hazardous Substances (OSPAR, 1998a), which sets out, *inter alia*:

- A definition of hazardous substances;
- the objective of the OSPAR strategy with regard to hazardous substances;
- the timeframe in which this objective should be achieved.

In Annex 5 of this strategy, a substance is defined as 'persistent' if its conversion, or the conversion of its degradation products, is slow enough to permit long-term occurrence and widespread distribution in the marine environment. This definition brings into play the concept of degradation products that may be more or less persistent than the parent substance.

At the same meeting, Ministers declared in the Sintra Statement (OSPAR 1998b) that the OSPAR Commission would:

- Develop a dynamic selection and prioritisation mechanism (DYNAMEC) in order to tackle initially the substances and groups of substances of most concern;
- use this mechanism to up-date, by 2000, the current OSPAR List of Chemicals for Priority Action, (comprising 15 substances/ groups of substances).

In line with the provisions, definitions and requirements set out in the strategy, the purpose of the DYNAMEC mechanism can be described as a tool to enable the OSPAR Commission, in a transparent manner and on the basis of sound scientific information, to:

- select those hazardous substances that need to be addressed by the Commission under the strategy;
- identify the hazardous substances which should be given priority in OSPAR's work.

The DYNAMEC mechanism consists of a variety of inter-related steps and procedures, which use the P, B and T criteria (OSPAR, 2000) for the substances of interest. The application of these criteria should both reflect the hazardous characteristics of substances or groups of substances, and give priority to their actual or potential occurrence and effects in the marine environment. PBT criteria are now in place for individual chemicals, and have been used as part of the process to draw up a list of priority hazardous substances (OSPAR, 2000).

Criteria for persistence in the OSPAR Strategy with regard to Hazardous Substances

The persistence of a substance reflects not only the potential for long-term exposure of organisms, but also the potential for the substance to reach the marine environment and be transported to remote areas. To assess persistence in the marine environment, in the context of the OSPARStrategy with regard to Hazardous Substances, an approach was suggested that allowed the use of different types of available information on the biodegradability of a substance. In this approach, three different levels of information were defined:

Level 3: experimental data on persistence in the marine environment;

Level 2: other experimental data, mostly from standard biodegradation tests;

Level 1: data from biodegradation estimation models.

It must be noted that this proposal reflects existing knowledge on biodegradation, and was considered by the Informal Group of Experts (IGE) as a pragmatic approach to make optimal use of the available data and methods. The IGE also considered that more research was needed to provide a better estimate, from existing biodegradation tests, of persistence in the marine environment. Moreover, where relevant, other degradation mechanisms such as hydrolysis and photolysis should be taken into account.

The implication of this procedure (from level 2), is that substances that pass a ready biodegradability test are considered to have an environmental half-life in freshwater and seawater of less than 50 days. Substances that do not fulfil the pass criteria for ready biodegradability, but meet the criteria for inherent biodegradability, are considered to have a half-life of greater than 50 days in freshwater and seawater.

This tiered approach in the use of information relating to the persistence of a substance is recommended by the IGE, since the use of data relating to degradation under environmental conditions, must be preferable to the use of standardised laboratory test data or estimated data. However, it should be noted that level 3 data are rarely available and interpretation for risk assessment can be difficult due to their site-specific nature.

The majority of the criteria proposed by OSPAR have now been incorporated into the revised TGD.

2.2.3 Overview of persistence criteria

The persistence criteria proposed by various organisations are summarised, as halflives, in Table 6. However, a half-life (t¹/₂) is the term used to characterise the rate of a first order reaction. Within a biodegradation context, a half-life time t₅₀ is the time taken, excluding lag phase, to reach 50% degradation. For first order kinetics t₅₀ = t¹/₂. Determination of the scientific basis for these persistence criteria is not clear-cut. For example, from a scientific perspective, there is no obvious cut-off point that clearly identifies a half-life of 2 months as the best (or most appropriate) persistence criterion for water. The UNEP POPs criteria have been widely used as the basis for other, more recent, persistence criteria, and were established by considering the environmental persistence of the twelve POPs. Since there is widespread scientific consensus that POPs are persistent substances, it would seem reasonable to use the data addressing their abiotic and biotic degradation as a starting point for development of generic persistence criteria.

There are a number of questions to be answered in developing a 'universal' set of persistence criteria:

- 1. What is the objective for setting the persistence criteria? Is it to
 - Screen large numbers of compounds to prioritise those requiring a more detailed (risk) assessment?
 - Select chemicals whose entry to the (marine) environment is undesirable?
- 2. What are the relevant environmental compartments that should be included?
 - Atmosphere (water, particles)?
 - terrestrial (soil, porewater)?
 - freshwater (water, sediment)?
 - marine (water, sediment)?

In particular should water and sediment be considered as separate compartments ? and should criteria for half-lives in soil and sediment be different?

- 3. What test methods will be used to determine compliance with the persistence criteria?
- 4. If there are several values of half-life, some of which are above a persistence threshold and some below, which value should be used?
- 5. What default approaches will be used in the absence of test data?
- 6. Are the criteria used for the different purposes in 1 and 2, and/or derived by test or default approaches, consistent?

Organisation	Air	Water	Soil	Sediment	Test result	Other
OSPAR (selection V)		T½ > 50 days			Not readily biodegradable	
UNEP	T/2 > 2 days	T½ > 2 months	T½ > 6 months	T//2 > 6 months		otherwise sufficiently persistent
				to be of concern		
EC PBT strategy		T½ > 60 days in		T½ >180 days in		
		marine water or		marine sediment or		
		T1⁄2 > 40 days in		T½ >1 20 days in		
		fresh water		freshwater sediment		
EC vPvB strategy		T½ > 60 days in		T½ >180 days in		
		fresh water		freshwater sediment		
EC ESR/NONS					Not inherently	the TGD is being revised and
					biodegradable	will incorporate the PBT and
						vPvB criteria
EC VMP			T1/2 > 30 days			
			(phase 1)			
			T½ > 60 days			
			(phase 2, tier A)			
EC PP	T1/2 > 2 days	T½ > 2 months	T½ > 6 months	T/2 > 6 months		
UK DETR		T½ > 2 months	T½ > 6 months	T½ > 6 months	Not readily biodegradable chemicals raised equivalent	reasons to believe that the
		concerns				
Environment Canada	T1/2 > 2 days	T½ > 182 days	T½ > 182 days	T1⁄2 > 365 days		
US-EPA OPPT		T½ > 2 months	T½ > 2 months	T// ₂ > 2 months		three tier test strategy - ready
		(controls)	(controls)	(controls)		biodegradability, shake-flask
		T½ > 6 months	T½ > 6 months	$T/_2 > 6$ months		die-away, sediment/water microcosm
		//	1 1	1		

Table 6: Summary of persistence criteria

2.3 Conclusions

There are many regulatory persistence criteria (Table 6) for application to substances, but very few definitions of persistence (Table 7).

Table 7: Definitions of a persistent substance

Origin	Definition
UNEP POPs	"hazardous chemicals that resist degradation by physical, chemical or biological pathways."
OSPAR	"its conversion or the conversion of its degradation products is slow enough to permit long- term occurrence and widespread distribution in the marine environment."
ICCA	"the ability of a chemical to stay unchanged in the environment for a long time."

Quantitatively, regulators currently define persistence in terms of the half-life of a substance in the environment. A substance can have a half-life for each environmental compartment and an overall value for the environment (see Sections 7 and 8).

Consideration of the existing criteria (see Sections 2.2.1 and 2.2.2), many of which were developed using a group of technical experts, suggests that there are some differences in the views of experts on what are appropriate persistence criteria. Combining of the quantitative definitions of persistence (or persistence criteria) adopted by the organisations cited above, produces the following extremes of half-lives (Table 8).

Table 8: Least and most precautionary criteria proposed for persistence

					- h
	Air	Freshwater	Seawater	Soil	Sediment
Least	T½ > 2 days	T½ > 182 days	T½ > 182 days	$T^{1/2} > 6$ months	T½ > 365 days
precautionary					
Most	T½ > 2 days	T½ > 40 days	T½ > 50 days	T½ > 30 days	$T^{1/2} > 2$ months
precautionary	,				

There is consensus for the air compartment on the appropriate persistence criterion to be used, namely a half-life of two days based on long-range transport considerations. For the other compartments, the differences range from a factor of about 3.5 for seawater to 6 for sediment. A half-life of 30 days will result in a decrease to less than 0.1% of the initial concentration over a period of one year, whereas a half-life of a year will result in a decrease to 50% of the initial concentration. Clearly neither of these values is right or wrong, but a shorter half-life will lead to a smaller propensity for a substance to accumulate in the environment. In the first instance the issue should not be focused on assigning a half-life to describe persistence, it should be consideration of how persistence is measured or inferred.

The biggest problem is not associated with the half-life values given above; it is with how they are assigned. The current regulatory approaches are focused on standard biodegradability tests largely developed as screens to identify substances that are not persistent. The recent revision of the TGD (EC, 2003) states that when assessing persistence, their method reflects existing knowledge on biodegradation and should be considered as a pragmatic approach to make optimal use of the available data and methods. This approach is advocated by ECETOC via the strategy proposed in the remainder of this report.

ECETOC proposes the following definition of a persistent substance based on the discussions within this section.

'A persistent substance is one that is resistant to abiotic and/or biotic degradation under both aerobic and anaerobic conditions'.

This definition has no 'quantitative' element to indicate how long a substance must persist for that persistence to be significant. This quantitative aspect is developed later in this report.

3. FACTORS AFFECTING PERSISTENCE: LABORATORY AND FIELD CONSIDERATIONS

3.1 Introduction

The persistence of a chemical in the environment is determined by the intrinsic properties of the substance, the physico-chemical characteristics of the system or environmental compartment, and the ability of the chemical to undergo degradation. Clearly, the ability of a chemical to be degraded abiotically is related intimately to the intrinsic properties of the substance and the physico-chemical characteristics of the system or environmental compartment (see Section 6). This section will examine the factors that influence the rate and extent of biodegradation in the laboratory and field. These factors create the uncertainty in extrapolating between laboratory tests and environmental compartments, and in predicting whether a substance persists in the environment or not. Before considering biodegradability testing strategies, these factors need to be considered with respect to inferring persistence. Issues to be considered include:

- Persistence and ready biodegradability
- the half-life concept;
- microbial diversity, growth and adaptation;
- substance concentration;
- cometabolism;
- temperature;
- oxygen content;
- bioavailability;
- metabolism by higher organisms.

3.2 Persistence and ready biodegradability

Legislation and environmental policy makers apply 'pass' or 'fail' criteria to standardised biodegradability tests measuring percentage biodegradation over a specified period of time. Subsequently, default rate constants are then applied to these pass/fail criteria to produce single medium environmental half-lives. This is described in detail in Section 2. However, it must be emphasised that whilst the scientific basis for this approach and the default rate constants in current use cannot be supported scientifically, it may still offer the most practical approach for handling ready biodegradability test data. Specific concerns related with the application of environmental half-lives to such data are addressed later within this section.

There is currently no formal mechanism within ready biodegradation test methodologies for calculating the persistence of a substance, since rate constants are not measured directly. However, it is possible for rate constants to be derived from these standard tests with only minor modifications. The modifications relate to the number of data points, as no precise sampling regime is specified (other than to carry out a sufficient number of samples to allow the percentage removal in the 10-day window to be assessed). It is questionable as to whether there is any merit in attempting to derive kinetic rate constants, first-order or any other kinetic description, for such biodegradability studies.

As defined by OECD (1992a), ready biodegradability describes substances that would undergo rapid and ultimate degradation in all environmental compartments. These tests serve this purpose well, and whilst existing pass criteria may be too stringent for studies with respirometric endpoints (60% for CO_2 evolution and oxygen demand), the current default rate constants for substances passing these tests may be adequate upon refinement in the light of increased comparisons between laboratory and field data. Consequently, the need to provide measured rate data from such tests is difficult to justify. These tests are simple screening procedures and a 'pass' in one of these tests, or one with only minor modifications, should be used to assign a substance as nonpersistent.

Substances that fail to meet the 'pass' criteria for ready biodegradability, still have the potential to undergo biodegradation in the environment. Consequently, no substance should be designated 'persistent' based only on the negative outcome of a ready biodegradability study, and any test-based strategy to assess the persistence of a substance in the environment needs to consider this point carefully (see Section 8). Given the stringent nature of these screening tests, the majority of substances fail to meet the criteria for ready biodegradability. For these substances there are two clear requirements:

- Data generated from inherent biodegradability studies needs to be treated in a more pragmatic manner (see Sections 4 and 8);
- a series of new or modified tests are required that can demonstrate the potential for biodegradation to occur whilst maintaining environmental realism (see Section 5).

3.3 The half-life concept

The concept of half-life time originates from the description of the physical phenomenon that radio-active isotopes show an exponential decline of the intensity of radiation over time. The application of this concept for biological processes has also become widely used in pharmacology, as biological half-lives are frequently used to express the disappearance of a drug from the body.

The use of the half-life concept for biodegradation in the environment has become familiar in the context of pesticide residues in soil. In some cases the concept fits well with empirical data because the rate of biodegradation is dependent on the rate of de-sorption, and this is a physical phenomenon that follows a first order type of reaction. Alexander (1999) reviewed the application of kinetic expressions to biodegradability data. The persistence of chemicals is almost always expressed in terms of the biological half-lives (see criteria in Section 2). There are a number of reasons why the concept of biological half-lives may not always apply for characterising the biodegradability of chemicals. In particular:

- Biodegradation kinetics show a lower and an upper threshold in relation to the substance concentration;
- at low concentrations of the substance the appropriate micro-organisms may be unable to maintain themselves. A minimum energy profit is necessary to support maintenance energy and compensation of losses or;
- by growth on other substances (co-metabolic growth and degradation) kinetics can follow a zero order type of reaction;
- at relatively high concentrations of the substance the enzymatic systems will be saturated. Biodegradation rates at that level will be zero order for the substance concentration;
- in between these upper and lower threshold levels, the rates will normally follow a second order type of kinetics. Thus, the rate is not only dependent on the substance concentration, but also on the density and growth of the relevant bio-mass fraction (competent organisms) in the system;
- in dynamic systems such as rivers or treatment plants, the capacity for biodegradation is a result of adaptation of the micro-flora to the dynamics of the system. The dynamics of the population (e.g. the required relative growth rate to compensate all losses) will determine the residual concentration of the substance at a steady state. Systems will adapt to any load until this residual concentration is reached. Thus the biological half-life will be variable and dependent on the load and the system dynamics;
- current biodegradation test methods use substrate concentrations in the range 10 100 mg l⁻¹ or where radio-labelled samples are used, at the 50 μg l⁻¹ level. However in the freshwater and marine environments the majority of substances are in the ng l⁻¹ range. As a consequence, the kinetic interpretation of die-away curves in laboratory tests refers to the zero order for substance (above the upper threshold and first order for specific bio-mass density), whereas the environmental conditions refer to the second order condition or zero order condition below the lower threshold;
- if first order rates are observed in heterogeneous systems (soil, sediment, suspended solids, solid surfaces) these refer often to physical processes such as de-sorption or mass transfer between liquid and solid. These rate constants are the result of the property of the environment (e.g. mass of adsorbent, turbulence) in combination with the adsorptive characteristics of the substance;

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. 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 for describing a measurement of biodegradability or persistence. A kinetic description is required that reflects the overall distribution of rates in the environment, independent of the type(s) of reaction kinetics operating (Sections 7 and 8).

3.4 Microbial diversity, growth and adaptation

3.4.1 Diversity

Micro-organisms dominate the biogeochemical cycles with an immense biodiversity and functional diversity. Current estimates suggest that only approximately 5% of the total number of micro-organisms in the environment have been described (Cloete, 1997), reflecting man's ability to culture only a small fraction of the wider microbial community. Consequently, when working with laboratory cultures, such as are used in biodegradability tests, only a narrow range of microbial species and functional capabilities will be present. The impact of scaling effects due to working with low total test volumes compounds this further and can result in highly variable test data (Thouand *et al*, 1996).

The fact that distinct variations occur in the ability of an inoculum to biodegrade a substance, means that the persistence of a compound will be strongly influenced by the organisms with which it comes into contact. This is a crucial point when considering the biodegradation and persistence of compounds in either laboratory tests or in the environment as a whole. For example, a chemical may persist in the environment because it does not come into contact with an organism capable of degrading it, or the species present are not capable of adapting to degrade it. Under laboratory test conditions the same may be the case.

Microbial cells contain large quantities of molecules from simple monomers or building blocks to complex polymers. Most monomers or building blocks are acquired from the environment in an available form, or synthesised within the cell from simpler molecules.

In contrast, larger molecules or macromolecules are always synthesised in the microbial cell. Water accounts for approximately 90% of the wet cell weight and macro molecules a further 9.6%. Table 9 summarises the dry cell weight composition of a typical prokaryotic cell based on studies of *Escherichia coli* and *Salmonella typhimurium* (Neidhardt *et al* 1996).

Molecule	% Dry cell weight	Molecules per cell	Different types
Macro-molecules	96	24,610,000	2,500
Protein	55	2,350,000	1,850
Polysaccharide	5	4,300	2
Lipid	9.1	22,000,000	4
DNA	3.1	2.1	1
RNA	20.5	255,500	660
Monomers	3.5		350
Amino acids and precursors	0.5		100
Sugars and precursors	2		50
Nucleotides and precursors	0.5		200
Inorganic ions	1		18

Table 9: Chemical composition of a typical microbial cell

As discussed above, most micro-organisms acquire their nutrients directly from the environment. Table 10 compares the principal nutrients obtained from the environment with those generally provided within laboratory systems. In addition to the macronutrients outlined in Table 10, microbial cells also require trace nutrients and vitamins. Failure to provide all the essential nutrients for any specific microbial population (including cometabolites), together with not establishing the correct physiological conditions (e.g. pH, temperature,), will result in no microbial growth (for growth-linked biodegradation) or cometabolism (for non-growth-linked biodegradation). Consequently, assessing biodegradation under highly defined nutrient and physiological conditions may be restrictive.

Element	Natural sources found in the environment	Chemical amendments to mineral media
Carbon	CO ₂ or organic compounds	Test chemical, glucose, malate, acetate, pyruvate, etc
Hydrogen	H ₂ O, organic compounds	H ₂ O, test chemical (if it contains hydrogen) or other organic compounds
Oxygen	O ₂ , H ₂ O, organic compounds	O ₂ , H ₂ O, test chemical (if it contains oxygen) or other organic compounds
Nitrogen	NH ₃ , NO ₃ ⁻ , N ₂ , organic nitrogen compounds	Inorganic: NH ₄ Cl, (NH ₄) ₂ SO ₄ , KNO ₃ , N ₂ Organic: Amino acids, nitrogen bases of nucleotides, nitrogen-containing organic compounds
Phosphorus	PO ₄ ³⁻	KH ₂ PO ₄ , Na ₂ HPO ₄
Sulphur	H ₂ S, SO ₄ ²⁻ , organic sulphur compounds, metal sulphides	NaSO ₄ , Na ₂ S ₂ O ₃ , Na ₂ S, cysteine or other organic sulphur compounds
Potassium	Aqueous K ⁺ , potassium salts	KCl, KH ₂ PO ₄
Magnesium	Aqueous Mg ²⁺ , magnesium salts	MgCl ₂ , MgSO ₄
Sodium	Aqueous Na ⁺ , NaCl or other sodium salts	NaCl
Calcium	Aqueous Ca ²⁺ , CaSO ₄ or other calcium salts	CaCl ₂
Iron	Aqueous Fe ²⁺ or Fe ³⁺ , FeS or other iron salts	FeCl ₃ , FeSO₄

Table 10: Chemical nutrients in nature and mineral media

3.4.2 Microbial Growth

Population growth is an essential component of microbial function, as any given cell has a finite life span. Consequently, a species can only be maintained in a given environment as a result of continued population growth (Madigan *et al*, 1997). In laboratory experiments using batch cultures, microbial growth occurs at the expense of the test chemical (growth-linked biodegradation). In microbiological terms, such tests represent simple enrichment cultures that encourage the growth of a competent microbial population. Such culturing techniques usually result in the enrichment of several competent micro-organisms that out-compete the wider microbial community present in the original inoculum. This competition is exaggerated in standard biodegradation tests through the use of a single growth-limiting test substance at relatively high concentrations.

Microbial growth can be divided into four distinct phases. These are:

- Lag phase;
- exponential phase;
- stationary phase;
- death phase.

Environmental and other nutritional factors can significantly affect any of these stages. Although in many cases it is the lag phase and exponential phase for growth-linked degradative processes that are the dominant factors when considering persistence. It must also be recognised that substances can be biodegraded in the absence of growth. Non-growth-linked degradative processes are often referred to as cometabolism or secondary substrate utilisation. Cometabolism will be considered later within this section.

3.4.2.1 Lag phase

Growth and mineralisation usually proceed after an initial lag period, which may be as brief as a few minutes or hours, or as long as a few days or several months. The length of the lag period is difficult to predict, and the exact events occurring during a lag period cannot always be explained. In addition, it can be difficult to relate a lag period observed in laboratory culture to microbial activity in the field. The length of a number of lag periods reported in the literature are summarised in Table 11.

Table 11: Lengths of lag phases reported for several organic chemicals (Alexander, 1994)

Chemical	Environment	Length of lag phase
Numerous aromatics	Soil	10 - 30 h
Dodecyltrimethylammonium chloride	Fresh water	24 h
4-Nitrophenol	Water-sediment	40 - 80 h
Amitrol	Soil	7 days
Chlorinated benzenes	Biofilm	10 days - 5 months
DNOC	Soil	16 days
Pentachlorophenol	Stream water	21 - 35 days
Mecoprop	Enrichments	30 - 37 days
NTA	Estuary	50 days
Halobenzoates	Anaerobic sediments	3 weeks - 6 months
2,4,5-T	Soil	4 - 10 weeks

Within a biodegradation study, the lag phase is observed by a delay in oxygen uptake, removal of dissolved organic carbon (DOC) or CO_2 production. Specific events that may contribute to, or be occurring during the lag period may include (Alexander, 1994; Hales *et al*, 1996):

- Enzyme induction;
- proliferation of competent populations from a low initial concentration;
- development of microbial consortia;
- predation by protozoa;
- horizontal gene transfer of catabolic capabilities on plasmids;
- genetic mutation.

A lag phase is only absent when exponentially growing cells are transferred or subcultured between identical media and conditions. The requirement for using a nonadapted inoculum in an OECD ready biodegradation study usually results in the presence of a lag phase, even though it may not be observed due to low sampling frequency. Extended lag periods that result in a chemical failing an OECD ready biodegradation study, may result in the chemical being classified as persistent under some legislative proposals.

OECD describes the ready biodegradation tests as having little potential for adaptation to occur due to the low biomass associated with the test and the 28-day duration. However, the use of a relatively high test chemical concentration (up to 100 mg l⁻¹) together with a low concentration of biomass (2 - 30 mg dry suspended solids per litre) does exert a powerful selective pressure for growth of the fastest competent micro-organisms and adaptation. The 28-day test period does restrict the specific types of adaptation that may be manifest by the presence of a lag phase. Within this time frame the appearance of new microbial genotypes is highly unlikely. However, the formation of new genotypes, and the acquisition of plasmids containing catabolic genes, may be the most important process of adaptation involved in overcoming persistent chemicals in the environment. Consequently, when predicting the persistence of a chemical, test systems that encourage all types of adaptation, or test regimes using pre-adapted inocula must be considered. It is such systems that will offer the greatest environmental realism, and the least uncertainty, in extrapolation from laboratory to field.

Enzyme induction

Micro-organisms produce numerous enzymes independent of presence of the specific test substance. These enzymes are known as constitutive enzymes and are usually involved in mainstream anabolic reactions. In contrast, inducible enzymes are formed only when its specific substrate, or a structurally related chemical or metabolite, is present. Many enzymes involved in the initial catabolic reactions during biodegradative processes are inducible, e.g. the dehalogenase enzymes that are responsible for the liberation of halide ions from halogenated aliphatic compounds (Alexander, 1994).

The enzyme induction process is usually rapid, yet lag phases can often last days or weeks. Thus it is unlikely that enzyme induction contributes to extended lag periods resulting in persistence, unless other nutrient or environmental factor are limiting (Richmond, 1968; Alexander, 1994).

Proliferation of competent populations from a low initial concentration

The stringent nature of standardised ready biodegradation tests is largely dictated by the introduction of a low biomass concentration. Consequently, the microbial biodiversity introduced into the test system may also be low, especially for test systems with a low total volume (≤ 100 ml). Alexander (1994) suggested that the lag phase was more apparent than real, and that the observed lag phase might be a function of the limit of chemical detection, rather than a biological phenomenon. This could be the case for OECD ready biodegradation studies, where the non-specific chemical endpoints used are relatively insensitive, and the limit of detection can be 10% of the theoretical maximum endpoint.

Where the observed adaptation period does reflect the need to establish a critical population size, in order to detect a change in chemistry, any changes that enhance the rate of microbial growth will reduce the length of the lag phase. The reverse is also true. Alexander (1994) has reviewed the possible causes for the failure of an inoculum to proliferate. These include:

- Absence of a competent degrader;
- limiting nutrients;
- suppression by predators and parasites;
- the need for cometabolites;
- chemical concentration was below the threshold concentration;
- need for an alternative carbon source;
- temperature, pH, salinity and redox conditions fall outside the physiological range of the competent micro-organism(s);
- presence of toxins;
- development of a microbial consortium.

It is widely perceived that a chemical is biodegraded by a single species of microorganism. Whilst this would give the competent micro-organism, with the fastest growth rate, a distinct competitive advantage, it is not always the case. For complete biodegradation of certain substances to occur, a consortium of micro-organisms is required. For example, Senior *et al* (1976) described a community of primary and secondary utilisers within a chemostat that degraded the herbicide Dalapon. This study also demonstrated the potential for genetic mutations to arise spontaneously in laboratory culture, where a secondary utiliser acquired an extant dehalogenase and became a primary utiliser.

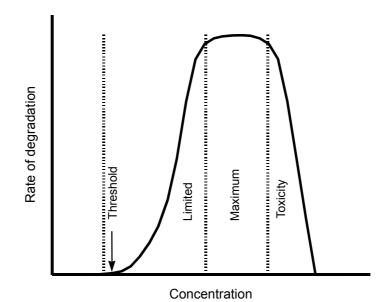
Standardised biodegradation tests, with a single input of a low biomass concentration within a small total test volume, restrict the potential for competent microbial consortia to be established, due to the introduction of a low microbial diversity. Consequently, a prolonged lag period may be observed, or biodegradation of the substance may not occur.

Chemical concentration

Both high and low substance concentrations can affect the length of a lag period. Figure 3 demonstrates the nominal response between substance concentration and the rate of biodegradation (or population growth).

The concentration of any given substance can be increased gradually until a threshold concentration is reached which represents the concentration at which biodegradative activity can be detected. Below this threshold point, competent microbial populations may not be able to sense the specific substance in the environment or derive sufficient maintenance energy from the chemical to survive. After this point, as the concentration of the substance increases, the rate of biodegradation (or growth) also increases. This relationship continues until a maximum rate of biodegradation (or growth) is reached. Above a certain concentration, the rate of biodegradation (or growth) starts to decrease, usually due to some form of toxic event. The relationship between substance concentration and the rate of biodegradation (or growth) is chemical specific. When testing for biodegradability, at a single chemical concentration, it is difficult to know exactly to which point of the curve the test relates (Figure 4). Testing at too high a concentration where toxicity or inhibition is occurring will reduce growth and biodegradation rates and may increase the length of the lag phase. Testing at too low a concentration may also produce a similar result.

Figure 4: An illustration of the relationship between the initial substance concentration and the rate of biodegradation



32 ECETOC TR No. 90

The concentration of a substance is also a major limitation on extrapolating laboratory data to the field situation. It has been postulated that concentrations of less than 1 µg l⁻¹ are not capable of inducing enzyme production (Hanne *et al*, 1993). A review by Alexander (1999), summarised in Table 12, supports this observation of Hanne *et al* (1993). The threshold for growth of micro-organisms capable of degrading chemicals is an important factor, which may impede the prediction from laboratory results (Zehnder and Schraa, 1988). The interaction between the substrate concentration and growth is not necessarily linear at low concentrations. Tests using 4-nitrophenol showed that at 10 µg l⁻¹ doses, a slow degradation rate was observed that did not increase upon addition of another $10 \,\mu g \,l^{-1}$. However, when the first dose was $60 \,\mu g \,l^{-1}$ or higher, a second dose was degraded at a higher rate (Van Veld and Spain, 1983). This factor is difficult to model, as often the subtraction of a lag phase is arbitrary and first order kinetics do not apply (Wesnigk *et al*, 2001). Even where microbial populations do exist that can degrade substances at low concentrations, the kinetics often differ from those at higher concentrations, with decreased rates and potentially reduced levels of mineralisation and more carbon being incorporated into biomass. Van Veld and Spain (1983) demonstrated this with 4-nitrophenol, where low concentrations were degraded after a lag period of 13 days, with slow mineralisation to a maximum of 60% after 35 days. At higher levels after the same lag phase duration, mineralisation was rapid, with a maximum of 70% being achieved after only 20 days. Measurements of bacteria capable of degrading 4-nitrophenol showed that there were only significant numbers present in samples containing the higher concentrations of test chemical, after the initial lag phase.

Chemical	Concentration (μg l ⁻¹ or μg kg ⁻¹)
2,4-D	2.2
Sevin	3.0
Aniline	0.1
4-Nitrophenol	1.0
2,4-Dichlorophenol	2.0
Styrene	2.5
Phenol	0.0015
Carbofuran	10 (soil)
2,4,5-T	100 (soil)
Dichlorobenzenes	0.2-7.1

Table 12: Reported	threshold	concentrations	(modified from A	Alexander, 1999)
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Biodegradation testing in the laboratory, using standardised protocols, is unlikely to use a chemical concentration below the threshold value. However, the existence of a threshold concentration is of great significance, and a factor that could result in the persistence of chemicals in the environment. All work to date has focused on the existence of threshold values for chemicals introduced as the sole or main carbon source into mineral media containing an excess of other essential elements. It is unknown if the threshold phenomenon is restricted to carbon sources or extends to include nitrogen, phosphorus and sulphur (Alexander, 1999). If threshold concentrations do exist in the wider environment, and current laboratory-based evidence suggests that these values are chemical specific, a balance between chemical monitoring, biodegradation testing and chronic toxicity testing needs to be reached.

Predation by protozoa

Numerous test systems use inocula derived from wastewater treatment plants and environmental waters, which contain a rich diversity of protozoan species. It is uncertain whether the lag period and microbial growth rate are affected by protozoan predation. Wiggins *et al* (1987) concluded that adaptation periods in many natural waters are unlikely to be affected by protozoa, however predation may contribute to intra-test variability associated with replicate flasks.

Horizontal gene transfer of catabolic capabilities on plasmids

In addition to the genetic material in a single circular chromosome, bacteria have other smaller genetic elements known as plasmids. Whilst plasmids are not considered to be crucial to the survival of the cell, they can play a role in biodegradation. Enzymes catalysing the degradation of specific compounds may be encoded by chromosomal and plasmid genes (Alexander, 1999).

Plasmids have been reported containing genes for the enzymes involved in the degradation of alkylbenzene sulphonate, benzoate, chlorobenzoate, chloroacetate, 2,4-D, naphthalene, phenanthrene and toluene. A number of these plasmids are transmissible, and can pass between different bacterial strains (Alexander, 1994; 1999).

The exchange of plasmids between bacteria is likely to be occurring throughout a biodegradation study, although no attempts have been made to quantify transfer frequency. In addition, the transfer of plasmids from a micro-organism unable to grow within a test system, to another organism capable of growth, could bring about biodegradation. The role of horizontal gene transfer via plasmids must not be underestimated.

Genetic mutation

Genetic mutation can occur naturally through small errors in DNA replication (Alexander, 1994), or by DNA damage caused by environmental stress (chemical, temperature, pH or UV exposure). Natural mutation rates are both low and random and do not account for the reproducible lag periods observed in short-term laboratory culture (Wiggins *et al*, 1987; Chen and Alexander, 1989; Linkfield *et al*, 1989; Spain *et al*, 1980; Fournier *et al*, 1981).

Stress induced mutation in the environment, resulting in new competent microbial populations in the field, is difficult to measure and quantify. However, the emergence of microbial populations with new catabolic competencies cannot be ignored, although the time taken for such capabilities to evolve is difficult to predict. A link between these mutation events and gene transfer has yet to be established (Alexander, 1994).

3.4.2.2 Log or exponential phase

The exponential phase of growth describes the binary fission process of microbiological growth where an individual cell divides into two identical daughter cells. It is during the exponential phase of growth, in batch systems, that the growth limiting substance is metabolised. In the case of an OECD ready biodegradation study the growth limiting substance that has been introduced as the primary source of carbon.

The rates of exponential growth vary greatly and are both chemical and micro-organism specific. The rates of growth are also influenced by environmental conditions (pH, temperature, trace nutrients and redox potential) and the concentration of the rate-limiting substrate, as discussed above.

3.4.2.3 Stationary phase

In any batch culture system, such as an OECD ready biodegradation study, growth cannot occur indefinitely. Once the test substance has been exhausted, or a waste product of growth builds up to an inhibitory concentration, growth will slow until it equals the rate of cell death. In the stationary phase there is no net increase or decrease in cell numbers. In a biodegradation study, the stationary phase corresponds to the plateau observed for CO_2 evolution, oxygen uptake or DOC removal.

It is important to recognise that inhibition of microbial populations is a natural phenomenon. Madigan *et al* (1997) illustrated this through a simple theoretical calculation. Given that a typical bacterial cell weighs approximately 10⁻¹² g and a generation time of 20 minutes, 48 hours of exponential growth would result in a microbial population that would weigh 4000 times that of the planet. This demonstrates the potential catabolic power of microbial populations and their ability to respond, or evolve, to change extremely rapidly.

3.4.2.4 Death Phase

When the period of incubation or exposure extends beyond the stationary phase, the rate of cell death will eventually exceed the rate of cell growth. Within a biodegradation study, the death phase is usually referred to as a period of cell or biomass turnover where CO_2 evolution or oxygen demand is low.

3.4.2.5 Laboratory tests providing opportunities for adaptation

Ready biodegradation screening tests derive inocula from sources that have been pre-exposed to low levels of industrial input, thus minimising the potential for using pre-adapted microbial populations. However, the potential for adaptation in the environment is real, it is a natural phenomenon and must be considered when assessing the persistence of a substance. Consequently, test regimes are required that either encourage adaptation or use adapted inocula. These tests should be available for use with all substances and not just those released continuously. At present two test regimes that allow the used of pre-exposed inocula have been ring tested. These are:

- The river die-away ring test (ISO, 2002a) using aniline and 4-chloroaniline;
- the modified headspace CO_2 test (ISO, 1999a) to determine the inherent biodegradability of surfactants.

River die-away ring test (ISO, 2002a)

A ring test using aniline (readily biodegradable) and 4-chloroaniline (borderline ready biodegradable) was carried out using the ISO 2002a river die-away method by seven laboratories (Nyholm and Torang, 1999). All laboratories used ¹⁴C-labelled compounds, with some participants carrying out the test at high (ca. 100-600 μ g l⁻¹) and low (1-20 μ g l⁻¹) concentrations, with adapted and unadapted organisms. First order rate constants (K_{non-adapted} and K_{adapted}) were derived for all of the tests. In every case the rates obtained for exposures using adapted inocula were greater than those using unadapted inocula.

The modified headspace CO₂ test to determine the inherent biodegradability of surfactants (ISO, 1999a).

A ring test was carried out to investigate the effects of adaptation using a modified version of the (ISO, 1999a) headspace CO_2 method with and without a 7-day pre-exposure. The test was conducted with the following surfactants:

- 'Hard' anionic (branched dodecylbenzene sulphonate, TPBS);
- 'Soft' anionic di-iso-octylsulphosuccinate-sodium salt;
- Cationic hexadecyl-trimethyl ammonium chloride;
- Non-ionic iso-nonyl phenol (ethoxylate);
- Amphoteric coco-amido-propyl, dimethyl hydroxysulpho-betaine.

No kinetic interpretation of the data was conducted as samples were only taken after 28 days incubation, however several interesting results were obtained. There was significant variation in results within and between individual laboratories. Pre-exposure did not have uniform effects on the level of biodegradation achieved or on its variability, although in general, the degree of biodegradation was increased after pre-exposure. It was concluded that a longer period of adaptation might be required before more consistent results are obtained.

Other factors limiting the use of standard laboratory tests

There are many factors that can influence the predictability of degradation in nature. These factors may interact producing additive, synergistic, or antagonistic effects. Historically, studies have tried to identify and quantify single factors, but the identification and even more, the quantification of interactions is in its early stages. Other specific factors influencing degradation test measurements such as cometabolism, temperature, oxygen, bioavailability and metabolism by high organisms are considered below.

