



**Guidance Document on Regulatory  
Physico-Chemical Testing in the United Kingdom**

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## 1. Introduction.

This guidance document has been produced by the Regulatory Physico-Chemical and Characterisation Group with support from the UK Analytical Partnership (UKAP), as an aid to those involved in the notification of new chemicals. The aim is to clarify some of the areas where there is frequent misunderstanding between notifiers, Contract Research Organisations (CROs) and the Competent Authority, with a view to smoothing the review of notification dossiers, improving the data quality and therefore allowing notifications to be accepted more quickly. The document will be of use to technical and scientific staff conducting physico-chemical tests, to notifiers from industry who may be unfamiliar with notification requirements or the purpose of the notification tests and to scientific staff within industry who may be required to produce information for a notification.

In cases where a CRO is responsible for producing a package of notification tests on behalf of their client, it is reasonable to expect the client to provide them with certain information on the substance to be tested in order to ensure that the tests conducted are scientifically valid and the results correctly interpreted. The types of information that may be requested are set out in Table 1. This document explains the importance of the physico-chemical tests in the overall notification scheme and how information from the client is used in preparation and review of the notification dossier.

Forthcoming changes to European chemical legislation and the proposed REACH scheme (Registration, Evaluation and Authorisation of Chemicals) will probably lead to changes in the present schemes for new and existing chemicals. However, the principles for obtaining, interpreting and applying physico-chemical data will remain the same.

<b>Minimum Information</b>	<b>Additional Information</b>	<b>Information for notification dossier and risk assessment</b>
Identity and chemical structure	Dissociation constant(s)	Amount of substance to be placed on market
Composition and identity of impurities	Any available information on chemistry e.g. hydrolysis products	Details of manufacture and use processes
Spectra and/or analytical data and methods		Potential exposure to humans and the environment
Physchem data appropriate to notification level		Waste quantities and method of disposal

Table 1. Information Requirements for Regulatory Testing and Notification

## 2. Notification of New Substances.

Before a chemical is placed on the EU market, it is important to ensure that all the appropriate regulatory requirements have been met. Various regulations apply to different types of substance, including:

- Medicinal products
- Cosmetic products
- Foodstuffs
- Pesticides and biocides
- New and existing chemicals

The main focus of this document is the regulation of new chemical substances, although many of the same principles apply to other substance types.

Under the present legislation, “new” substances are those which are not listed in the European Inventory of Existing Commercial Chemical Substances (EINECS). EINECS contains all substances which were on the EC market between 1 January 1971 and 18 September 1981. Any substance not on this list (with the exception of certain polymers) and which is not covered by one of the other areas of legislation must be “notified”, whereby certain information must be presented to the Competent Authority (the Health and Safety Executive and the Environment Agency (acting on behalf of DEFRA) jointly hold this position in the UK). The “notifier” is either the EC manufacturer or, if the substance is manufactured outside the EC, the importer. The importer may then distribute the substance to customers throughout the EC. In some cases, it is appropriate to make a “sole representative” notification, when a notifier can cover several importers under a single notification. In the UK, this legislation is implemented in the Notification of New Substances Regulations (NONS)<sup>1</sup>.

The actual information needed is dependent on the amount of substance being placed on the market, but in general can be summarised as:

- Technical data (e.g. substance identity, purity and identity of impurities)
- Commercial information (e.g. the intended use of the substance, the amount of material being manufactured or imported)
- Physico-chemical properties
- Toxicological properties
- Ecotoxicological properties and environmental fate data
- Proposed classification and labelling for the substance based on the properties determined

In addition, for any substance which is classified as “dangerous”, it is also necessary to provide a material safety data sheet and a risk assessment must be conducted. The risk assessment considers the risks to humans and the environment at all stages of the life-cycle of the substance within the EC. The definitive risk assessment is prepared by the Competent Authority, but any available information should be presented in order that the assessment is as accurate as possible. If relevant information is not available, the Competent Authority must use default values set out in the EU Technical Guidance Document<sup>2</sup>. The outcome of the assessment may lead to additional testing on the substance or risk reduction activity.

The information is submitted to the Competent Authority in a standardised format and is assessed to ensure that: the data are in compliance with the relevant regulations; that the substance is correctly labelled and that the substance will be used without unacceptable risks to workers, the general public or the environment. Only once the Competent Authority is satisfied that the data are in compliance with the regulations can the substance be placed on the market.