3.5 Cometabolism

Not all catabolic processes are growth-linked. Biologically mediated transformations can occur in the absence of growth. The term cometabolism is often used to describe the transformation of organic compounds by micro-organisms that are unable to use the substance for growth, or as a source of energy (Alexander, 1967). Two types of reactions are possible. In one, the cometabolised substance is transformed in the presence of a second compound that supports growth. In the second, the substance is transformed in the absence of a second substrate (Horvath, 1972). The inability of the degrading micro-organisms to grow on the cometabolised substrate usually results in very slow transformation. It must also be emphasised that cometabolism typically results in only the parent substance undergoing primary biodegradation, and consequently results in the formation of metabolites that may or may not be degradable. Alexander (1981) gives three different explanations for why an organic substrate does not support growth:

- The initial enzyme does not convert the substance to a product that could be further transformed by other enzymes. Therefore no metabolic intermediates that can be used for biosynthesis and energy production are produced from that substance. The basis of this explanation is the fact that many enzymes are quite unspecific and act on several structurally-related compounds;
- the initial substrate is transformed to products that inhibit the activity of other enzymes in the mineralisation pathway or that suppress growth;
- the organism needs a second substrate in order to bring about some particular reaction.

Cometabolic transformations are of great environmental significance because of the potential of the organic products to accumulate (Horvath, 1972). Whether or not the parent compound is toxic, the converted products may be harmful. However, as a result of the great diversity of micro-organisms, it is possible that the cometabolised products of one species could be transformed by a second species to a metabolite that can enter common metabolic pathways and be mineralised. Also, a compound that is cometabolised at one concentration, might be mineralised at another one (Wang *et al*, 1984), and a compound that is cometabolised in one environment, may be mineralised in another (Fournier, 1980). These final points could influence significantly the persistence of a substance measured either in the environment or during laboratory testing. It is probable that cometabolism will be more important at low test substance concentrations where it may have to be relied upon (at least initially), as the main degradative process, and there may be insufficient test chemical present to promote bacterial growth.

The route of mineralisation, either via growth or cometabolism, will have a major effect on biodegradation. The main difference should be that a cometabolic rate does not increase over time, it is likely to decline as a function of the concentration of the growth substrate and substance being cometabolised. Robertson and Alexander (1994) demonstrated this to be the case using simazine and carbofuran which do not support growth, and 2,4-D and propham (IPC) which initially showed a lag phase, then an increase in degrader numbers and in mineralisation rate. A second addition of test sample was only mineralised faster in the last two cases.

In extrapolation and decision making, it is essential to know if a substance can be mineralised with growth. In such cases quantitative degradation in nature can be expected, although a prediction of the length of time this will take can be difficult. If a substance is only cometabolised, extremely slow rates of biodegradation are to be expected, with the possibility of production of recalcitrant metabolites. There is no current standard laboratory test that actively encourages cometabolic processes to occur. The design of ready biodegradation studies actively discourages such processes, because the substance is present as the sole source of carbon at a relatively high concentration.

3.6 Temperature

For abiotic tests, the rate of degradation is controlled by temperature, and this is described by the Arrhenius equation which enables calculation of degradation rates at varying temperatures. For biodegradation studies, there is conflicting evidence regarding the way in which micro-organisms react to temperature. Literature examining this aspect is limited, but Widdel (1985) stated that the greatest variety of sulphate-reducing bacteria is to be found at moderate to low temperatures, and that many anaerobes live at low temperatures (never above 10°C) all the year round because of stratification. Furthermore, a field study by Gschwend *et al* (1982) does not support temperature dependent biodegradation. It was noted that alkylbenzene degradation increased only slightly during the summer, and that temperature was not the only factor influencing this increase.

When biodegradation experiments have been performed under cold (1-4°C) and temperate (10-13°C) conditions with inocula collected from Arctic and temperate locations respectively, temperature was not the critical issue (Schaaning *et al* 1997; Whyte *et al* 1998).

Rivkin et al (1996) examined the relationship between temperature and specific growth rates (SGR) in the marine environment where approximately 90% is permanently at <4°C. Based on the analysis of published data, where approximately 50% of the observations were from environments <4°C they found the mean (0.39-0.42d⁻¹) and median (0.25-0.29 d⁻¹) SGR of bacteria from cold (<4°C) and warm (>4°C) were not significantly different. They concluded that the growth rates for bacteria from cold and temperate oceans are similar at their respective ambient temperatures. Arnosti et al (1998) carried out a series of experiments to investigate whether relative temperature responses differ between the microbial communities of temperate and permanently cold environments. They compared the temperature responses of the microbial communities at two temperate sites, to the temperature responses at two permanently cold Arctic sites, using carbon turnover rates. They concluded that temperature in the marine environment did not affect biodegradation rates, as bacterial populations were fully adapted to these conditions, and that half-lives as short as those from standard tests could be observed, even when the temperature was close to zero centigrade. Other studies support their hypothesis that carbon turnover rates in Arctic environments are not necessarily slower than in temperate environments (Wheeler et al, 1996; Hodson et al, 1981), while Meyer-Reil and Köster (1992) concluded that rates of enzymatic hydrolysis in pelagic sediments are regulated by organic matter concentration and not by temperature.

Whether degradation does or does not occur at extremely low temperatures is not the main issue. The most important issue, when considering the measurement of persistence using standard test methods based on a single temperature, is that there is a substantial body of evidence showing that seasonal trends in bacterial activity occur in temperate zones. Consequently, if degradation tests are to be carried out at temperatures that reflect the range of environmental temperatures encountered, then great care should be taken to ensure that the microbial inoculum is obtained from a relevant environment.

Temperature also has an impact on the adaptation of bacteria and the effect of low temperature has been shown to be more pronounced under conditions of low chemical concentration. For example, uninduced microbial communities incubated with low concentrations of 4-nitrophenol showed no degradation, whereas, for tests with higher 4-nitrophenol additions, adapted communities degraded the chemical in only a slightly longer time at 10°C than at 20°C. Mineralisation of nitrilotriacetic acid has also been shown to be more rapid and complete at higher temperatures (Palmisano *et al*, 1991).

The most important issue regarding temperature is that the inocula used in the tests are used within their natural temperature range. Table 13 summarises the different microorganisms classified based on their temperature range. The choice of incubation temperature must cover the range at which psychrophilic and mesophilic organisms can proliferate to maintain environmental realism with respect to temperature and microbiota.

Temperature Class	Description
Psychrophile/facultative psychrophile	Optimal growth is at 15°C or lower, maximal temperature is ca. 20°C, and minimal temperature is 0°C or lower.
Psychrotroph	Capable of growing at 5°C or lower, maximal temperature normally above 25 to 30°C; term in this case is misnomer because it does not indicate nutritional characteristics.
Mesophile	Generally defined by optimal temperature for growth, which is approximately 37°C; frequently grows in the range 8 to 10°C and from 45 to 50°C.
Thermophile	Grows at 50°C or above.
Hyperthermophile	Grows at 90°C or more, although optimal temperature for growth is generally above 80°C; maximal growth of pure cultures occurs between 110°C and 113°C although the maximum may increase as further research is done.

Table 13: The classification of micro-organisms based on their temperature optimum and tolerance

3.7 Oxygen content

Oxygen is a prerequisite for most mineralisation processes. It is not usually a limiting factor for environmental waters unless the concentration of the substrate is excessively high. Indeed low temperature incubations of environmental waters offer to increase oxygen availability further. However, in soils and sediments, oxygen limitation can be a very important factor. The water content in a soil is inversely proportional to its gas content. Therefore, in water-saturated soils, the potential for aerobic biodegradation to occur is greatly reduced if mixing does not result in an influx of oxygen. In addition, the porosity of soils will decrease with grain size, which will in turn reduce the rate of

oxygen exchange within the soil. No optimum water content can be defined as, in different soils, an optimal degradation is achieved with different water contents (Wesnigk *et al*, 2001). An example of this is provided by the degradation of tetrachlorobenzene. The lowest rates of tetrachlorobenzene degradation were observed at 100% saturation in all soils. In sandy soils of high porosity, similar rates of tetrachlorobenzene degradation were observed at 70% and 40% saturation owing to the ease of oxygen diffusion. However, in fine soils, degradation was most rapid in soil with only 40% moisture content (Keskin, 1994).

Table 14: The classification of micro-organisms based on their oxygen requirements

Classification	Description
Aerobe	Capable of using oxygen as a terminal electron acceptor; can tolerate a level of oxygen equivalent to or higher than the 21% present in the atmosphere and has a strict respiratory-type metabolism.
Anaerobe	Grows in the absence of oxygen; some anaerobes have a fermentative type metabolism; others may carry out anaerobic respiration in which a terminal electron acceptor other than oxygen is used.
Facultative anaerobe	Can grow either aerobically or anaerobically - characteristic of a large number of genera of bacteria including coliforms such as <i>Escherichia coli</i> .
Microaerophile	Capable of oxygen-dependent growth but only at low oxygen levels; cannot grow in the presence of a level of oxygen equivalent to that present in an air atmosphere (21% O ₂).

3.8 Bioavailability

One of the main limitations associated with carrying out sediment and soil tests is obtaining a material that is representative. Soils and sediments contain widely ranging proportions of organic carbon, clay minerals and sand in them, which will control the bioavailability of the test substance as well as potentially affect microbial growth. To overcome this to some extent, the soils and sediments used in these tests need to be fully characterised to allow the best possible data interpretation. Additionally, a number of replicate tests should be carried out using different sources of soil and sediment to obtain an estimate of the likely range of degradation results.

There are few examples of a transfer of experiments performed in the laboratory to the pilot scale. On a larger scale the degradation process is often restricted by suboptimal water content, e.g. dryness or insufficient oxygen supply accompanying a high water content, as well as low bioavailability of the degradable substance or other nutrients. As a consequence, conditions encountered in the natural environment often do not lead to such positive results as those obtained under controlled laboratory conditions (Braun *et al*, 1994). Although single factors have been investigated, the complex interactions between these cannot be covered by laboratory experiments (Goldstein *et al*, 1985; Stucki and Alexander, 1987).

The toxicity of xenobiotic substances to the autochthonous soil microbial ecology was shown to be higher in smaller samples than in large-scale experiments (Malkomes, 1985), probably as a result of a the presence of a greater variety of micro-organisms in larger samples. Abiotic factors may also be important including the effects of water drainage in natural soils compared with disturbed soil columns (Stoller *et al*, 1975). A major problem arises with soil and sediments exposed to xenobiotic substances for a longer period of time prior to remediation (e.g. older contaminated deposits, etc.) so that complex sorption processes have already taken place. These time-dependent reactions cannot be easily simulated under laboratory conditions and no standard methodologies exist (Viswanathan *et al*, 1978).

Sorption

Sorption is caused by a number of different mechanisms, such as van der Waals forces, charge transfer complexation, hydrogen bonding and hydrophobic interactions (Bollag, 1992). All adsorption processes, except for covalent bonds, are reversible; substances covalently bonded to the humus matrix are called 'bound residues'. Sorption and degradation processes are dependent on each other, with limitation of degradation processes by sorption being found to differ with different bacterial strains. As a consequence, standard laboratory tests using sediment or soil from a single source will not be representative of the environment as a whole where many factors control the bioavailability and hence potential to degrade of the test chemical. Such factors include:

- The type and quantity of organic carbon present;
- clay mineral fraction;
- redox potential;
- · microbial community associated with the particulate phase and
- particle size distribution.

For aqueous tests using inocula derived from sewage effluent, suspended particulate concentrations are low and, unless a test compound is particularly susceptible to adsorption, i.e. has a very high partition coefficient (K_p) or high organic carbon:water partition coefficient (K_{oc}), then sorption is unlikely to prove significant as the vast majority of the substance will be in the dissolved phase (Section 7).

However, in tests using hydrophobic or poorly water soluble substances where the K_p or K_{oc} of a compound is greater than 10^4 and solids are present, (e.g. sediment tests, or sewage treatment simulations), sorption of the test substances to particulates will become significant and potentially result in reduced bioavailability. Additionally the likelihood of adsorption to test vessel walls will be increased, thus further decreasing the availability of the compound and leading to a potential under-estimation of biodegradation. However, it must be noted that substances that adsorb to solids in a laboratory test environment will show exactly the same tendency in the real environment. The problem with adsorption is mainly related to the fact that a substance may be seen to 'fail' a test due to reduced availability. There are two main possibilities for overcoming the issue of chemical adsorption during testing:

- Carry out the test under conditions where sorption is minimised. This could include keeping particulate matter concentrations to a minimum and 'de-activating' vessel surfaces (e.g. by silanisation of glass or the use of PTFE vessels);
- take bioavailability into account as one of the test criteria.

The first option provides a solution to overcome difficulties whilst testing, however for compounds prone to adsorption, results would only provide a 'best case' estimation of environmental persistence. In reality it is probable that the persistence would be greater due to the presence of a higher solids loading in the environment, leading to reduced bioavailability.

The second option would provide a much more realistic estimation of environmental persistence, but is significantly more difficult to work into a test methodology. Current EC proposals for guidance on persistence in soils provide bioavailability criterion which state that "a plant protection product is considered persistent and authorisation not granted pending further investigations if during tests in the field it persists in the soil for more than one year (i.e. $DT_{90}>1$ year and $DT_{50}>3$ months); and if during laboratory tests the chemical forms non-extractable residues in amounts exceeding 70% of the initial dose after 100 days with a mineralisation rate of less than 5% in 100 days" (EC, 2000b).

Non-extractable is defined as "chemical species originating from pesticides used according to good agricultural practice that cannot be extracted by methods which do not significantly change the chemical nature of these residues". This criterion is included to ensure that there is no accumulation of the product in soils receiving repeated applications. Widespread use of such a criterion for chemicals entering the environment at much lower concentrations, and via more diffuse pathways, is obviously not appropriate. A better approach would be to apply a 'bioavailability factor' to be applied to degradation data, prior to applying a pass or fail judgement. The derivation of a bioavailability factor would have to be carefully considered and based upon sound scientific practice.

However, data are available linking the octanol:water partition coefficient (K_{ow}) of neutral compounds to their bioaccumulation into organisms, and derivation of a multiplication factor for use within biodegradation tests based on the K_{ow} of a substance may be possible.

The overall significance of particulate-associated chemicals is subject to much debate within the scientific community (Beck et al, 1995; Pignatello and Xing, 1996; Bosma et al, 1997). Lack of bioavailability results in increased persistence, but the chemical is less prone to bioaccumulate and so its overall risk to the environment is often thought to be of no concern (Kahn, 1982; Calderbank, 1989). Conversely, sorbed materials may be released when there are changes in environmental parameters such as pH, moisture content and cation exchange capacity in soils and sediments. Similarly, they can be released by changes in ionic strength, pH, suspended solids loading and dissolved organic carbon levels in waters (IUPAC, 1984; Cheng and Koskinen, 1986; Ebing, 1987). It must also be acknowledged that partitioning processes are the result of equilibrium between two phases, which is largely reversible. Hence, if particulate material containing an absorbed chemical is transported away from its source and comes into contact with an aqueous phase depleted in that chemical, then in order to maintain the equilibrium partition coefficient, dissolution from the particulates must occur. Thus, the ultimate availability of a chemical will be largely controlled by physical transportation, although the small fractions likely to be released from bound residues are considered to have no additional significance from the regulatory viewpoint (SCP, 1999).

3.9 Metabolism by higher organisms

Although metabolism of substances by micro-organisms, and specifically bacteria, is generally considered the major route of biotic degradation in the environment, metabolism by higher organisms can also occur to varying degrees depending on the organism, the substance and the environment in which it occurs.

The persistence of a chemical will be influenced by two possible mechanisms within higher organisms:

- Persistence may be reduced by an increase in metabolism relative to that which would have occurred outside of the body (e.g. in food, in sediment or water etc);
- persistence may be increased through bioaccumulation of the substance without metabolism, (i.e. it is simply stored in fatty tissue and is not available for degradation either within the body of the organism or outside in the environment). Metabolism may however occur further along the food chain.

The second point is an important one when considering the persistence of a chemical, as simple uptake and bioaccumulation and/or biomagnification only indirectly influence a substance's persistence if it has not been degraded. Metabolism within the organism will directly affect the substance's persistence. As a consequence, bioaccumulation data alone are insufficient to assess persistence in higher organisms.

Absorption, metabolism and clearance must be considered for a full assessment of the significance of metabolism in higher organisms. Uptake and elimination indirectly influence persistence by controlling the supply and removal of the chemical in the organism, thus regulating the period in which metabolism may occur.

Metabolism by invertebrates in the aquatic environment

There is a growing body of evidence that organic chemicals can be metabolised by small invertebrates in water, sediment and soil. Persistent compounds, such as PAHs, can be metabolically transformed within the P450 mixed-function oxygenase system. For most taxonomic groups, the details of the enzymes involved, and the metabolites produced during transformation of xenobiotic chemicals such as PAHs remain poorly understood (Forbes et al, 2001). For chemicals that partition to sediment, deposit-feeding infauna may serve to reduce contaminant concentrations in the sediment and pore water by metabolism. The decline in worm body burden of chemical contaminants has been attributed to bacteria living in association with invertebrates, either in the digestive tract or on the body surface (Forbes et al, 1996). Recent studies however, have shown that certain polychaetes, for example Capitella capitata, are capable of metabolising fluoranthene in their own right (Forbes et al, 2001) and are generally more effective than other aquatic invertebrates at metabolising compounds such as PAHs. Capitella capitata, however, is a specialised species of worm, which thrives at highly contaminated sites where densities of up to 500,000 m³ can be reached. This ability could make them significant contributors to the degradation of sediment-associated contaminants, but since the tests were carried out at very high fluoranthene concentrations (554 μ g g⁻¹), which do not necessarily reflect realistic environmental levels, the results should be treated with caution. The freshwater amphipod Hyalella azteca has been shown to metabolise anthracene at a rate of about 2% of the body burden per hour (Landrum and Scavia, 1983). This rate is much greater than that found for the freshwater amphipod Pontoporeia hoyi (Landrum, 1982), but is lower than that for the midge larvae Chironomous riparius (Gerould et al, 1983).

Metabolism by vertebrates in the aquatic environment

There are numerous examples of organic chemicals being metabolised via both oxidative and reductive mechanisms by fish in the aquatic environment (Corner and Harris, 1976; Addison, 1976; Lang *et al*, 1997; Nordone *et al*, 1998), with rapid metabolism of nonpersistent chemicals occurring. Acrolein, an aquatic herbicide, was rapidly metabolised by freshwater catfish and sunfish, although the mechanisms were quite different, leading to large variations in the concentration of the various metabolites produced. It was noted, however, that the compound had already been shown to be rapidly degraded by microbial populations (Nordone *et al*, 1998). More persistent chemicals, such as PAHs, PCBs and nitrotoluenes, have also been shown to be metabolised by fish (Smeets *et al*, 1999). The rates of metabolism are generally lower than for terrestrial organisms, which may be a result of temperature differences rather than variations in metabolic processes (Addison, 1976). In many cases metabolism is achieved by oxidation catalysed by cytochrome P450 monooxygenase enzymes (Lang *et al*, 1997; Smeets *et al*, 1999).

Relative significance of metabolism by higher organisms

There is no doubt that higher organisms are capable of metabolising chemicals, using similar enzyme-induced mechanisms. The main difference in the degradation potential of micro-organisms as compared with higher level organisms, is that bacteria have more varied mechanisms for degrading chemicals under a wide variety of environmental conditions. Evidence also seems to suggest that microbial degradation is a more rapid process than metabolism by higher organisms, probably due to shorter adaptation times and more rapid growth rates. Therefore although appreciable metabolism occurs in higher organisms, it is not a process that is as significant as microbial degradation.

3.10 Conclusions

At present there is no robust mechanism for assessing the persistence of a chemical substance in the environment. A number of factors need to be considered to make such an assessment including:

- The intrinsic physico-chemical properties of the substance;
- the physico-chemical characteristics of the system or the environmental compartment, and how the chemical interacts with the system;
- the ability or potential of the chemical to undergo abiotic and biotic degradation;
- how the system or environmental compartment determines whether degradation occurs, and at what rate.

Whilst the intrinsic properties of the substance and its potential to degrade can be tested within the laboratory with a reasonable degree of ease, the greatest challenge lies with determining accurately the behaviour of the substance in the environment and the rate at which it is eliminated through degradative processes, especially biodegradative processes. It must also be recognised that a one-off estimation may not be sufficient for biodegradation when the potential for adaptation exists.

There is currently no formal mechanism within ready biodegradation test methodologies for calculating the persistence of a substance, since rate constants are not directly measured. However, it is possible for rate constants to be derived from these standard tests with only minor modifications. These modifications relate to the number of data points, as no precise sampling regime is specified other than to carry out a sufficient number of samples to allow the percent removal in the 10-day window to be assessed. Whether there is any merit in attempting to derive kinetic rate constants, first-order or any other kinetic description, for such biodegradability studies is questionable. The Task Force (TF) recommends that alternatives to the half-life concept for describing degradation in the environment are given due consideration, as the kinetics occurring, the laboratory and environment are much more complex and variable. An alternative approach is described in Sections 7 and 8.

As defined by OECD (1992a), ready biodegradability describes substances that would undergo rapid and ultimate degradation in all environmental compartments. This is a purpose that these tests serve well, however a fail in these tests alone should not be used to assign a substance as persistent.

In order to assess persistence with a greater degree of accuracy, additional screening tests are required that maximise environmental realism without comprising the biodegradative potential Consequently, the TF recommends that tests that allow for the natural phenomenon of microbial adaptation are urgently required, as well as a regulatory framework in which data from these tests can be used in a pragmatic manner. Adaptation is a term used to describe a number of different biological events that occur in the environment that may or may not occur in laboratory test systems. Such events include enzyme induction, proliferation of competent populations from a low initial concentration, development of microbial consortia, horizontal gene transfer of catabolic capabilities and genetic mutation. Test protocols are required that allow the potential for all these adaptive processes to be realised. Tests systems with increased inoculum densities may reduce scale effects that result in extended lag periods, or failure for biodegradation to occur, due to low densities of competent micro-organisms, and especially if degradation is dependent on a microbial consortium (see Section 5).

The TF also recommends that the bias towards growth-linked biodegradation studies (through working at relatively high substance concentrations and low biomass concentrations) needs to be addressed, with protocols that can measure non-growth linked degradative processes such as cometabolism. Other factors requiring specific attention when considering new laboratory test protocols, and accounting for the uncertainty in extrapolating from laboratory to field, include:

- Bioavailability and partitioning;
- the use of realistic substance concentrations;
- temperature, pH, salinity and redox conditions;
- nutrient status;
- light;
- predation.

Datasets are required to determine the relative importance of these parameters for each environmental compartment.

Finally, biodegradation is not always mediated by micro-organisms. Higher organisms also play an active role in the metabolism and detoxification of substances in the environment. Currently, this is not considered as part of the environmental risk assessment process. Accordingly, the TF recommends that a review be conducted to assess the contribution made by higher organisms in the metabolism of chemical substances in the environment.

4. EXTRAPOLATION FROM LABORATORY AND FIELD DATA

The parameters that influence degradation in the environment (see Section 3), increase the uncertainty in extrapolating from the results of standard laboratory-based studies to degradation in the field. Nevertheless, there have been a number of attempts to assess the potential of carrying out such an operation. This section will compare and contrast the published degradation data that are available for standard laboratory tests with environmental data or simulation-type data.

4.1 Extrapolation from standard tests

One study assessing the effectiveness of extrapolating mineralisation rates from ready tests to activated sludge, river water and soil environments, provided contrasting results (Federle *et al*, 1997). Nine substances with diverse physico-chemical properties were degraded in the ready test (modified Sturm) and in each of the environmental compartments studied. In all cases the rates of degradation observed in the ready biodegradation test were slower than those for the other compartments under investigation. In this instance, the ready test acted as an effective screening test. On average, the rates observed in the ready test were 8.1, 2.5 and 1.2 times lower than the rates in activated sludge, river water and soil tests respectively. There was also significant variability in degradation rates between different substances, with scaling factors used to extrapolate from the ready biodegradation test data ranging from 1.7 - 19 for activated sludge, from 0.1 - 5.6 for river waters and from 0.3 - 2.8 for soil. Statistical analysis (using SRC-QSAR software) was conducted for each substance with respect to biodegradation test data, solubility, octanol:water partition coefficient (K_{ow}) and molecular weight (Table 14).

There was no statistical correlation between the ready test data and those performed in environmental media. Overall, the data suggested that although mineralisation rates were strongly correlated with solubility in ready tests, this factor is less important under more realistic environmental conditions, probably owing to the elevated test substance concentration used for the ready test. Moreover, this finding suggests that the rate of biodegradation observed in a ready test is merely a reflection of a chemical's solubility. Consequently, the rates determined in the ready biodegradation study may have little or no reflection of the true rate(s) of biodegradation in the environment (Federle *et al*, 1997).

Scaling factor	Molecular	Log K _{ow}	Solubility	Log solubility
	weight	0.00**	0.54	0.0.4**
Ready to activated sludge	0.42	0.88**	-0.54	-0.94**
Ready to river water	-0.03	0.82**	-0.45	-0.69*
Ready to soil	0.58	0.50	-0.50	-0.6
Activated sludge to river water	-0.56	-0.04	0.07	0.30
Activated sludge to soil	0.23	-0.37	0.03	0.37
River water to soil	0.24	-0.57	0.33	0.45

Table 14: Correlations (r) between scaling factors and physico-chemical properties of test substances (adapted from Federle et al, 1997)

* significant (p<0.05)

** significant (p<0.01)

Although Struijs and van den Berg (1995) reported that the rates of biodegradation are correlated with the relative concentration of micro-organisms present, Federle *et al* (1997) could not demonstrate a strong relationship between biomass concentration and the rate of biodegradation in this study.

Other workers found that screening test data did not always correlate with data generated in more realistic test systems. Larson and Payne (1981) showed that biodegradation rates of nitrilotriacetic acid (NTA) and linear alkylbenzene sulphonate (LAS) in a CO₂ screening test were similar to those observed in natural waters. In contrast, the rates of mineralisation of octadecyltrimethyl ammonium chloride and dioctadecyldimethyl ammonium chloride were much slower in the screening test than in natural waters. The former substances are highly soluble, while the latter are poorly soluble or insoluble. However, other researchers (Gledhill *et al*, 1991) attributed poor biodegradation data with LAS and methyl ester sulphonate at higher test substance concentrations to microbial toxicity and not to poor solubility. Furthermore, rapid rates of biodegradation of these compounds were observed in river water/sediment microcosms (Gledhill *et al*, 1991). In conclusion, both solubility and toxicity may influence results observed in standardised tests using unrealistically high test substance concentrations.

Boethling *et al* (1995) compared the aerobic rates of biodegradation in freshwater with those generated in surface soil. Results for primary biodegradation were shown to vary between a factor of 0.13 for 4-chlorophenol and 5.22 for pentachlorophenol, whereas results for ultimate biodegradation varied between 0.049 for aniline to 6.66 for acrylamide. Biodegradation data for di-n-butyl phthalate for many different environmental media were also provided (Table 16).

Conditions	Primary (P) or ultimate (U)	Mean half-life (d)	Range (d)	No. of tests
Marine water aerobic	Р	2.0	0.7 - 9.8	4
Marine water:sediment aerobic	Р	2.0	0.6 - 7.5	5
Freshwater:aerobic	Р	1.4	0.9 - 2.8	4
Freshwater:sedimentaerobic	Р	2.8	2.3 - 3.4	3
Freshwater:sedimentaerobic	U	6.5	4.0 - 13.0	14
Soil: aerobic	Р	4.8	4.8 - 4.9	2
Soil: aerobic	U	55.9	11.0 - 106	8
Soil: anaerobic	Р	22.0	-	1

Table 16: Biodegradation data for di-n-butyl phthalate (from Boethling et al, 1995)

The data in Table 16 illustrate that the half-lives for di-n-butyl phthalate covered a wide range (up to an order of magnitude), even under supposedly similar test conditions. Comparison of biodegradation rates in marine and freshwaters showed that it was not possible to apply a single factor to predict one from the other. The rates of biodegradation in marine to freshwaters varied from 0.19 - 10.1 for primary degradation and from 0.071 - 7.8 for ultimate degradation. Comparison between tests with and without the addition of sediment suggested that in general, sediment enhanced biodegradability. However, this is by no means a hard and fast rule, as other studies have shown that sorption to sediment inhibits biodegradation (Steen *et al*, 1980 and Flenner *et al*, 1991). This illustrates further the potential complications with extrapolation of data between environmental media.

4.2 Defaults and standard ready test data

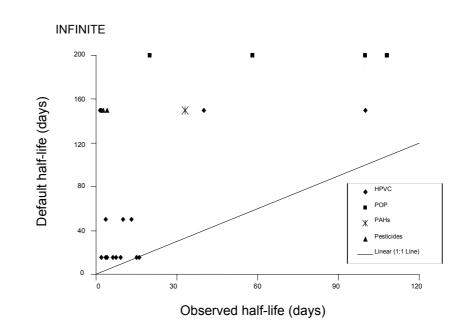
The EU Technical Guidance Document (EC, 2003) proposes a set of environmental halflives for chemicals based on the pass or failure of standard laboratory tests for ready and inherent biodegradation (Tables 3 and 4). In order to assess the accuracy of these assumptions, a comparison of laboratory and field data for a range of chemicals is presented in Table 17, alongside the half-life for freshwater/soils that would be attributed to them under the TGD. A random selection of between 30 and 40 chemicals was taken from easily accessible compendia of laboratory and field data (e.g. IUCLID, 2000; Howard, 1989) and a list of pesticide data (Egli, 2000). The selected chemicals covered a range of persistence from readily biodegradable, to those classified as POPs. Chemicals classified as POPs were included in the comparison in order to provide extreme persistence values, which are translated into half-lives of infinity under the EU TGD. Where a range of halflives have been measured, as a consequence of performing tests under varying conditions, an average value was compared with the default figures.

4.2.1 Aqueous data

4.2.1.1 Freshwater

The data shown in Table 17 are not intended to be an exhaustive list, but are presented to provide a comparison between default values and field and/or laboratory data, using a representative selection of substances. Comparison of the TGD default half-life for freshwater, with the results of the river water die-away test and other freshwater data, shows that the default value is invariably significantly longer than the measured test value. Where the 10-day window criterion in the ready test was fulfilled, the TGD default half-life value of 15 days is generally longer than measured values. A closer examination of the data shows that, for most of the compounds selected, measured half-life data derived from testing were in the region of 0.5 - 0.1 of the assigned default value. Furthermore, in the case of biodegradation, the test data generally correspond to mineralisation rather than parent disappearance, which leads to an even greater discrepancy between measured and default values. Chemicals exhibiting measured persistence values of less than 100th of the default value were almost exclusively persistent organic pollutants (POPs), which were given an infinite degradation half-life under the TGD scheme, although evidence of environmental degradation was available. A breakdown of default versus actual data is provided in Figure 5, showing a comparison between different groups of compounds (e.g. POPs, high production volume chemicals, PAHs).

Figure 5: Observed versus EU TGD default half-lives for freshwater biodegradation



Note: Measured half-lives shorter than default rate are above the line; measured half-lives greater than default rate are below the line.

Table 17: Re _l	ported half-live	Table 17: Reported half-lives in waters, sediments and soils for a range of substances	s and soils for a π	ange of suk	stances				
Substance	Biodegradation test passed	River water die-away standard test (half-life)	Non standard aquatic tests (half-life)	Sediment (haff-life)	Soil	Kp (I kg ⁻¹)	Half-life range)	TGD default half-life water /soil*	Abiotic half-life
Adipic acid	10-day window ¹	3.5 days (700 mg l ⁻¹) ³				<100 ^{2#+}	3.5 days	1 <i>5 /</i> 30 days	
Allyl alcohol	10-day window ⁴				4 to 8 days ⁷¹	<100 ^{70#+}	4-8 days	15 / 30 days	
Acrylamide	10-day window ⁶	6.25 days (90% degradation) ⁶ 1 day (acclimated) ⁶	<8 days ⁷		1-14 days ⁷³	0.54 ^{72#+}	1-14 days	15 / 30 days	
Acrylic acid	10-day window ⁸		1-7 days ⁹		1-7 days ⁷⁵	<100 ^{74#}	1-7 days	15 / 30 days	
Benzene	10-day window		1.8 days (lab) 1.4-3.5 days (field) ³⁷				1-4 days	15 / 30 days	
Benzoic acid	10-day window ¹⁰		0.2-3.6 days ¹²		< 7 days ⁷⁷	<100 ^{76#}	0.2-7 days	15 / 30 days	
Butylbenzyl phithalate	10-day window ²³	1.5 days RDA at 1 mg l ⁻¹ 2 days ⁶⁹ 2 days ⁶⁹	0.3 days in RDA at 1 μg 1 ⁻¹⁶⁶ 1.4 days, lake microcosm ⁸⁶ <3 days river microcosm ⁶⁹		1-7 days	850 ^{24#}	1-7 days	15 / 300 days	0.75 days photodeg
2-Cresol	10-day window ²⁵	100% 2-7 days ²⁶	2-28 days ²⁶		100% 8 days ¹¹	1 ^{13#}	2-28 days	15 / 30 days	
4-Chlorophenol	10-day window ^{31,86}	Complete removal at 1 ppm level after 13 days in acdimated water ²⁶	1,00 to 0 ppb in 15 days ³²	3 days ⁸⁷	84% loss in 12 days complete deg in 3-9 days in silt loam ⁸⁸	0.8-24 ⁸¹	3-15 days	15 / 30 days	
Di-butyl phthalate 10-day window	10-day window	<3 days (primary biodegradation) 5.8 days	0.87 days at 1 µg l ⁻¹ 1.4 days estuary ⁶⁶	5 days in RDA with low sediment 2.4 days in sediment microcosm	2-23 days	634	2-23 days	15 days	0.9 days photodeg
Di-butylbenzyl phthalate	10-day window	<3 days (primary biodegradation)			1-7 days		1-7 days	15 days	
* Where there is conflicting da	* Where there is conflicting data the worst case is used.	vorst case is used.							

 $^{\sharp}$ Assumes 5% organic carbon. $^{+}$ Recent studies confirm this substance to be readily biodegradable.

Table 17: Re	ported half-live.	Table 17: Reported half-lives in waters, sediments and soils for a range of substances (cont'd)	s and soils for a ru	ange of subs	tances (cont	(þ			
Substance	Biodegradation test passed	River water die-away standard test (half-life)	Non standard aquatic tests (half-life)	Sediment (half-life)	Soil	Kp (I kg ⁻¹)	Half-life range)	TGD default half-life water /soil*	Abiotic half-life
Di-ethyl 2 hexyl phthalate	10-day window	30 days in RDA at 1 mg 1 ⁻¹ 24	3.5 days in RDA at 1 µg 1 ^{-1 66}	24-100 days 0.4 days in estuarine sediment ¹²⁰	2-70 day Iysimeter -21 days loam 53 days sand ¹¹⁹	16500 estimate	2-70 days	15 days	0.44 days photodeg ¹¹⁹
Di-ethyl phthalat	Di-ethyl phthalate 10-day window	4.3 days MITI inoculum ¹²¹	0.4 days in RDA at 1 µg l ^{-1 66}		3-56 days		3-56 days	15 days	2.4 days photodeg ¹¹⁹
Di-isononyl phthalate	10-day window	<3 days (primary biodegradation)					< 3 days	15 days	0.35 days photodeg ¹¹⁹
Di-methyl phthalate	10-day window	0.23 days MITI inoculum ¹²²	22 0.5 days in RDA at 1μg 1 ⁻¹ (uninoculated river water) ⁶⁶		 9 days in aqueous suspension 1.7 days garden soil at 30°C¹¹⁹ 		< 3 days	15 days	14.4 days photodeg ¹¹⁹
Ethanol	10-day window	0.3-1 day			0.1-1 day		0.1-5 days	15 days	5 days
Linear alkylbenzene sulphonate	10-day window		0.25-4.3 days		12.8-21 days ³⁵ 1000-4000 ³³ 0.25-21 days	1000-4000	³³ 0.25-21 days	15 days	
Nitrilotriacetate	10-day window ⁵²	Rate 0.5 mg l ⁻¹ h ^{-1 53}	4-29 days ⁵⁴		1hr to 5 days ¹⁰⁴ 0.3 ^{103#}	4 0.3 ^{103#}	0.1-29 days	15 / 15 days	
Phenol	10-day window ²⁶	100% 2-4 days ²⁶	9 days (estuarine) ⁵⁹		2-5 days ¹¹²	2-7.4 ^{111#}	2-9 days	15 / 30 days	
Vinyl acetate	10-day window ⁶⁵					1-381		15 / 30 days	7.3 days (hydrolysis) 67 T _{1/2} = decreases in alkaline moist soils due to hydrolysis ⁴⁵
Benzyl chloride	Ready ⁵					0.9 ^{80#+}	0.6-19 days	50 / 90 days	0.6 - 19 days (hydrolysis) ¹⁵
Di-isodecyl- phthalate	Ready	<1 day (primary biodegradation)					< 3 days	50 days	0.3 days photodeg ¹¹⁹
* Where there is	* Where there is conflicting data the worst case is used.	orst case is used.							

* Where there is conflicting data the worst case is used. # Assumes 5% organic carbon.
* Recent studies confirm this substance to be readily biodegradable.

Persistence of Chemicals in the Environment

Table 17: Rep	orted half-live:	Table 17: Reported half-lives in waters, sediment	nts and soils for a range of substances (cont'd)	ange of sub	stances (cont'	d)			
Substance	Biodegradation test passed	River water die-away standard test (half-life)	Non standard aquatic tests (half-life)	Sediment (half-life)	Soil	Kp (I kg ^{.1})	Half-life range)	TGD default half-life water /soil*	Abiotic half-life
Ethylene oxide	Ready ⁴	Biodeg rate = hydrolysis rate = 12-14 days ³⁸			1.9-2.1 days ⁹⁵	0.8 ^{95#}	2-14 days	50 / 90 days	hydrolysis rate = 12-14 days ³⁸
Linear alkylbenzene	Ready	4-15 days ⁵⁸			7-22 days ⁶²	440		50 / 900 days	
2-Nitrotoluene	Ready ^{44,49,50#}	Estimated 3.2 days ⁵¹			>64 days ¹⁰²	6-21 ^{81#}	3-64 days	50 / 90 days	
Toluene	Ready ¹²⁴		1.7-6.9 days ³⁷						
Toluene-2,4- diamine	Ready ⁶⁰				>64 days ¹¹³	6-21 ^{81#}		50 / 90 days	
Bis (2- chloroethyl) ether	Inherent ^{16,17}	28-180 days ¹⁸	40 days ¹⁹		40 days ¹⁹	1.2 ^{81#}	28-180 days	1 <i>5</i> 0 / 300 days	
Bisphenol A ^{+, 22}	Inherent ^{20,21}	0.5 - 1.8 ²²	<4 days (acclimated) ²⁰		0.5-1.8 dαys ²²	16-76 ^{81#}	0.5-4 days	1 <i>5</i> 0 / 300 days	
Dichloroaniline	Inherent 126		5.5 days (90%ile) ³⁷						
1,2- Dichlorobenzene	Inherent ^{27,28}	0.3 - 3 days ²⁹	28-180 days ³⁰		6.3% deg in alkaline soil in 10 weeks ⁸⁵	4-6 ⁸⁴	0.3-180 days	1 <i>5</i> 0 / infinite	
Glyphosate	Inherent ³⁹		4 days ⁴⁰	13-29% degraded in 45 days ⁴⁰	30-40 days ⁴⁰	<100 ⁴⁰	4-45 days	1 <i>5</i> 0 / 300 days	
Naphthalene	Inherent ^{46,47}		3-63 days ⁴⁸ 2-3 days ⁴²	5hrs - > 88 days ¹⁰⁰	100 days ¹⁰¹	41-120 ^{99#}	2-100 days ^{101, 4;}	2-100 days ^{101, 42} 150 / 3000 days	\$
Nonylphenol	Inherent	8-9 days 6-26 days in seawater					6-29 days		
Octylphenol	Inherent	12.4 days							
17β-estradiol	Inherent			0.2-9 days			0.2-9 days		
<u>17α-</u> ethinylestradiol	Inherent			17 days			17 days		
* Where there is conflicting da # Assumes 5% organic carbon. + Recent studies confirm this s	* Where there is conflicting data the worst case is used. # Assumes 5% organic carbon. + Recent studies confirm this substance to be readily bid	* Where there is conflicting data the worst case is used. # Assumes 5% organic carbon. + Recent studies confirm this substance to be readily biodegradable.							

Persistence of Chemicals in the Environment

Table 17: Repo	orted half-lives	Table 17: Reported half-lives in waters, sediment	's and soils for a range of substances (cont'd	nge of sub	stances (cont'	d)			
Substance	Biodegradation test passed	River water die-away standard test (half-life)	Non standard aquatic tests (half-life)	Sediment (half-life)	Soil	Kp (I kg ⁻¹)	Half-life range)	TGD default half-life water /soil*	Abiotic half-life
Phenanthrene	Inherent ⁴¹		15 days ⁴²		2.5 - 800 days ^{1 10}	1 9 0 ^{108#} - 66000 ^{109#}		1 <i>5</i> 0 / 30000 days	T _{1/2} = 59-69 days (photolysis) ⁵⁷
Tetrachloroethylene	Inherent ¹²⁷		4 days (90%ile) ³⁷						
Triallate	Inherent ⁶¹		2-3 days (water in water/sediment) ⁶¹		3-88 days ¹¹⁵	100 ^{114#}	3-88 days ¹¹⁵	50 / 300 days	
1,2,4- trichlorobenzene	Inherent ^{41,63}		0.3 - 3 days (river) ²⁹ 58 days (marine) ⁶⁴		28 -180 days ³⁰	34 -250 ^{116#} 0.3-180	[#] 0.3-180	1 <i>5</i> 0 / 3000 days	
1,1,1,- trichloroethane	Inherent ¹²⁵		2.3 days (90%ile) ³⁷						
Xylene	Inherent ¹²⁴		8.7 days (90%ile) ³⁷						
Cyprodinil	None ⁸²				23 days (field) 28 days (lab) ^{82#}	1.	23-28 days ⁸²		
IQ	None					1 666 30 ^{83#}		Infinite	81 days (hydrolysis) 12 years under cid conditions ⁹
Difenconazole	None ⁸²				50 days (field) 176 days (lab) ⁸²		50-176 days ⁸²		
2,3,7,8-TCDD Dioxin	None		969-2089 days (estimated) ³⁴	500-600 days ⁹¹	1945225 ^{90#}	23387 ^{89#}	Infinite	Infinite	
Benzo'a'pyrene	None				60 - 530 days ⁷⁹ 500- 1000	, 500- 1000000 ⁷⁸	0.5-530 days ⁷⁹	Infinite	0.4 hr - 13 days (photolysis) ¹⁴
Hexachlorobenzene None ⁴¹	∋ None ⁴¹		0.3-2000 days ⁹		1 530 days ⁹⁷	500-5000 ^{96#}	#5	Infinite	
Hexachloroethane	None ^{43,44}		28 - 180 days ⁴⁵		Spiked concentration <lod 330d<br="" after="">in sandy aquifer, 28-180 days⁹⁸</lod>	10-48 ⁸¹ d		Infinite	
Metalaxyl	None ⁸²				27 days (field) 47 days (lab) ⁸²	1.3 ^{82#}	27-47 days ⁸²	300 days	

* Where there is conflicting data the worst case is used. ${}^{\sharp}$ Assumes 5% organic carbon.

56

JUDSIGLICE	Biodegradation	River water die-away	Non standard	Sediment (half-life)	Soil	Kp (I kg ⁻¹)	Half-life range)	TGD default half-life water	Abiotic half-life
			(half-life)					/soil*	
Ethylene oxide	Ready ⁴	Biodeg rate = hydrolysis rate = 12-14 days ³⁸			1.9-2.1 days ⁹⁵	0.8 ^{95#}	2-14 days	50 / 90 days	hydrolysis rate = 12-14 days ³⁸
Metolachlor	None ⁸²				15 days (field) 16 days (lab) ⁸²	16 ^{82#}	15-16 days ⁸²	300 days	
Oxasulfuron	None ⁸²				5 days (field) 8 days (lab) ⁸²	0.003 ^{82#}	5-8 days ⁸²	300 days	
Penconazole	None ⁸²				56 days (field) 195 days (lab) ⁸²	103 ^{82#}	56-195 days ⁸²	3000 days	
PCB-15	None		58 days ⁵⁵					Infinite	
PCB-52	None		20 days ⁵⁵					Infinite	
PCB-86	None		108 days ⁵⁵ , biodeg estimated 138 years ⁵⁶	14 years ¹⁰⁷		2944 ^{105#} - 52000 ^{106#}		Infinite	
Primisulfuron	None ⁸²				11 days (field) 30-63 days (lab) ⁸²	0.03 ^{82#}	11-63 days ⁸²	300 days	
Propiconazole	None ⁸²				72 days (field) 71 days (lab) ⁸²	103 ^{82#}	71-72 days ⁸²	3000 days	
Prosulfuron	None ⁸²				11 days (field)	0.01 ^{82#}	11-132 ⁸²	300 days	
Pymetrozine	None ⁸²				13 days (field) 5 days (lab) ⁸²	0.01 ^{82#}	5-13 days ⁸²	300 days	
Octachloro dibenzodioxin	None			10 years ⁹³		41500- 400000 ^{92#}	4-18 days ³⁶ #	Infinite	4-18 days via photolysis ³⁶ 102 years - photoreduction ⁹⁴
Terbuthylazine	None ⁸²				36 days (field) 79 days (lab) ⁸²	32 ^{82#}	36-79 days ⁸²	300 days	
Vinyl chloride	None ⁶⁸		5.8-16.1 days ³⁷	28 days ¹¹⁷		0.02-3 ^{81#}	5-28 days ^{37, 117}	Infinite	Photoxidation 2-3 days ¹¹⁸

* Where there is conflicting data the worst case is used. $^{\#}$ Assumes 5% organic carbon.

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Clearly when, using a system where environmental default data are extrapolated from laboratory tests that do not necessarily reflect field conditions, safety factors must be applied to offer suitable protection. Default values of 15 days for readily biodegradable substances that pass the 10-day window should therefore be considered precautionary (providing a safety factor of at least 3). The same applies to substances passing the ready test but not the 10-day window, where defaults of 50 days for fresh and estuarine waters also provide safety factors of at least 3. Figure 6 shows the worst-case measured halflives for substances that are classified as readily biodegradable (from the data in Table 17). All the substances have measured half-lives of less than 25 days in non-standardised river die-away type studies. The majority also have half-lives in soil of less than 25 days. Some of the biggest differences between TGD allocated default values and experimentally determined half-lives occur for those substances which only pass the inherent test. Figure 7 illustrates the measured half-lives for substances that are classified as inherently biodegradable. The figure shows that from the limited data available, >80% of the substances have half-lives of less than 50 days. The TGD default half-life for these substances is 150 days in freshwater, which is much greater than some of the measured half-lives provided in the literature. For example, a half-life of only 4 days was reported for glyphosate and 3 - 63 days for naphthalene. Other inherently degradable substances also appear to have much lower half-lives if sediment is present (triallate 2-3 days) or once adaptation has occurred (bisphenol A < 4 days). This suggests that default values based on inherent test data may provide an excessively large safety factor. Though the data cited herein are insufficient to support firm conclusions, they suggest that careful consideration of the basis upon which the default values have been derived is needed, especially for inherent biodegradability test data. This may be achieved by carrying out a larger-scale data gathering exercise than that performed in this report.

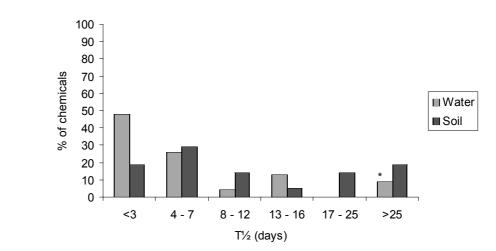
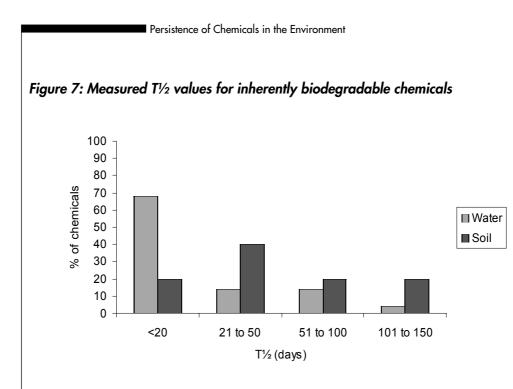


Figure 6: Measured T¹/₂ values for readily biodegradable chemicals

* Worst case value from a range of measured data (4-29 days) and a substance tested above its solubility (at low concentration $T\frac{1}{2} = 4$ days)



4.2.1.2 Marine water

Limited biodegradation data are available for the marine environment. It was therefore not possible to compare measured half-lives with those of at least 50 days recommended in the TGD. Longer default half-lives for the marine environment are used on the assumption that numbers of degrading bacteria are lower in the marine environment, and that they are less well adapted to chemical degradation. However, there is evidence to suggest that marine bacteria may be as efficient in degrading chemicals as those in the freshwater environment. A review of studies of the biodegradation of substances in the marine environment showed that there were conflicting data regarding the relative rates of degradation in freshwater and marine environments (ECETOC, 1993; ECETOC, 2001). In general, readily biodegradable substances showed a similar rate of degradation, with less biodegradable chemicals exhibiting degradation rates approximately four times slower in saline waters. It was concluded however, that chemicals shown to be readily biodegradable in freshwaters were also biodegradable in marine environments (ECETOC, 1993).

Based on the data obtained for freshwater, combined with research cited in Evans *et al* (2001) default values may overestimate significantly (in excess of what may be considered an adequate safety margin) environmental half-lives in marine waters. Further research would be needed to confirm this.

4.2.2 Terrestrial data

Whilst the data presented in Table 17 are not exhaustive, some useful preliminary conclusions can be drawn on the comparison between default values and field and/or laboratory data. Data in Table 17 for soil and sediment show a similar outcome to that for freshwater. The choice of partition coefficient bands in the TGD means that most

substances fall into the K_p<100 category, (equivalent to a K_{oc} value of 2000 [log K_{oc}=3.3] assuming a 5% organic carbon content). In most cases the TGD default half-life values are significantly greater than measured values in the environment. Environmental data for most chemicals shows that default values overestimate persistence by a factor of between 2 and 10 (Figures 5 and 8).

For example, readily degradable chemicals that partition strongly to the sediment (e.g. phthalates), or inherently biodegradable substances that have a weak affinity for soil (glyphosate) are given a default value of 300 days, which appears to be an overestimate of their environmental persistence. Laboratory degradation tests for these type of substances show half-lives in the region of 7 - 60 days. Data for other such substances would be required, however, before a stronger conclusion could be drawn. As previously discussed for water, safety factors of these magnitudes may well be inappropriate, even if using a precautionary approach.

For soils, unlike water, there are a large number of substances where environmental half-lives are at least 10 times shorter than default values. In many cases these are pesticides that were not readily biodegradable, but were (or were assumed to be) inherently biodegradable. As with aqueous data, compounds defined as having infinite half-lives on the basis that they fail the inherent biodegradability test, have been reported to degrade in the environment. These substances fall into the category of field data being in excess of 100 times the default value. The data therefore suggest that further assessment may be required of the criteria on which default half-lives are based.

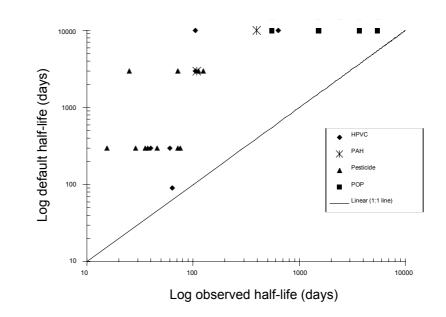


Figure 8: Observed versus EU TGD default half-lives for soil biodegradation

Note: Measured half-lives shorter than default rate are above the line; measured half-lives greater than default rate are below the line.

Moreover, the TGD (EC, 2003) assigns default rates based on a combination of ready and inherent biodegradability test data and the partition coefficient (K_p) of the substance. This assumes that substances that are strongly adsorbed to soil are less bioavailable, and hence more persistent. It is important to note however, that partitioning is an equilibrium process between the dissolved and particulate phases. For a substance that has a high K_p but is readily biodegradable in water, the concentration of the compound in the dissolved phase will have to be continually supplemented by desorption from the solids to maintain the equilibrium. The half-lives of these types of compounds would probably be significantly shorter than the 1 - 10 years assigned under the TGD.

4.3 Discussion and conclusions

Based on the data presented, the default half-life criteria applied to both ready and inherent biodegradations tests are longer than those observed in the environment or compartment specific simulation tests. This observation holds true for the degradation of some high production volume chemicals (HPVCs), POPs, PAHs and pesticides in freshwater and terrestrial environments. One possible reason for some of the discrepancies between measured and default data may be for substances where abiotic processes represent a significant route of degradation (e.g. vinyl acetate, dioxins, DDT). Conditions under which ready and inherent biodegradation tests had been carried out, may not have been suitable to mimic environmental photolysis/hydrolysis processes. As a consequence, persistence in the environment has been shown to be less than that suggested from biodegradation test data. Consequently, the use of measured abiotic and biotic degradation rates (T½ values) is required to predict accurately the overall persistence of a substance in the environment.

At present there are insufficient data to compare rates observed in standard marine/freshwater biodegradation studies to the marine environment. ECETOC believes that this data gap needs to be filled to reduce the uncertainty in extrapolating from standard freshwater/marine biodegradation studies to the marine environment. Based on such data, the proposed default rate constants for the marine environment based on ready biodegradability may need to be refined.

In ready biodegradation test systems using elevated substance concentrations, the rate of biodegradation appears to be closely correlated to the solubility of the substance for a number of test substances. Clearly, this factor is less important in the environment, and measured rates obtained in the laboratory test may be longer than those observed in the field. Therefore ECETOC recommends that, where possible, the biodegradability of a substance should be assessed at concentations below its water solubility.

One of the major problems highlighted when collating these datasets were the endpoints that were measured for biodegradation. These ranged from parent compound to dissolved organic carbon (DOC) removal, oxygen demand (OD) and carbon dioxide evolution. These endpoints are clearly non-equivalent (see Section 5). However, as persistence should be assigned to the parent compound, T¹/₂ values for DOC removal, OD and carbon dioxide evolution will err on the side of caution.

Overall, the data presented in this section demonstrate that to estimate the true persistence of a substance in the environment, significantly more thought has to be given to how the data generated in the standard test methodology are used, and in particular how the default values are derived.

To predict accurately the global persistence and behaviour of substances, persistence data are likely to be required for several environmental compartments. Selection of appropriate compartments should be based on the application and fate of the substance in question (see Section 7). Ideally a structured approach should be used to determine the most sensitive and exposed habitat, and the fate of the substance tested in depth in the resulting compartment(s) to derive advice for management purposes.

The information on the ready and inherent biodegradation of a selection of organic compounds shown in Table 17 and Figures 5-8 demonstrates the difficulty in assigning a default half-life based on ready and inherent biodegradation data as used in the TGD (EC, 2003) for persistence in freshwaters and soils. In particular:

- For compounds that pass the ready biodegradation test within the 10-day window, a default value of 15 days is generally an over estimate compared with river die-away tests, but is of the same order;
- for readily biodegradable compounds that fail the 10 day window a default value of 50 days is also likely to be an over estimate.

The greatest discrepancies occur for the substances that are either inherently biodegradable or fail the ready tests altogether. For example, a half-life of less than 4 days has been measured for Bisphenol A in water tests, but the original laboratory tests suggested it was only inherently biodegradable (the studies by Klecka *et al* (2001) have clearly shown that Bisphenol A is readily biodegradable). The variability in standard test data however, makes extrapolation difficult and inaccurate. A similar over estimation was assigned to 2-nitrotoluene and bis (2-chloroethyl) ether, with river die-away tests giving a half-life of only 3 and 35 days respectively, compared with the default of 150 days. One substance (vinyl chloride) that failed both the ready and inherent tests has been shown to degrade in the environment in a period of weeks, which is contrary to the infinite persistence assigned via the TGD. This may also be true of other substances which fail standard biodegradation tests.

5. IMPROVEMENTS TO EXISTING BIODEGRADABILITY TEST METHODS

The current suite of ready biodegradation test methods (OECD, 1992a) were designed to identify those substances that would undergo rapid and ultimate degradation in the environment. They provide a relatively simple and inexpensive technique to identify substances that do not require further information regarding their biodegradability, as they will not persist in any environmental compartment (see Section 4 for supporting data). However, substances that do not meet the 'pass' criteria may still have the potential to undergo biodegradation in the environment. No test substance should be designated 'persistent' based solely on the negative outcome of a ready biodegradability study.

Before formulating improvements to these existing biodegradation protocols, or recommending novel approaches to studying biodegradability, the factors identified in Section 3 require careful consideration. In designing a new predictive test, it is important to ensure that it can simulate all relevant conditions and/or support all loss processes that might occur in the environment in question. Critical factors include, but are not limited to, concentration of test substance, physic-chemical form of test substance, temperature, light intensity, pH, redox potential, level of microbes, physiological history of the microbes, growth rate of the microbes compared to residence time, presence of other substrates to support cometabolism or use of the test chemical as a secondary substrate. Some of these factor affect the kinetics of the reaction, while others affect their occurrence. All of these conditions must be maintained in a manner that permits a sufficient signal to noise ratio to monitor loss of the chemical, formation of metabolites, whether it be activated sludge, surface water sediment or soil. Many of these issues have been addressed in Section 3.

The remainder of this Section will focus on the endpoint(s) to be measured; the source, pre-treatment and density of the inoculum; the assignment of the pass/ fail criteria; the inherent variability of the test data generated; and environmental realism. There is scope for improving the existing tests, and for proposing alternative tests with increased biodegradation potential and environmental realism, so as to enable rates of degradation to be measured.

What rate should be measured?

Existing tests allow rates of parent compound removal, release of CO_2 , O_2 uptake and DOC removal to be measured. However, it must be emphasised that the pass criteria are not equivalent even for the same substance. This observation is especially true at low initial test substance concentrations (Snape and Evans, 1997) (Table 18). In studies comparing the time taken to reach the pass criteria for glucose, aniline, 4-nitrophenol, and 4-chloroaniline, the pass criterion for DOC removal was always exceeded earlier than that for CO_2 evolution. Whilst this is not surprising, since a cell prior to complete mineralisation must take up a substance, it does have severe consequences for the enforcement of the 10-day window and the assignment of default first-order rate constants.

To infer, from degradation and biodegradation studies, whether a substance is persistent, a minimum number of endpoints must be identified. For biodegradability testing the minimum endpoint must be parent compound disappearance (i.e. primary biodegradation). In addition, terminal metabolites need to be characterised such that their toxicological properties can be assessed relative to that of the parent substance. In the case of complete aerobic mineralization, the terminal metabolite would be carbon dioxide. It must also be recognised that metabolites may have markedly different physicochemical properties to that of the parent compound.

Where biodegradation studies use radiolabelled substances, a mass balance must be obtained. The minimum data required to achieve this mass balance must include: carbon dioxide evolved; residual organic carbon in solution; volatile carbon (usually volatilised during sampling); and carbon associated with particulate/ solid matter.

Table 18: A comparison of the time taken to achieve the pass criteria for ready
biodegradability in terms of CO2 evolution and DOC removal for different
concentrations of glucose, aniline, 4-nitrophenol and 4-chloroaniline (Snape
and Evans, 1997)

Initial concentration Time taken to achieve pass criteria (mg C I ⁻¹) for CO ₂ evolution (d)		Time taken to achieve pass criteria for DOC removal (d)	
Glucose			
10	5, 4	2, 1	
1	7	<1	
0.01	13	<1	
0.001	20, failed	<1, <1	
Aniline			
20	-	3	
10	-, 7	3, 3	
1	-	3	
0.1	-	3	
0.01	-, 10.5	3, 4	
0.001	-	6.5	
4-Nitrophenol			
10	14	12.5	
1	7	6	
0.1	14	12.5	
0.01	12	10.5	
4-Chloroaniline			
10	25	22	
1	failed	27.5	
0.1	failed	27	
0.01	failed	failed	

5.1 Source and density of inoculum

The inherent intra- and inter-laboratory variability of biodegradation data is a major source of concern that also needs to be addressed. The OECD 301 series of ready biodegradation tests have been standardised largely on parameters that do not have the greatest impact on the biodegradation data being generated; these are typically the use of a standardised mineral media, robust sampling regime, and the measurement of DOC removal, CO_2 evolution or O_2 demand. By far the greatest sources of variability introduced into these tests are the source and concentration of inocula, and conducting semi-specific chemical analyses (for DOC, TIC and O_2 demand) near the limits of detection. The use of an inadequate number of replicate vessels and time points only acts to compound these inherent intra- and inter-laboratory variabilities.

Preparation and standardisation

Currently, there is little guidance given on the preparation and standardisation of inoculum sources other than the clear stipulation that ready biodegradability tests must use inocula derived from a source that has minimum industrial input, and no previous exposure to the test substance (something that clearly cannot be guaranteed). Inocula can be derived from sewage sludges, river water, soil or a composite of each. However, in practice, only a single biodegradability test is conducted with the inoculum derived from only a single source.

Prior to their use in biodegradability testing, inoculum sources are typically washed to remove exogenous carbon and re-suspended in mineral media, usually as a percentage (for river water or secondary effluent) or as a defined quantity of dry suspended solids (for activated sludges). At this point the inoculum can be acclimated to the test conditions, or the test substance can be introduced and the biodegradation test can begin.

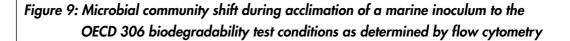
Acclimation

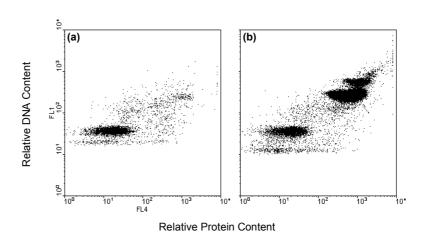
Acclimation periods exist to serve two distinct purposes: the reduction of exogenous carbon in the test and control flasks, thus improving analytical precision, and the reduction in intra-test variability between replicate flasks. To achieve these goals two approaches exist.

The most widespread form of acclimation is basically an ageing process where the inoculum is suspended in the test media for a period of up to seven days under the standardised incubation conditions (temperature and agitation). The test substance is added after this ageing period.

The second process is restricted to the MITI protocol (OECD, 1992b) and involves an extensive period of pre-culturing. Inocula are derived from at least ten sources and are blended. The rationale for using more then ten sites is to maximise microbial biodiversity. This composite inoculum is then fed daily with supplements of glucose, peptone and phosphates for a minimum period of one month prior to use. Using a number of methods Forney et al (2001) investigated the impact of these pre-culturing procedures on the inoculum composition and function. These investigations included assessments of substrate utilisation patterns and phospholipid fatty acid profile analysis (PLFA) to profile communities in different inoculum sources. The community impacts of pre-culturing were also assessed by 16S rRNA profiling using denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment analysis. By these studies, Forney et al (2001) convincingly demonstrated that inocula derived from different sources were markedly different and the source from a single location was also subject to temporal variations. Pre-culturing resulted in changes in community structure such that the final cultured inoculum used for biodegradability testing did not resemble the source inoculum. The implications of this on biodegradability testing needs further consideration.

Preliminary investigations using flow cytometry to examine the impact of ageing on marine inocula have demonstrated large shifts in the microbial community (Figure 9).





Plot (a) shows inoculum at time zero Plot (b) shows inoculum at time 160 hrs Data generated by Dr Bernhard Fuchs, Max Planck Institute, Bremen, under the Cefic LRI Persistence Programme (http://www.cefic.org/lri). Specific phylogenetic groups of marine bacteria have characteristic protein to DNA ratios that can be resolved by flow cytometric analysis. The data demonstrate that a 7-day period of ageing results in a significant shift in the bacterial composition of the inoculum i.e. the acclimation period imposes a selective pressure. Consequently, when the test substance is introduced after acclimation, the microbial population has become atypical of the environment. These flow cytometric studies clearly demonstrate that acclimation procedures have an impact on the microbial diversity inoculum; this in turn may have an impact on the outcome of the biodegradability test result.

To ensure environmental realism, the impact of acclimation practices on microbial diversity, viability and the test outcome must be examined in greater depth. Whilst current practices for acclimation may reduce exogenous carbon, and thus improve analytical precision, they may also reduce or increase the potential for biodegradation to be observed. This only offers to increase the uncertainty in the extrapolation of laboratory biodegradation test data to the environment. Acclimation procedures should be selected and standardised to serve the two purposes stated above, without reducing environmental realism and the potential for demonstrating biodegradability, or they should be omitted completely.

Pretreatment

With the exception of acclimation periods, inocula are rarely pre-treated prior to use in biodegradation testing. However, the introduction of three further pre-treatment stages may serve to reduce the inherent variability within and between biodegradation tests. These pre-treatment stages are dispersion, filtration and numeration and concentration (Thouand *et al*, 1995; Ingerslev and Nyholm, 2000).

- Dispersion will improve the homogeneity of the inoculum by dispersing aggregates of bacteria. Dispersion is particularly useful for biodegradability tests conducted with activated sludge inocula. Gentle sonication or homogenisation would achieve such dispersion although care must be taken not to reduce the viability of the inoculum through cellular disruption;
- Filtration will remove protozoans that graze on bacteria during acclimation periods (for those tests where they are in use) and extended lag phases. For batch biodegradation studies the presence or absence of one or two protozoa between replicate test flasks may have a marked outcome on the biodegradation test result. Filtration will also remove other fines or particulates that may result in different microbial densities between replicates. A course nylon filter (approximate 5µm pore size) will be sufficient. Numeration, by either total counts or viable counts (colonyforming units; CFUs), will enable the inoculum density to be standardised quantitatively. This will help reduce the variability introduced into the test flasks, especially in tests using sacrificial bottles. Numeration and a most probable number (MPN) approach to testing may also be used to infer the specific fraction of biomass competent of biodegrading the test substance to within one order of magnitude.

• Concentration, either by tangential filtration or centrifugation, will reduce scale effects apparent in laboratory-based biodegradation studies. These scale effect are especially apparent for tests using low working volumes of river and marine waters that have low initial microbial cell densities.

Pre-exposure / adaptation

Adaptation is actively discouraged within the current biodegradation testing strategy, and any data derived from a test that has the potential for adaptation to occur, is used in an overcautious manner. However, adaptation is a natural phenomenon, and any modification to existing biodegradability tests or novel testing protocols must reflect this natural phenomenon by allowing the use of pre-exposed sources of inocula. This is particularly true for substances that are to be released on a continuous basis. In order to assess the persistence of a substance, the potential for biodegradation to occur must be maximised without compromising environmental realism. The use of tests with inocula pre-exposed to the test substance, or prolonged semi-continuous and continuous biodegradability studies will serve this purpose.

The rate at which micro-organisms adapt to enable them to degrade a chemical is one of the critical factors that determine whether a chemical will persist. However it is also the factor with the greatest uncertainty, especially when extrapolating to environmental behaviour from laboratory test data. As a result, adaptation has been the subject of much debate within the scientific and regulatory communities. In general, the weight of evidence suggests that pre-adapted populations will degrade test chemicals at a greater rate; this is usually due to the enrichment of competent microbial populations (Thouand *et al*, 1996).

The concept of adaptation has been illustrated by, for example, Saltzmann (1982) and Massie *et al* (1985), who studied the potential for heterotrophic microbial populations to degrade hydrocarbons around the oilfields in the North Sea. They demonstrated that the total microbial population in waters and sediments contaminated with hydrocarbons did not increase, rather, it was the case that the existing population adapted their metabolic activity to degrade the nutrient input. Selection pressure from the degree of contamination meant that the rate of degradation was found to increase with decreasing distance from the platforms. Bauer and Capone (1988) provided evidence to show that, in marine sediments, degradation rates of a number of PAH's were influenced by pre-exposure (2 weeks) to benzene, naphthalene and anthracene. They suggested that the enhanced degradation was a result of either populations with broad substrate specificities or common biodegradation pathways.

Biodegradability tests restrict substance exposure to a defined range of micro-organisms, these can be as low as 10^5 cells per litre (< 10^4 cells in the test flask) for marine waters. This exposure regime is extremely stringent, and the probability of a competent degrader or consortium being present is low, especially for biodegradability tests that have a low total volume. These tests contain only the most abundant microbial populations present in the inoculum at the time of collection. In the environment, a chemical substance, especially one that resists degradation and partitions between different environmental compartments, will be exposed to an infinitely larger diversity of micro-organisms than can ever be simulated in a laboratory test. Consequently, any concerns regarding the substance (F) to inoculum (M) ratio (F:M) are unfounded when assessing a chemicals' persistence. The issues of pre-exposure and the requirement for a low inoculum concentration are confined to determining ready biodegradability and do not infer persistence. To assess the persistence of a chemical substance, environmental realism must be maximised, however the potential for biodegradability to occur must not be compromised. This can be achieved either through exposing the substance to a greater number and diversity of micro-organisms or through the use of pre-exposed inocula for substances that are likely to be released in a continuous manner.

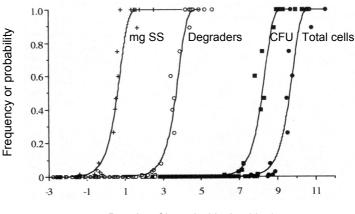
Thouand *et al* (1996) investigated the impact of inoculum density on the probability of p-nitrophenol biodegradation by activated sludge and river water inocula. For both river water and activated sludges, Thouand *et al* (1996) demonstrated that the probability of p-nitrophenol biodegradation was related to the initial density of the inoculum (Figure 10). Initial inoculum densities for activated sludge of 10^{10} cells per litre, as determined by a total cell count, demonstrated a probability of one for p-nitrophenol degradation. This observation was also demonstrated for river water inocula (Thouand *et al*, 1996). The number of specific degraders associated with the inoculum source was determined by a MPN approach. Clearly, at higher inoculum densities, the probability of having a competent micro-organism or appropriate microbial consortium increases. Thouand *et al* (1996) demonstrated that a minimum of approximately 10^{10} cells per litre were required to maximise the probability for p-nitrophenol degradation. However, this approach is likely to be chemical substance specific, with readily biodegradable substances such as glucose requiring far fewer cells per litre to have a probability of one. It must also be acknowledged that parallel studies have not been conducted using marine inocula.

The impacts of scale effects related to the microbial inoculum density and diversity have also been investigated by Ingerslev and Nyholm (2000). An alternative to increasing inoculum density, via pre-concentration (Thouand *et al*, 1996), is to increase the size of the test vessel or to utilise semi-continuous or continuous test systems. Ingerslev and Nyholm (2000) investigated the importance of the test volume on the lag phase in biodegradability studies. Using river water and activated sludge as the inocula, the impact of test volumes from 1.8 ml to 100 l were investigated on the biodegradability

of p-nitrophenol and 2,4-dichlorophenoxyacetic acid. Ingerslev and Nyholm (2000) concluded that a sufficient total amount, as well as a sufficient concentration of specifically degrading micro-organisms or consortia of bacteria, must be present initially for biodegradation to commence. Therefore, incubations will low volumes and consequently low microbial densities or unrepresentative microbial diversities, will fail randomly. Volumes of 50 ml or less containing river water were observed to have increased lag times and an increased probability of biodegradation not taking place (Ingerslev and Nyholm, 2002). These volumes may be higher for inocula derived from pristine environments and marine waters where the microbial diversity and concentration may be somewhat lower. Recently, Curtis et al (2002) estimated that the least abundant microbial taxon in marine waters, if equally distributed, would only be present in every 10 cubic kilometres. Clearly, the issue of scale effects, through working at low test volumes and low initial microbial densities, does impact laboratory-based biodegradability screening and thus inference of persistence. Consequently, new robust testing procedures are required that improve realism with respect to the diversity of microbial species present such that scale or laboratory artefacts are minimised.

In addition to studying the impact of the initial inoculum density on the probability of biodegradation, Thouand *et al* (1996) also determined the impact of pre-exposure on the probability of p-nitrophenol biodegradation. Thouand *et al* (1996) concluded that if the inoculum source was pre-exposed to p-nitrophenol for a period of five days, the initial inoculum density could be reduced from 10^{10} to 10^5 cells/ litre whilst still ensuring the maximum potential for biodegradation to occur.

Figure 10: Experimental frequency of positive p-nitrophenol biodegradability tests (data points) and probability model (solid line) according to the density of activated sludge inocula quantified by total suspended solids (mg SS per litre), specific p-nitrophenol degraders (per litre), cultivable bacterial (CFU per litre) and total bacterial cells (per litre)



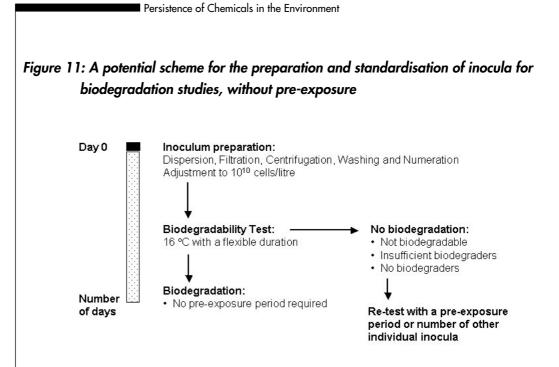
Density of inocula (decimal log)

Potential improvements to existing biodegradation screening tests

For new substances not satisfying the criteria for ready biodegradability, and existing substances for which there are no ready biodegradation data, test systems are recommended that either allow the use of elevated biomass concentrations (Figure 11) or pre-exposed inocula (Figure 12). These biodegradability tests can be environmental compartment specific if required, can be conducted within existing ready biodegradation test apparatus, and have the added benefit of allowing probabilistic biodegradation assessments to be made. In Section 8, a probabilistic-type approach is advocated, because measured rates of biodegradation in the laboratory cannot be used directly as an environmental half-life ($T^{1/2}$), and no single rate can be used to describe biodegradation in the environment. The $T^{1/2}$ will not be single number, but will cover a range or distribution of values that will reflect the different degradation kinetics and rates observed in all environmental compartments (see Section 8). A suggested approach to determine biodegradability, with elevated biomass concentrations and pre-exposure based on the work of Thouand *et al* (1996) is outlined below. For reasons of practicality the elevated biomass approach was preferred over the use of elevated test volumes.