Further information on the notification scheme is available on the HSE website:

[www.hse.gov.uk/hthdir/noframes/nons/](http://www.hse.gov.uk/hthdir/noframes/nons/)

The risk assessment Technical Guidance Document may be downloaded from the European Chemical Bureau's website:

<http://ecb.jrc.it/>

### **3. Physico-chemical Testing.**

#### **3.1 What are the Physico-chemical Tests?**

As part of the notification process, it is necessary to submit certain physico-chemical data on a substance. At the so-called Base Set level (substances manufactured or imported between 1 and 10 tonnes per year) the following are required:

- Melting temperature
- Boiling temperature
- Relative density
- Vapour pressure
- Surface tension
- Solubility in water
- Octanol-water partition coefficient
- Flash point
- Flammability (including pyrophoric properties and flammability on contact with water)
- Explosive properties
- Self-ignition temperature
- Oxidising properties
- Particle size distribution

Additionally, two of the environmental fate tests:

- hydrolysis as a function of pH
- adsorption/desorption coefficient

will be considered in this document.

For polymeric substances, some polymer specific data are needed (e.g. molecular weight distribution, number average molecular weight), and some additional tests (e.g. water extractivity, extractivity with hexane) may be required. Specific guidance on polymers is available from the HSE website.

These tests should be carried out according to recognised tests methods under conditions of Good Laboratory Practice. However, the most important consideration is that the test is scientifically valid and in some cases it is possible to present non-standard data to the Competent Authority provided that this is justified. For the majority of tests, the so-called Annex V methods (or their OECD equivalents) are used. The original Annex V methods<sup>3</sup> (A1, A2 etc.) were published in Commission Directive 67/548/EEC and updates and new methods are added via adaptations to this directive, published in the Official Journal of the European Commission. The European Chemical Bureau's website can be consulted to ensure that the most recent version of a method is being used (<http://ecb.jrc.it/testing-methods/>).

One test for which there is no Annex V method is particle size determination. Guidance on appropriate methods is available from HSE. Presently, there is no Annex V method for oxidising properties of liquids. However, a new method A21 for liquid oxidisers is due to be adopted in the 29<sup>th</sup> Adaptation to Technical Progress. The UK Competent Authority is likely to accept this method in advance of the publication of this document.

### **3.2 Why are these tests so important?**

It is very important that the physico-chemical data are accurate because they are pivotal to the whole of the notification process, from designing the remainder of the test package to the risk assessment. The order in which the tests are carried out should be given some consideration, as the results of each test can influence how the others are conducted. For example, a positive result in an explosivity test precludes the need to perform flammability testing, indeed it would be dangerous to do so. In some cases, more than one technique may be described in the test method and the one used should be chosen carefully to ensure that it is suitable for the substance in question.

The "hazardous" physico-chemical properties (flash point, flammability, explosive and oxidising properties and self ignition temperature) are used mainly for the purposes of safe handling and hazard communication i.e. classification and labelling. Results of the other tests are vital in designing the appropriate toxicological and ecotoxicological test packages and also for risk assessment calculations. A summary of the overall significance of the tests is given in Table 2.

Test	Impact on other PC tests	Impact on Toxicology	Impact on Ecotoxicology	Impact on Risk Assessment
A1 Melting temperature	Choice of method for flash point/flammability, autoflammability, oxidising properties, explosive properties (sensitivity to friction). If decomposition occurs during the melting point study, a boiling point need not be measured.			
A2 Boiling temperature	Related to vapour pressure. Affects classification as "highly flammable" or "extremely flammable".			Consider process temperature for risk assessment.
A3 Relative density				Fire-fighting measures: H <sub>2</sub> O extinguishers not suitable if $D_{4}^{20} < 1$ .
A4 Vapour pressure	Extra care needed to minimise vapour losses. Related to boiling point.	Choice of dermal or inhalation exposure route for acute toxicity test. Route of exposure for sub-acute toxicity test. Exposure and excretion routes for toxicokinetic assessment.	Choice of test method for biodegradation test. Closed/covered vessels for ecotoxicity tests.	Calculation of vapour exposure for human health risk assessment. Calculation of Predicted Environmental Concentrations (PECs) for environmental risk assessment- vapour pressure is a key parameter in determining environmental fate and behaviour. Determination of atmospheric behaviour as for exposure of man via the environment calculations.
A5 Surface tension	Suitability of methods for Kow and Koc determination for surface active substances.	Cellular disruption		Environmental fate.
A6 Water solubility	A5 not applicable for WS < 1 mg/l. Need to prepare 90% saturated solution (up to a maximum of 1 g/l) for A5 test. Time to achieve saturation can be relevant to solution preparation for A5. Water solubility affects concentration used in hydrolysis test.	Toxicokinetic behaviour.	Sample preparation for ecotoxicity tests.	Environmental classification and labelling. PEC calculations – water solubility is a key parameter in determining environmental fate and behaviour.
A8 Partition coefficient (Kow)	Generally, substances with a high log Kow will be hydrophobic and have low water solubilities. Substances with negative log Pow will be hydrophilic and have high water solubilities.	Toxicokinetic behaviour. Suitable vehicle for toxicity studies. Prediction of dermal absorption.	Choice of test method for biodegradation test (some are not suitable for substances which have high adsorption) High log Kow may lead to losses in ecotoxicity tests through adsorption. Bioaccumulation and adsorption potential. Toxicity prediction.	Environmental classification and labelling. PEC calculations – log Kow is a key parameter in determining environmental fate and behaviour and is used as a surrogate for bioaccumulation potential in the absence of bioaccumulation tests.
Hazardous properties				Classification and labelling. <u>Human health risk assessment.</u>
Particle Size		Choice of dermal or inhalation exposure route for acute toxicity test. Toxicokinetic assessment.		Estimation of dust exposure for human health risk assessment.
C7 Hydrolysis	Interpretation of results for surface tension, Kow and Koc tests and water solubility.	Exposure to hydrolysis products <i>in vivo</i>	Preparation of test solutions for ecotoxicity tests. Interpretation of ecotoxicity results.	Influences PEC calculation
C18/C19 Adsorption/desorption coefficient (Koc)			As for log Kow	log Koc is a key parameter in determining environmental fate and behaviour.