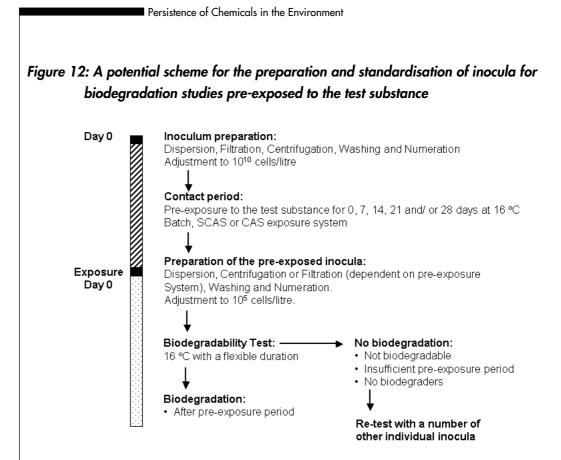
a) Increased inoculum density

The elevated biomass test is applicable for assessing biodegradability in sewage treatment, river water and marine waters. The initial choice of inocula/inoculum could be based on the use and release pattern of the substance in order to maximise the potential for environmental realism. Inocula should be used within 24 hours of collection to minimise laboratory selective pressures, pre-treated according to Thouand *et al* (1996) to reduce variability, and introduced into the test flask with a final cell density of 10¹⁰ cells/litre as determined by a total cell count (Figure 11). For typical sewage sludges this will represent a 100-fold dilution. However for typical river waters (10⁶ cells/litre) and marine waters (10⁵ cells/litre) a concentration procedure is required. Membrane filtration (0.22µm) represents the best option. The biodegradation test has no defined duration, however, if there is no evidence of biodegradation after 28 days, it is recommended that the test is terminated and repeated with another inoculum source or with a pre-exposed source of inocula (Figure 12). The use of no more than ten different sources of inoculum is recommended.



b) Adapted inoculum

The use of pre-exposed inoculum offers to enrich a competent microbial population, thus mimicking the selection process that would occur in the environment. The approach is recommended for substances that will be released into the environment on a continuous basis, such that adaptation will occur in the environment through continued exposure. The initial choice of inocula/inoculum could be based on the use and release pattern of the substance. This inoculum should be pre-treated and introduced into the test flask with a final cell density of 10¹⁰ cells/litre as determined by a total cell count (Figure 12). The contact or pre-exposure period can be varied (pre-exposure periods of 0, 7, 14, 21 and 28 days are recommended) after which the 'adapted' inoculum must be dispersed, filtered or centrifuged, and washed prior to the test exposure. The varied exposure periods will provide some indication of the ease with which the competent microbial populations were enriched. The washing procedure is required to remove any residual test substance or metabolites. To compensate for the possible enrichment of competent microbial populations, a reduced cell density of 10^5 cells/litre is recommended for the test exposure. Care must be taken to ensure that the scale of the pre-exposure system is sufficient to provide the requisite density of cells for the test exposure. The pre-exposure test system used can be batch, semi-continuous or continuous, but must not exceed 28 days and the test substance concentration must be the same as that in the test exposure. The biodegradation test has no defined duration, however if there is no evidence of biodegradation in the test exposure after 28 days it is recommended that the test is terminated and repeated with another inoculum source (Figure 12). The use of no more than ten different sources of inoculum is recommended.



The use of alternate batch test systems that have a benthic (sediment) component could also be used for assessing biodegradability in freshwater-sediment and marine-water sediment environments (ISO 14592-1).

Semi-continuous and continuous test systems

Without doubt the inherent biodegradability tests (OECD 302 series) and simulation biodegradability tests (OECD 303 series) allow a greater degree of microbial adaptation to occur than the ready biodegradation tests. This arises largely through the use of increased biomass levels, longer test periods and higher test substance concentrations (strong selective pressure). These inherent tests provide conditions that increase the potential or probability for biodegradation to occur and the data given in Section 4 suggest that the default values currently assigned to data generated from these tests are too conservative.

Some recent batch and continuous biodegradation protocols have been accepted to simulate biodegradation in surface waters (ISO 14592-1 and 14592-2). These still have a number of practical limitations, which need to be addressed to ensure their wider applicability within a biodegradation framework. These limitations arise from their reliance on and poor use of ¹⁴C-labelled material.

Alternate semi-continuous and continuous test systems are required that improve the potential for biodegradability to be assessed in all environmental compartments (marine, freshwater and terrestrial). These systems need to maximise biodegradation potential and environmental realism. This realism must reflect biological and chemical interactions between water bodies and sediment, and the physico-chemical status of the environmental compartment. Strategies for inoculum reintroduction, to maximise microbial diversity and viability, are required especially for marine and freshwater biodegradability testing. In addition such strategies would also ensure that other limiting nutrient resources are replenished. Perhaps the most suitable test system is the semi-continuous activated sludge (SCAS) apparatus. This test system, and the cascading river-bed, may offer suitable starting points, especially for marine and freshwater studies. However, the value of conducting such studies within the current regulatory framework needs urgent clarification to ensure that optimum value of the data is realised.

5.2 Test chemical concentration

Because of the insensitivity of the analytical techniques used, current ready tests are generally carried out at concentrations that are orders of magnitude higher than those usually encountered in the environment through general use (particularly the marine environment). This situation could give rise to false negatives arising from either the potential for toxic responses or solubility/mass transfer effects. A significant improvement in current testing could be achieved by performing tests at more realistic substance concentrations. This is likely to necessitate the use of specific chemical analysis or radiochemistry in order to attain the required lower limits of detection. Careful selection of test concentrations would have to be made based on substance specific properties (e.g. solubility or analytical limits of detection) to obtain a balance between realism and practicality.

5.3 Test temperature

Most standard biodegradation test methods specify ambient temperatures in the range 20-25°C. Even the OECD 306 method (OECD, 1992c) for simulating biodegradation in marine waters specifies temperatures in the range 15-20°C. These temperature ranges are only observed in tropical regions or in the mid-summer of temperate climates. Tests conducted at more environmentally-realistic temperature may therefore provide better estimates of environmental persistence, through this does have practical and cost implications within the laboratory. For example, test durations may need to be extended and temperature- controlled rooms or incubators with cooling units will be necessitated and these cooling requirements will have considerable energy implications. When selecting test temperatures, it is critical, to take steps to ensure that the microbial populations used in the inocula are within their natural temperature range. This recommendation could be applied to all tests, ready, and simulation, but would be particularly applicable to the latter. An incubation temperature of 16°C would improve environmental realism, and encourage the proliferation of both psychrophilic and mesophilic bacterial populations.

5.4 Duration of test, required number of replicates and the frequency of sampling

The data produced during existing ready biodegradability testing need to be statistically more robust, reflecting the inherent stringency of the tests. Often triplicate analyses prove inadequate. To improve the interpretability of the data generated, it is suggested that at least five replicates should be used for control flasks (those containing the inoculum and not the test substance) and test flasks. The availability of these additional data will enable outlying data to be disregarded on a statistical basis with a greater degree of certainty. For screening tests with increased biomass concentrations and those using pre-adapted inocula (Section 5.1), triplicate analyses should be adequate since these procedures will reduce the variability experienced at day 0 with existing tests.

The current ready test methodologies only provide vague descriptions of sampling frequency, but the TF considered there is little benefit in increasing the sampling frequency beyond once a week. The main objective of the ready test is to determine whether the pass level is achieved within the test duration, it is not designed to generate measured rates. In contrast, the number of time points should be maximised in biodegradation tests with increased inoculum densities or adapted inoculum, such that rates of degradation can be derived. These time points should be concentrated on the degradation phase to increase the confidence in the rates observed. Similar considerations are required for semi-continuous and continuous biodegradation test systems.

The TF recommends that in tests where the onset of biodegradation has occurred within the initial 28-day period, but where the plateau has not been reached, the test durations should be extended until degradation ceases. Continuous and semi-continuous tests should have undefined test duration.

5.5 Conclusions

Ready biodegradation test methods were designed to identify those substances that would undergo rapid and ultimate degradation in the environment. They provide a relatively simple and inexpensive technique to identify substances for which further information regarding their biodegradability is not needed, as they will not persist in the environment. However, substances that do not meet the 'pass' criteria may still have the potential to undergo biodegradation in the environment. Consequently, the TF believes that no test substance should be designated 'persistent' based solely on the negative outcome of a ready biodegradability study.

A minimum number of endpoints must be identified to infer 'persistence' from degradation and biodegradation studies. For biodegradability testing, the minimum endpoint must be parent compound disappearance. In addition, any accumulating terminal metabolite(s) must be identified; in the case of complete aerobic mineralisation the terminal metabolite would be carbon dioxide. The persistence and toxicity of any stable metabolites would have to be addressed separately in isolation from the parent compound.

The TF recommends that the impact of acclimation procedures on inoculum composition and viability, linked to the outcome of the biodegradation test result, needs to be addressed. In addition, strategies for inoculum reintroduction to maximise microbial diversity and viability are required, especially for marine and freshwater biodegradability.

Adaptation is a natural phenomenon; however existing tests provide a minimum opportunity for adaptation to occur. In order to assess the persistence of a substance, every realistic opportunity for biodegradation to occur should be allowed, this includes the use of semi-continuous and continuous test systems, and inocula pre-exposed to the test substance. The potential for adaptation to both new or existing chemicals is identical; it is the use pattern (continuous or intermittent) and environmental loading of a chemical that will have the greatest impact on the time taken for adaptation to occur in the environment. The TF recommends that pre-exposure regimes, and the use of inoculum densities that maximise the probability for biodegradation, must be used to determine the persistence of chemicals.

The issue of biomass concentration requires careful consideration. For reasons of cost effectiveness, replication, routine analysis and automation tests volumes used in biodegradability are decreasing. These decreases in test volumes are creating scale effects and laboratory artefacts that result in poor reproducibility between replicate flasks and an increased number of false negatives with respect to biodegradation. To reduce these scale effects, the requirement to maintain a strict ratio of substance to microbial density must be addressed, and test systems allowing the used of elevated biomass levels should be ring tested.

It must be recognised that not all studies can be conducted using radioisotopes or specific chemical analysis. However, biodegradation studies that use radiolabelled test substances must demonstrate a mass balance. The minimum data required to achieve this mass balance must include: carbon dioxide evolved; residual organic carbon in solution; volatile carbon (usually volatilised during sampling); and carbon associated with particulate/ solid matter. A single test concentration for all substances cannot be supported. The TF recommends careful selection of test concentrations based on substance specific properties (e.g. solubility or analytical limits of detection) to attain a balance between realism and practicality.

6. ABIOTIC DEGRADATION PROCESSES

As discussed in the previous sections of this report, the fate of a chemical in the environment is determined by a combination of physical, chemical and biological processes. The abiotic processes that affect a chemical in the environment include distribution, transportation and degradation.

The former two processes may be considered, in effect, as the partitioning behaviour of a compound. The physico-chemical properties of a compound affect its inherent behaviour, and thus distribution and partitioning in the environment. The physico-chemical nature of a compound can therefore determine the concentration of that compound in any particular phase or compartment in the environment. These aspects of fate are considered in Section 7. Degradation processes are concerned with the transformation of a substance into other compounds in the environment. Previous sections within this review have focused on biologically-mediated degradative processes and the factors that impact on biodegradation; the remainder of this section will briefly review abiotic degradation.

The environment is best considered as having different phases, which are represented by different environmental compartments. Thus the gas, liquid and solid phases are represented as atmospheric (gas), aqueous (liquid) and soil or sediment (solids) compartments, respectively. Each type of abiotic degradation mechanism has a varied degree of importance for each environmental compartment, consequently the process and the rate at which it occurs needs to be considered with respect to the environmental compartment in which the substance resides.

The main abiotic degradation processes to be considered are:

- In water: hydrolysis, photolysis and oxidation processes;
- in soil: hydrolysis and oxidation processes;
- in the atmosphere: oxidation through reaction with the hydroxyl radical and direct photolysis.

This section will focus predominantly on hydrolytic and photolytic degradative processes. Both of these processes are generally considered to have first order rate kinetics, with relatively short environmental half-lives, that would be compatible with a DT_{50} range approach to describe degradation in all environmental compartments.

6.1 Hydrolysis

Hydrolysis describes the reaction of a substance (RX) with water to form a new carbonoxygen bond by cleavage of the original carbon-X bond. The overall reaction is the direct replacement of X by a hydroxyl group. The reaction is described by the following equation:

$$RX + H_2O \rightarrow ROH + HX$$
 Equation (1)

Hydrolysis can be considered as the ionisation of RX followed by nucleophilic attack by water, and can be distingushed from other reactions of organic compounds with water such as acid-base reactions, hydration of carbonyls, addition to carbon-carbon bonds and elimination (Harris, 1982a). By its very nature hydrolysis is important for chemicals that are to be found in aqueous environments (freshwater, marine water, sediments, and to some extent soils) and thus the solubility of the chemical in water is important. Only a few chemical classes can undergo hydrolysis, namely alkyl halides, esters and ester-like compounds such as amides, carbamates, epoxides and lactones, phosphoric acid esters and thioesters (Table 19; Hemond and Fechner, 1994). Other functional chemical classes are resistant to hydrolysis (Table 19).

Functional groups susceptible to hydrolysis		Functional groups resistant to hydrolysis	
Alkyl halides anhydrides	Lactones	Alkanes	Aromatic nitro
	Lactams	Alkenes	compounds
Amides	Nitriles	Alkynes	Aromatic amines
Amines	Phosphinic acid esters	Benzenes	Alcohols
Carbamates	Phosphonic acid esters	Polycyclic aromatic halides	Phenols
Carbonates	Phosphoric acid esters	Heterocyclic polycyclic	Glycols
Carboxylic acid esters	Sulfonic acid esters	aromatic halides	Ethers
Epoxides Esters Imides	Sulfuric acid esters Urea	Halogenated aromatics/PCBs Aldrin and related halogenated hydrocarbon pesticides	Aldehydes Ketones Carboxylic acids Sulfonic acids

Table 19: Types of functional groups that show susceptibility and resistance to hydrolysis. (Adapted from Harris (1982a) and Hemond and Fechner (1994)).

While rates of abiotic hydrolysis reactions can be slow when compared to biologicallymediated hydrolysis (Connell, 1997), abiotic hydrolysis has been found to be a significant process for some compounds (Walker *et al*, 1988).

6.1.1 Kinetics

Hydrolysis is considered as a first order reaction [Equation 2], although it is actually a pseudo first order expression best considered as the summation of acid- and base-catalysis in addition to the nucleophilic attack by water [Equation 3]:

$$-d[RX]/dt = k_T[RX]$$
 Equation (2)

$$k_{T} = k_{H}[H^{+}] + k_{0} + k_{OH}[OH^{-}] + \sum_{i} k_{HAi}[HA] + \sum_{j} k_{Bj}[Bj] \qquad \text{Equation (3)}$$

which simplifies in weak buffers to:

$$k_T = k_H [H^+] + k_0 + k_{OH} [OH^-]$$
 Equation (4)

The term k_0 is the rate constant for neutral hydrolysis and should be written in terms of a second order rate constant:

$$k_0 = k_{H_2O}[H_2O]$$
 Equation (5)

However, since the concentration of water in aquatic systems is usually relatively constant (~ 55.5M), k_0 is treated as a pseudo first order constant. Therefore hydrolysis is dependent on the pH of the aquatic environment at a given temperature (Mabey and Mill, 1978; Equation 4). Rate constants for the hydrolysis of many substances are available and can be easily determined in the laboratory. Thus, given the pH of a particular environment, the hydrolysis of a given compound can be predicted with a reasonable degree of accuracy. In environments where sorption is a factor the hydrolysis rate is reduced proportionally to the remaining dissolved concentration of the compound.

6.1.2 Measurement of hydrolysis rate

The measurement of the hydrolysis rate for a given substance should take into consideration the rate law, the products of reaction and its temperature dependence (activation energy). Experimentally the depletion of RX, or accumulation of X, can be determined in dilute aqueous buffer over time. The use of dilute aqueous buffer allows the H⁺ and OH⁻ concentrations to be fixed over the course of the experiment so that pseudo first order conditions are achieved, as described in the equations below:

$$-d[RX]/dt = k_{H}[H^{+}][RX] + k_{0}[RX] + k_{OH}[OH^{-}][RX]$$
Equation (6)
$$-d[RX]/dt = k_{T}[RX]$$
Equation (7)

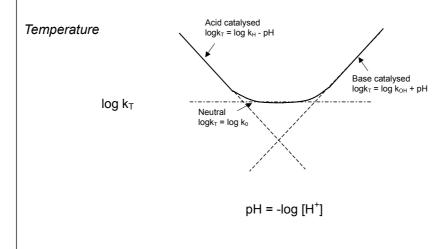
Throughout the experimental period, small aliquots of the test solution can be withdrawn and the depletion of RX, or appearance of X, measured by any suitable method. These include chromatographic analysis (e.g. GC, HPLC) or the measurement of absorbance of UV or visible light at a wavelength appropriate for the substance being measured. A linear plot of the logarithmic concentration of RX over time indicates that presumed first order reaction has occurred, where the slope is equal to $-k_T$ and the intercept is the logarithmic initial concentration of RX. The concentration of RX used is higher than that expected in the environment, typically at least 0.001M, probably for ease of detection. However, since the kinetics follow first order rates, this should not be important.

6.1.3 Factors influencing hydrolysis

pН

Figure 13 shows the strong effect that pH has on the rate and extent of hydrolysis. Neutral hydrolysis typically occurs between pH 5 - 8, which is the pH range normally encountered in natural aqueous environments. Below pH 5, acid-catalysis occurs and above pH 8, base-catalysis occurs. In order to determine the effect of the constants k_H and k_{OH} on the overall (k_T) hydrolysis constant, experiments are usually conducted at various pH values; typically 5, 7 and 9 (Howard, 1990; Howard *et al*, 1991), or 4, 7 and 9 (OECD, 1981a), and at pH 1-2 for physiological functions (OECD, 1981a). Accurate measurements for pH are required especially in determinations of kH and kOH, since an uncertainty in the pH value of ± 0.02 can lead to an error of about 5% of the rate constant (Harris, 1982a).

Figure 13. Schematic diagram of the effect of pH on hydrolysis rate.



In common with many chemically-mediated reactions, hydrolysis is dependent on temperature; an increase in temperature is associated with an increase in the rate of hydrolysis. Thus, it is recommended that an accurate and precise temperature is maintained while determining the rate of hydrolysis. This is generally, in the 0 - 50 °C range; a 1 °C change in temperature can give a 10% change in k_T , whereas a 10 °C change can cause a 250% change in k_T (Harris, 1982a). Thus, it is often recommended that the test temperature be maintained within ± 0.1 °C of the desired value (OECD, 1981a). The OECD Guidelines (OECD, 1981a) advocate that preliminary tests on substances with unknown hydrolytic stability are conducted at a temperature of 50 °C. If the compound is hydrolytically stable at 50 °C, no further tests are required and the substances, it is recommended that the test is completed at two temperatures between 0 and 40 °C.

Hydrolytic data for many chemicals are generally reported within temperature ranges of 0 - 50 °C, but most typically around 20 or 25 °C (Howard, 1990; Howard *et al*, 1991; Mabey and Mill, 1978). Care must be taken when conducting tests at higher temperatures since other reaction mechanisms, such as elimination reactions, may occur (Harris, 1982a).

The temperature dependence of k_T can be expressed by the Arrhenius equation [Equation 11] and plotting log k versus 1/T, or by using expressions based on Eyring reaction rate theory (Harris, 1982a). Given the dependence and sensitivity of k to temperature, care must be taken when extrapolating values of k beyond those temperatures measured. However, when extrapolating, the values may be considered as order of magnitude estimates. Order of magnitude estimates are generally acceptable for environmental situations and are compatible with existing model estimates.

$$k = A e^{\frac{E_A}{RT}}$$
 Equation (11)

where E_A is the activation energy (kcal mol⁻¹), R is the gas constant (1.987 cal deg.mol⁻¹), and T is the temperature (K).

Other factors

Other factors that must be taken into consideration when measuring the rate of hydrolysis, include ensuring that no other abiotic degradation mechanisms such as photolysis and oxidation can take place (OECD, 1981). In addition, the buffering solution must be carefully selected to minimise its impact on the rate of hydrolysis, and its reaction type particularly in acid or base conditions. The buffers usually chosen have a low ionic strength and usually provide a good approximation of natural freshwater environments. A list of suitable buffers is given in the OECD Test Guideline 111 (OECD, 1981).

The solubility of a substance is also important. If it is relatively insoluble in water OECD recommend that a half saturated solution in water is prepared. Alternatively, a solvent solution could be prepared provided that the solvent only represents <1% of the solution (OECD, 1981). Care must be taken where co-solvents are used as these can have a dramatic impact on the rate of hydrolysis compared to aquatic only systems (Harris, 1982a).

Elucidating the products of hydrolysis reactions is important because the hydrolysis product(s) may have different inherent properties to the parent molecule. Typically, hydrolysis products have an increased polarity due to the substitution of one group with a hydroxyl group. Such compounds tend to be more soluble, and stable, and may have an increased or decreased biological impact. The hydrolysis products are also usually more prone to biotransformation (Connell, 1997). Products can be determined by the chromatographic techniques mentioned above or more comprehensively studied using radio-isotopically labelled substances.

6.1.4 Estimates of hydrolytic rates

There are methods available based on structure-activity relationships for estimating hydrolytic rates for certain substances, in particular the linear free energy relationship (LFER) concept (Harris, 1982a). This uses a set of constants for substituent groups to model the rate of hydrolysis. However, due to the limited validated dataset only a limited number of substances can be investigated in this manner (Harris, 1982a). In addition, the rates for acid-, neutral- and base-hydrolysis need to be estimated individually. As the validated dataset is limited, it is difficult to quantify and qualify the errors involved in this estimation method, it should be considered that these structure-activity relationships only estimate rates of hydrolytic degradation within an order of magnitude at best.

Howard *et al* (1991) used a graphical exposure modelling system for estimating hydrolysis rates (US-EPA PCHYDRO-PGEMS, 1987). The US-EPA produced several programs for the estimation and/or extrapolation of hydrolysis rate data to other conditions (EXAMS, Burns and Cline, 1985; WASP, Ambrose *et al*, 1987; RATE, Hamrick *et al*, 1992; and FATE, Kollig *et al*, 1993). Some of these programs use activation energy values, measured or assumed, to extrapolate measured hydrolysis rate data at one temperature to other temperatures by using a form of the Arrhenius equation. These data appear to agree closely with experimentally determined rates of hydrolysis (Hamrick *et al*, 1992). The FATE program incorporates estimation routines that are based on chemical structure theory and correlations to provide quantitative structure-activity relationships (QSAR) (Kollig *et al*, 1993).

As with all (Q)SAR data, measured values from experimental determinations cannot be replaced by estimations. However, a greater degree of certainty can be placed on estimates of chemically based degradation mechanisms (e.g. hydrolysis), than those mediated biologically.

6.1.5 Prediction and extrapolation of hydrolysis rates

Conditions for experimentally determined hydrolysis rates appear to be sufficiently realistic for extrapolation to many freshwater environments; this is supported by the relatively few studies where natural environments have been studied (Mabey and Mill, 1978; Harris, 1982a). Indeed, the computer-based estimates detailed above may enable realistic extrapolations from measured laboratory-based data to the range of environmental conditions present in the field, for certain classes of substances (e.g. RATE, Hamrick *et al*, 1992). However, in complex environments (especially those with multiple sub-compartments where intrinsic chemical properties allow for complex interactions), the rate of hydrolysis may be strongly influenced. This again dictates the careful use of estimated data from (Q)SARs in environmental exposure assessment.

Reference data

Experimentally and estimated hydrolysis rates for many existing compounds have been compiled in the primary literature and various publications (Freed, 1976; Mabey and Mill, 1978; Palm, 1975-1979; Harris, 1982a; Howard, 1990; Howard *et al*, 1991; Roberts, 1998), some of which are available on the Environmental Fate Data Bases of the Syracuse Research Corporation (Howard *et al*, 1986) and the databases of the US-EPA (e.g. FATE, Kollig *et al*, 1993). Whenever possible these sources also give the transformation product(s) (Roberts, 1998; FATE, Kollig *et al*, 1993). Kollig *et al* (1993) has also highlighted the problems associated with obtaining data from secondary sources or databases.

6.2 Photolysis

Photolysis describes the transformation or degradation of a substance by light. This mechanism is also referred to as photodegradation or phototransformation. In the environment, photolysis is directly mediated by sunlight, which can act to transform substances in two ways. Firstly, by the direct absorption of light by a substance followed by a subsequent photochemical reaction (direct photolysis) and secondly, by the absorption of light by other substances or materials in the environment that then react with the substance (indirect or sensitised photolysis). Both processes may involve the generation of free radicals. In aquatic environments the rate of photodegradation is strongly influenced by the colour, clarity and DOC of the environmental or test water(s).

Generally the rate of photolysis can be described by first-order kinetics with respect to light intensity and substance concentration. The presence of fines or sediments can directly affect light attenuation and provide a surface area for the sorption of the substance and thus influence photolysis rates. Direct photolysis has been the subject of intensive laboratory investigations; indirect photolysis has received less attention.

6.2.1 General theory

Photolysis is dependent on the characteristics of both the substance and the incident light. Light is electromagnetic radiation, which can be described in terms of particles (photons) or waves. The first law of photochemistry states that only the light absorbed by a molecule can produce photochemical change in the molecule (Wayne and Wayne, 1999). If a substance absorb lights, the radiation energy absorbed can cause a number of molecular transitions that depend on the energy associated with the particular wavelength(s) of light absorbed. The energy is best described as a quantum of light or photon, where each photon of light has a discrete energy value expressed by the following equation:

$$E = hv$$
 Equation (12)

where E is energy in Joules, h is Plank's constant (6.63 × 10^{-34} J), and v is frequency (Hertz, or cycles per second, s⁻¹). The above equation relates energy to wavelength since:

 $c = v\lambda$ Equation (13)

where c is the constant velocity of light through a vacuum $(3 \times 10^8 \text{ ms}^{-1})$ and λ is the wavelength of light (expressed in metres).

The radiation energy absorbed must be greater than that required for bond dissociation if a substance is to undergo transformation. From the above equations it can be deduced that shorter wavelengths of light possess greater energy than those with longer wavelengths. When considering the photodegradation in the environment, only those wavelengths encountered at the earth's surface and lower atmosphere are important. Therefore, those wavelengths of light possessing sufficient energy to initiate transformation within the ecosphere are within the ultraviolet (UV) and visible (VIS) spectrum of light (290 - 750 nm).

Absorption can be measured and quantified using the Beer-Lambert law, which relates absorbance to concentration at a given wavelength for a chemical in solution (Equation 14):

 $I = I_0 \cdot 10^{- \epsilon.c.l}$

Equation (14)

Equation (15)

and

 $A = \varepsilon c I = \log(I_0/I) = -\log T$

Where A is absorbance (optical density), I_0 is the intensity before absorption (quanta or energy s⁻¹ or photons cm² s⁻¹), I is the intensity after absorption (units as previous), ϵ is the molar extinction coefficient of the substance (L mole⁻¹ cm⁻¹), c is the concentration (mole L⁻¹), I is the optical path length (cm) and T is transmission (T = I/I⁰).

The above equation can be adapted for gas phases with the use of natural logarithms and concentration in molecules/cm³ (N), to give:

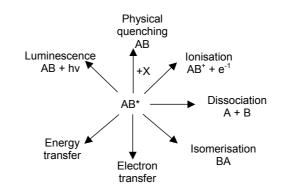
$$\ln (I/I_0) = \sigma N I$$

Equation (16)

where σ is the absorption cross section (cm² molecule⁻¹).

Molecules in their natural state exist at their lowest energy, their so-called ground state. When a molecule absorbs a quantum of light, it is photochemically activated and is considered to be in an electronically excited state. In this state, the excited molecule may react, use or lose its excess energy in a number of different competing ways (illustrated in Figure 14), one of which is transformation or degradation. These transformation or degradation reactions include dissociation, reaction (oxidation) and isomerisation. In addition, some of the primary reaction processes (Figure 14) may also initiate some subsequent secondary reactions.

Figure 14. Competitive reaction pathways for the loss of energy from an excited substance (adapted from Wayne and Wayne, 1999).



The second law of photochemistry (Stark-Einstein) states each photon can only activate one molecule in the primary process of a photochemical sequence. This law provides another useful concept in photochemistry, the quantum yield (Equation 17). The quantum yield is a substance-specific property that is a measure of the efficiency by which a substance converts photon energy into energy for chemical conversion. The sum of the quantum yield for primary processes is unity (i.e. 100% conversion). However, a quantum yield of greater than one may be obtained and suggests the occurrence of secondary reactions or chain reaction mechanisms. In the vapour state quantum yields are wavelength dependent, whilst in the liquid state, quantum yields are generally wavelength independent. Clearly this has implications for the atmospheric and aquatic environments.

$\mathbf{F} =$	number of molecules that photoreact	Equation (17)
	number of photons (quanta) absorbed	Equation (17)

6.2.2 Measurement of direct photolysis rate

Experiments show that, when concentrations of substances are very low (as is generally the case for environmental pollutants), first-order rate expressions usually describe the kinetic data:

 $- d[C]/dt = k_p[C]$ Equation (18)

thus

 $[C]_t = [C]_0 e^{-kt}$ Equation (19)

where [C] is the concentration of the substance of interest at time t and at time 0, and k_p is the overall photolysis rate constant.

In natural waters, the overall rate constant is equivalent to the sum of the direct and indirect photolysis rate constants, k_d and $k_{s'}$ respectively. Direct photolysis is usually the predominant process in aqueous systems (Zepp, 1980). From the above equations the overall half-life of the substance may be determined:

 $DT_{50} = 0.693/k$ Equation (20)

The substance is usually irradiated by a light source that closely resembles solar irradiation, or else, by sunlight itself. The disappearance of the parent compound in solution or vapour state is usually followed over time by any suitable analytical technique (e.g. GC, HPLC, NMR-spectroscopy). The solution may be made using distilled water, a weak ionic buffer (as for hydrolysis) or natural water. A plot of ln [C] over time gives a straight line with a slope of $-k_{p'}$ from which the overall photolysis half-life may be calculated. Other methods for determining the rates of photolysis in aquatic systems have been comprehensively reviewed by Roof (1980a,b) and Zepp (1980).

6.2.3 Estimation of direct photolytic rates

Initial screening of substances for direct photolysis rates may be accomplished by estimation. Primary screening may only include knowledge of a substance's absorption spectrum. To undergo photodegradation the substance must be capable of absorbing light within the UV/VIS spectrum (>290 nm). The overall absorbance spectrum of a substance may be inferred from its constituent functional chemical groups, which act as chromophores having specific maximum absorption wavelengths and associated molar extinction coefficients (for examples see Harris, 1982b).

Further simple, quantitative estimation methods, involving little experimental input, may also be used to determine direct photolysis rate constants. Essentially, the overall rate constant can be derived from:

- Knowledge of the spectral overlap between the radiation that a substance absorbs and the incident solar radiation;
- the quantum yield of the substance.

The former factor is a measure of the rate of absorption of sunlight, which has been extensively modelled by Zepp and colleagues (Harris, 1982b; Mill, 1980; Zepp, 1980). Knowledge of the solar irradiance ($E^{av}{}_{\lambda}$; known also as the solar 'light intensity' or flux of solar energy), at a particular wavelength is required. Thus, photolysis rates are proportional to the rate of light absorption (I_{λ}) and the quantum efficiency ($\varphi\lambda$):

$$(d[C]/dt)_{\lambda} = I_{\lambda}\phi_{\lambda}$$
 Equation (21)

Specific absorption spectra

In direct photolysis;

$$I_{\lambda} = k_{a\lambda} [C]$$
 Equation (22)

where $k_{a\lambda}$ is the rate constant for absorption of light of wavelength $\lambda.~k_{\alpha\lambda}$ is also defined as:

$$k_{a\lambda} = 2.303 \varepsilon_{\lambda} E^{av}{}_{\lambda}$$
 Equation (23)

where ε_{λ} is the molar absorption coefficient and $E^{av}{}_{\lambda}$ is the average solar light intensity (the term is divided by constant j, that is equal to 6.02×10^{20} when $E^{av}{}_{\lambda}$ is expressed in units of photons cm⁻² s⁻¹, or unity, when $E^{av}{}_{\lambda}$ is expressed in units of millieinsteins cm⁻² s⁻¹). Tables of values for $E^{av}{}_{\lambda}$ are available or may be computed using a program described in the same paper and elsewhere (Zepp and Cline, 1977, EXAMS; Burns and Cline, 1985). Parts of these tables are available from other sources (Harris, 1982b; Roof, 1980a,b; Zepp, 1980). Thus, from Equation 21;

$$-(d[C]/dt)_{\lambda} = \varphi_{\lambda} k_{a\lambda} [C]$$
 Equation (24)

The overall rate of absorption of light in sunlight is the sum of the absorption rates over all the wavelengths absorbed by the compound. As previously stated, the quantum yield in water is usually wavelength independent above 290 nm, and so the overall photolysis rate may be calculated from the equation below:

$$-(d[C]/dt) = \varphi k_a [C] = k_p[C]$$
Equation (25)

The overall photolysis rate can thus be determined by relatively simple experimental procedures. Firstly, the molar absorption coefficient of the compound is determined simply by measuring the UV/VIS spectrum (290 - 750 nm) of the compound at different dilutions in water ($10^{-2} - 10^{-6}$ M; Harris, 1982b) or in vapour samples in air. Secondly, the quantum yield may be measured at one wavelength, or by assuming a specific solar quantum yield (Harris, 1982b; Mill, 1980). An assumed maximum value of unity is also used for some estimates (e.g. Armbrust, 2001), and here the rate is equal to the k_a (Zepp, 1980).

Quantum yield

As previously stated, quantum yields are wavelength dependent in vapour state, and wavelength independent in aqueous phase (Zepp, 1980). For aqueous samples the quantum yield is usually determined in the laboratory by observing the photolysis rate using monochromatic light. A low concentration of the substance is used in a solution, or vapour, with an absorbance of less than 0.02 AU, where the sample is aerated or in air. The rate is determined by correlation with a reference substance with a known value of φ , in the same apparatus. The reference substance is known as an actinometer and absorbs all of the incident radiation, several examples are given by Zepp (1980). Physical actinometers are also available, which are often calibrated using chemical actinometers. The reaction rate for the substance is given by:

Rate =
$$\varphi k_a [C] = 2.303 \text{ A } I_{0\lambda} \epsilon I \varphi[C]/V$$
 Equation (26)

where A is the fixed area of the cell, l is the pathlength, V is the volume and $I_{0\lambda}$ is the monochromatic light intensity. The other symbols have been detailed before.

The term (2.303 A $I_{0\lambda}I/V$) can be measured directly using the actinometer, which absorbs all of the incident radiation. The above equation can be integrated to give:

$$\ln \left(\left[C \right]_0 / \left[C \right]_t \right) = \varphi k_a t \qquad \text{Equation (27)}$$

and

$$\varphi = \ln \left([C]_0 / [C]_t \right) / k_a t \qquad \text{Equation (28)}$$

By plotting ln [C] against time the slope gives φ :

 $\varphi = - \text{slope/k}_a$ Equation (29)

A detailed account of the procedure for determining quantum yield is given by Zepp (1978). The quantum yield may also be determined by exposing the substance of interest and the actinometer (or another reference chemical with known quantum yield) to a light source on a rotating apparatus known as a 'merry-go-round' (Mill, 1980; Zepp, 1980). The ratio of the slopes of the plots for ln [C] against time for both solutions /vapours can give the quantum yield:

$$\phi_{\rm P} = \frac{\text{slope C}}{\text{slope R}} \frac{\varepsilon_{\rm R} \phi_{\rm P}}{\varepsilon_{\rm P}}$$
Equation (30)

Similarly, the above kind of ratio can be calculated from the direct photolysis rate constant determined in sunlight (Mill, 1980; Zepp, 1980):

$$\varphi_{\rm P} = \frac{k_{\rm p} k_{\rm a}^{\ R} \varphi_{\rm R}}{k_{\rm R} k_{\rm a}}$$
Equation (31)

Overall rate

The overall direct photolysis rates for many substances in aqueous and soil environments have been determined experimentally. Some studies have measured only the overall direct photolysis rate (Marsella *et al*, 2000; Sanz-Asensio *et al*, 1999), whereas other studies have determined the quantum yield (Krieger *et al*, 2000) for a particular substance in a particular environmental compartment. A comprehensive list of substances whose quantum yield has been measured is given by Harris (1982b), where the substances are grouped by chemical class.

Until recently, it was considered that photochemical reactions were too complicated to derive meaningful quantum yields and overall direct photolytic rates by structure-activity relationships (Harris, 1982b). However, in the past few years quantitative structure-property relationships (QSPRs, similar to (Q)SARs) have been developed based on correlations of quantum structural descriptors of different classes of substances (Peijnenburg *et al*, 1992; Chen *et al*, 1996, 1998a, b, 2000a, b, 2001a, b).

These researchers have elegantly demonstrated that QSPR can be used to estimate the direct photolytic rates and quantum yields for classes of substances such as; polycyclic aromatic hydrocarbons (PAH), substituted aromatic halides and polychlorinated dibenzodioxins and furans (PCDD/F), using sophisticated statistical analyses and a molecular modelling package (ALCHEMY). Their results have been validated by comparisons of experimentally determined rates and yields from widely cited traditional data sets (e.g. Smith *et al*, 1979; Zepp and Schlotzhauer, 1979), and may possibly have good predictive abilities (Chen *et al*, 2001a, b). Modelled estimates appear to be good and are within an order of magnitude of the experimental values. Further research of this type into other classes of substances is much needed as these methods seem promising, fast and potentially predictive.

6.2.4 Factors influencing photolysis

In aqueous and soil systems, experiments are usually conducted using transparent containers with a well chosen artificial polychromatic light source or in sunlight. Gaseous phase experiments are usually conducted using a smog chamber (Roof, 1980a,b; Connell, 1997).

Light source

It is essential that the light source is chosen carefully. The main requirement is that the light source mimics those wavelengths likely to be encountered in the environment, i.e. the light source must be polychromatic, having a spectrum that generates wavelengths within the 280 - 750 nm range. There is a wealth of information on photolysis rates and quantum yields at wavelengths of less than 280 nm (typically 254 nm) from early photochemical studies, but these data are considered to be irrelevant (Harris, 1982b; Zepp, 1980). Commonly used artificial light sources include medium-pressure mercury vapour lamps, sunlamps, fluorescent blacklights and xenon lamps. Often the light from these sources requires filtering using various media (typically Pyrex glass) to remove light of lower wavelengths (<280 nm; Zepp, 1980). In the experimental determination of photolysis rates, xenon lamps are considered the best artificial light source, although in his critical review of light sources, natural sunlight was considered the best source for rate experiments (Zepp, 1980). A comprehensive review of the factors influencing the choice of light source is given by Zepp (1980).

For the determination of quantum yields in aqueous/soil systems, monochromatic light may be used as quantum yield is wavelength independent. Mercury lamps often produce distinct spectral lines and are ideal for this purpose and are typically employed using filters. The quantum yields of gases are often determined using lasers (Wayne and Wayne, 1999).

Light and dark controls are always run simultaneously to observe the effects of hydrolysis and/or biodegradation if soil or sediments are used. Photolysis rates are usually appreciably faster than hydrolysis rates.

Despite the recommendations of Zepp (1980) and others (Mill, 1980) to use natural sunlight, many researchers still use artificial sources (Armbrust, 2001; Marsella *et al*, 2000; Sanz-Asensio *et al*, 1999).

The effects of media

The estimation and experimental determination of photolysis rates in aqueous phase, are usually conducted in distilled water, weak buffers or natural waters (Roof, 1980a,b; Zepp, 1980). The same caveats with regards to solubility for hydrolysis rates, apply here. If substances are relatively insoluble in water, weak polar solvents may be used for the determination of rates by estimation (i.e. measuring the molar absorption coefficient). Both distilled water and weak buffers allow direct photolysis rates to be measured without undue influence of indirect processes due to radical formation. If weak buffers are used the rates may be determined at a constant temperature and one (pH 7) or more pHs (e.g. 5 and 9). This experimental set-up is attractive, since it is similar to standard hydrolysis rate tests (OECD, 1981) and allows the simultaneous acquisition of other rate kinetics. However, some caution is required for ionisable substances, as their quantum yield and adsorption characteristics will differ in their neutral and ionised forms. Sometimes photolytic rate data for a substance is acquired for both pristine and natural solutions. Comparisons of this type have shown that the rates determined in pristine solutions are similar to those in natural water; estimation methods may thus be used with a certain degree of confidence (Zepp and Cline, 1977). However, if the natural water shows significant absorbance and/or contains many particulates, these factors need to be addressed and a correction applied. This may be done experimentally (by comparison with pristine solutions) or by estimation (Roof, 1980a,b; Zepp, 1980). The relevance and inadequacies of these methods for poorly water soluble substances also needs to be assessed in greater detail to ensure their appropriateness for inferring persistence.

Light attenuation, scattering and absorption

Distilled water is transparent to solar radiation whereas many bodies of natural water have some opacity to wavelengths above 280 nm (Roof, 1980a,b; Zepp, 1980). Natural water contains various dissolved and suspended matter that can influence both the incident radiation and its effects. Natural light attenuation is different for different bodies of water, and each type of system possesses a characteristic spectrum. Thus lakes and oceans are often clear and blue, eutrophic lakes are green (suspended algae) and muddy rivers are brown (suspended particles/sediment). Water is generally transparent to blue light and attenuates shorter wavelengths. Attenuation coefficients may be measured as a function of absorption coefficients and are wavelength dependent (Zepp, 1980). Light scattering is wavelength independent and considered unimportant in aquatic systems; it may be measured if necessary (Zepp, 1980). One of the greatest influences on light attenuation in aquatic systems is caused by an increase in depth. Zepp and Cline (1977) accounted for the influence of depth in their estimate model. Absorption may also lead to quenching (Roof, 1980a,b) and sensitisation (Roof, 1980a,b; Zepp, 1980). This is usually caused by the presence of a plethora of organic and inorganic substances especially particular matter (clays), iron-complexes and humic substances.

The attenuation of light by clouds is considered to be less than a factor of two (Zepp, 1980). In the atmosphere, light scattering is caused by airborne particulate matter and dust and increases with decreasing wavelength. However, such particles may act as sites for increased photocatalytic activity by the adsorption pollutants (Parlar, 1980).

Temperature

Artificial light sources can often generate significant amounts of heat and thus require good cooling systems so that the effects of temperature are minimised. Even so, in aqueous systems, the spectral distribution and flux intensities are more important factors than temperature. In the gaseous phase temperature has a significantly larger effect that can be modelled using the Arrhenius equation.

Photosensitisation and quenching

Photosensitisation and quenching occurs when a non-target substance absorbs the incident light and either transfers its excitation to the target substance (sensitisation), or else dissipates that energy in other ways (quenching). Photosensitisation is thus distinguishable from photooxidation events following ionisation and the formation of free radicals. These reactions essentially result in the generation of highly reactive radicals. Photosensitisers in natural water include humic substances (Gonenc and Bekbolet, 2001; Gong *et al*, 2001) and iron-complexes (Roof, 1980a,b; Pozdnyakov *et al*, 2000). Photoquenchers include particulates such as clays (Roof, 1980a,b; Aguer *et al*, 2000; El-Nahhal *et al*, 2001). The effect of quenching is thought to occur through screening.

The effects of photosensitisation have been exploited to generate systems for the elimination of polluting substance from water and wastewater. The photocatalysts typically used are titanium oxide and sodium decatungstate, in the presence of UV or sunlight (Texier *et al*, 1999a, b; Sabate *et al*, 2001; Prevot *et al*, 1999).

Adsorption

In addition to their ability to cause quenching, clays, soils and sediments can greatly decrease the photolysis rate by adsorption of the substance, thus partitioning the substance into the sediment layer and causing screening (Aguer *et al*, 2000; Gong *et al*, 2001). These effects are also a function of depth.

6.2.5 Prediction of photolysis, uncertainties and variation

For relatively soluble substances with absorption spectra in the UV/VIS range, and in relatively clear waters with little or no sediment, estimate methods provide a reliable means of predicting the overall degradation rate due to photolysis. Indeed, newer computational methods employing QSPRs seem to provide a promising and potentially more effective means of estimating the overall rate. These criteria would also appear to apply for the overall direct photolysis rates in vapour samples.

For soils or opaque waters (e.g. containing soils, sediments or clays), it would be more appropriate to determine experimentally the overall photolysis rate. Factors such as attenuation, adsorption and partitioning, quenching and screening, photosensitisation and biodegradation become more important. Thus, experiments need to be designed carefully to consider fully the impact of these aspects on the photolysis rate.

Furthermore, only measurement of the degradation rate of the parent substance has received attention in this report. It must always be remembered that photolytic product(s) may undergo further abiotic and biotic degradation or else remain persistent (Krieger *et al*, 2000). It is thus important that studies into photodegradation pathways are also performed (Sanz-Asensio *et al*, 1999; Samanta *et al*, 1999; Ellis and Mabury, 2000; Singh *et al*, 2002). The products may be identified by suitable analytical techniques as discussed for hydrolysis. In the future, such studies may elucidate the mechanisms by which particular substances photodegrade, potentially allowing for product prediction. Photolytic products from particular substances are given in a number of publications (Harris, 1982b; Roof, 1980a,b; Roberts, 1998).

6.3 Indirect photooxidation or chemical oxidation

Chemical oxidation is due to the free radical oxidation, which, in the presence of light, can be treated as photooxidation. In this section direct photolysis or photooxidation of a substance is considered separately (see above) from oxidation due to other free radicals. The oxidation of substances by free radicals, singlet oxygen $({}^{1}O_{2})$ or ozone (O_{3}) is an important degradative process for many substances in the atmosphere and natural waters. The general equation for oxidation reactions is given below:

$$AY + OX \rightarrow$$
 Products and $R_{OX} = k_{OX}[AY][OX]$ Equation (32)

Oxidation is the removal of electrons from an electron rich molecule, by an electron deficient molecule (oxidant). Oxidants found in the environment include peroxy radicals (RO_2°) , hydroxy radicals (HO°) , singlet oxygen $({}^{1}O_2)$, ozone (O_3) , and photoexcited triplet diradicals $(R^{\circ}O^{\circ})$. Prediction of the rate of oxidation requires knowledge of the identity and quantity of oxidants in the environmental compartment, the rate of oxidation by the oxidant with a specific site in the substance, and the kinetic law for each substance.

In aquatic systems, RO_2^{o} and 1O_2 play a more important role than HO^o radicals (Mill, 1980). These transient oxidants originate from the organic humic matter in natural waters. Their reactivities range over twelve orders of magnitude and their concentration in natural waters varies enormously. Other transient oxidants and stable oxidants are also present, but as yet their quantitative importance has not been established (Mill, 1980).

 $\rm HO^o$ and $\rm O_3$ are the important radical species in the troposphere, as determined both experimentally and by estimates (Mill, 1980). These radicals also play an important role (involving the dissociation and eventual reoxidation of $\rm NO_2$) in the photochemical cycle that leads to urban smog.

6.3.1 General theory

The rate of oxidation of a substance (R_{OX}) is related to the concentration of both the substance and the oxidant:

$$R_{OX} = k_{OX} [C] [OX]$$
 Equation (33)

Where k_{OX} is the specific second order rate constant for oxidation at a specific temperature (usually expressed in units of M⁻¹ s⁻¹), and [C] and [OX] are the concentrations of the substance and oxidant respectively. The overall reaction rate is the sum of the oxidation rate reactions with each kind of oxidant:

$$R_{OX(T)} = [C] \Sigma k_{OXn} [OX_n]$$
Equation (34)

In most cases the oxidant concentration is assumed to remain constant throughout the experimental determination and so Equation 34 simplifies to a pseudo first order rate:

$$R_{OX(t)} = \Sigma k'_{OXn} [C]$$
 where $k'_{OXn} = k_{OXn} [OX_n]$ Equation (35)

Giving;

$$\ln [C^0]/[C_t]) = \Sigma k'_{OXn} t \qquad \text{Equation (36)}$$

and

$$DT_{50} = \ln 2/\Sigma k'_{OXn}$$
 Equation (37)

Thus the degradation half-life of a substance can be determined by the sum of the measured rate constants for the different oxidants, determined by the disappearance of the substance over time (Mill, 1980). The overall rate expression is usually expressed as:

$$R_{OX} = k_{RO2} [C] [RO_2^{\circ}] + k_{RO} [C] [RO^{\circ}] + k_{OH} [C] [HO^{\circ}]$$
 Equation (38)

Although many different kinds of R_0^2 and R_0^o radicals exist they are usually considered together with the additional assumption that the structure of R has little effect on the rate (Mill, 1980).

The measurement of rate constants also requires knowledge of the specific site of reaction on the molecule, since free radicals will attack a number of different functional groups.

Four processes are known to occur for oxidation by the RO₂°, RO°, and HO° radicals (Mill, 1980) they are;

H-atom transfer by RO_n^{o} ; $RO_n^{o} + CH \rightarrow RO_nH + CH_3^{o}$

(the reactive carbon radical then undergoes a chain reaction resulting in stable products and oxygen).

Addition to double bonds by HOº or RO2º;

HO^o or $RO_2^{o} + H_2C=CH_2 \rightarrow RO_2CH_2-C^{o}H_2$ or $HOCH_2-C^{o}H_2$

HO^o addition to aromatics; HO^o + Ar \rightarrow ArOH^o

 RO_2 transfer of O-atoms to certain nucleophiles; $RO_2^{o} + NO \rightarrow RO^{o} + NO_2$

Measurement of k_{RO2} for RO_2°

The chain reaction in the H-atom transfer process utilises oxygen in a complex chain event (Mill, 1980). This enables the oxidation rate to be measured in terms of oxygen uptake at CH bonds:

 $-dO_2/dt = R_{O2} = (Ri/2k_t)^{1/2} k_{RO2} [RH]$ Equation (39)

where Ri is the rate of radical formation, k_t is the rate constant for the termination reaction and RH is the concentration of any CH bond. Since the former two values are usually unknown the rate constants are determined in relation to another reference compound whose rate constants are defined (usually t-BuO₂H).

A similar expression is used for the determination of oxidation rate constants at NH and OH bonds in phenols or amines (Mill, 1980), but where the loss of the phenolic compound is measured over time.

Measurement of k_{HO} in air

The measurement of this oxidant rate constant requires the production of HO^o radicals, whose loss is then measured over time in excess substance (RH). The rate can be followed spectroscopically at 304 nm and the absolute rate constant can be calculated directly:

$$k_{OH} = \ln [HO]_0 / [HO]_t / t$$
 Equation (40)

The rate constant of the reaction of a substance with OH° radicals can be measured in laboratory with a good degree of accuracy by two methods. Firstly, the relative rate constant measurements, where the substance studied is compared with one or more reference compounds for which the rate constant is well established (this type of experiment can be carried out in an atmospheric chamber where disapperance of both the substance to be studied and the reference compounds monitored at the same time with analytical techniques such as FTIR and GC/MS). The other commonly-used method is laser pulse photolysis with laser induced fluorescence detection. This method uses two laser beams, one produces a pulse at an appropriate wavelength to produce an initial OH° concentration in the media by photolysis of a precursor e.g. H_2O_2 . The second laser beam is used to produce the OH° radical fluorescence which is detected by a light amplifier. Concentration of the substance to be studied is put in excess and can be considered as constant during the experiment.

Similar measurements can be made in aqueous solutions, although the OH^o radical oxidation is not considered important in these systems.

Measurement of k_{OX} for 1O_2 oxidations

The measurement of k_{OX} for ${}^{1}O_{2}$ involves measurement of rate constants expressed in the kinetic scheme below:

Sen \rightarrow light ? Sen*		
$\mathrm{Sen}^* + \mathrm{O}_2 \rightarrow \mathrm{Sen} + {}^1\mathrm{O}^2$	(ϕk_T)	
$^{1}\text{O}_{2} + \text{C} \rightarrow \text{CO}_{2}$	(k _{OX})	Equations (41 - 45)
$^{1}\text{O}_{2} + \text{S} \rightarrow \text{S} + \text{O}_{2} \text{ or SO}_{2}$	(k _s)	
$^{1}\text{O}_{2} \rightarrow ^{3}\text{O}_{2}$	(k _d)	

where Sen^{*} is a triplet sensitiser (usually a dye), C is the substance of interest, S is a chemical that competes at a known rate with C for ${}^{1}O_{2}$ and k_{d} is the rate of deactivation (radiationless). The value of k_{OX} can be measured from known values of k_{d} and k_{s} . The experiment is conducted in competition with the reference substance (S) and the loss of the chemical and/or the sensitiser is followed. The k_{OX} is derived from subsequent ratios of first order reaction rates (Mill, 1980).

Extrapolation to the environment

With knowledge of the individual oxidant rate constants, the overall removal rate of a substance can be calculated, if the concentration of the oxidants in the atmosphere of interest is known. The factors influencing removal rate include temperature, air quality (particulates effect scattering), and latitude and longitude. A correction for some of these factors can be applied using appropriately selected values for estimation. Extrapolation to the aquatic environment will also be influenced by particulates. Other factors influencing photooxidation in the aquatic environment include the concentration of the oxidants, temperature, depth and colour.

6.3.2 Estimation of total loss

In atmospheric determinations, the tradition has been to estimate the rate of loss, or mean residence time, of a substance by several means, usually in the troposphere (Lyman, 1982). The estimate methods usually follow the form:

residence time, $\tau = Q/E = Q/R$ Equation (46)

where Q is the total mass in the compartment, E is the overall sum emissions and R is the overall losses at steady state (Lyman, 1982).

When the removal rate is a first order rate process, then;

$$DT_{50} = 0.693 \tau$$
 Equation (47)

Other estimation methods given by Lyman (1982) are derived from the same experimentally determined rate constants as discussed above. These constants have been determined for many substances, at a specified temperature; comprehensive lists are provided by Lyman (1982). Often the estimates can be corrected for temperature using the Arrhenius equation. By using the values listed for the concentration of various oxidants in the atmosphere (Lyman, 1982), which varies from place to place and with the quality of the air, the overall removal rates of a substance can be determined. The error involved in such estimates cannot be calculated as the removal of substances, in the environment, is difficult to assess.

Another means of calculating the residence time is by using Junge's correlation, which shows that there is a correlation between the mean standard deviation of a substances concentration in the atmosphere and tropospheric residence time. Structure-activity relationships have been reported for oxidation reactions for certain classes of compounds using, amongst others, Hammett type relationships (e.g. phenols and amines; Mill, 1980) and fragmentation methods (Atkinson, 1987; Atkinson and Carter, 1984; Mill, 1989). Rates for many compounds have also been published (Howard, 1990; Howard *et al*, 1991; Lyman, 1982; Mill, 1980; Roberts, 1998).

An excellent tiered system for determining the atmospheric indirect photooxidation of compounds has been proposed, which incorporates the initial use of structure-activity relationships for determining dominant pathways. Once the dominant pathways have been established, the second tier involves laboratory measurements, followed finally by simulation tests of those processes considered important (Klein, 1980).

6.4 Conclusions

The degradation of many compounds by abiotic processes appears to follow first order, or pseudo first order, rate kinetics. The resulting half-lives can be related to a range of $T^{1/2}$ values to reflect the ranges of abiotic degradation rates evident under different environmental conditions.

There is an enormous incentive to have estimation models available for assessing degradation, so much so that the uncertainties inherent in those currently available are being readily overlooked. Advocating the use of (Q)SARs and other estimation methods for assessing abiotic degradation should include details of their specific limitations for use. Due to the time involved with performing the standard tests, the development of estimation methods is a highly attractive goal. However, (Q)SAR-type models, developed to predict the results of the currently available tests (which require water solubility), should be assessed for their pertinence for hydrophobic compounds before being recommended.

For certain types of substances (Q)SAR predictions may be appropriate for screening persistence such that P3 and P4 compounds can be identified (as far as hydrolysis and photolysis are concerned) with a fair degree of certainty. However, distinguishing P1 (least reactive) from P2 may be more difficult, as the current tests, and thus (Q)SARs, may not be capable of assessing the abiotic degradability of such substances. While estimated data may be appropriate for screening purposes, more accurate (experimental) data should be used for chemical substances for which potentially high persistence is indicated. Currently available standard methods for measuring hydrolysis and photolysis are both appropriate for water-soluble chemicals. However, most of the chemicals that are likely to be of principal concern with respect to persistence are hydrophobic, and development of appropriate methods for measuring the abiotic degradation of these compounds is needed.

In summary:

- Tools are not yet available with which to assess the abiotic environmental degradation of most of those chemicals that are likely to be of greatest concern with respect to their persistence;
- some well-developed, standardised tests are available for measuring abiotic degradation of water soluble chemicals in some environmental media;
- estimation methods exist that can be used to give an indication of the hydrolysis and photolysis of some compounds. Guidelines for the use of estimated data should clearly state the limitations of their scope (which substances and for what ends).

7. MODELLING

7.1 Introduction

As discussed in previous sections of this report, various regulatory agencies have proposed different criteria to define persistence. Most of these rely on estimates of halflives in individual environmental media such as air, water and soil. However, the environment consists of several compartments between which chemicals may partition. This means that reliance on single media half-lives alone can result in errors in identifying whether a substance is actually likely to be persistent since it may not be present realistically in the compartments of concern (Webster *et al*, 1998).

As a consequence, several proposals have been made to incorporate information from multi-media fate and transport models (MFTMs) into evaluations of persistence, since these can take into account the multi-phase partitioning and fate of chemicals. This section describes the underlying basis for MFTMs and provides a foundation for the recommended strategy, outlined in Section 8.

More specifically, this section will:

- Provide a summary of the concepts on which MFTMs are based;
- discuss the types of available MFTM's recommended for evaluation of persistence, including their strengths and limitations;
- summarise the key outputs of MFTMs, relevant to chemical persistence, which can be used within a practical and rational evaluation strategy (Section 8).

A basic outline of the conceptual underpinnings and assumptions of MFTMs is given in this section; more comprehensive descriptions can be found in the referenced works. In particular, in the context of evaluating overall persistence, the reader is referred to the background papers and final report generated from the 2001 OECD workshop on the use of Multimedia Models for Estimating Overall Environmental Persistence and Long-Range Transport in the Context of PBTs/POPs Assessment (OECD, 2002).

In the context of this report, perhaps the most important output from MFTMs, is information which can indicate the 'target' environmental compartments in which a substance is most likely to be found, under a given (realistic) emission scenario. This information may be used to focus laboratory testing (such as measurement of degradation) relevant to the compartment(s) into which the substance is most likely to be released or migrate. In addition, MFTMs can also be used to provide an estimate of overall persistence of the substance, or overall half-life, taking into account partitioning behaviour and release pattern. While, in a regulatory context, there are no generally accepted rules for interpreting overall persistence values, the concept of overall persistence provides an attractive alternative to assessing persistence on the basis of single media half-lives. There are, however, a number of difficulties associated with the use of MFTMs. These include the large uncertainties in the values of input parameters (particularly degradation rate constants, to which persistence is particularly sensitive, and partition coefficients) and the simplistic nature of the models. Model calculations must always be viewed as crude estimates rather than precise predictions, and consequently the results should be interpreted with a degree of caution.

MFTMs are frequently used to estimate environmental concentrations, as part of a chemical risk assessment process. They are also used to predict the potential for long range transport (LRT), which is often calculated in parallel with the evaluation of persistence. Whilst we recognise these broader applications - and some of their limitations, it should be emphasised that the focus of this section is on chemical persistence.

7.2 Review of use of modelling to estimate persistence

7.2.1 Elements of multi-media fate models

7.2.1.1 Basic concepts

Most MFTMs have common objectives and are based on common concepts. They attempt to describe the behaviour of chemicals in a hypothetical or evaluative environment. The evaluative environment is divided into a number of conceptual compartments, each representing a single medium (e.g. water, air, soil and sediment) with dimensions which are in proportion to those in the 'real' world. The fate of a chemical in this environment is predicted on the basis of the assumption of conservation of mass, and on the thermodynamics of the system, taking into account the pattern of release and the degradation characteristics of the substance. A substance entering the environment will tend to distribute itself between the compartments, attaining a state of equilibrium in which the ratio of concentrations in each phase corresponds to its partition coefficients.

The use of an evaluative model depicting a hypothetical, but typical, environment, was first described by Baughman and Lassiter (1978), and later developed by Smith *et al* (1977, 1978). Mackay (1979) and Mackay and Paterson (1981, 1982) advanced the development of the evaluative model by introducing the concept of fugacity as a criterion for equilibrium. Many MFTMs now use fugacity concepts and the term 'fugacity model' is used almost synonymously with MFTMs; a brief introduction to this concept is therefore warranted. Fugacity is essentially the tendency of a substance to 'escape' from a particular medium. It has the units of partial pressure (Pa) and is proportional to concentration. Chemicals which migrate between phases are attempting to establish an equilibrium state that is traditionally defined using the ratio of concentrations in each phase of a multi-phase system. It can be shown that an alternative equilibrium criterion, is when fugacity is equal in all phases. Mackay-type MFTMs use equations which express

the partitioning behaviour of chemicals in terms of fugacity. This has a number of computational and conceptual advantages, details of which (together with the formulations) are well described by Mackay (2001). The most recent and widely-accepted fugacity model, which has been developed by Mackay and co-workers, is the Equilibrium Criterion or EQC model (Mackay *et al*, 1996).

A basic simplifying assumption of most models is that substances are homogeneously distributed throughout each individual compartment, i.e. concentrations are equal in all locations within a compartment. This is often referred to as the 'well mixed' or 'continuously stirred tank' (CST) assumption. Although this assumption is rarely completely valid, it is often an acceptable working model.

As a consequence of the CST assumption the mass of chemical in any compartment, [M(g)], is simply the product of the compartment volume, $[V(m^3)]$ and the concentration $[C \text{ g/m}^3]$.

A mass balance equation can be written for the compartment which states that

Rate of inventory change = Input rate - output rate

Where rates are expressed in units of g/h.

This equation predicts the time course of the concentration change as a result of changes in inputs and outputs. An alternative simplification is to assume steady-state conditions (input equal output) under which the left side of the equation becomes zero.

For the purposes of evaluating persistence, input processes (emissions), degradation reactions (abiotic and biotic) and inter-media transfers (e.g. exchanges between water and sediment, volatilisation to air from land and water, and wet and dry deposition from the atmosphere to land and water) should be included; advective inflows and outflows (chemical transfer into or out of a compartment without change in phase - e.g. dispersion by the wind) should not, since this merely represents relocation of the substance.

The main outputs of MFTMs within the context of evaluating persistence are: (1) the proportion of total steady state mass in each compartment and (2) a prediction of the mean residence time or overall half-life of a chemical in the whole evaluative environment.

7.2.1.2 Number of compartments

The number of media required in MFTMs has been addressed at length in the SETAC publication edited by Klecka et al (2001). The three primary compartments in the environment are the atmosphere, water and solid media (including soils, bottom sediments and biota). The minimum segmentation is thus into these three media. However, the most common segmentation is a four-compartment system of air, water, soil and sediment. This is used in the EQC model of Mackay et al (1996), which is also implemented in EPIWIN (Syracuse Research Corporation) (Meylan, 1999). There is a general consensus that this is adequate for most screening purposes. More compartments may be added (e.g. upper and lower layers in the atmosphere, epilimnion and hypolimnion in lakes or different horizons in soils) if justified by the spatial scale of the application or by the detail of output required. For example, if there is a desire to treat areas which differ in population density or industrial activity, it may be appropriate to include extra compartments with these characteristics as is done in the SimpleBox EUSES model (EC, 1996). More complex models may also be required to simulate processes such as global distillation (net chemical volatilisation in tropical and temperate regions and net deposition in high latitudes).

Many models also contain sub-compartments in 'bulk' media (i.e. particles or aerosols in air; suspended solids and biota in water; air and water in soil; solids and water in sediment). It is generally assumed that the chemical present within sub-compartments of a bulk medium achieves thermodynamic equilibrium, even if equilibrium is not established between bulk media themselves.

7.2.1.3 Partitioning behaviour

Partitioning between media is described, at least for many organic substances, by dimensionless partition coefficients which represent the ratio of chemical concentrations at equilibrium in the respective environmental media. Values for these partition coefficients can be used to indicate into which media a chemical is likely to partition. However, limits in intermedia transport rates may mean that equilibrium is never reached, and in such cases the ratios of concentrations between compartments may be quite different from those that would be expected from the respective partition coefficients. Partition coefficients can be measured experimentally or estimated, either from physicochemical properties (such as aqueous solubility, vapour pressure, boiling point and melting point) or from chemical structure. If (Q)SARs are used, care must be taken to ensure that they are valid for the chemicals being considered (see also Section 7.2.1.7).

The dimensionless air-water partition coefficient, $K_{aw'}$ can be calculated from Henry's Law constant (H), by dividing H by the product of the universal gas constant, R, and the absolute temperature of the system being modelled. H is the ratio of the saturated vapour pressure of the substance at a particular temperature, and the aqueous solubility at the same temperature. Values of H range from >100 Pa m³/mol for highly volatile substances, to <10⁻⁶ Pa m³/mol for low-volatile substances. Some partition coefficients (e.g. K_{aw}) in marine waters may be different from those in freshwaters, although this is not usually taken into account in MFTMs (and is just one of many simplifications made in these models).

Octanol is frequently used as a surrogate for natural organic matter, lipids and waxes. The octanol-water partition coefficient, K_{ow} , describes the hydrophobicity of a substance and is defined as the ratio of the concentration of a substance in octanol to its concentration in water at equilibrium. Values of K_{ow} range from between $10^{-3} - 10^7$ and are typically expressed as a base-10 logarithm. An organic chemical with a log K_{ow} value >3 will often have a high affinity for lipids in biological, media and for the organic carbon fraction of soil and sediment. This is not always straightforward, however, since the type of chemical and environmental factors such as pH may be important, and care should be taken when using K_{ow} to predict partitioning characteristics for substances such as ionisable compounds.

The K_{oc} value (organic-carbon to water partition coefficient) is a measure of the relative sorption potential of organic chemicals to organic carbon. Several empirical relationships have been developed for estimating K_{oc} based on other properties, such as K_{ow} . Most notable is that suggested by Karickhoff (1981), i.e. $K_{oc} = 0.41 \text{ K}_{ow}$. More recently Seth *et al* (1999) suggested that, on average $K_{oc} = 0.35 \text{ K}_{ow}$, but highlighted that there will be considerable variability around this relationship as a consequence of the nature of the organic matter present. Thus K_{oc} can be as high as 0.9 K_{ow} and as low as 0.14 K_{ow} . However, it should be recognised that K_{oc} is not necessarily a good predictor of adsorption to solids. Particular care must be taken with some substances (e.g. ionisable compounds).

The octanol-air partition coefficient, $K_{oa'}$ is used to predict the partitioning behaviour of a substance between organic matter (including soils, vegetation and some aerosol particles) and the atmosphere. Values of K_{oa} can range between 10^{-1} and 10^{13} and can either be measured or estimated from the relationship $K_{oa} = K_{ow} / K_{aw}$.

It should be noted that the experimental methods used to measure partition coefficients are limited in terms of the range of values in which accurate measurements are possible. For instance, the reliability of markedly high K_{ow} (>10⁷) and K_{oa} (>10¹²) values may be suspect. However, the absolute values for substances with extreme partition coefficient values, indicating preferential partitioning to one environmental medium, may not be

important to the model output. For example, a non-polar organic substance with a $K_{ow} > 10^7$ will always be associated with the organic phase. Thus, it makes little difference to the persistence of a substance if the value of K_{ow} is 10^8 or 10^9 . On the other hand, differences in intermediate values (10^3 to 10^6) can affect significantly the model output, and therefore the uncertainty associated with these values should be considered.

7.2.1.4 Degradation

Although degradation kinetics in the environment are often complex, consisting of a range of interacting abiotic and biologically mediated processes, most MFTMs simplify these into a bulk behaviour for each compartment, based on the assumption of first order kinetics. The limitations with this approach are discussed in Section 3 and will be elaborated on later in this section. First order kinetics relate the rate of reaction to concentration i.e.

$$dC/dt = -k.C$$
 Equation (48)

where *C* is the concentration (g/m^3) in a given compartment, *t* is time and *k* is the degradation rate constant in that compartment (time⁻¹). Note that *k* is effectively assumed to be the sum of individual first order rate constants for different processes (e.g. biodegradation + hydrolysis + photodegradation). Defining the initial concentration (at *t* = 0) as *C*₀ this equation has the solution.

$$C = C_0 \cdot \exp(-k.t)$$
 Equation (49)

If C/C_0 is 0.5 then $t\frac{1}{2} = \ln(2)/k$ where $t\frac{1}{2}$ is the degradation half-life.

The above equations only consider the effect of degradation on concentration in a single compartment. In MFTMs, the equations also take into account simultaneous emissions and intermedia partitioning.

The mass balance equation for a single compartment, in which the only loss is by reaction, is represented by

$$dM/dt = E - k.V.C$$
 Equation (50)

where *M* is the total mass in the compartment (g), *E* is the rate of emission (g/h) and *V* is the volume of the compartment (m^3). Under the assumption of steady-state we get

$$E = V.C.k$$
 Equation (51)

From this *C* can be calculated when *E*, *V* and *k* are known. The average residence time of the chemical in the compartment, τ , is calculated as *M* / *E* or 1 / *k* or t¹/₂/0.693. This time τ is often referred to as the 'characteristic time'. It is the time required for the chemical to decay to exp(-1) or 37% of its original mass, and is thus longer than the half-life, specifically 1.44 times longer. These equations essentially form the basis for the definition of overall persistence and are discussed further in Section 7.2.3. However, it is important to remember that they apply only to steady state conditions.

7.2.1.5 Level I, II, III and IV models

A series of increasingly complex calculations can be applied in MFTMs. Mackay and co-workers have employed the terminology Level I to IV to represent this series and this has been widely adopted.

Level I models merely show the relative equilibrium partitioning of a conserved (i.e. non-reacting) substance in a multi-media setting. They assume that full equilibrium and steady-state apply in a closed system and do not yield any information on persistence. A common fugacity applies to each medium.

Level II models include degrading reactions and can include advective losses (open system), but assume that all media are at steady-state and in thermodynamic equilibrium. Again, a common fugacity applies to each medium. The mode of entry of a substance to the environment (i.e. the proportion of the total emission which enters each medium) is irrelevant, because the substance is assumed to come into equilibrium immediately after introduction to the system.

Level III models assume steady-state (i.e. conditions are constant with time), but compartments are not at thermodynamic equilibrium, because inter-media transport rates may limit substance migration. This more closely reflects 'real world' situations in which complete equilibrium between phases is rare. As a consequence of non-equilibrium, different fugacities apply to each medium. Substance mode of entry information is now needed and this can alter significantly the overall residence time (persistence) and the predicted proportion of steady state mass in each medium.

Level IV models are dynamic or non-steady-state in nature. They are most often used to determine the length of time it will take for concentrations to change as a result of changing rates of emission. The time-course of chemical emissions into each medium is, therefore, needed. In general, level III models are preferred for the estimation of the environmental persistence of chemicals. This is because thermodynamic non-equilibrium conditions frequently exist for substances discharged into the environment (i.e. distribution between phases is not the same as would be expected from the respective partition coefficients), which level I and level II models are unable to predict. However, since they still assume steady state conditions, level III models are easier to set up and run than level IV models. In taking into account the important limiting effects of intermedia transfers, level III (and higher) models can potentially give a more realistic prediction of chemical distribution (and overall persistence) for a given distribution of emissions. However, mode-of-entry information is required. Whilst in some cases it is possible to estimate to which compartments a substance is most likely to be emitted (based on use and release patterns), this is not always the case. When this is not possible, running a level II model (Gouin *et al*, 2000) is recommended (particularly for screening), over blind guesses of mode of entry scenarios (which may incorporate biases).

7.2.1.6 Application to different chemical types

Many thousands of chemical substances are used in commerce. These can be grouped into a variety of classes for the purposes of environmental modelling (Mackay *et al*, 1996).

- Relatively hydrophobic, non-polar organic substances (e.g. halogenated and polycyclic aromatic hydrocarbons). These partition appreciably into all media and have measurable values of K_{aw}, K_{ow} and K_{oa}. MFTMs can usually be used for these substances.
- Substances with zero or negligibly small vapour pressures (e.g. certain surfactants and metals). They do not partition appreciably into air but are soluble to a measurable extent in water. MFTMs can usually be used for these substances.
- Substances which do not have a measurable solubility in water, but do partition significantly into the atmosphere (e.g. certain silicones and higher molar mass hydrocarbons). MFTMs can usually be used for these substances.
- Substances which do not partition appreciably into either air or water (e.g. some dyes, pigments, waxes and polymers). As a consequence, MFTMs cannot usually be used. Substances which speciate (e.g. acids or bases that form ionic species) are also a problem. In some cases, existing single-species models can be used, but extreme care is necessary and customized evaluation is usually required. For such substances, care must be exercised when using quantities such as K_{ow} and K_{oc} to predict partitioning behaviour, in certain cases they may not be applicable.