Table 2. Summary of Use of Physico-Chemical Tests in the Notification of New Substances

### **3.3 How do the results of the physico-chemical tests affect each other?**

The individual test results are related to each other in various ways. For example:

The water solubility and hydrolysis tests are closely linked, as an indication of solubility is needed to select an appropriate test concentration for the hydrolysis study, while knowledge of the hydrolytic behaviour of a substance is necessary to be able to interpret the results of a solubility study, since solubility is measured over a period of up to 72 hours.

The results of the water solubility and hydrolysis tests should be available before determination of surface tension, partition coefficient (K<sub>ow</sub>) and adsorption coefficient (K<sub>oc</sub>) are conducted. The surface tension test is not applicable for substances with water solubility below 1 mg/l and the water solubility value is needed in order to determine the appropriate concentration used in this test (90% of saturation, up to a maximum of 1 g/l). Meanwhile the surface tension result is needed to be able to judge if a K<sub>ow</sub> study is valid.

For hydrolysable substances, use of the HPLC methods for determination of K<sub>oc</sub> or K<sub>ow</sub> is advisable as they are faster than the wet-chemistry methods, although the HPLC methods may not be applicable for some types of substance (e.g. metal complexes and surface active substances). Also, the HPLC methods are more appropriate for substances that are poorly soluble in water and octanol. The results should also be checked to ensure that they are not conflicting. For example, a highly water-soluble substance is unlikely to have a high partition coefficient value.

Similarly, the melting point, boiling point and vapour pressure results must be checked for consistency. For example, a high melting-point solid is unlikely to have a high vapour pressure at ambient temperatures. The melting and boiling point results must also be considered when selecting the temperature range over which vapour pressure measurements are made, to ensure that no phase transitions occur during the determination.

The vapour pressure study is an area where care must be taken over the selection of a suitable test method, as the techniques described in Annex V are applicable over only certain ranges of vapour pressure. If the wrong technique is selected, the result may be deemed invalid and the notification delayed or rejected while the test is repeated.

The melting point result is also important in choosing the appropriate hazardous property tests. The A9 flash point and A15 autoflammability tests are used for liquids and low melting-point solids, while the A10 flammability and A16 relative self-ignition tests are appropriate for higher melting-point solids. For substances which melt in the 50 – 150 °C range, further consideration must be given to the decision and the behaviour of a substance in the melting point study (DSC method) must be examined.

### **3.4 Which order should the physico-chemical tests be conducted in?**

There are two important factors which must be taken into account when deciding the order of physico-chemical tests. Firstly, the safety of the scientist conducting the tests is paramount. Secondly, the impact of results on other tests should be considered. For the Base set, the following test order is preferred:

## **4. Regulatory Requirements.**

### **4.1 Top Level Testing.**

A13 Pyrophoric Properties: This can take the form of either a theoretical screening test (based on experience in handling and use, giving consideration to the chemical structure) or full testing. A positive result would remove the need for further flammability tests and would make any other tests extremely difficult to perform.

### **4.2 Second Level Testing.**

Within each group, the tests should be performed in the order shown. There is no requirement for the groups to be conducted in any particular order, although it is useful to have the melting point result in selecting appropriate flammability and autoflammability tests.

#### **Group 1**

A12 Flammability (contact with water): This can take the form of either a theoretical screening test (based on experience in handling and use, giving consideration to the chemical structure) or full testing. A positive result would remove the need for further water-based physico-chemical testing on the substance. Further toxicological or ecotoxicological tests may be required on the reaction products.

C7 Hydrolysis and A6 Water Solubility: Information on hydrolysis potential can be useful in interpreting the results of the water solubility test and conversely, knowledge of the water solubility is useful in setting the starting concentrations for the hydrolysis tests. It is therefore beneficial to run these tests concurrently, with a “screening” hydrolysis result (perhaps based on chemical structure), being available before reporting the results of the solubility test.