7.2.1.7 Limitations of MFTM's

There are a number of limitations for the use of MFTMs. These tend to be associated with chemical type (some chemicals cannot be evaluated with 'standard' MFTMs), availability of input data (particularly degradation rate constants and partition coefficients), parent compound versus ultimate biodegradation, and poor validation.

Problems due to lack of data

Many substances that need to be assessed for persistence do not have readily available, measured physico-chemical or reactivity data. If measured values are not available, (Q)SARs can be used to calculate partition coefficients or degradation rates in different media on the basis of other physico-chemical properties and/or chemical structure. Although some estimation programs are very good, care should be taken to ensure that the relationships applied are appropriate and have been validated for the all the chemicals being evaluated (i.e. specific models will be required for certain classes of chemicals).

The Estimation Programs Interface for Windows (EPIWIN) is a software package developed by the Syracuse Research Corporation (Meylan, 1999), which brings together ten separate estimation programs into one easy-to-use package. These programs estimate various properties based on chemical structure: e.g. melting point, boiling point, vapour pressure, water solubility, K_{ow} , $K_{oc'}$ simple narcosis toxicity, Henry's Law constant and degradation rate constants for air, water, soil and sediment. Potentially, these estimated properties can be used to perform MFTM calculations (Meylan, 1999), and the relative ease with which the data can be obtained makes it an attractive tool.

It should be noted that (Q)SARS may not always be reliable and care should be exercised when using estimated data (e.g. (Q)SARS for physico-chemical properties tend to be much more reliable than those for biodegradation). ECETOC (2003) summarised the status of the four most commonly used models (Biowin, Topkat, Multicase and Catabol) available for predicting aerobic biodegradability. The conclusion, confirmed at the Setubal (Q)SAR workshop (Cefic, 2002), was that current models based on the MITI database, and used to predict 'non-ready biodegradability', may be acceptable for priority setting and even, in some cases, a classification scheme. However the models were not suitable to support calibration of exposure models for use in risk assessment (i.e. environmental half-lives). There is an urgent need to develop reliable (Q)SARs for predicting aerobic biodegradation. This would be greatly facilitated by the development of simple, cost effective test methods to provide more realistic estimates of ultimate biodegradation in waters, soil, sediments and sewage treatment plants.

There are no models currently available to predict anaerobic biodegradation. The availability of (Q)SARs for predicting the abiotic half-lives of organic chemicals in the aquatic, marine and terrestrial environments is also limited (ECETOC, 2003). However, there are reliable (Q)SARs that are applicable to a wide range of chemical classes for estimating abiotic half-lives in the atmosphere.

Parent compound versus ultimate degradation

It is important to realise that MFTMs only describe the degradation of a parent compound to other unidentified species (primary degradation) and not complete conversion to $CO_{2'}$ water and salts (ultimate degradation). Although models have been developed describing the 'joint persistence' of a substance based on the parent compound and its metabolites (e.g. Fenner *et al* (2000) using a Level IV three-compartment model), there appear to be no generally-applicable models that predict ultimate biodegradation or mineralisation. Such models would obviously require information on all degradation products and their properties' since metabolites may have different partitioning properties and behaviour to those of their precursors. Because most biodegradation screening tests are designed to give an indication of complete mineralisation (by measuring CO_2 efflux, DOC removal or oxygen demand), there may be an inconsistency between that which the models are simulating and the rate constants which are provided by standard tests. This inconsistency will almost always be conservative since it always takes longer for complete mineralisation to occur than for less complete chemical transformations.

Thus the overall persistence, calculated using MFTMs (primary), may contrast with definitions of persistence discussed earlier in this report (complete mineralisation).

Validation

Overall persistence is a chemical property which cannot be measured easily. This makes full validation of MFTMs, within the context of the evaluation of environmental persistence, difficult, if not impossible. It also poses a problem for defining classes of persistence based on overall residence time. In principle, MFTMs can be 'validated' by assessing their ability to predict environmental concentrations. However, the paucity of good monitoring data means that such comparisons are not common.

It should be noted that the results from inter-model comparison exercises should not be considered as validation, although attempting to explain any differences in the results from different models is often useful, and may help to improve the consistency of predictions.

7.2.2 Available multi-media models

Table 20 lists a number of MFTMs that can be used to estimate persistence. The reader is referred to the primary references for detailed descriptions of the individual models. The list is grouped into 'Single Unit World' models and more complex 'Connected Unit World models'. These are briefly discussed below.

Table 20: MFTMs that can be used to predict chemical persistence

Model	Reference	Description		
Single unit world models				
Limiting Law	Müller-Herold, 1996 Müller-Herold <i>et al,</i> 1997	Three-compartment Level II model. A 'limiting law' is used for determining persistence (an upper boundary for persistence).		
EQC	Mackay <i>et al</i> , 1996	Level I, II and III models in a single program. Area 100 000 km ² .		
TaPL3	Beyer <i>et al,</i> 2000 Webster <i>et al,</i> 1998	Level III model designed specifically to calculate persistence and long range transport potential.		
GOU	Gouin <i>et al,</i> 2000	An equilibrium approach that uses partitioning data to identify key half-lives.		
SimpleBox 1.1	van de Meent, 1993	Level III unit world model with no advection. This model has been modified and incorporated into the EUSES program .		
WAN	Wania, 1998	A three-compartment Level III unit world model.		
BENN	Bennett <i>et al,</i> 1999	A three-compartment Level III unit world model.		
PEN1	Pennington and Ralston, 1999	A four-compartment Level III model with no advection.		
CalTOX	McKone, 1993	Level III multi-compartment evaluative model designed for Californian conditions. Includes an extensive human exposure assessment (particularly from hazardous wastes).		
ChemCAN	Mackay et al, 1991	Level III regional model parameterised for Canada.		
HAZCHEM	ECETOC, 1994	A five-compartment Level III model developed for the European chemical industry. Similar to Simple Box and ChemCAN in concept (ECETOC, 1994).		
CoZMo-POP	Wania <i>et al,</i> 2000	Non-steady state multi-compartment regional model		
Linearly connected unit wo	orld models			
GloboPOP	Wania and Mackay, 1993 Wania and Mackay, 1995 Wania and Mackay, 1999 Wania <i>et al</i> , 1999 Wania, 2000	A dynamic, multi-compartment, linearly connected global distribution model for organic pollutants. Includes 9 distinct climatic zones, each of which includes 6 environmental compartments.		
SimpleBox 2.1	Brandes et al, 1996	Nested version, of the SimpleBox 1.1 Level III model consisting of 5 spatial scales. Has been incorporated into EUSES.		
BETR	Macleod <i>et al,</i> 2001 Woodfine <i>et al,</i> 2001	A regionally-segmented linearly-connected contaminant fate model of North America. consists of 24 regions that are characteristic of distinct ecological zones in North America, where each region includes 7 environmental compartments.		

Model	Reference	Description		
Circular Multi-box models				
SCHE	Scheringer, 1996,1997	Circular three-compartment multi-box model for evaluating persistence and long-range transport.		
ChemRange	Held, 2001	A continuous version of the SCHE model solved analytically.		
Other models				
PEN2	Pennington, 2001	Heuristic approach is used for determining overall persistence.		
CART	Bennett <i>et al,</i> 2000	A classification and regression tree analysis (CART for determining whether a substance is persistent o non-persistent based on partitioning and reactivity data.		
EPIWIN	Meylan, 1999	Estimated physical-chemical property and reactivi data are used to determine persistence.		
FENN	Fenner <i>et al</i> , 2000	A Level IV three-compartment evaluative model, applied to parent compounds and their transformation products.		

Table 20: MFTMs which can be used to predict chemical persistence (cont'd)

7.2.2.1 Single unit world models

All the single unit world models are structurally similar and, if parameterised similarly with respect to chemical properties and the environment, should yield essentially identical results (Cowan *et al*, 1995).

The specific formulation of the model used will depend on the objectives of the application. It may be that predictions are required for a particular region (e.g. Europe or North America), in which case a model with environmental characteristics (e.g. dimensions and climatic conditions) tailored to this region will be most appropriate. However, for the most part, a common evaluative 'benchmark' environment will be sufficient, especially for screening. Since, in principle, it is preferable to use the simplest available model that can generate the desired results, multiple connected unit world models are not normally needed until the later stages of assessment.

7.2.2.2 More complex models

Simple MFTMs have been developed to include more environmental compartments (e.g. vegetation and biota) or to split single compartments into two or more (e.g. vertical segmentation into layers of the atmosphere, oceans, soil or sediment). For example, it has been increasingly argued that vegetation should be included as a separate compartment, especially for 'semi-volatile' substances such as PCBs in which the air-vegetation-agricultural animals-dairy products-humans exposure route may be important.

McLachlan and Horstmann (1998), Cousins and Mackay (2001) and Wania and McLachlan (2001) concluded that it is important to include vegetation for some substances of high K_{oa} or low K_{ow} . However, there is still a lack of adequate predictive capability concerning partitioning to vegetation, and few data exist on reaction rates in vegetation.

Similarly, there are a number of circumstances where substances, following ingestion or absorption, can be readily metabolised within the organisms (see Section 3.9).

In some models, a number of 'unit worlds' (each consisting of a set of compartments) are combined to give a network of linearly connected or nested units. This allows a better description of the fate of a substance in a spatially heterogeneous region, and can be useful for assessing the effect of spatially variable environmental parameters(such as temperature) over large areas, or even on a global scale. Examples are given in Table 20.

Obviously, it is possible to compile complicated assemblies of compartments and subcompartments and thus to build complex models. However, as the number of compartments and inter-compartmental transport processes increase, models are becoming increasingly more complex and demanding of input data. Ultimately, a compromise must be sought between high complexity (which may yield greater fidelity but with greater data demands) and low complexity (which may yield inaccurate results but which may be easier to run for multiple chemicals). When complex models are being considered as replacements for simpler ones, a key question should be: What improvement, if any, do these complex models give over simpler models, especially in data poor situations? Inclusion of additional compartments or multiplication of 'unit worlds' is perhaps best left for higher tier risk assessment stages in which a more detailed evaluation is required. At that stage there should be sufficient information on the partitioning characteristics of the chemical, to enable efforts to be focused on the key media in which greater segmentation is justified. Industry is currently funding a research project as part of the Cefic Long range Research Initiative (LRI) (http://www.cefic.org/lri) to progress these ideas.

No matter how complex models appear, they are only approximations of reality. Both the input data and how predictions match real world behaviour will be subject to errors. Some degree of uncertainty will always exist in the results, and there should be an awareness of the probable sources of the major uncertainties.

7.2.3 Model Output

7.2.3.1 Realistic Presence

A key output from MFTMs is the fraction of the total mass of chemical in the evaluative system that is predicted to occur in each compartment (F_i). This is sometimes referred to as a prediction of realistic presence. The greater the value of F_i , the more important is the respective compartment from the point of view of persistence. Such information may be employed to prioritise the compartments for which measured data (e.g. on degradation and partitioning behaviour) should be collected ('media of concern'). Similarly, it may be used to justify the omission of testing for those compartments in which the realistic presence is likely to be low (e.g. $\leq 5\%$).

7.2.3.2 Definition and calculation of overall persistence

Within the context of estimating the environmental persistence of chemicals, one of the most important outputs from MFTMs is the overall reactive residence time of the chemical, or overall persistence (P_{ov}). This value effectively encapsulates the net effect of the reactivity of a chemical in individual media (usually defined in terms of single-media half-lives), partitioning behaviour and the distribution of emissions. Although agreement has not yet been reached on its significance within the regulatory community, P_{ov} has great potential for providing objective guidance for the classification of chemical persistence.

Overall, persistence can be defined as the total mass of chemical in the environment (soil + sediment + water + air), divided by the sum of the degradation rates for all compartments (Webster *et al*, 1998). Under the steady state assumption, and ignoring advective losses, total inputs (emissions) = total outputs (degradation rates). Thus

$$P_{ov} = \Sigma M_i / E$$
 Equation (52)

where M_i (g) is the mass of chemical in compartment (*i*) resulting from a total emission rate E (g/h). Assuming first order kinetics in each medium with rate constants, k_i , it follows that the rate of reaction in each medium is (k_i . M_i) (g/h), and the total rate is $\Sigma(k_i.M_i)$. At steady state, this must equal the emission rate, E, thus,

$$E = \Sigma(k_i M_i) = \Sigma M_i / P_{ov}$$
 Equation (53)

But $M_i / \Sigma M_i$ is $F_{i'}$ the fraction of the total mass in each medium, thus,

 $\Sigma(F_i,k_i) = 1 / P_{ov}$ Equation (54)

If the rate constant, k_i , is replaced by its reciprocal $1/k_i$ or τ_i (the characteristic time), it follows that,

$$1 / P_{ov} = \sum (F_i / \tau i)$$
 Equation (55)

Since, under first order kinetics, $t\frac{1}{2} = (\ln(2).\tau_i)$, overall persistence and the prediction of realistic presence may be related by

$$P_{ov} = \frac{1}{\ln(2) \cdot \sum (F_i / t_{1/2i})}$$
 Equation (56)

It should be noted that steady state conditions are assumed in the derivation of the above equations.

7.2.3.3 Relationship of Pov to single media half-lives

The overall half-life of the system $(T^{1/2}_{ov})$ may be defined (Gouin *et al*, 2000) as

$$\frac{1}{T_{1/2}^{ov}} = \sum_{i=1}^{n} \frac{F_i}{t_{1/2i}}$$
 Equation (57)

i.e.

$$T_{1/2 ov} = \frac{1}{\sum_{i=1}^{n} \frac{F_i}{t_{1/2i}}} = \ln(2).P_{ov}$$
 Equation (58)

Thus, under steady state conditions, the contributions of individual media half-lives to $T\frac{1}{2}_{ov}$ are weighted by F_i . Since a half-life is conceptually easier to deal with than the mean residence time, and since reaction rates in individual media are often discussed in terms of half-lives, $T\frac{1}{2}_{ov}$ provides a useful output from MFTMs.

The analysis presented above (and the relationship between overall residence time and overall half-life in a multi-media system) is only valid for steady state conditions. Under non-steady state conditions, (e.g. under time varying inputs or if inputs suddenly ceased) the fraction of the total mass in each compartment would change as a result of differences in reaction rates in each medium. The change in total mass over time would not follow a first order (negative exponential) curve, especially when the reaction rate is much more rapid in one compartment (to which a reasonable proportion of the chemical partitions), than in the others. The term $T^{1/2}_{ov}$ can thus be regarded as a 'pseudo half-life', since it represents the average time taken for 50% of a substance emitted into a steady state system to degrade, but does not represent the time required to degrade 50% of the total mass under transient (time varying) conditions.

It is instructive to compare the $T^{1/2}_{ov}$ values with individual media half-lives for a number of chemicals and a number of emission scenarios. $T^{1/2}_{ov}$ values derived using a level III model as part of the Cefic-LRI Persistence project (AstraZeneca, 2002) are shown in Table 21, together with the individual media half-lives, assuming 100% release to each medium respectively. The results show that $T^{1/2}_{ov}$, derived by assuming 100% emission to each of the three main environmental compartments (air, water and soil), can differ dramatically from the single medium half-life for that compartment as a consequence of transport and partitioning mechanisms. For example, B(a)P and DDT releases to both water and air result in $T^{1/2}_{ov}$ values that are much greater than would be expected from their individual half-lives (due to partitioning to sediment and deposition to surface compartments respectively). Naphthalene, on the other hand, has a shorter $T^{1/2}_{ov}$ for release to water and to soil than would be expected from its respective aquatic and terrestrial half-lives alone, as a consequence of volatilisation to air, where it is degraded more rapidly. Predicted $T^{1/2}_{ov}$ values for phenol, caffeine and PCP indicate that these chemicals behave as would be expected from their individual compartment half-lives.

Substance	Water		Air		Soil	
	t ½	T ¹ /2 _{ov}	t ½	T ¹ /2 _{ov}	t½	T ¹ /2 _{ov}
Phenol	9.00	8.95	0.63	0.76	0.15	0.14
Caffeine	8.33	8.32	8.33	8.32	8.33	7.91
Naphthalene	24.00	8.78	1.00	1.04	18.00	8.26
РСР	0.21	0.35	31.29	24.32	31.29	31.25
B(a)P	0.04	0.35	0.88	53.83	60.00	60.01
DDT	229.17	1958.83	7.08	659.88	708.33	707.70

Table 21: Comparisons between single media half-lives and T½_{ov} (days) for 100% emission into the same medium for six chemicals

7.2.3.3 Uncertainty and sensitivity analysis

As with all models, a degree of uncertainty is frequently associated with many of the input variables required by MFTMs. Depending on the substance and emission pattern, some parameters may be more important than others, and it is these parameters for which a good understanding of uncertainty is required. Hence, overall persistence of a substance is often strongly influenced by uncertainty in the degradation rate constants for the environmental media into which the substance is most likely to partition. This uncertainty is due, in part, to the wide range of degradation reactions to which chemicals in the environment are subjected, including abiotic transformations (such as hydrolysis, photolysis and chemical REDOX reactions), and biotic processes (such as aerobic and anaerobic biodegradation). Laboratory tests are frequently unable to isolate the contributions of individual mechanisms to the overall behaviour of a chemical which, in turn, often leads to difficulties in assigning appropriate rate constants.

Furthermore, most MFTMs bulk individual mechanisms into single rate constants for individual media, and assume that degradation occurs according to simple first order kinetics. In reality, reactions often do not obey first order kinetics, and this will immediately introduce errors and uncertainties into the predictions. These transformation processes and the issues surrounding them, (including typically how they are measured and the uncertainties associated with those measurements), are discussed in earlier parts of this report (Sections 3 and 6).

Measured data, derived using a standard method, are generally preferred for a model over estimated rate constants (e.g. based on (Q)SARs) or expert judgement. When measured data report a wide range of values, the frequency distribution of values should be examined and geometric, rather than arithmetic, means should be used, if appropriate.

Sensitivity analysis (quantifying the relative effects of changes in model parameters on model output) may be useful in understanding model uncertainties, since it is important to quantify uncertainty for those parameters to which the model is sensitive. For example, if a substance partitions 95% to water, the degradation rate in soil or sediment will make little difference to the prediction of overall persistence, hence the uncertainty in those parameters is largely irrelevant (see Section in 7.2.3.1). Conversely, using the same example, it would be much more important to characterise accurately the degradation rate in water.

7.3 Conclusions

MFTMs can provide a valuable contribution to the evaluation of chemical persistence in the environment by allowing information on chemical properties (partitioning behaviour and typical degradation rates), environmental characteristics and mode of entry, to be combined in an objective and scientifically defensible manner. They also allow evaluation of the contribution of each compartment to overall persistence. In so doing, they may be used as a guide in testing programmes (for both media-specific halflives and partitioning data). More specifically, testing effort should be focussed only on those compartments in which a substance is likely to have a realistic presence (i.e. where the fraction of the total steady state mass is greater than 5% as a consequence of the emission distribution and partitioning behaviour). For substances which partition significantly to a number of compartments, the predicted proportion of total mass in each compartment, at steady state, may also be used to prioritise testing in those compartments where the substance is likely to be most abundant. In the opinion of the TF, simple (single unit world) models with four compartments (air, water, soil and sediment) provide the most appropriate tools for the evaluation of persistence. If mode of entry data are available, level III models are preferred since they attempt to account for the often important effects of limitations in inter-media transport on the overall behaviour of a chemical, but are not so complex that they require extensive input data. If mode of entry data are not available, then level II models can provide a reasonable, and unbiased, first step towards identifying those compartments in which testing should be focussed. The use of more complex models should be reserved for higher tier (targeted) assessments of persistence, where such assessments are warranted.

Although there is clearly a place for MFTMs in the designation of environmental persistence, they should be used carefully and with an awareness of their limitations and shortcomings. Further work is needed to ensure that the models, and the interpretation of their results, are based on sound scientific principles and are appropriately validated (Fox *et al*, 2002). Examples of where more work is needed include: improving model applicability to chemicals beyond the non-polar non-ionising substances and polar non-ionising substances, for which there is currently the most confidence; improving the description of input data (such as deposition rate to surfaces); improving the description of partitioning and degradation related to vegetation; assessing the importance of the OH-radical degradation pathway for chemicals that are associated with aerosols or particles; establishing accepted methodologies for incorporating uncertainties in input variables (OECD, 2002a).

It is also important to bear in mind that environmental persistence is not, itself, a measure of environmental risk and should, therefore, not be considered or used in isolation (Fox *et al*, 2002). Persistence is just one property contributing to the environmental behaviour and impact of a chemical. The calculation of persistence should be an intermediate step towards a more detailed risk assessment rather than as an end in itself (Fox *et al*, 2002).

Where important information (e.g. emission rates, mode of entry or chemical properties) is scarce or highly uncertain, it is important that these uncertainties in model inputs are recognised when interpreting model output (which should ideally be associated with some estimation of uncertainty) and that more accurate data should be utilised as soon as they become available in order to refine model predictions.

The topics and factors described in this section provide support for the strategy recommended in Section 8.

8. A TEST-BASED STRATEGY TO IDENTIFY PERSISTENCE CLASSES FROM DEGRADATION MEASUREMENTS

The persistence of chemical substances in the environment cannot be measured directly. It can only be inferred from the continued presence of a substance in the environment or the lack of observed degradative data in the laboratory. However, a predictive, test-based framework is urgently required, which combines good quality empirical data with multi-media model assessments, and which identifies those substances with the potential to persist in the environment. Such a framework is needed to maximise the use of existing data and promote the generation and use of new data in a pragmatic fashion. As part of a wider PBT assessment, a robust and effective framework to assess the overall persistence of a chemical substance will allow the effective prioritisation of substances such that the number of toxicological and bioaccumulation studies may be reduced.

Extrapolation is a major component of any strategy to assess the fate and persistence of a chemical in the environment. It is not possible to measure degradation under all environmental conditions and for all environmental compartments. It is therefore necessary to attempt to relate laboratory test results, often from limited degradation studies, representative of one or more environmental compartments, to removal rates in multiple environmental compartments. Sections 3-5 discussed the issues and limitations associated with extrapolation of data from currently-available biodegradation tests to the environment. Section 6 addressed abiotic degradation mechanisms and indicated that for certain types of chemical substances, estimated or measured abiotic rates of degradation could be predictive for specific environmental conditions. The use of multimedia models to identify compartments of interest for specific chemical substances, where insufficient data exist, has also been described (Section 7). This section proposes a test and model-based strategy to prioritise effectively substances based on their persistence in the environment.

It is important to recognise that the environment is dynamic, and the inherent conditions of any given compartment are constantly in a state of flux, thus making it impossible to identify a distinct rate of degradation, biotically or abiotically. In addition, degradation cannot be described by a single type of reaction kinetics, it can only be described by a range of rates that reflect changes in environmental conditions, and the different types of kinetics that may be operating at any given point in time. Consequently, this section will advocate the use of a range of half-life ($T\frac{1}{2}$) values to describe the degradation and persistence of a substance in the environment (see Section 3). The ranges of $T\frac{1}{2}$ values reflect the range of reaction kinetics that may be taking place together with any spatial and/or temporal differences in reaction rate(s) that may exist. Although, for simplicity, most multi-media models assume that degradation proceeds according to first order kinetics (i.e. $T\frac{1}{2}$ values are converted to first order rate constants), reaction kinetics in the environment may not behave in this way.

There is sufficient scientific knowledge, based on experimental measurements and model estimations, to identify those substances that will undergo rapid degradation in the environment or those that will persist for a long period of time. However, there is a need to identify, as effectively and as accurately as possible, potentially persistent substances, since the majority of substances will lie between these extremes of degradability. Such substances may, for example, be degradable within a certain range of environmental conditions and within a certain range of substance concentrations in a specific environmental matrix. For these chemicals in particular, a more-structured and environmentally-realistic assessment will be required. Such an assessment will need to take into account the quality of degradation data, the environmental behaviour and partitioning of the chemical substance, emissions data and pattern (if available), and the environmental relevance of the data.

A pragmatic, science-based strategy has been developed by the TF to assess the persistence of a substance (Figure 16). This represents the best approach, based on current knowledge and it is described in detail in Section 8.2. The strategy maximises the use of existing degradation test data, from standard and non-standard test systems, and promotes the generation of new data from novel and emerging test regimes. The persistence of a substance has been divided into four distinct categories ranging from 'easy degradable' (P4) to 'persistent in the environment' (P1). Categorisation of a substance into one of these four categories is based on the T½ range into which the substance is predicted to fall. The T½ is derived from a combination of biodegradation test results and the test system used, the potential for biodegradation to be realised, and the abiotic half-life. Substances categorised as P4 and P3 in the screening stage are considered to be of no further concern (T½ <50 days); whereas, substances categorised as P2 and P1 should be subjected to more detailed scrutiny in the confirmatory stage. The remainder of this section will outline the rationale for this testing strategy.

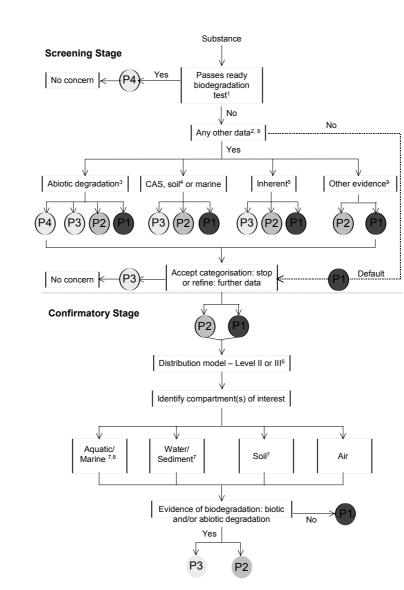


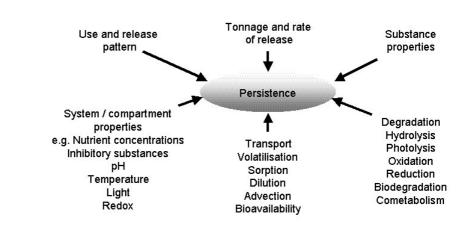
Figure 16: A test-based strategy to determine the persistence of a chemical substance in the environment

- ¹ Includes phytodegradation, although current database is limited, phytotransformations in soil and water environments should be considered.
- ² QSAR predicts fail go to Confirmatory Stage
- ³ Abiotic degradation testing can be applied at all stages, the usefulness will depend on the specific substance and the distribution between the environmental compartments. Abiotic degradation may be combined with degradation to assess persistency.
- ⁴ T¹/₂ for soil is measured directly in laboratory soil die-away tests or in field studies.
- ⁵ Passing an inherent or simulation test has been defined in the specific OECD guidelines.
- ⁶ If emission data is available
- ⁷ Option to expose to 0, 7, 14, 21 and 28d for continuously released substances
- ⁸ Enhanced biomass studies for batch marine and river water studies
- ⁹ Monitoring data

8.1 T¹/₂ Distribution ranges

The fate and distribution of a substance into the various environmental compartments is the combined result of the intrinsic properties of the substance, the emission scenario (distribution of emissions to different environmental compartments), the physicochemical characteristics of the receiving environment (e.g. hydrodynamics, temperature, transport properties, sorption, nutrient status), the microbiological activity capable of metabolising the substance, and the strength of the different abiotic degradation mechanisms to which the substance is susceptible (see Figure 17 and Section 3).

Figure 17: Factors controlling persistence



All the factors described above, and listed in Figure 17, are interdependent, and combine to determine not only whether degradation will occur, but also the rate at which it occurs. Any one of these factors in isolation has the potential to limit the overall rate of degradation, or even prevent it occurring. The relative effect of each of these factors on the rate and extent of degradation, may be compartment specific. Thus, under certain environmental conditions, and for specific types of chemical, abiotic degradation rates determined in the laboratory are more easily extrapolated to the field, than biodegradation rates, which are highly uncertain (see Section 6).

This level of uncertainty reflects the:

- Nature and inherent limitations of laboratory-based biodegradation tests (see Sections 3 and 5);
- dynamic, heterogeneous and adaptive nature of the environment.

Whilst it is possible to measure distinct rates of degradation in laboratory-based test systems, the validity of these rates for predicting degradation in the 'real' world will always be questionable. This is because they always reflect a specific type of reaction kinetics resulting from a prescribed test protocol. A distinct measured rate will probably not account for all types of reaction kinetics, and the range of conditions, that the substance will experience in the environment. It is therefore difficult to identify realistic and generally applicable biotic or abiotic degradation parameters. An alternative to single rate parameters, is to consider a range of values that reflect changes in environmental conditions and the different types of kinetics that may be operating. Consequently, the use of a distribution of T¹/₂ values to describe the degradation and persistence of a substance in the environment is recommended. This reflects how the fate and exposure of agrochemicals has been addressed for a number of years (EC, 1991).

8.2 Strategy for predicting persistence of substances in the environment

ECETOC proposes a systematic approach to estimating persistence (see Figure 16). The first step, 'Screening Stage' (Figure 18), maximises the use of all existing degradation test data towards eliminating all substances for which fairly rapid degradation can be assumed and which, therefore, represent no further concern. Based on the data presented in Section 4, such substances will have a T¹/₂ of less than 50 days.

The second step 'Confirmatory Stage' assesses the persistence of a substance in more detail. Model assessments are required to determine how the substance partitions in the environment based on its physico-chemical properties (which govern partitioning behaviour) and emission data (if available). Based on model predictions, 'targeted' testing may be required to confirm whether a substance should, or should not, be considered as 'persistent'.

STEP ONE - Screening stage

To maximise the use of existing information in this screening step, it is proposed to make effective use of all available abiotic and biotic degradation data from standard or non-standard tests, and from (Q)SARs. The most difficult task is the allocation of a substance to a given persistence class (Figure 18). This allocation to the persistence category requires careful consideration of the:

- Power of the degradation study;
- observed result (rate and extent);
- endpoint measured (primary or ultimate).

For screening purposes, sediment and water are considered as a single compartment, on the assumption that a substance will partition rapidly between the two phases, and that its concentration in the water and sediment phases will be at or near equilibrium. Degradation in one phase will result in a decline in concentration in the other phase, as equilibrium is re-established. Consequently, in this screening stage, it is proposed that there should not be separate criteria for fresh or salt waters or sediments, and that T¹/₂ data in either water or sediment is considered as a demonstration of degradation in the aquatic compartment. It is therefore considered unnecessary to have measured degradation data in the individual environmental compartments (i.e. fresh water, salt water, sediment, soil, air) in the screening stage. Data presented in Table 17 support this recommendation (Section 4).

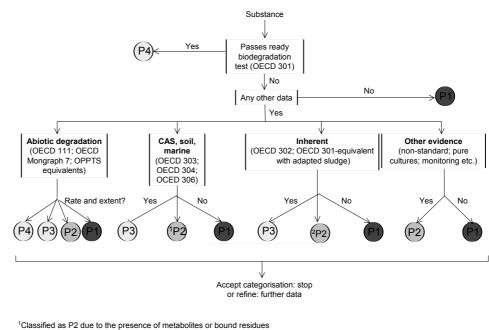
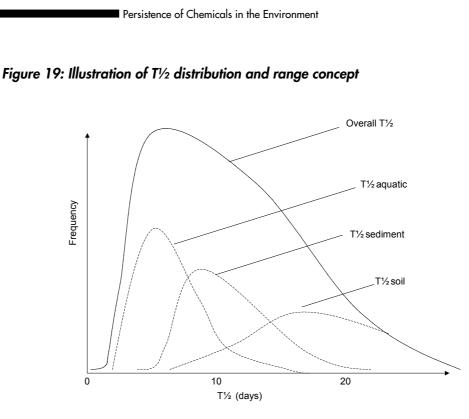


Figure 18: STEP ONE - Screening stage

¹Classified as P2 due to the presence of metabolites or bound residues ²Achieved 70% degradation outside the guidance outlined in the TGD (Section 2.3.6.4)

In the screening stage, the substance is allocated to one of four categories. These categories are defined by a range of $T_2^{1/2}$ values that reflect the overall removal from the environment (i.e. they include $T_2^{1/2}$ distribution curves for waters, sediments, soils and air).

Figure 19 is a simple illustration of this concept. The shape of the overall T¹/₂ distribution and the T¹/₂ distribution for individual compartments will depend on the physicochemical properties of the substance, the compartment, and the factors described in Section 8.1.



The four categories of substances proposed are P1, P2, P3 and P4 with P1 being the most persistent and P4 being the most rapidly degradable. Beek *et al* (2001) and the Dutch Strategy on Management of Substances (SOMS, 2002) also advocate the use of four persistency classes based on the relative power of the test, the result, the endpoint measured, the extent of degradation and the potential for bound residues or metabolites to exist.

The approach recommended by ECETOC builds on the structures proposed by Beek *et al* (2001) and included in SOMS, (SOMS, 2002), in that it is not restricted to the use of existing standardised tests at the screening and confirmatory stages. This enables the effective prioritisation of substances, based on all available degradation data (standard and non-standard). In addition, the ECETOC scheme provides an effective framework in which data from new, targeted, environmentally-realistic and relevant test systems can be used effectively at the confirmatory stage (see Step 2 below). Clearly, such tests will require ring-testing or regulatory approval.

The approaches presented by ECETOC, Beek *et al*, (2001) and SOMS, (2002) make a clear distinction between substances that are of concern with respect to persistence, and those that are not. In addition, the ECETOC scheme provides a framework under which substances of concern can be further evaluated with respect to their persistence. The T¹/₂ ranges proposed in the text below are based on the data presented in Section 4. These ranges should be reviewed and revised if necessary, as more data become available.

The approach outlined below is based on results that are derived from laboratory and field data. In this approach it is not necessary to derive individual T¹/₂ values for each separate compartment. The T¹/₂ ranges proposed are considered to be a distribution of the range of T¹/₂ values predicted for each of the separate environmental compartments (see Appendix B). The confidence with which the probability of degradation can be extrapolated from a laboratory test to the 'real world' is related to our understanding of degradation mechanisms under environmental conditions. For biodegradation, this knowledge is strongest for the fresh water environment and for soils; the confidence here is therefore highest.

Ρ4

A pass result from any of the current standard ready tests (OECD, 1992a), or a ready test with minor modifications, for example, prolonged test duration, reduced substance concentration, or the use of silica etc. to prevent toxicity. Substances passing these tests have a very high potential for biodegradation that will be manifested in all environmental compartments. Such substances are classified as P4 and are predicted to have a T¹/₂ distribution within the range of 1-25 days for all environmental compartments. They should not be considered as 'persistent'. Alternatively, abiotic degradation data or abiotic degradation (Q)SAR estimations for suitable substances, demonstrating a T¹/₂ distribution in the range of 1-25 days, will also be classified as P4.

P3

A substance that fails the standard or modified ready test, but passes either a sewage simulation test (e.g. Husmann or porous pot; OECD, 2001 a,b); an inherent biodegradation test (OECD, 1992d; according to the criteria described in Section 2.3.6.4 of the TGD); a ready test using an adapted inoculum (adaptation period of up to 28 days maximum); a standard laboratory soil study (OECD, 1981b); or a standard marine biodegradability study (OECD, 1992c) can be classified as P3. These results indicate a strong potential for adaptation and growth-linked biodegradation. Based on the data in Section 4, such substances would have a predicted T¹/₂ distribution in the range of 5-50 days, and they should not be considered 'persistent'. Alternatively, abiotic degradation data or abiotic degradation (Q)SAR estimations for suitable substances, demonstrating a T¹/₂ distribution in the range of 5-50 days, would also result in a substance being classified as P3.

P2

A substance that passes any of the standard inherent tests (OECD, 1992d; series without satisfying the criteria described in Section 2.3.6.4 of the TGD, EC, 2003) would be predicted to have a T¹/₂ distribution in the range of 10-150 days and would initially be assigned P2. Substances that only partially degrade in sewage simulation (OECD, 2001 a,b), soil studies (OECD, 1981b) or marine studies (OECD, 1992c) and where metabolites or bound residues may be an issue are also classified as P2 in the screening stage. Substances demonstrating slow or partial abiotic degradation, where there is reason to believe that degradation may not occur in the environment, are also classified as P2.

Results from water/sediment simulations (standard or non standard), anaerobic studies and additional evidence from non-standardised biodegradation studies (e.g. pure culture studies, co-metabolism studies) suggesting that reasonable breakdown might be expected in the environment, would also result in a substance being classified as P2. P2 covers the widest T¹/₂ range because of the higher level of uncertainty in the probability of degradation being manifested in the environment. A substance falling into this category may be of potential concern and further investigation will be required for substances classified as P2 in the screening stage (see Step 2 below).

P1

Substances which fail any of the above tests, substances for which no evidence of degradation can be found, or substances which are predicted to have a T¹/₂ greater than 150 days, should be classified as P1 (persistent). For those substances which do not have any degradation data available, data should be generated according to the procedures described in Step 2.

In addition, any P4-P2 substance that is found, in a robust environmental monitoring programme over a 24-month period, to be increasing in concentration as a result of normal usage, should be reassigned as P1.