A5 Surface Tension: This test requires knowledge of the water solubility and saturated solution equilibrium time prior to being conducted. If a substance hydrolyses then the time scale of the test should be such that significant hydrolysis has not taken place.

A8 Partition Coefficient: This test should not be performed on surface-active substances as they interfere with partitioning. Thus, a knowledge of surface tension is required prior to testing. If the test substance is only mildly surface active, a shake-flask method may be valid. A preliminary test should be conducted and observations of foaming or emulsion-formation made. These would indicate that the method was not suitable.

#### **Group 2**

A14 Explosivity: This can take the form of either a theoretical screening test (based on structural indicators, oxygen balance calculations and evidence from DSC experiments) or full testing. A positive result would remove the need for any further tests in this group. Further information can be found in “Bretherick’s Handbook of Reactive Chemical Hazards”<sup>4</sup>.

A9/10/11 Flammability, A15/16 Autoflammability: These tests can be conducted in either order, although the physical form of the test substance must be known in order to select an appropriate method. A positive result in the flammability test, leading to classification, removes the need to perform a test for oxidising potential.

A17 Oxidising Potential: This test can usually be omitted on the grounds of a lack of structural alerts for oxidising groups (see Bretherick). It should not be performed on flammable or explosive substances.

### **Group 3**

A1 Melting Point: Observations from the melting point test can directly affect both the boiling point and the vapour pressure tests. For example, if there is decomposition on melting, a boiling point determination is not required. If the melting point is  $> 360^{\circ}\text{C}$  then a calculated vapour pressure is sufficient. The melting and boiling point tests can often be combined by using Differential Scanning Calorimetry (DSC).

A2 Boiling Point: This property is closely related to vapour pressure and for some substances a single test can provide both end points.

A4 Vapour Pressure: It is important to have knowledge of both the melting and boiling points prior to measuring the vapour pressure, to ensure that an appropriate temperature range is chosen, i.e. one in which there is no phase transition and the substance is stable. The result should correlate with the boiling point.

### **4.3 Lower Level Tests.**

A3 Relative Density  
Particle Size Distribution

For VIIB reduced notifications (i.e. up to 1 tonne per annum), the full package of physico-chemical tests is not required; therefore this testing order cannot be followed. However, the chemical structure should be examined for any alerts for properties such as explosivity.

## **5. Application of Test Data.**

### **5.1 How do the results of the physico-chemical tests affect the toxicology and ecotoxicology tests?**

There are various ways in which the physico-chemical properties of a substance can affect other parts of the notification test package. For toxicological testing, water solubility and the potential for hydrolysis are important in selecting suitable solvents for dosing *in vitro* and *in vivo* studies. The identification of hydrolysis products may also have animal welfare implications, for example if rapid evolution of hydrochloric acid fumes occurs. The toxicological testing package at Base Set level includes two acute toxicity tests. The acute oral toxicity test is mandatory, but the choice of exposure route for the second acute test (dermal or inhalation) is dependent on the results of the vapour pressure or particle size study. The route chosen should reflect the most likely route of exposure to humans during use of the substance and therefore the inhalation test is most appropriate for substances with high vapour pressure or a high proportion of particles of inhalable size.

At Base Set level, it is also necessary to provide an assessment of the toxicokinetic behaviour of a substance (i.e. absorption, distribution, metabolism and excretion *in vivo*). The results of the

toxicity tests are used for this purpose, but the results of the physico-chemical studies are also needed in order to predict how the substance is transported *in vivo*, particularly if no effects are seen in the toxicity tests.

The results of the water solubility, vapour pressure and hydrolysis tests are crucial in selecting an appropriate method of media preparation for the ecotoxicity studies. It is generally considered that only the dissolved fraction of a substance in water is bioavailable. In most cases, it is important that aquatic test organisms are exposed only to test solutions at or below the limit of solubility in the test media. The presence of undissolved material can also have adverse physical effects on aquatic test organisms such as clogging of gills or light inhibition. The water solubility test can provide a useful indication of the expected water solubility in test media, although the solubility in pure water and test media may be very different due to the presence of dissolved ions. In cases where the solubility limit has been exceeded, it is important to provide solution analysis on filtered and unfiltered samples (or centrifuged and uncentrifuged), to determine the bioavailable concentration.

Results of the hydrolysis and vapour pressure studies are needed to ensure that the test concentrations are maintained during the study. The fate of a substance in water will affect the test media preparation and exposure regimes, for example aquatic toxicity testing of a volatile substance may require a sealed system and/or flow-through regime. For further guidance, refer to OECD Guidance Document #23 on Aquatic Toxicity Testing of Difficult Substances and Mixtures<sup>5</sup>.

The physico-chemical test results are also needed in order to select the appropriate test method for the biodegradation study, as some techniques are not appropriate for volatile, poorly soluble or highly adsorptive substances.

## **5.2 How do the results affect classification and labelling?**

Results of the hazardous physico-chemical tests are used directly for classification of substances as “flammable”; “highly flammable”; “extremely flammable”; “explosive” or “oxidising”, along with the appropriate symbols, “R” and “S” phrases for labelling purposes.

The results of the water solubility and Kow tests, along with the biodegradation study, are important in assigning classification and labelling of substances as “dangerous for the environment”. In addition to the acute toxicity to aquatic organisms, determined in the Base Set test package, long-term effects and persistence are of concern. Kow is used as an indication of bioaccumulation potential in organisms, while biodegradation potential is indicative of the persistence of a substance in the environment. The use of abiotic degradation half-lives (e.g. from the hydrolysis test) may also be useful to determine persistence. Substances which are “very toxic” to aquatic organisms are classified as “dangerous for the environment” regardless of Kow and biodegradation potential. However, those substances which are “toxic” or “harmful” are usually only classified if they also indicate concern for bioaccumulation or persistence. Even in cases where no acute toxicity is observed in the Base Set studies, poorly water-soluble substances require the risk phrase R53 “May cause long-term adverse effects in the aquatic environment” if they are not readily biodegradable and have a log Kow greater than 3, since there is a possibility for long-term effects that were not detected in the acute toxicity tests. Since the environmental classification is so dependent on these physico-chemical test results, it is vital to ensure that accurate determinations are made.

Full details on classification and labelling of dangerous substances can be found in Annex VI of EC Council Directive 67/548/EEC, updated in the 28<sup>th</sup> Adaptation to Technical Progress 2001/59/EC. In the UK, the requirement for classification and labelling is covered by the CHIP regulations, details of which are available on the HSE website.

### **5.3 How are the results used for risk assessment?**

The risk assessment combines the hazardous properties of the substance with an exposure assessment to determine the risk posed to humans and the environment from all stages of its life-cycle. This includes manufacture, transport, use and disposal of the substance. A detailed description of the EU risk assessment process for new and existing substances is given in the Technical Guidance Document. Using appropriate storage and handling techniques can usually mitigate any concerns regarding hazardous physico-chemical properties. Toxicology results are used to judge other hazards to human health, but the vapour pressure and particle size determination are required to estimate the likely exposure that will occur to humans both in the workplace and in consumer use. Exposure estimates are usually calculated using the EASE model (from the risk assessment Technical Guidance Document), or other appropriate models, and volatility or the size and nature of particles are crucial to estimating inhalation exposure. Particle size is also important for determining the likely dermal exposure. The physical state of a substance at the process temperature is also an important consideration for determining likely hazards therefore melting and boiling temperatures are needed.

The environmental risk assessment examines the potential for release of the substance to the environment, the ultimate fate and behaviour of the substance in the environment and, in combination with the hazardous properties, evaluates the risk to each compartment of the environment. A “Predicted Environmental Concentration” (PEC) is calculated, using estimated releases and the physico-chemical properties of the substance, and is compared with the “Predicted No Effect Concentration” (PNEC), calculated from the results of the ecotoxicity studies. The PEC/PNEC value indicates if there is a concern and if any additional testing is needed to further investigate the ecotoxicological properties of the substances, or if risk reduction measures should be implemented. A PEC/PNEC value below 1 indicates no immediate concern.

As indicated in table 2, several parts of the environmental risk assessment rely heavily on the use of physicochemical properties. The following examples are used to demonstrate this.

Very often, waste streams from industrial processes and the waste produced from public use of a product will not be released directly into environmental waters, but will be released to drain and enter a waste water treatment plant (WWTP). The behaviour of the substance within the WWTP is controlled by its degradability and its physico-chemical properties. This part of the risk assessment estimates the proportion of substance that is degraded, lost to air, released in aqueous effluent and associated with sewage sludge (which may subsequently be spread on agricultural land). Generally, these estimates are initially made by means of the SimpleTreat model, described in the risk assessment Technical Guidance Document. The model uses a measure of biodegradation (usually from the ready biodegradability test), Henry’s Law constant (derived from vapour pressure, molecular weight and water solubility) and either Kow or Koc.

Properties such as water solubility, Kow, Koc, vapour pressure and degradation also determine the distribution and ultimate fate of a substance in the environment. For example, stable, non-volatile substances with high water solubility and low Koc are likely to remain in the aquatic compartment,

whereas a poorly soluble substance with high Koc is likely to be associated with the terrestrial compartment. Vapour pressure can be used to calculate exposure to plants from the atmosphere and determination of atmospheric behaviour (e.g. vapour/particle partitioning) is necessary for an assessment of exposure to humans via the environment. Koc is also used to calculate other solid-water partition coefficients (e.g. soil, sediment and suspended matter) which are essential in calculating a risk to soil and sediment.

Since the physico-chemical values are so fundamental to the environmental risk assessment, it is important that these are accurate and appropriate for the substance being assessed, not least as the outcome of the assessment could potentially lead to costly long-term ecotoxicity studies or risk management.