STEP 2 - Confirmatory stage

For a substance classified as P1 or P2 on the basis of available degradation data or in the absence of such data, more detailed information is required with regard to its persistence in the environment. The scheme outlined in Figure 20 is also appropriate for new substances that, based on (Q)SBR predictions or expert judgement are not expected to pass a ready biodegradability study.

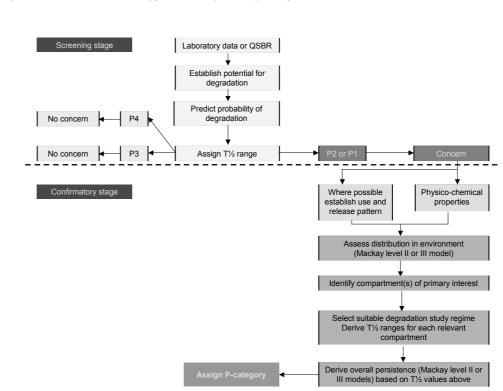


Figure 20: Overall strategy for categorising the persistence of a substance

Persistence of Chemicals in the Environment

Testing at this stage should be targeted to the specific environmental compartment(s) of interest, taking into account, if available, any information on the partitioning behaviour and release pattern (actual or anticipated) of the substance. For substances where no degradation or partitioning data exist, (Q)SAR estimations should be used to assist in targeting the relevant environmental compartments. At this stage, MFTMs, (see Section 7) may be used to help guide testing and eventual classification. If there are no degradation data or reliable (Q)SARs, then a level I model may be used to give an indication of those compartments to which the substance is most likely to partition. This can then be used to prioritise testing. However, level I models give no information about environmental persistence.

If degradation data (or reliable (Q)SARs) are available, then higher level models may be used. If mode of entry data exist (the proportion of total emission to each environmental compartment), a level III model should be used to derive (1) the fraction of total steadystate mass which would be expected in each environmental compartment (giving a general indication of the relative importance of each compartment) and (2) the overall half-life of the substance. If accurate mode of entry data are not available, a level II model may be used to give the same outputs. It should be noted, however, that the output from level II and level III models might differ as a consequence of the sometimes unrealistic assumption in level II models, of complete equilibrium between phases. Testing priority should be given to those compartments which are expected to contain most of the substance of interest. Half-lives for media in which a realistic presence (>5%) is expected, should be considered within the confirmatory stage (Section 7). Once the environmental compartment(s) of interest have been identified, an appropriate test protocol should be employed to maximise information regarding the fate and behaviour of the substance in that compartment. The prediction of the overall persistence (P_{ov}) or overall half-life ($T^{1/2}_{ov}$) produced by level II and level III MFTMs, potentially can also be used within the evaluation system, although the threshold criteria have yet to receive regulatory acceptance. The TF suggests that using MFTMs a reasonable screening threshold for persistence would be $T^{1/2}_{ov} > 60$ days, which corresponds with the individual half-life thresholds adopted by may regulatory authorities (e.g. US-EPA).

For substances that are continuously discharged into the environment, or those with discrete local discharges, pre-exposure periods of between 0 and 28 days should be considered to demonstrate the ability of an inoculum to adapt to the test substance. In addition, for substances being discharged predominantly to the marine and freshwater environments, careful consideration must be given to ensuring that an adequate representation of the relevant microbial community is present in the test system. Section 5 has already described reports of spatial effects being introduced into laboratory test systems, especially those with small volumes. Consequently the TF recommends that where possible, either elevated biomass tests are used for batch tests, or that a semicontinuous or continuous test design is considered (see Section 5).

The TF recognises that there are relatively few compartment-specific degradation protocols that have been ring-tested and accepted as standards. This deficiency should be addressed as a matter of priority.

If no evidence of degradation is observed in compartment-specific tests, the substance should be designated P1, and it should be evaluated with respect to its bioaccumulative and ecotoxicological properties. Where evidence of degradation is observed, the substance should be characterised as either P2 or P3. Substances should be classified as P3 if their measured T¹/₂ distribution in the test system is <50 days and there is no evidence of residual metabolites or bound residues. Substances which have a T¹/₂ distribution of >50 days, or which are only partially degraded, and give rise to the presence of residual metabolites or bound residues, should be classified as P2. Substances designated as P2 should be evaluated with respect to their bioaccumulative and ecotoxicological properties, but with a lower priority than those designated as P1, unless any metabolite or residue that is produced is thought to have an increased impact.

8.3 Conclusions

The degradation of chemicals in the environment is complex and proceeds by a combination of different processes, at different rates and with different kinetics. It is, therefore, extremely difficult to extrapolate single degradation rates or type of reaction kinetics to describe the behaviour of a substance in the environment. Degradation is best described by a distribution or range of T¹/₂ values that reflect the ability of a substance to be degraded in all environmental compartments. The T¹/₂ ranges presented in this section are based on currently available laboratory and field data (Section 4; Appendix B). As new data become available, especially for the marine environment, these T¹/₂ ranges need to be reviewed and revised as necessary.

The present system of defining a substance as 'persistent' or 'non-persistent' on the basis of the pass / fail of a ready biodegradation test needs to be refined. A persistence strategy is urgently required that can identify substances that pose a long-term threat to the environment. In this way, subsequent bioaccumulation and ecotoxicology studies can be prioritised and focused accordingly. To achieve this aim, the TF recommends four-persistency classes P1, P2, P3 and P4, with P1 the most persistent and P4 the most rapidly degradable. Each of these persistency classes has a distinct range of T¹/₂ values associated with it. Substances that are assigned P4 and P3 are not considered to be 'persistent' and pose no long-term threat to the environment; whereas, substances assigned P1 and P2 should be assessed in greater detail.

The strategy for assessing persistence must make effective use of all the available standard or non-standard degradation and partitioning data, and promote the active development of new, focused, degradation test protocols that can reduce the uncertainty in degradation assessments. In addition, wherever possible tests should be designed and conducted to reflect release pattern and distribution of a chemical in the environment. The two-tiered testing strategy presented in this section seeks to integrate the effective use of existing data, and the generation of high quality environmentally relevant data where appropriate, with model assessments to serve these goals.

9. CONCLUSIONS AND RECOMMENDATIONS

This review considers the current definition of persistence of chemicals in the environment, the factors that influence their degradation, methods currently used to measure degradation and the extent to which current standard degradation tests can be used to infer 'persistence'. The review highlights the fact that a single, scientifically-agreed definition of 'persistence' does not exist and that regulatory bodies evaluate the persistence of chemical substances in an inconsistent manner. Some of the major issues highlighted by this review and recommendations for future research are summarised below.

Methods to determine ready biodegradability were originally designed as screening tests to identify chemical substances that would undergo rapid and ultimate degradation in all environmental compartments. This is a purpose that they serve very well; substances that pass these tests can be assigned, with a high degree of certainty, as being nonpersistent through normal usage. Ready biodegradation tests provide a relatively simple and inexpensive technique to identify substances that do not require further information regarding their biodegradability. However, many substances that do not meet the pass criteria for ready biodegradability still have a high potential to undergo biodegradation in the environment. Therefore, ECETOC proposes that no substance should be designated as being 'persistent' based solely on the negative outcome of a ready biodegradability study. The current reliance of assigning persistence, largely on the outcome of these tests, clearly justifies the need for a new, pragmatic, test-based framework (supported by multi-media modelling) to classify a chemical substance as persistent or not. If such a system is correctly implemented, subsequent bioaccumulation and ecotoxicological studies can be focused on those chemical substances that are truly persistent, and the number of whole organisms tests can be effectively prioritised and potentially reduced.

Persistence is defined, in the majority of regulatory assessments, on the basis of single media environmental half-lives. In the case of biodegradability testing, a half-life value is assigned to different environmental compartments based on the pass or fail outcome of the test, rather than by determining a measured degradation rate. These tests are generally carried out under conditions that do not adequately reflect the real environment and thus extrapolation of their results to the environment is difficult. The TF questions the merit of attempting to derive any kinetic description(s) from screening tests, such as ready biodegradability studies, due to their distinct lack of realism, the existence of test system artefacts and their stringent nature. However, there exist test systems that offer improved environmental realism, and these tests should be employed in determining realistic rates of degradation.

The rate of degradation in the environment is not usually restricted to first order kinetics and cannot be described using a single first order rate constant (or half-life). Degradation kinetics are determined by a number factors, both environmental and physico-chemical, and degradation in the environment may occur as a result of a number of different processes, operating at different rates, and obeying different kinetics. These will be subject to both temporal and spatial variation. The TF concludes that alternatives to the half-life concept for describing degradation in the environment, must be given due consideration. As a first step towards addressing this issue, it is proposed that the persistence of a substance in the environment should be described by a distribution of degradation rates. This distribution attempts to account for spatial and temporal differences in the rates and kinetics of degradation across all environmental media. It is not reliant on a single half-life value, as currently proposed in most regulatory criteria, and does not give weight to one single environmental medium over another (e.g. marine degradation data over freshwater data, as in the revised TGD (EC, 2003)). The distribution ranges and persistency classes proposed by ECETOC in this review are based on the available published data. However, as additional data from tests with improved environmental realism become available, there will be a need to review and refine the (numerical) persistency classes presented.

Any strategy to infer the persistence of a chemical substance in the environment should make effective use of all available standard or non-standard degradation and partitioning data; it should also promote the development of new, targeted, test protocols towards decreasing the uncertainty in degradation assessments. In addition, where possible, tests should be designed and conducted to reflect release patterns and the distribution of a chemical in the environment. The two-tiered testing strategy presented in this report seeks to integrate the effective use of existing data, and where appropriate the generation of high quality environmentally-relevant data, with model assessments to serve these goals.

The strategy proposed by ECETOC (see Section 8) comprises a screening stage and a confirmatory stage and is applicable to both new and existing chemicals. Where degradation data exist, the strategy seeks to maximise their use when assessing the persistence of chemical substance. A structured testing strategy is also proposed, covering the situation where no data exist or where existing data are insufficient or inadequate. The strategy incorporates improved tests, including the use of pre-exposed inocula for substances that are released continuously, and enhanced biomass levels for marine and freshwater studies. A major change to the current test-based approach used to assess persistence is proposed. In the confirmatory stage, the effective use of multi-media modelling, is advocated, to target testing in specific environmental compartments, where significant realistic presence is predicted. Multi-media modelling may also be used at the confirmatory stage, to assess the overall persistence of a chemical (i.e. in the environmental as a whole) on the basis of its emission scenario, partitioning characteristics and media-specific removal or degradation rates. This can help to confirm classifications that are based on measured data and assist in prioritisation of testing programmes based on an objective and holistic approach. In brief, this strategy maximises the use of existing degradation data, from standard and non-standard tests, and promotes the generation of new data from appropriate novel and emerging tests.

The new laboratory test systems proposed in Section 5 take into account the factors discussed in Section 3, such as microbial adaptation, microbial diversity and low substance concentration. The impact of scale effects through working at reduced volumes in the laboratory also needs careful consideration, especially with regard to ensuring sufficient representation of the broader microbial biodiversity in laboratory biodegradation tests. Research is also required to understand the quantitative impact of the different factors governing biodegradation rates in the environment i.e. physical properties of the molecule and the system constraints (laboratory and environmental). For new substances that do not satisfy the criteria for ready biodegradability and for existing substances for which there is no ready biodegradation data, test systems have been recommended that either allow the use of elevated biomass concentrations or pre-exposed inocula. These biodegradability tests can be conducted within existing ready biodegradation test apparatus and have the added benefit of opening the way for probabilistic biodegradation assessments to be made. Robust and validated test systems are required for all environmental compartments. At present, there are insufficient data to compare rates observed in standard marine/freshwater biodegradation studies with those in the marine environment. ECETOC concludes that the marine environment should be given priority and research carried out to either towards reducing the uncertainty in extrapolating from standard freshwater / marine biodegradation studies to the marine environment, or to demonstrate the need for marine studies to be conducted for all substances. Based on the generation of such data, the T1/2 values proposed in this document may need to be refined.

Tests that allow microbial adaptation are urgently required, as well as a regulatory framework in which data from such tests can be used. Adaptation is a natural phenomenon, especially for substances released continuously. However, existing tests provide minimum opportunities for adaptation to occur. The integration of this concept in the testing procedure and overall testing strategy must be focused such that this potential is realised. To assess the persistence of a substance, every realistic opportunity for biodegradation to occur must be allowed, this includes the use of semi-continuous and continuous test systems, and inocula pre-exposed to the test substance. The potential for adaptation is identical for both new and existing substances. However, the use pattern (continuous or intermittent) and environmental loading of a substance will control the time taken for adaptation to occur in the environment. The TF recommends that pre-exposure regimes, for substances continuously released, and the use of inoculum densities that do not compromise the potential for biodegradation must be used to determine the true persistence of a chemical substance. The TF recognise the need to have these test protocols validated and ring-tested as a matter of priority.

In ready biodegradation test systems, using elevated substance concentrations, the rate of biodegradation for a number of test substances appears to be closely correlated to the solubility of the substance. This will hold true for any laboratory-based test conducted above the solubility level. Clearly, this factor is less important in the environment and rates of degradation observed in the laboratory test may be lower than those observed in the field. Therefore, whenever possible a substance's biodegradability should be assessed below its limit of aqueous solubility.

Degradation data in existing databases need to be critically evaluated with respect to the degradation system used (standard or non-standard), and the endpoints measured. Data reviewed in Section 5 has highlighted the fact that the semi-specific measurements for biodegradation (dissolved organic carbon removal, carbon dioxide evolution and oxygen demand) are non-equivalent. Consequently, chemical substances are currently being classified as persistent within national and international databases on a non-uniform basis. The imbalance between measured and estimated data also needs to be assessed.

In some cases the default values which are applied for biodegradation, especially for a substance which passes an inherent test but not a ready test, should be revised downwards but this can only be done on the basis of more extensive comparisons between laboratory and field data. A series of laboratory tests need to be carried out to distinguish the relative contribution of abiotic and biotic degradation rates for a number of substances and their breakdown products. With the availability of improved degradability test systems, data need to be generated to provide a comprehensive database to relate laboratory tests with expected environmental T¹/₂. The results of any new test systems must also be exploited to update statistical distributions of T¹/₂ where appropriate. Finally, the persistence criteria described in Section 8 should be refined in the light of these experimental data.

GLOSSARY

Primary biodegradation

The structural change (transformation) of a chemical substance by micro-organisms resulting in the loss of chemical identity.

Ultimate aerobic biodegradation

The breakdown of a chemical substance by micro-organisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralisation) and the production of new biomass and organic microbial biosynthesis products.

Mineralisation

The breakdown of a chemical substance or organic matter by micro-organisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present.

Lag phase

The time from the start of a test until adaptation of the degrading micro-organisms is achieved and the biodegradation degree of a chemical substance or organic matter has increased to a detectable level (e.g. 10 % of the maximum theoretical biodegradation, or lower, dependent on the accuracy of the measuring technique).

Primary substrate

A collection of natural carbon and energy sources that provide growth and maintenance of the microbial biomass.

Secondary substrate

A substrate component present in such low concentration, that by its degradation, only insignificant amounts of carbon and energy are supplied to the competent microorganisms, as compared to the carbon and energy supplied by their degradation of main substrate components (primary substrates).

Degradation rate constant

A first order or pseudo first order kinetic rate constant, k (d^{-1}), which indicates the rate of degradation processes. For a batch experiment k is estimated from the initial part of the degradation curve obtained after the end of the lag phase.

$T^{1/2}_{ov}$

A kinetic independent expression that describes a range or distribution of degradation rates that may be following different kinetic processes. For the simplicity of multi-media modelling this value equates to a range of half-life values.

Half-life, $t^{1/2}$ (d)

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 $t^{1/2} = -\ln 2/k$.

Dissolved organic carbon (DOC)

That part of the organic carbon in a sample of water which cannot be removed by specified phase separation, for example by centrifugation at 40000 ms⁻² for 15 min or by membrane filtration using membranes with pores of 0.2 μ m - 0.45 μ m diameter.

Advection

Physical transport or movement of a substance with its medium (air, water, sediment).

Bioavailability

The ability of a substance to interact with the biosystem of an organism. Systemic bioavailability will depend on the chemical or physical reactivity of the substance and its ability to be absorbed through the gastrointestinal tract, respiratory surface or skin. It may be locally bioavailable at all these sites. *

EINECS

European Inventory of Existing Commercial Chemical Substances: a list of all chemicals either separately or as components in preparations supplied to a person in an EC Member State at any time between 1 January 1971 and 18 September 1981.

Emission

Release of a substance from a source, including discharges into the wider environment. *

Environmental compartments

Subdivisions of the environment which may be considered as separate boxes, and which are in contact with each other. A simple model would separate the environment into air, water, and soil, with biota, sediment (bottom and suspended), layering of water bodies, and many other refinements being allowed if data to support their inclusion are available. Concept from Mackay (1991).

Existing chemicals

Chemicals listed in the EINECS (EU legislation). See also EINECS.

Fate

Disposition of a material in various environmental compartments (e.g. soil or sediment, water, air, biota) as a result of transport, partitioning, transformation, and degradation. *

IUCLID

Programme developed by the European Chemicals Bureau to facilitate access to and query of data on existing substances. Currently contains data on HPVC chemicals. See also EINECS.

Model

A formal representation of some component of the world or a mathematical function with parameters which can be adjusted so that the function closely describes a set of empirical data. A *mathematical* or *mechanistic* model is usually based on biological, chemical or physical mechanisms, and its parameters have real world interpretations. By contrast, *statistical* or *empirical* models are curve-fitted to data where the mathematical function used is selected for its numerical properties. Extrapolation from mechanistic models (e.g. Pharmacokinetic equations) usually carries higher confidence than extrapolation using empirical models (e.g. the logistic extrapolation models). A model that can describe the temporal change of a system variable under the influence of an arbitrary 'external force' is called a *dynamic* model. To turn a *mass balance* model into a dynamic model, theories are needed to relate the internal processes to the state of the system, expressed e.g. in terms of concentrations. The elements required to build dynamic models are called *process* models. *

Monitoring

Long-term, standardised measurement, evaluation, and reporting of specified properties of the environment, in order to define the current state of the environment, and to establish environmental trends. Surveys and surveillance are both used to achieve this objective.

Parameterise

The allocation of values to the variables.

Steady-state

The non-equilibrium state of a system in which matter flows in and out at equal rates so that all of the components remain at constant concentrations (dynamic equilibrium). In a chemical reaction, a component is in a steady-state if the rate at which the component is being synthesised (produced) is equal to the rate at which it is being degraded (used). In multi-media exposure models and bioaccumulation models it is the state at which the competing rates of input/uptake and output/ elimination are equal. An apparent steady-state is reached when the concentration of a chemical remains essentially constant over time. Bioconcentration factors are usually measured at steady-state. *

Verification

Compare predicted with measured values, and test assumptions and internal logic of the model. This includes 1/ scientific verification that the model includes all major and salient process; 2/ the processes are formulated correctly; and 3/ the model suitably describes observed phenomena for the use intended.

* From Van Leeuwen and Hermens, 1996

APPENDIX A: SUMMARY OF METHODS FOR DETERMINING DEGRADATION RATES AND BIODEGRADABILITY

Marine Waters

OECD Guideline 306

OECD Guideline 306 (OECD, 1992c), is a simulation test which is a variant of the modified OECD Screening Test (OECD, 1992e), available for assessing biodegradability of individual substances in seawater. Method 306 (which corresponds to ISO Standards; ISO, 1994a and ISO, 1994b and OPPTS, 1998) can be carried out as either a shake flask or closed bottle method and the only micro-organisms added are those colony-forming heterotrophic bacteria in the test seawater to which the test substance is added. Relatively high concentrations of the test substance must be used because of the poor sensitivity of the analytical method for DOC. This in turn necessitates the addition to the seawater of mineral nutrients (N and P), the low concentrations of which would otherwise limit DOC removal through oligotrophic effects.

The test guidelines state that "the results from this test are **not** designed to be taken as indicators of ready biodegradability but are to be used specifically for obtaining information about the biodegradability of chemicals in marine environments".

The US-EPA equivalent to this OECD (OPPTS, 1998) also uses natural seawater both as the aqueous phase and as the source of micro-organisms. In an endeavour to conform to the methods for ready biodegradability in fresh water, the use of ultrafiltered and centrifuged seawater was investigated, as was the use of marine sediments as inocula. The investigations were unsuccessful, however, and natural seawater pre-treated to remove coarse particles was therefore used as the test medium.

In the current method, a minimum of five samples is required to describe the time-course of degradation for a substance of interest, but no fixed time schedule for sampling can be stated as the rate of degradation is variable. If DOC analyses are performed immediately after sampling, the next sampling occasion should be considered on the basis of the results of the previous analytical determinations. If samples for DOC analyses are to be preserved for measurement later, more than the minimum of five should be taken. The last sample taken should be analysed first and by working backwards, with a judicious selection of samples for analysis a good description of the biodegradation curve can be obtained using a relatively small number of analytical determinations. If no degradation has taken place by the end of the test, no further samples need be analysed. The test can be ended before day 60 if there is an obvious plateau on the degradation curve. In contrast, if degradation has begun by day 60, but has not reached a plateau, the test can be extended (provided real-time analysis takes place). If toxic effects are expected or possible it is advisable to include an inhibition experiment in the test design.

The characteristics of the test method are summarised in Table A.1. Failure to satisfy the test criteria (namely >70% DOC removal or >60% reduction in Theoretical Oxygen Demand (ThOD)) does not preclude the potential for biodegradability in the marine environment, but rather indicates that further study is needed. Other standard degradation methods developed for testing in fresh water have been modified and adapted to marine conditions. These methods are the DOC die-away test (ISO, 1994a; OECD, 1992f), the closed bottle test (ISO, 1994b; OECD, 1992g), the two-phased closed bottle test (ISO, 1997a), the CO₂ evolution test (ISO, 1999b; OECD, 1993h) and the CO₂ head space test in sealed vessels (ISO, 1999a). International Standard (FDIS) 16221 (ISO, 2001) also provides guidance for the determination of ultimate aerobic biodegradability in the marine environment by aerobic micro-organisms in static aqueous test systems.

Fresh Waters

Simulation Tests

There are several standard simulation tests that can be applied to organic chemicals to provide more environmentally realistic biodegradation data. Two ISO methods evaluate the biodegradability of organic substances in natural waters by aerobic micro-organisms:

- 1. A shake flask batch test with surface water or surface water/sediment suspensions (ISO, 2002a);
- 2. A continuous flow river model with attached biomass (ISO, 2002b).

These tests have been designed specifically to provide information on biodegradation behaviour and kinetic rates of degradation for a test compound at low concentrations (normally below 100 μ g l⁻¹). In such circumstances the low concentrations of organic substances means they serve as secondary substrates to natural carbon which constitutes the primary source. The expected kinetics of degradation in these tests are first order non-growth kinetics because the degrading micro-organisms obtain the major part of their energy and carbon from the primary substrates and not from the secondary substrates. First order kinetics imply that the specific rate of degradation is constant and independent of the concentration of the test compound.

ISO Standard 14592-1 (ISO, 2002a) is applicable to (a) natural surface water free from coarse particles to simulate a pelagic environment ('pelagic test') or (b) surface water amended with suspended solids/sediments of 0.1 - 1.0 g l⁻¹ dry weight (suspended sediment test) to simulate a water/sediment interface or a water body with resuspended sediment material. This test cannot be used to provide evidence of ultimate biodegradability, which is better assessed using other standardised tests. The US-EPA equivalent standard method is OPPTS (1998).

ISO Standard 14592-2 (ISO, 2002b) is applicable to natural waters by means of a continuous flow river model with attached biomass and can be used for organic chemicals which (under the conditions of the test and at the chosen test concentration) are:

- Water soluble;
- quantitatively detectable with appropriate analytical methods or available in radiolabelled form;
- non-volatile from aqueous solution (for example Henry's Law Constant < 1 Pa m³ mole⁻¹);
- not photolysed;
- not inhibitory to the micro-organisms of the test system.

Whereas the shake-flask tests give preliminary information about the fate of a test compound, the US-EPA also proposes a sediment/water microcosm biodegradation test (OPPTS 1998), that provides more environmentally realistic scenarios. Microcosms may be used to replicate many of the processes affecting the fate of a chemical in complex ecosystems. These model systems provide an opportunity to manipulate various test conditions and to observe the effects of these alterations and their interactions on fate processes. Compared with field investigations, microcosms are more easily replicated so effects of environmentally variability can be studied more readily. The fact that microcosms can be used to examine the significance of various fate processes (e.g. hydrolysis and biodegradation), thus making it possible to focus on critical processes and consider site-specific environmental situations.

Primary Biodegradability

The OECD has developed a screening test to assess the primary biodegradability of surfactants (EC 1973a,b; 1982a,b). It is carried out over a period of 19 days in a shaken, open flask containing an aerobic micro-organism inoculum of 10^{2-3} viable cells/ml and an initial test compound concentration of 5 mg l⁻¹. Removal is measured by using non-specific colorimetry, or in some cases, specific chemical analysis. If the percentage removal is greater than 80%, no further testing is required. If the removal rate is less than 80%, the confirmatory test is applied where a value of more than 80% primary biodegradability must be reached before the surfactant may be marketed. The confirmatory test uses either the UK porous pot system or the Husmann apparatus (3 litre aeration vessel). Briefly, the surfactant (10-20 mg l⁻¹) is added to the influent synthetic sewage (2.5 g l⁻¹). The retention time of the sewage is 3 hours, while the mean retention time of the sludge is 6 to 10 days. The test unit may be run for a maximum settling in period of 6 weeks followed by a steady operation for 3 weeks during which the effluent is analysed for primary biodegradation.

Ready and Inherent Biodegradability Tests

Table A.2 summarises the test conditions of OECD Guidelines (OECD, 1992a) and ISO Standards (as defined in ISO, 1997b) that permit the screening of chemicals for ready biodegradability in an aerobic aqueous medium. The OECD Guidelines (with the corresponding ISO Standards) are:

- 301A: Dissolved Organic Carbon (DOC) Die Away Test (ISO, 1994a)
- 301B: Carbon Dioxide (CO₂) Evolution Test (Modified Sturm Test) (ISO, 1999b)
- 301C: Modified MITI I (Ministry of International Trade and Industry, Japan) Test (No corresponding ISO Standard)
- 301D: Closed Bottle Test (ISO, 1994b)
- 301E: Modified Screening Test (ISO, 1994a)
- 301F: Manometric Respirometry Test (ISO, 1999c).

In all these tests a solution or suspension of the test substances in a mineral medium is inoculated and incubated under aerobic conditions in the dark or with diffuse light over a 28-day period. OECD ready biodegradation tests use 'unadapted' inocula, whereas the OECD inherent tests are designed to allow for a greater degree of adaptation. Test substances, which are soluble in water to at least 100 mg l⁻¹, can be assessed by all methods, provided the substances are non-volatile and non-adsorbing. The test inoculum may be derived from a variety of sources such as surface waters, unchlorinated sewage treatment works effluents, activated sludge or a mixture of these. If activated sludge is used for the DOC Die-Away (301A), CO₂ Evolution (301B) and Manometric Respirometry (301F) methods, it should be taken from a treatment plant or laboratory scale unit receiving predominantly domestic sewage. Inocula from other sources, usually yielding lower cell densities, have been found to result in a greater scatter of results. For the Modified OECD Screening (301E) and Closed Bottle (301D) methods, a more dilute inoculum without sludge flocs is needed and the preferred source is a secondary effluent from a domestic waste water treatment plant or laboratory-scale unit. For the MITI (I) method, the inoculum is derived from a mixture of sources.

Two standard inherent biodegradation test procedures have been developed: the Zahn-Wellens test and the semi-continuous activated sludge (SCAS) test which has been adapted for use with soluble (OECD, 1981c) and volatile and insoluble chemicals (OPPTS 1999) (Table C1). The Zahn-Wellens batch test requires pre-washed activated sludge (1g 1^{-1}) and a relatively high test concentration (50-400 mg DOC 1^{-1}). The test is carried out in a 3-4 litre reactor with a sludge volume of 2 litres which is continuously aerated and stirred. This test has a high potential for biodegradation since there is a high concentration of biomass. Biodegradation is followed by the difference in DOC or COD analysis between a control and test reactor. In contrast the SCAS test is operated on a semi-continuous fill and draw cycle that involves the addition of fresh sewage sludge containing the test compound (20 mg DOC 1^{-1}) once per day. The sludge concentration of dissolved oxygen above 2 mg 1^{-1} . The sludge retention is 36 hours.

Since the added sewage sludge is mineralised within 8 hours, there are 16 hours for endogenous respiration and attack on the test compound before the reactor is inoculated with fresh sewage micro-organisms. On a daily basis, micro-organisms progress through the cycle of substrate saturation followed by substrate depletion, thus offering a good opportunity for adaptation to the test substance. The adaptation period may be as long as 6 months. Biodegradation is monitored by a comparison of DOC analysis between test and control vessels. A negative result in the SCAS test may be used to indicate that a test compound may be environmentally persistent (assuming toxic effects have been eliminated). However, with the Zahn-Wellens, where the period of adaptation is significantly less than the SCAS test, a negative result does not rule out biodegradability in an adapted environment.

In general the methods apply to organic chemicals which are:

- Water-soluble under the conditions of the test used;
- poorly water-soluble under the conditions of the test used, though special measures may be necessary to achieve good dispersion of the compound;
- non-volatile, though volatile substances can be assessed provided that an appropriate test with suitable conditions is used;
- not inhibitory to the test micro-organisms at the concentration chosen for the tests. The presence of inhibitory effects can be determined as specified in this standard.

The conditions described in ISO Standard 16221 (ISO, 2001) do not always correspond to the optimal conditions for allowing the maximum degree of biodegradation to occur and methods for degradation in freshwater (ISO, 1997b) and at low exposure concentrations (ISO, 2002 a,b) are applicable.

The pass levels for the ready biodegradability methods are 70% removal of DOC and 60% of ThOD or ThCO₂ production for respirometric methods. The pass levels are lower in respirometric methods since, as some of the carbon from the test substance is incorporated into new cells, the percentage of CO₂ produced is lower than the percentage of carbon being used. These pass values have to be reached in a 10-day window within the 28-day period of the test, except where mentioned below. The 10-day window begins when the degree of biodegradation has reached 10% DOC, ThOD or ThCO₂ and has to end before day 28 of the test. Substances which do not attain the pass level within the 28-day period are not deemed to be readily biodegradable. The 10-day window does not apply to the MITI method (301C). The value obtained in a 14-day window would be acceptable in the Closed Bottle method if it is considered that the number of bottles necessary to evaluate the 10-day window caused the test to become impractical.

Abiotic Degradation Test Methods

A number of degradation tests are dedicated to abiotic processes such as hydrolysis, photolysis and sorption characteristics (Table A 3).

Sorption methods

Although sorption itself is not a degradation process, it has a major influence on the availability of organic substances for biodegradation. Thus strongly sorbed chemicals may be effectively removed from biodegradation. Therefore, sorption mechanisms have to be considered when assessing the persistence of a substance. The methods available vary significantly in their complexity, from the OECD 121 test to estimate the organic carbon:water partitioning (Koc) on soil and sludge using HPLC to more sophisticated techniques using a range of soils (e.g. OECD, 2000; OPPTS 835.1220 (OPPTS, 1998)).

The simplest of the screening tests use chromatographic techniques to estimate a chemical's leaching potential. Before 1968 methods of investigating the mobility (and potential bioavailability) of non-volatile organic chemicals within soils were based on field analysis, soil adsorption isotherms and soil columns. In 1968 Helling and Turner introduced soil TLC as an alternative procedure; it is analogous to conventional TLC, with the use of soil instead of for example silica gels or oxides, as the absorbent phase. This type of test now provides the most basic standard test used to determine sorption of chemicals to soil (OPPTS 835.1210) (OPPTS, 1998). Soil TLC offers many desirable features. Firstly, mobility results are reproducible. Mass transfer and diffusion components are distinguishable, and the method has relatively modest requirements for chemicals, soils, laboratory space and equipment. A chemical extraction/mass balance procedure can be incorporated into the test to elicit information on degradation and chemical transformations occurring at colloid interfaces. This method is similar to the OECD 121 test which utilises high performance liquid chromatography to determine the organic carbon:water partition coefficient for organic chemicals sorbed to soil and sewage sludge, the main difference being that the HPLC tests use a standard chromatographic phase rather than actual soils or sediments.

The OPPTS 835.1220 method (OPPTS, 1998) is a derivation of the OECD 106 test to determine sediment and soil adsorption/desorption isotherms. The tests advocate using three types of soils of varying physico-chemical characteristics from an acidic sandy to a slightly alkaline loamy soil with isotherms being determined for both adsorption and desorption. Such tests are more complex but provide more environmentally relevant sorption data than simple chromatographic tests.

Hydrolysis

Standard methods for the determination of hydrolysis have been developed to meet the requirements of regulators, including the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TCSA) in the USA. Basic methods provide hydrolysis rate data as a function of pH, but standards such as OECD, 1981a and OPPTS 835.2130 (OPPTS, 1998), a derivative of the OECD test, evaluate hydrolysis as a function of pH and temperature. All techniques investigate hydrolysis of the test substance over the range of pHs likely to be encountered in the environment (pH 4-9) and under more acidic conditions (pH 1-2) for physiological purposes. The methods require the chemical to be soluble and non-volatile. Surface controlled reactions can sometimes predominate over bulk solution hydrolysis, especially in the soil environment and this may result in different degradation rates than would have been predicted from these simple tests.

Photolysis

There is a range of standard test methods designed to assess the photolytic degradation of a chemical under a range of simulated conditions. The method used as a first tier screening level test is OPPTS 835.2310 - Maximum direct photolysis rate in air from UV/visible spectroscopy (OPPTS, 1998). This method provides estimates of the maximum direct photolysis rate constant and minimum half-life of chemicals in the atmosphere in sunlight as a function of latitude and season of the year. Tests using this method are carried out in the gas/vapour phase using a suitable gas absorption cell.

The next level of test methodology is the determination of the direct photolysis rate in water by sunlight (OPPTS 835.5270) (OPPTS, 1998). This technique offers a more environmentally realistic photolytic degradation simulation and is based on principles developed elsewhere (Zepp and Cline, 1977). The method describes a two-tiered screening level approach for determining direct photolysis rate constants and half-lives of chemicals in water in sunlight. The first tier is as described in test OPPTS 835.2310 (OPPTS, 1998). The second tier is applicable to the direct photolysis of chemicals in an homogeneous dilute solution, with absorbance less than 0.05 in the reaction cell at all wavelengths greater than 290nm and at shallow depths (<0.5m). The results are applicable to direct sunlight photolysis of chemicals in air-saturated pure water. Results derived from the test provide environmentally relevant rate constants at low absorbance and shallow depths as a function of latitude and season of the year.

Another methodology is a US-EPA method to measure the indirect photolysis of chemicals (OPPTS 835.5270) (OPPTS, 1998). Chemicals dissolved in natural waters are subject to two types of photoreaction, (1) the direct absorption of sunlight to excite a molecule leading to decomposition, and (2) reaction of the dissolved chemical via chemical or electronic excitation transfer from light-absorbing humic species in the natural waters. In contrast to direct photolysis, this photoreaction is governed initially by the spectroscopic properties of the natural water. Overall photodegradation is a combination of both these processes, with the possibility of them occurring simultaneously. Experiments are undertaken in both pure water (to provide a degree of direct photolysis) and natural water (to give total photolysis), with the indirect photolytic degradation being the difference.

Tests for terrestrial environment

A number of laboratory tests, both aerobic and anaerobic, have been developed to determine the degradation of chemicals in the terrestrial environment ranging from simple screening tests to more complex simulations (Table A 5).

Standard soil tests

Tests that assess the abiotic behaviour of chemicals in soil (i.e. sorption potentials) have been discussed above.

A number of standard laboratory tests have been developed to assess the degradability of chemicals in the terrestrial compartment. The conditions employed in some of the most relevant tests are outlined in Table A 5. Standard soil(s) are normally employed, although the soil from the site of interest, or a similar soil is often recommended. Test substance concentrations vary quite widely, with some requiring radiolabelled chemicals. Analytical measurements range from loss of chemical only, to monitoring metabolites and determining mineralisation from radiolabelled CO₂ emissions. Some tests set data quality objectives, whereas others give only general guidance, relying on the user to select appropriate experimental design.

Most of the tests do not specify whether they are intended to simulate biodegradation in the unsaturated or saturated zones. Anaerobic conditions will only be commonly encountered in the saturated zone, however, most of the soil anaerobic biodegradation tests do not include groundwater, with the exception of anaerobic biodegradation in the subsurface US-EPA test (OPPTS 835.5154) (OPPTS, 1998). In contrast to the OECD ready biodegradability tests, none of the soil tests explicitly define 'biodegradable' from the test results. Although the soil tests do not attempt to define persistence, some regulators define persistence based on soil test results, or by using the freshwater OECD ready biodegradability test.