## **6. Other Considerations.**

### **6.1 Difficult” Substances.**

“Difficult” substances are defined, for the purposes of aquatic toxicity testing, in OECD Guidance Document #23<sup>5</sup>. Ecotoxicity testing of difficult substances will not be dealt with here. The OECD guidance document should always be consulted before conducting any ecotoxicity testing on a difficult substance. No parallel document exists for physico-chemical tests, but some types of substance should be given special consideration during testing and reporting of results to ensure that the data are meaningful. Substances considered “difficult” from the physico-chemical point of view are substances which are:

- poorly water soluble
- volatile
- unstable (photolytically, hydrolytically or to oxidation)
- ionisable
- highly adsorbing (high Koc/Kow) or
- mixtures.

For very poorly water-soluble substances, it is not always possible to develop a suitably sensitive analytical method to obtain an accurate assessment of water solubility or hydrolysis. In such cases, it is important that the Limit of Quantitation (LOQ) is sufficiently low as to be useful for ecotoxicity testing and the risk assessment. For example, simply quoting water solubility < 100 mg/l is not helpful as this would usually be the maximum level at which an acute ecotoxicity study would be conducted. If the hydrolysis test is not deemed possible due to the analytical method not being sensitive enough to detect a 10% loss of test substance, this should clearly be explained in the notification dossier. Filtration or centrifugation of test solutions in the ecotoxicity tests is recommended under these circumstances. Filtration may be problematic for substances with high Koc as the substance may become adsorbed to filters. In such cases, centrifugation is preferable, or filters should be “pre-conditioned” by passing a volume test solution through and discarding the filtrate prior to filtration of the definitive sample.

For volatile and unstable substances, the main concern is minimising loss of test substance. This may be achieved, for example, by using closed or covered vessels, conducting tests under dark conditions, and by using semi-static or flow-through dosing regimes in the ecotoxicity tests. For hydrolysing substances, the OECD document contains guidance on how the ecotoxicity test solutions should be prepared. These are not hard and fast rules and the Competent Authority should always be consulted prior to testing unstable substances. In general, for substances with a

hydrolytic half-life greater than three days, the parent substance should be tested, possibly using a flow-through system, or with media renewal at appropriate intervals to maintain adequate test concentrations. For substances with half-life less than one hour, the parent should be allowed to degrade before addition of the test organisms. Testing for substances with a half-life value between one hour and three days should be decided on a case-by-case basis, with input from the Competent Authority. Reporting of the hydrolysis result should be undertaken with these cut-off values in mind and, if possible, quoting of results such as  $t_{1/2} < 1$  day should be avoided. It is also useful, where possible, to identify degradation products as these may have a significant impact on the toxicity of the substance.

Rapidly degrading substances can present difficulties in determining water solubility and surface tension. The reported surface tension result is likely to be for a mixture of parent compound and degradant(s) and it may only be possible to provide a short-term visual assessment of solubility.

The main difficulty with ionisable substances is that small changes in pH can greatly affect physico-chemical properties such as  $K_{ow}$  and  $K_{oc}$ . These tests should ideally be conducted at a pH where the substance is in its non-ionised form, achieved by buffering the mobile phase in the HPLC methods, or by buffering the aqueous phase if the shake-flask method is used for  $K_{ow}$ . However, buffering should only be used within a pH range relevant for the environment. Detailed guidance on the treatment of ionisable substances, salts and zwitterions is available in a document produced by the Environment Agency – “Regulatory Compliant log  $K_{ow}$  for Ionisable Substances and Salts”<sup>6</sup>

It is possible that different components of mixtures have significantly different behaviour in the physico-chemical tests and therefore also *in vivo* and in the environment. It is therefore important to ensure that the results presented for the physico-chemical tests represent each component rather than the mixture being treated as a single component. For simple mixtures where the components are known and easily identifiable, this may mean presenting individual values for water solubility,  $K_{ow}$ , hydrolysis and  $K_{oc}$ . For complex mixtures, the HPLC methods are ideal for determination of  $K_{ow}$  and  $K_{oc}$ , and a range of values should be presented, with an indication of the proportion of substance within a given range (e.g. > 90% of components have  $\log K_{ow} > 6$ ), to allow the significance of these results to be reflected in the risk assessment. It may not be possible to separately quantify the water solubility of components in very complex mixtures and, as a last resort total peak area or total organic carbon measurements can be used as an overall estimate.

## 6.2 Are there any occasions when tests can be omitted?

In principle, all of the physico-chemical tests are required for a notification to be considered in compliance with the regulations, and it is at the discretion of the Competent Authority to request any test they consider necessary for adequate assessment of the substance. The Competent Authority should always be consulted before deciding whether a test is required or not. If a test is omitted, a detailed explanation of the reason why the test was omitted should be included in the notification dossier. However, in practice there are some cases where certain tests may be omitted from the test package. For example:

- Melting and boiling point studies are commonly incorporated into a single test using Differential Scanning Calorimetry (DSC).
- Vapour pressure study may be omitted if the melting point is  $> 360\text{ }^{\circ}\text{C}$ .
- Tests for pyrophoric properties and flammability on contact with water are routinely omitted, as these properties are uncommon and are likely to have been recognised prior to a notification being required.
- It is becoming increasingly common to omit tests for explosive and oxidising properties based on chemical structure, oxygen balance and behaviour in a DSC determination.
- The surface tension test is not applicable for substance with water solubility below 1 mg/l.
- The hydrolysis test may be omitted if a suitably sensitive analytical method cannot be developed to detect a loss of 10% of the test substance in solution.
- Particle size determination is not appropriate for liquids and waxy solids, or for substances that are only ever to be supplied in the form of a liquid preparation.
- In some cases, there may be technical reasons why a particular test is inappropriate and the Competent Authority may agree to omit the test.
- The properties of a substance may mean that conducting some physico-chemical tests is unsafe and, with consultation with the Competent Authority, these tests can be omitted.

## 6.3 What analytical considerations should be made to ensure that the tests are valid and useful?

Certain of the physico-chemical tests require analytical determinations to be made as part of the study. These are the surface tension, water solubility, Kow, Koc and hydrolysis tests. For determination of surface tension, analysis is needed to ensure that the correct solution concentration is used (this should be 90% of saturation up to a maximum of 1 g/l). In the water solubility test, dissolved concentrations are determined using external standards in a suitable solvent and similarly for the hydrolysis test, test solutions are analysed at various time intervals using freshly prepared standards to determine any loss of parent test material. The Kow and Koc tests may be performed using the HPLC methods, where the analytical conditions are set out in the appropriate test guideline. The system is calibrated using reference standards of known Kow or Koc and the measurement based on retention time. If the shake-flask method for Kow or the batch equilibrium method for Koc determination is used, solution analysis using external standards is required.

Ideally, the analytical technique used should be substance-specific, preferably GC or HPLC with an appropriate method of detection. Mass spectroscopy is the detection method of choice, as

positive substance identification may be made and problems such as co-eluting or overlapping peaks can be overcome. However, other techniques such as UV/Vis or fluorescence detection for HPLC or FID, ECD or FPD for GC are acceptable. The method should be shown to give a linear detector response for use with a single set of standards, or a suitable calibration used. In some cases, for example for highly coloured substances, it may be possible to use a simple spectrophotometric method. If any sample preparation steps are required, such as concentration using liquid-liquid or solid phase extraction, recovery data, conducted either pre-study or concurrently with the test, are needed.

In some cases, standard chromatographic techniques may not be appropriate and other methods such as GPC, ion chromatography, atomic absorption or emission or electrochemical techniques may be used. Non-substance specific methods are of limited use for the hydrolysis test or for mixtures. In the case of mixtures, individual components should be identified as far as possible and the use of "total peak area" should be avoided. The HPLC methods for Kow and Koc determination are ideal for mixtures as values for individual components can be obtained from a single analysis.

Any analytical method used should be suitably validated and the validation results presented in the test report. Any useful background information available from literature or other sources should be included either in the study report or notification dossier.

## **6.4 Other information which is helpful in conducting tests and reviewing the notification dossier.**

### **6.4.1 Spectra**

Provision of spectral data is a requirement of the notification regulations. Spectra are used not only to confirm the identity of the tested substance but for comparison with other substances for which data sharing enquiries are submitted to the Competent Authority. It is therefore necessary to provide, if technically possible, UV/Vis (including the effect of acid and alkali), IR and NMR (for an appropriately selected nucleus) spectra, and if available mass spectroscopy, on all substances including mixtures and polymers. Guidance on acquisition and presentation of spectroscopic data is available on HSE's website.

For substances such as inorganic crystals, other data such as X-ray diffraction are useful. If these or other non-standard techniques are used, some explanation and interpretation of the data are helpful to the reviewer.

### **6.4.2 Composition**

Again, provision of compositional information is a requirement of the notification. As a minimum, this should include a typical chromatogram, where appropriate, indicating the main component(s) and impurities. However, the results of any routine analyses performed such as elemental analysis, determination of inorganic impurities not detected by chromatography or weight loss on drying are helpful. In particular, it is important to ensure that the composition of components and organic impurities is given in the notification dossier as weight percent and not area percent from the chromatographic analysis.

In the case of mixtures, the provision of additional information is of particular importance. Elemental analysis is essential if total organic carbon content is to be used as a method of

analysis for any of the notification tests. If identification of components of a complex mixture cannot be provided, other information such as the identity and proportion of starting materials and reaction conditions can assist the reviewer. A short report from the synthetic chemists containing any available information on the chemistry of the product and some attempt at structure elucidation would be ideal.