Non-standard soil tests

There are a number of studies that have attempted to simulate the natural rate of biodegradation of chemicals in the terrestrial environment (Table A 5). Other studies have been designed to evaluate the potential for increasing degradation rates in contaminated soils by adjusting the conditions (augmentation). Tabak *et al* (1995) and Fu *et al* (1996) incorporated the effects of oxygen and chemical diffusion limitations (not covered in standard tests), and demonstrated that these effects can be quite significant. Other non-standard tests differ from the standard ones in that they employ historically contaminated soil that may contain adapted micro-organisms. This may result in increased tolerance to toxic chemicals and increased degradation rates, as well as reducing any lag time before biodegradation commences. Tests such as the EPA biodegradation treatability studies (EPA, 1991) use only historic contaminants, whereas others, such as the RTDF protocol (Anon., 1995), also use spiked chemicals. By using historically contaminated soils bioavailability of chemicals will generally be lower than in spiked soils, which will lower the fraction of chemical that can be biodegraded, but conversely may potentially reduce the ecological impact of the chemicals.

In attempting to understand the factors influencing biodegradation rates, these studies also normally include detailed characterisation of the soil and often profile changes in the microbial community during the degradation (e.g. using genetic techniques, such as 16S rRNA profiling, Davis *et al*, 1999).

Other standards

The standard methods discussed thus far are the most popular currently used to measure degradation of chemicals in the environment. In most cases they can be applied to any substance provided it is non-volatile and sufficiently soluble. There are however, a number of other standards that exist for specific applications (e.g. BSI and ASTM methods). Additionally there are numerous methods available that are very similar in methodology to those produced by the OECD ones, but have been published by other organisations (e.g. BS/ISO, ASTM), these too, have been summarised in Table A.6.

Method	Test duration	Inoculum	Test conditions	Measurements	Limitations
Simulation Tests for Marine Waters	- Marine Waters				
OECD 306 (OECD, 1992c) ISO 7827 and 10707, (ISO, 1994 a,b) (OPPTS 835.3160)	Up to 60 days	Micro-organisms ² in test seawater	Agitation in the dark or diffuse light under aerobic conditions at 15-20°C. Concentrations 5-40 mg DOC I ⁻¹	Dissolved organic carbon ¹ (DOC)	Test chemical must be non- toxic at test concentrations, soluble and not sorbed by vessel. Closed bottle test subject to interference from nitrification. High nutrient concentrations with respect to seawater
Simulation Tests for	Simulation Tests for Freshwater and Sediment Systems	ediment Systems			
ISO 14592-1 (ISO, 2002a) (OPPTS 835.3170)	No fixed duration	Micro-organisms in surface water samples filtered through 100 um filter for a 'pelagic test' which may be amended with an aerobic sediment slurry from the study site for a 'suspended sediment test'.	Agitation in the dark or diffuse light under aerobic conditions at field temperature or 20-25°C	Specific chemical or radio-chemical analysis (and DOC or TOC if possible) giving 1st order rate	Test substance has to be non-toxic, non-volatile and soluble. Site specific with respect to sediment. Sorption to sediment may be mis-leading if ¹⁴ C not used.
ISO 14592-2 (ISO, 2002b)	No fixed duration but <60 days	Micro-organisms in surface water	Natural diffuse daylight or constant illumination of artificial white light (400-700 nm) with an energy of 50 uE/m²/s at the water surface	Specific chemical or radio-chemical analysis giving 1 st order rate const.	Test substance has to be non- toxic, non-volatile and soluble. Site specific with respect to sediment if used - glass beads may not be representative of sediment. Sorption to sediment may be mis-leading if ¹ ⁴ C not used.
OPPTS 835.3180 Sediment/water microcosm	Less than 60 days	Less than 60 days Natural microbial assemblage.	Sediment microcosms using intact intact cores with (semi) continuous water replacement. ¹⁴ C labelling at environmentally realistic levels recommended.	Chemical analysis of transformation products or ¹⁴ CO ₂ analysis where labelling used.	Test substance has to be non- toxic, non-volatile and soluble. Site specific with respect to sediment. Sorption to sediment may be mis-leading if ¹⁴ C not used.

Table A.1 Summary of the characteristics of the simulation tests and ready and inherent biodegradability methods

152 ECETOC TR No. 90

Table A.1 Summary of the characteristics of the simulation tests and ready and inherent biodegradability methods (cont/d)	Test conditions Measurements Limitations	Static test with natural water and Chemical analysis of Simulates suspended sediment sediment, with non-volatile ¹⁴ C transformation only. Test substance has to be labelled compounds at natural products or ¹⁴ CO ₂ non-toxic, non-volatile and levels. I compound the sediment sediment. Sorption to sediment may be mis-leading if ¹⁴ C not used.		From a Test and control pots filled with Primary biodegradation Test substance has to be soluble, inoculum and 10-20 mgC/1 test biodegradation non-volatile, not sorbed to vessel aubstance. a vessel determined by test or sludge and non-toxic at test chemical removal, conc. DOC analysis provides measure of ultimate biodegradation.		:ells/ml) Agitation in the dark or diffuse light DOC removal Test substance has to be soluble, under aerobic conditions at 20-24°C non-volatile, not sorbed to vessel treatment works effluents or activated or sludge and non-toxic at test sludge	cells/ml) Agitation in the dark or diffuse light CO ₂ production Test substance must be non-toxic l sewage under aerobic conditions at 20-24°C at test concentration. It is to be according t	ells/ml) Agitation in the dark under aerobic O ₂ uptake Test substance has to be non- l sevage conditions at 24-26 °C to subject to interference from subject to interference from intrification.
haracteristics of the simulation tes	Inoculum			activated sludge mixed liquor from a domestic plant.		Up to 28 days Micro-organisms (~10 ⁷ -10 ⁸ cells/ml) surface waters, unchlorinated sewage	Micro-organisms (~10 ⁷ -10 ⁸ cells/ml) surface waters, unchlorinated sewage treatment works effluents or activated sludge	Micro-organisms (~10 ⁷ -10 ⁸ cells/ml) surface waters, unchlorinated sewage treatment works or industrial effluents or activated sludge
nmary of the c	Test duration	Less than 100 days	dability Tests	At least 21 days	ability Tests	Up to 28 days surface waters, u	Up to 28 days	Up to 28 days
Table A.1 Sun	Method	OECD (OECD, 2002)b Draft method	Primary Biodegradability Tests	OPPTS 835.3220 (UK Porous Pot Method, Painter and King, 1978)	Ready Biodegradability Tests	OECD 301A (OECD, 1992f) ISO 7827 (ISO, 1994a)	OECD 301B (OECD, 1992h) (ISO 9439, OPPTS 835.3120)	OECD 301C (OECD, 1992b)

Method	Test duration	Inoculum	Test conditions	Measurements	Limitations
OECD 301D	Up to 28 days	Micro-organisms (~10 ⁵ cells/ml) in	Agitation in the dark under aerobic	O ₂ uptake	Test substance has to be non-
(OECD, 1992g) ISO 10707 (ISO, 1994b)		surface waters or unchlorinated sewage treatment works effluents.	conditions at 20-24°C.		toxic at test concentration, subject to interference from nitrification.
OECD 301E (OECD, 1992e) ISO 7827 (ISO, 1994a)	Up to 28 days	Micro-organisms (~10 ⁷ - 10 ⁸ cells/ml) in unchlorinated sewage treatment works effluents.	Agitation in the dark or diffuse light under aerobic conditions at 20-24°C.	Dissolved organic carbon ¹ (DOC)	Test substance has to be soluble, non-volatile, not sorbed to vessel or sludge and non-toxic at test conc.
OECD301F	Up to 28 days	Micro-organisms (~10 ⁷ - 10 ⁸ cells/ml)	Agitation in the dark or diffuse light	O ₂ uptake	Test substance has to be non-toxic
(OECD, 1992i) ISO 9408 (ISO, 1999c)		in surface waters, unchlorinated sewage treatment works effluents or activated sludge.	under aerobic conditions at 20-24°C.		at test concentration, subject to interference from nitrification.
ISO 14593 (ISO, 1999a)	Up to 28 days	Inoculum of aerobic mixed micro- organisms (approx 10 ⁷ -108 cells/l)	Batch culture, aerated aquatic test using the test chemical as the sole carbon source at 20-25°C. Assesses ultimate biodegradation.	CO ₂ production giving	Test substance must be non-toxic at test concentration.
ISO 11734 (ISO, 1995)	Up to 60 days	Washed digester sludge at 1-3 /l in amended anaerobic medium, containing a redox indicator in sealed vessels.	Batch culture with test concentration of 20-100mg l ⁻¹ as OC, at 35°C. Assesses ultimate biodegradation.	Total gas production (CH ₄ + CO ₂) using a pressure transducer and DIC.	Test substance must be non-toxic at test concentration.

Table A 1. Summary of the characteristics of the simulation tests and ready and inherent hisdeared chility methods (cont/d)

- - :	-	-	3	:	
Method Test duration	Test duration	Inoculum	Test conditions	Measurements	Limitations
OECD 302A (OECD, 1981c) Modified SCAS (OPTS 835.3210)	Months (offen 120 days)	Settled domestic sewage and activated sludge.	Test chemical (20 mg DOC l ⁻¹) aerated with settled domestic sewage and activated sludge (ca. 2500 mg l ⁻¹ TSS) for 23h at 20-25°C. Aeration stopped, sludge settled and supernatant removed. Fresh sewage and test chemical are added and the cycle repeated. ¹⁴ C-radiolabelled chemicals can be used for increased sensitivity	DOCPotentially ¹⁴ C	Test substance must be non-volatile, not lost by foaming and non-toxic at test conc. Sorption potential test needs to be determined.
OECD 302B (OECD, 1992j) Zahn Wellens (OPPTS 835.3200)	28 days	Inoculum of 200 - 1000 mg I ⁻¹ (TSS) of activated sludge.	Aerated batch culture, using the test chemical as the sole carbon source (50 - 100 mg l ⁻¹ DOC) and with the inoculum at 20-25°C. Assesses ultimate biodegradation.	DOC or COD or Specific analysis for primary transformations	Test substance must be non-volatile, not lost by foaming and non-toxic at test conc. Sorption potential needs to be determined.
ОЕСР 302С (ОЕСР, 1981d) МП (II)	14-28 days	Aerobic mixed, specially grown, , unadapted micro-organisms at 100 mg l ⁻¹ (TSS or approx. 3 x 10 ⁷ - 3 x 10 ⁸)	Agitated batch culture, using the test chemical as the sole carbon source (30 mg ThOD/I) with inoculum. Assesses ultimate biodegradation.	O ₂ demand and possibly specific chemical analysis	Test substance must be non-volatile, not lost by foaming and non-toxic at test concentration.
OPPTS 835.3100 Aerobic aquatic biodeg	28 days after pre-adaptation	Pre-adapted inoculum	Agitated aerated aquatic test using using test chemical (10 mg l ⁻¹ DOC) pre-adapted inoculum from a medium concentration of aerobic mixed micro-organisms at 20-25°C. ¹⁴ C labelled compounds may be used.	DOC removal and hydroxide trapped CO ₂ ¹⁴ C provides mass balance phase distribution data.	Test substances mus be soluble and non-volatile.

Method	Test duration	Inoculum	Test conditions	Measurements	Limitations
Inherent Biodegradation Tests	adation Tests				
CONCAWE	56 days or until	Activated sludge inoculum (approx	Arerated, batch culture, using the test	CO ₂ evolution giving	Test substance must be non-toxic to
(1999) Inherent			chemical (20mg I ⁻¹ C) as the sole	% degradation	micro-organisms.
biodegrdation	plateau is reached		carbon source with inoculum at 20°C.		ı
of oil products			Assesses ultimate biodegradation.		
OPPTS	40 to 120 days	Settled domestic sewage and	Same principle as for OECD 302A	DOC.	Additional analytical
835.5045		activated sludge.	but with a volatiles trap on the	Specific analysis can	requirements.
Modified SCAS)	aeration unit and additional	provide primary	
test for insoluble			analytical requirements for trapped	transformation data.	
and volatile			volatiles and sludge solids.	Kinetic data and half-	
chemicals			20 mg l ⁻¹ DOC test concentration	life determination	
			at 20-25°C. ¹⁴ C labelled	available. >20%	
			compounds may be used.	removal of DOC	
				= inherent	
				biodegradation,	
				>70% = ultimate	
				biodearadation.	

Table A.1 Summary of the characteristics of the simulation tests and ready and inherent biodegradability methods (cont'd)

Table A.2 Summary of anaerobic degradation	ary of anaerok	bic degradation test methods			
Method	Test duration	Inoculum	Test conditions	Measurements	Limitations
Anaerobic Degradation Test Methods	ion Test Methods				
OPPTS 835.3400	Up to 56 days.	Sludge from an anaerobic sludge digestor.	Test sample concentrations at around $\rm CO_2$ and $\rm CH_4$ 50mg 1 ⁻¹ with tests carried out at 35°C. production measured	CO ₂ and CH ₄ production measured	Not applicable to toxic chemicals, reproducibility not yet fully
defined. Anaerobic				by pressure transducer.	Uses high concentrations of test
biodegradability of organic chemicals		Recommendations are for a well- mixed primary sludge from a digester with a retention time of			substances.
Table A.3 Summary of sewage treatment simul	ary of sewage	treatment simulation test methods	thods		
Method	Test duration	Inoculum	Test conditions	Measurements	Limitations
Sewage Treatment Simulation Tests	imulation Tests				
OECD 303A	Up to 12 weeks	Up to 12 weeks Aerobic sewage	Elimination of test chemicals	DOC or COD giving	Test substance must be water
(OECD, 2001)			(20 mg l ⁻¹ DOC) from continuously	% degradation	soluble and non-volatile.
Aerobic sewage			fed laboratory scale coupled sewage		

treatment units.

treatment: coupled unit test

Table A.4 Summary of abiotic degradation test	abiotic degr	adation test methods		
Method	Test duration	Test conditions	Measurements	Limitations
Abiotic Degradation Test Methods	hods			
OPPTS 835.2110 hydrolysis Up to 7 day. as a function of pH	Up to 7 day.	Tests carried out at pH 1.2, 4, 7, and 9 in 0.05M sterile buffer solutions. Hydrolytically unstable substances are tested at 2 temperatures between 0 and 40°C, others are put through a preliminary test at 50°C - chemicals stable under these conditions are considered hydrolytically stable (half-life greater than 1 year at 25°C). Those not stable are re-tested at between 0 and 40°C.	Disappearance of the chemical using specific chemical analysis to give hydrolysis rate and half-life.	Not applicable to insoluble or volatile chemicals.
OECD 111 (OECD 1981a) Hydrolysis as afunction of pH (equivalent to OPPTS 835.2110)	Up to 5 days	Sterile conditions, in the dark at various pH values. Test concentration 0.01M or 50% of solubility whichever lower at 50°C initially for accelerated test. Subsequently at 2 temperatures between 0 and 40°C, at least 10°C apart.	Disappearance of the chemical using specific chemical analysis	Not applicable to insoluble or volatile chemicals.
OPPTS 835.2310 Maximum direct photolysis rate in air from uv/vis spectroscopy	N/A	Test chemicals are vaporised and absorption measured between 270 and 800nm. From λ_{max} data it is then possible to apply look up tables and calculations to estimate the photolysis rate for specific latitudes at specific times.	Spectroscopic determination of chemical.	Only applicable to chemicals that absorb in the 290-800nm range. Method is only a 1 st level screening test.
OPPTS 835.2210 Direct photolysis rate in	Up to 28 days	Sterile, aqueous solutions at less than 50% of the water solubility concentration exposed to natural or simulated sunlight. water by sunlight.	Disappearance of test chemical and appearance of degradation products.	Sterile deionised water does not contain natural photosensitisers.
OPPTS 835.5270 Indirect photolysis screening test	16 days	Chemicals at less than one half of their solubility are dissolved in sterile solutions of pure water and synthetic humic water (ca. 60ppm DOC) and exposed to sunlight in quartz vessels. Samples are sacrificed on days 1, 2, 4, 8 and 16 until at least 20% conversion is reached. Data provides rate constants and proportion photolysis attributable to direct and direct photolysis.	Disappearance of test chemical and appearance of degradation products using specific analysis.	Chemicals need to be soluble at the test concentrations, without appreciable absorbance in the range 290-825nm, preferably with a half-life of >1d.

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Method	Test duration	Test conditions	Measurements	Limitations
Abiotic Degradation Test Methods	thods			
SETAC Aqueous hydrolysis Up to 7 days	Up to 7 days	Sterile, aqueous solutions at less than 50% of the water solubility concentration exposed to natural or simulated sunlight.	Disappearance of test chemical and appearance photosensitisers. of degradation products using specific analysis.	Sterile deionised water does not contain natural
OECD monograph No. 7 Guidance document on photolysis of chemicals in water	No specified	Sterile, Aqueous solutions at less than 50% of the water solubility concentration exposed to natural or simulated sunlight.	Disappearance of test chemical and appearance of degradation direct products using specific analysis.	Sterile deionised water does not contain natural photosensitisers.
OECD 121 (OECD, 2001c) N/A Estimation of Koc on soil and on sewage sludge using HPLC	N/A	Comparison of the test chemical at a concentration up to its solubility in the HPLC mobile phase, with standard chemicals using the correlation of known Koc values and retention times under defined HPLC conditions.	Retention time under defined HPLC conditions.	Not validated for all groups of chemicals. Has limited range of retention times and Koc values.
OPPTS 835.1110 Activated sludge isotherm test	Until sufficient partitioning has occurred	Freeze dried activated sludge is mixed, in varying ratios, with $CaCl_2$ amended solutions of the test chemical (1-25 mg l ⁻¹ or <50% of solubility or ¹⁴ C labelled material). Specific chemical analysis or ¹⁴ C radiolabel used to determine the partitioning until equilibrium is obtained. Blank $CaCl_2$ solutions are used to asses the desorption of the test chemical back into solution from the sludge.	Adsorption, desorption and Koc values.	Not suitable for insoluble or volatile chemicals. Chemical must be stable for the duration of the test.
OECD 106 (OECD, 2000) Soil adsorption/ desorption test (OPPTS equivalent 835.1220)	Up to 72 hours	A range of soils are mixed, in varying ratios, with calcium chloride amended solutions of the test chemical (1-25 mg 11 or <50% of solubility or 14 C labelled material). Specific chemical analysis or measurement of a 14 C radiolabel used to determine the partitioning of the chemical between the soil and liquid phases until equilibrium is obtained. Blank CaCl ₂ solutions are used to assess the desorption of the test chemical back into solution from the soil.	Adsorption, desorption and Koc values.	Not suitable for insoluble or volatile chemicals. Chemical must be stable for the duration of the test.

Table A.4 Summary of abiotic degradation test methods (cont'd)

			N N	1
Standard Tests				
OECD 304A	64 days	Batch culture at 20°C, using the test chemical at approximately	CO ₂ evolution	Uses disturbed soil.
(OECD, 1981B) Soil biodegradation (OPPTS 835.3300)		10 mg/kg as the sole added carbon source in sieved soil relying on the indigenous micro-organisms. Assesses ultimate aerobic biodegradation.		
OECD 307	100 days	20°C, using the test chemical at its expected	CO_2 and CH_4 evolution	Uses aerobic soil in anaerobic test
(OECD, 2002C) draft		e in	and formation of metabolites	
Anaerobic and aerobic transformation in soil		sieved soil relying on the indigenous micro-organisms. Anaerobic conditions induced by flooding. Assesses primary and ultimate aerobic/anaerobic biodegradation.		
ISO 14239 (ISO, 1997C)	100 days	Batch culture, using the test chemical its expected environmental	CO ₂ evolution	Uses disturbed soil.
Aerobic soil biodegradation		concentration as the sole added carbon source in sieved soil relying on the indigenous micro-organisms. Assesses ultimate aerobic biodegradation.		
OPPTS 835.1210 Soil	N/A - time	Test temperature is 23°C, using distilled-deionised water at pH7.	Measurement by zonal	Not suitable for volatile or
thin layer chromatography		es	extraction or plate scanning to detect parent compound.	insoluble compounds.
OPPTS 835.5154	100 days	Batch culture, using the test chemical (at concentrations up to its	CO ₂ and CH ₄ evolution	Uses aerobic soil in anaerobic test
anaerobic biodegradation in subsurface soils		solubility in water) as the sole added carbon source in sieved soil relying on the indigenous micro-organisms. Anaerobic conditions induced by flooding. Assesses primary and ultimate anaerobic biodegradation.	and formation of metabolites	
Non-Standard Tests				
US-EPA/CERCLA Guide for conducting biodegradation treatability studies	Duration required to remediation tarraets	Tiered approach using laboratory column studies at ambient field stemperatures with historically contaminated soil to simulate <i>in-situ</i> conditions, followed by field pilot studies.	Chemical and any toxic metabolite reduction.	Restricted to historically contaminated sites.

160 ECETOC TR No. 90

Table A.5 Standard test methods for the terrestri	test methods fo	r the terrestrial environment (cont'd)		
Method	Test duration	Test conditions	Measurements	Limitations
RTDF Natural attenuation microcosm test	As required to observe loss from spiked or historically contaminated site	Anaerobic and aerobic microcosms employing contaminated soils and groundwater, spiked with radiolabelled chlorinated solvents at field concentrations.	Chemical and metabolite analysis	Uses disturbed soils
RTDF Microbial profiling Several years	Several years	16S rRNA gene sequences, phospholipid fatty acid analyses and microbial plating used to study microbial communities in field aquifers and laboratory microcosms. Used for chlorinated solvents at field concentrations.	Identification of degrading species.	Identification of degrading Used together with degradation species.
Biotreatment measured by chemical and toxicity assays	25 weeks	Aerobic biological treatment at 20°C of historically contaminated soil using mixing to increase aeration. Chemical analysis plus plant, invertebrate and microbial assays compared. Used for PAHs at field concentrations.	Chemical analysis and toxicity reduction measurements	Aerated soil used only.

Table A.6 Other available standard methods

Method	Test conditions
BS ISO 14852:1999 (ISO, 1999a) Determination of ultimte aerobic biodegradability of plastic materials	Aerobic aqueous test suitable for all plastics. Biodegradability determined by analysis of CO ₂ evolution.
ASTM D5864-00 Standard test for determining biodegradation of lubricants or their components.	Aerobic aquatic test designed to address difficulties associated with testing insoluble materials and complex mixtures found in lubricants. Test is suitable for non-toxic, non-volatile lubricants.
ASTM D6139-00 Standard test for determining biodegradation of lubricants or their components (Gledhill shake flask).	Aerobic aquatic test for non-toxic, non-volatile fully formulated lubricants and their components on exposure to an inoculum. Ultimate biodegradation test measuring CO ₂ evolution. Designed to address difficulties in testing insoluble materials.
ASTM D6384-99ae1 Standard terminology relating to biodegradability and ecotoxicology of lubricants	Terminology covers definitions relating to biodegradability and ecotoxicology of lubricants and their components.
ASTM D5338-98e1 Standard method for determining biodegradation plastics under controlled composting conditions	Aerobic test to yield reproducible test results under controlled conditions designed to resemble composting environments. Test of provides a rate and percentage of conversion of carbon in the sample to CO_2 .
ASTM D5929-96 Standard test method for determining biodegradability of materials exposed to municipal solid waste composting conditions by	Designed to mimic municipal solid waste composting conditions. Acclimation time, cumulative oxygen uptake, carbon dioxide production and percent theoretical biodegradation monitored. Test does not establish the suitability of the composed product for any use. compost respirometry.
ASTM D6340-98 Standard method determining biodegradation of radiolabelled plastics under composting conditions	Aerobic test to determine the rate and degree of biological for oxidation of carbon in radio-labelled plastic in a composting environment containing simulated municipal waste or an controlled aqueous environment. Method A utilises a mixed culture derived from target environment (wastewater, sewage sludge, compost eluant, etc) where temperature, mixing and aeration are monitored and controlled. Suitably sensitive to determine biodegradation at environmental concentrations. Test B carried out with fresh compost and proceeds to an early mature stage with maintenance of temperature, aeration, and moisture.
ASTM D6002-96 Standard guide assessing the compostability of environmentally degradable plastics	Assesses the compostability of environmentally degradable for plastics under controlled laboratory conditions.
ASTM D6006-97a Standard guide assessing biodegradability of hydraulic fluid	Test for unused fully formulated hydraulic fluid. Tests available for for saerobic freshwaters, aerobic soil and anaerobic media. Test designed to simulate biodegradation under incidental environmental releases.
ASTM 1720-95 Standard test method for determining ready ultimate, biodegradability of organic chemicals in a sealed vessel.	Ready, ultimate biodegradability determined by analysis of CO ₂ evolution in sealed vessels for up to 28 days, containing dilute domestic sewage inoculum. If >60% degradation, it is assumed that the test compound will biodegrade in most aerobic environmental compartments. Applicable to compounds with greater than 25 mg-C/l solubility, HLC<10 ⁻² atm/m ³ /mole and toxicity of <10 mg-C/l. Measurements of CO ₂ evolution as dissolved inorganic carbon in liquid phase and gaseous CO ₂ in headspace.
ASTM D2667-95 Standard test method for biodegradability of sulphonates.	Test used to distinguish between branched chain alkylbenzene sulphonates and more readily biodegradable linear alkylbenzene alkylbenzene sulphonates.

Method	Test conditions
ASTM D5526-94 Standard test method determining biodegradation of plastic materials under accelerated landfill conditions.	Anaerobic test to determine the degree and rate of anaerobic for biodegradation of plastics in an accelerated landfill test environment. Decomposition occurs under dry (>30% solids) non-mixed conditions. Gaseous formation of carbon monitored. Does not simulate all conditions found in landfills - closely resembles those where gas generation is recovered.
ASTM E1625-94 Standard test method for determining biodegradability of chemicals in a semi-continuous activated sludge (SCAS).	Method based on OECD, soap and detergent industry association and ASTM D2667 tests to determine biodegradability or organic removability of non-volatile (HLC<10 ⁻³ atm/m/day), non-toxic organic chemicals using activated sludge from a domestic wastewater treatment plant. Test chemicals should be well characterised wrt to physical and chemical properties. Multiple replicates (3 or 4) used. Measurement by DOC, radiochemistry, or specific analysis. For analysis by DOC, biodegradation can only be claimed if other removal mechanisms are discounted by means of specific tests.
ASTM D5511-94 Standard test method for determining biodegradation materials under high solids anaerobic digestion conditions.	Anaerobic test using high solids (>30%) in a methanogenic inoculum derived from anaerobic digesters operating on pre-treated of plastic household waste alone under static non-mixed conditions. Designed to resemble conditions found in biologically active landfills. Percentage of carbon to carbon gas measured.
ASTM D5988-96 Standard test method for determining aerobic biodegradation in soil of plastic materials or residual plastic materials after composting.	Determines the degree and rate of aerobic biodegradation of synthetic plastics in contact with soil, or a mixture of soil and mature compost. Applicable to all non-toxic plastics.
ASTM E1196-92 Standard test method for determining anaerobic biodegradation of organic chemicals.	Anaerobic test carried out under methanogenic conditions. Suitable for non-toxic, low-volatility chemicals.
ASTM D5271-93 Standard test method for determining biodegradation of plastic materials an activated sludge wastewater treatment system.	Aerobic test that provides an index of plastic materials that are more or less biodegradable relative to a positive standard in an aerobic activated sludge environment (0.1 to 2.5 g l-1 mixed liquor volatile suspended solids). High solids loading ensures better precision and more likelihood of rapid adaptation or acclimation of the biomass. Applicable to all plastics that are not inhibitory to sewage micro- organisms.
ASTM D5209-92 Standard test method for determining aerobic biodegradation of plastic materials presence of municipal sewage sludge.	Aerobic test to determine the degree and rate of biodegradation of non-toxic, synthetic plastic materials, including formulation additives. Provides an index of plastic materials that are more or less in the biodegradable relative to a positive standard in an aerobic environment.
ASTM D5210-92 Standard test method for determining anaerobic biodegradation of plastic materials in the presence of municipal sewage sludge.	Anaerobic test to determine the degree and rate of biodegradation of non-toxic synthetic plastic materials, including formulation additives. Provides an index of plastic materials that are more or less biodegradable relative to a positive standard in an anaerobic environment.
ASTM D5247-92 Standard test method for determining the aerobic biodegradability of degradable plastics by specific micro-organisms.	Pure culture study for evaluating the biodegradation of degradable plastics in a submerged culture under aerobic conditions. Degradation evaluated by weight loss, tensile strength loss, percent elongation and changes I molecular weight distribution.

Table A.6 Other available standard methods

Table A.6 Other available standard methods (cont'd)

Method	Test conditions
ASTM E1279-89(R1995) Standard test method for biodegradation by shake-flask die-away method.	Test for assessing biodegradation in natural surface water samples in the presence and absence of natural sediment materials. Can also a provide limited information on the aerobic biodegradation rate (via 1 st order rate constant) for test compound, and sorption to sediment and vessel walls. Test concentrations dependent on chemical-specific analytical methodology. Applicable to non-toxic, soluble compounds that do not rapidly volatilise or decompose abiotically.

APPENDIX B: DEGRADATION RATE DISTRIBUTIONS

Venosa *et al* (1996) compared the rates of hydrocarbon biodegradation measured in the field and the laboratory (Table B1) and showed that in the field the rate of degradation for n-alkanes on a plot inoculated with an adapted microbial population was about 1.8 fold higher than the control plot. They also reported that the ratio of the first order rate constants (calculated from the field study) to those measured in sealed laboratory flasks (using the same (adapted) microbial populations collected from the shoreline study site) were generally very similar. However, since the marine biodegradation database is limited, the current ranges have been made sufficiently broad to account for this uncertainty. It is proposed that more data be generated in this area and that the T¹/₂ ranges be reviewed as data become available.

Although not discussed in detail in this review, there are considerable data on biodegradation rates in ground waters particularly for hydrocarbons and chlorinated solvents. Data from Venosa *et al* (1996) and the extensive review by Suarez and Rifai (1999) can be used to illustrate the DT₅₀ distribution approach that is proposed by the Task Force. The data for benzene (Suarez and Rifai, 1999) in particular (25 T¹/₂s) show the range of T¹/₂s that can be measured (from 0.3 days to infinity) in laboratory studies. With 23 rates available, the 90%ile is 1.8 days and the arithmetic mean is 0.3 days. The range reflects the effects of some of the variables discussed earlier (i.e. site specific conditions, redox conditions, electron acceptor regimes, temperature etc). Only three data are available for the field but here the range was only 1.4-3.5 days (see Table B1).

Table B2 lists the T¹/₂s derived from first order decay rates for a range of chemicals under aerobic and anaerobic conditions, and again demonstrates the ranges measured in the laboratory and field under different conditions.

	In Situ	Laboratory						
Substance	No of studies	Mean T½ (arithmetic)	90%-ile T/2	T//2 range	No of studies	Mean T½ (arithmetic)	90%-ile T/⁄2	T1⁄2 range
benzene	c	2.1		1.4-3.5	23	0.3	1.8	$0.3-\alpha$
toluene	c	с		1.7-6.9	13	2.6	1.9	0.4-43
m-xylene	1	1	1		4	4.2		1.6-86
o-xylene	S	11.6	1	6.9-17	7	7.1	2.6	1.8-86
p-xylene			1		m	3.3		1.6-86

Table B1: Summary of T/ss (days) derived from 1st order decay rates for BTX in laboratory and field studies

Substance No of studies Mean T/2 (arithmetic)							
		90%-ile T ½	T½ range	No of studies	Mean T½ (arithmetic)	90%-ile T // ₂	T½ range
benzene 26 2.1	1.6		0.3-α	104	86	28.8	7.8-α
toluene 16 2.6	1.8	~	0.4-43	106	3.0	1.6	0.2-α
ethylbenzene 0				69	4.7	3.0	0.1-α
m-xylene 4 4.3			1.6-86	73	11.2	3.3	1.4-α
o-xylene 10 8.1	8.7		1.8-86	70	46.2	18.7	3.2-α
p-xylene 3 3.3			1.6-86	47	18.7	9.7	1.5-α
dichloroaniline 7 14.4	5.5		5.3-50	18	138	43.3	15.8-α
dichloroethylene ¹ 5 1.5	0.4	-	0.5-8.6	25	173	10.0	3.5-α
dichloroethylene ² 8 1.5	0.8	~	0.6-α	19	36.5	57.8	2.5-α
trichloroethane 16 8.8	2.3	~	0.6-α	31	2.0	0.6	0.3-α
trichloroethene 29 2.0	0.5	10	0.4-α	56	8.1	31.5	0.2-α
tetrachloroethene 13 115	40.8	8.	12.8-α	23	10.2	3.7	1.7-α
vinyl chloride 8 1.2	6.5	10	5.8-16.1	ω	66	1.4	$1.3-\alpha$

Persistence of Chemicals in the Environment

Table B2: Summary of T1/2s, derived from 1st order decay rates, measured under aerobic and anaerobic conditions

Substance	Laboratory T½ (days)	Field T½ (days)
Naphthalene	0.9	2.3
C1-naphthalene	1.1	6.4
C2-naphthalene	2	15
C3-naphthalene	3	22
C4-naphthalene	3.9	31.5
Phenanthrene	1.9	15
C1-phenanthrene	3.3	23
C2-phenanthrene	4.3	34
C3-phenanthrene	6.9	46
Fluorene	1.9	14
C1-fluorene	2.8	24
C2-fluorene	4.1	32
C3-fluorene	4.9	39
Dibenzothiophene	2.2	16
C1-dibenzothiophene	3.5	30
C2-dibenzothiophene	4.7	37
C3-dibenzothiophene	7.2	53

Table B3: Comparison of marine laboratory and field data for PAH biotransformation (adapted from Venosa et al, 1996)

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No. 2	1,4-Dioxane
No. 3	Methyl Ethyl Ketone
No. 4	Methylene Chloride
No. 5	Vinylidene Chloride
No. 6	Xylenes
No. 7	Ethylbenzene
No. 8	Methyl Isobutyl Ketone
No. 9	Chlorodifluoromethane
No. 10	Isophorone
No. 11	1,2-Dichloro-1,1-Difluoroethane (HFA-132b)
No. 12	1-Chloro-1,2,2,2-Tetrafluoroethane (HFA-124)
No. 13	1,1-Dichloro-2,2,2-Trifluoroethane (HFA-123)
No. 14	1-Chloro-2,2,2-Trifluoromethane (HFA-133a)
No. 15	1-Fluoro 1,1-Dichloroethane (HFA-141B)
No. 16	Dichlorofluoromethane (HCFC-21)
No. 17	1-Chloro-1,1-Difluoroethane (HFA-142b)
No. 18	Vinyl Acetate
No. 19	Dicyclopentadiene (CAS: 77-73-6)
No. 20	Tris-/Bis-/Mono-(2 ethylhexyl) Phosphate
No. 21	Tris-(2-Butoxyethyl)-Phosphate (CAS:78-51-3)
No. 22	Hydrogen Peroxide (CAS: 7722-84-1)
No. 23	Polycarboxylate Polymers as Used in Detergents
No. 24	Pentafluoroethane (HFC-125) (CAS: 354-33-6)
No. 25	1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0)
No. 26	Linear Polydimethylsiloxanes (CAS No. 63148-62-9)
No. 27	n-Butyl Acrylate (CAS No. 141-32-2)
No. 28	Ethyl Acrylate (CAS No. 140-88-5)
No. 29	1,1-Dichloro-1-Fluoroethane (HCFC-141b) (CAS No. 1717-00-6)
No. 30	Methyl Methacrylate (CAS No. 80-62-6)
No. 31	1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2)
No. 32	Difluoromethane (HFC-32) (CAS No. 75-10-5)
No. 33	1,1-Dichloro-2,2,2-Trifluoroethane (HCFC-123) (CAS No. 306-83-2)
No. 34	Acrylic Acid (CAS No. 79-10-7)
No. 35	Methacrylic Acid (CAS No. 79-41-4)
No. 36	n-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9)
No. 37	Methyl Acrylate (CAS No. 96-33-3)
No. 38	Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3)
No. 39	Tetrachloroethylene (CAS No. 127-18-4)
No. 40	Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions

Special Reports

No. Title

- No. 8 HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances
- No. 9 Styrene Criteria Document
- No. 10 Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1)
- No. 11 Ecotoxicology of some Inorganic Borates
- No. 12 1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0)
- No. 13 Occupational Exposure Limits for Hydrocarbon Solvents
- No. 14 n-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document
- No. 15 Examination of a Proposed Skin Notation Strategy
- No. 16 GREAT-ER User Manual

Documents

- No. 32 Environmental Oestrogens: Male Reproduction and Reproductive Development
- No. 33 Environmental Oestrogens: A Compendium of Test Methods
- No. 34 The Challenge Posed by Endocrine-disrupting Chemicals
- No. 35 Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances
- No. 36 Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals
- No. 37 EC Classification of Eye Irritancy
- No. 38 Wildlife and Endocrine Disrupters: Requirements for Hazard Identification
- No. 39 Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach
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- No. 42 Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction
- No. 43 Contact Sensitisation: Classification According to Potency, A Commentary