For the purposes of notification, it is also important to ensure that the presence of water (including water of crystallisation) and other solvents is avoided or kept to a minimum, regardless of whether the final product to be marketed contains these. A batch of material should be prepared where these have been removed by suitable means, ensuring that the substance is not decomposed or otherwise altered under the conditions used. In the case of water, it is possible to use this batch only for the physico-chemical tests, as purity corrections can be made to allow for water content in the toxicological and ecotoxicological tests. If it is not possible to achieve this, evidence should be presented to the Competent Authority. If for any reason solvents are included in the notified material, this means that a change of solvent or supply of a more pure material may not be allowed without another notification being made. The cumulative tonnage of material supplied would also include the solvent; therefore higher tonnage levels are reached more quickly.

Unusual or unexpected results in some tests may indicate the presence of solvents in a substance so this should be highlighted as soon as possible. For example, a solid unexpectedly found to be highly flammable in an A10 test may contain a flammable solvent or the evaporation of water may be noted in a DSC trace.

It is also vital that identity and compositional information are available before the physico-chemical tests are conducted to ensure that appropriate considerations are made in developing analytical methods and designing tests, especially for “difficult” substances. Potential difficulties can often be anticipated from chemical structure therefore time and effort can be saved by providing this information in advance.

### **6.4.3 Polymers**

In the case of polymers, it is useful to include information on the synthetic process. For copolymers, this could include the ratio of monomers used and the structure of the final polymer (e.g. block, random or alternate structures). Details of the reaction scheme would also be of use.

### **6.4.4 Chemistry**

The research and development scientists responsible for a product are likely to have a better understanding of the chemistry of the substance than anyone else involved in the notification process. They may have information that is not explicitly required for the notification dossier, but is useful for interpretation and application of test results.

For example, measured pKa values for ionisable substances, or the isoelectric point for zwitterions can be used to ensure that the Kow and Koc tests are performed at the correct pH and the results used correctly in risk assessment calculations. If a measured value is not available, a calculated value, either from literature or computer estimation, could be included.

Information may also be available on the stability of the substance to degradation (oxidation, photooxidation, hydrolysis etc) and the identity of degradation products should be provided if possible. This would allow suitable test conditions to be employed, may help in the interpretation of toxicity and ecotoxicity test results and can be taken into consideration in the risk assessment.

Supplying analytical methods to the CRO before testing begins can also save time and the cost of developing new methods.

#### **6.4.5 Waste production and disposal.**

For the purposes of the environmental risk assessment, it is helpful if the notifier can access data on waste production and disposal at manufacturing and use sites. Monitoring data may be available for some substances in waste streams which can be used directly in risk assessment calculations. Otherwise, information may be available the frequency and quantity of plant washings, including analysis of the waste. For example, it is useful to know if a substance is batch produced, with cleaning after each batch, or if a continuous process with only intermittent cleaning is used, if washings are carried out with organic solvent, water or both, whether these washings are incinerated, added to a waste stream for on-site treatment, released to drain or direct to the environment.

#### **6.5 Non-availability of data.**

If these data are not available, the risk assessment calculations are performed using the default values set out in the Technical Guidance Document. However, the outcome of such an assessment may not be accurate and could result in additional testing being requested.

### **7. Conclusions.**

To summarise, it is important to understand the purpose of a notification and of the tests being conducted to support that notification. This understanding should clarify some of the information requested by CROs conducting the tests and the Competent Authority reviewing the notification dossier which may otherwise seem irrelevant or unnecessary. The more information that is made available to the CRO and the Competent Authority, the easier it is to ensure that the tests are relevant and the results interpreted correctly. Review of the notification and finalisation of the risk assessment are therefore more straightforward and there is no delay in the process to allow a substance to be marketed.

Often, concerns over confidentiality result in a reluctance to provide information. However, it should be noted that confidential information is not made available by the Competent Authority at any time.

### **8. Further Reading**

1. Notification of New Substances Regulations 1993, Guide to Notifiers (available from Health and Safety Executive, Magdalen House, Bootle Merseyside L20 3QZ)
2. EC Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances (available from <http://ecb.jrc.it/new-chemicals/> - soon to be updated)

3. Annex V Test Methods (available from <http://ecb.jrc.it/testing-methods/>)
4. Bretherick's Handbook of Reactive Chemical Hazards, 6<sup>th</sup> Edition, Peter Urben and Leslie Bretherick, Butterworth-Heinemann
5. OECD Series on Testing and Assessment Number 23 Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, ENV/JM/MONO(2000)6 ([http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/env-jm-mono\(2000\)6](http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/env-jm-mono(2000)6))
6. "Regulatory Compliant log Kow for Ionisable Substances and Salts"

Further useful documents will shortly be added to the Regulatory Physico-Chemical and Characterisation Group website at <http://www.chemsoc.org/networks/ukap/rpc.htm>

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