ecotoxicological testing of petroleum products: test methodology


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ABSTRACT

This report reviews the applicability of standard ecotoxicological tests for petroleum products and provides guidance on the choice of appropriate methodology.

KEYWORDS

Bioconcentration, biodegradation, dispersant, ecotoxicology, fish, hydrocarbons in water, infra-red, LC₅₀, LD₅₀, photodegradation, test methods, tier testing, water soluble fraction.

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SUMMARY

This is the second of two CONCAWE reports considering ecotoxicological testing of petroleum products. An assessment is made of the relevance and validity of the standard ecotoxicological test protocols for the assessment of the environmental hazards presented by oil products. The report concludes that in many cases it is technically feasible to adapt standard methods for aquatic toxicity, bioaccumulation and ready biodegradability, but that further work is needed, particularly to develop appropriate "inherent biodegradability" tests for oil products. A number of specific recommendations are made with the intention of promoting a uniform approach to the generation of ecotoxicological test data on petroleum products.
1. INTRODUCTION

In order to assess the impact that petroleum products may have on the environment, information on their fate and effects is required. Such data are generally obtained by testing products in a number of standard ecotoxicological tests which were originally designed for testing pure chemicals. However, the application of such tests to oil products presents a number of technical and scientific problems for the following reasons:

- Petroleum products generally have a low solubility in water and are often volatile. These properties, which are very important in determining the fate and behaviour of such products in the environment, present difficulties for the design of ecotoxicological tests.

- Petroleum products are highly complex mixtures of hydrocarbons, produced to conform to tightly specified performance criteria. The precise composition will vary depending on the source of the crude oil and the refining processes.

- The marketed products may contain additives whose purpose is to ensure that the products meet the required performance criteria. Here again, function rather than chemical composition is the key feature.

This is the second of two CONCAWE reports considering ecotoxicological testing of petroleum products. The previous report discussed the general background for the assessment of environmental hazard and recommended a tier testing approach for the acquisition of relevant data. A major question raised in that report was the relevance and validity of many of the standard ecotoxicological test protocols for obtaining the data needed to assess the environmental hazards presented by oil products.

The purpose of this report is to identify problems associated with the methodology and to provide guidance in the selection of appropriate test methods. The adoption of uniform or standard methods will provide a sound basis for the comparison of data from various sources and contribute to a better understanding of the ecotoxicological properties of petroleum products.
2. BACKGROUND

Any system for assessing environmental hazard must be based on a clear understanding of the potential hazards in question. In the case of petroleum products, there are already data on the effects of spills of both crude oil and oil products on the environment. A wide range of effects including physical fouling, tainting and toxicity have been studied, as has the long-term fate of crude oil and products in a diverse range of environments.

Petroleum and petroleum derived products find their way into each environmental compartment from natural sources and from human activities. In the tier testing scheme referred to above, the hazard that a product poses to each compartment is assessed on a case by case basis as data are progressively acquired. It should be emphasised that the initial screening tests in Tier 1 of the suggested sequence are not designed to reproduce actual environmental conditions. Rather, they are designed to obtain comparative information by testing all materials under essentially the same conditions, and to identify those products for which additional test data are required.

The standard ecotoxicological test protocols, such as the OECD guidelines for ecotoxicity, bioaccumulation and biodegradation testing, were designed to provide an initial indication of the environmental hazards presented by water soluble, non-volatile chemicals. These test protocols have been modified in a variety of ways, to allow their use with products, including mixtures, with low water solubility and high volatility. Data on a number of petroleum products have been obtained in this way and the practical experience gained will be called upon in this report. There is a clear need to define standard approaches to these modifications so that these data may be compared and interpreted meaningfully.

Ecotoxicity

Petroleum products can pose an environmental hazard for both aquatic and terrestrial organisms. This report will concentrate on Tier 1 aquatic toxicity tests, since these highlight some of the more fundamental issues that need to be addressed and because petroleum products are seen as likely to present the most immediate hazard to the aquatic environment.

Aquatic organisms may be exposed to an oil product in solution, as a dispersion or emulsion, and as the product itself, e.g. as an oil slick. The effects on the organisms that may result can be caused either by the intrinsic toxicity of the product, or by physical fouling.

Initial (Tier 1) ecotoxicological testing should assess the intrinsic toxicity of the product under standard test conditions. To develop a wider understanding of the environmental impact, it may subsequently be appropriate to determine the toxicity of the product under different conditions in Tier 2 or 3 tests. Such tests may be triggered by the nature of the product, for example by its tendency to emulsify in different environments, or by consideration of the type of exposure that may result from release or disposal.

Physical fouling is a consequence of spillages of many oil products and constitutes the most visible environmental effect of such incidents. The potential for a material to cause fouling can be assessed from the physical and chemical properties of the product and there is no need to confirm this experimentally. This aspect will therefore not be dealt with in this report.
Bioaccumulation

The toxic effects that oil products may have on the environment are not necessarily limited to the organism exposed directly to the material or to the instantaneous exposure concentration. Effects on organisms higher up the food chain are well documented for a number of chemical products and are the consequence of bioaccumulation over a period of time to concentrations capable of causing harmful effects. In addition, compounds that accumulate in the lipids of an organism may be released and reach toxic levels during fat mobilization. However, these processes may be counterbalanced by metabolism and/or depuration. The determination of the potential of a product to bioaccumulate is a necessary part of any assessment of environmental hazard and should be addressed via the tier testing approach.

Biodegradation

The fate of oil in the environment has been studied following spills of crude oil and some products. Most components degrade biotically and/or abiotically relatively quickly, although the higher molecular weight components such as those found in tar balls may have long environmental half lives. For volatile products, evaporation followed by photochemical transformation in air is an important mechanism in their removal. However, even volatile components will evaporate only slowly if they penetrate into soil or groundwater or are dispersed in water. Under these circumstances, biodegradation can play a part in their breakdown. For many of the less volatile hydrocarbons, biodegradation is likely to be the primary loss process and it is probably rate limited in nature by the low water solubility and molecular size of such compounds.

Following release to the environment, oils may be present in water, soil or sediments, which may be aerobic or anaerobic. No screening test for biodegradability can reflect a product’s behaviour in all of these environments. Following the tier testing approach, initial testing would be designed to establish the extent to which the product is biodegraded in an aerobic environment under standard conditions. This is a logical approach since the initial receiving environment of most spills is aerobic, being either soil or surface water. In addition, it is well established that oxygen is required for the metabolism of hydrocarbons, at least at environmentally significant rates in freshwater environments.
3. AQUATIC TOXICITY

Since petroleum products are generally mixtures of components varying widely in their water solubility it is often not possible to prepare a solution of the product in water by the direct addition of a measured quantity. Other means have therefore been devised to prepare aqueous media for aquatic toxicity testing. The results obtained from such tests are dependent upon the means used to prepare the exposure medium.

In a review of the literature on aquatic toxicity testing of petroleum products, Girling identified two different types of test media that have been used in previous work:

- Water accommodated fractions (WAF) - media containing only that fraction of the product which is retained in the aqueous phase after a period of mixing followed by sufficient time for phase separation.
- Dispersions - media containing a quantity of the product distributed uniformly throughout the water by either continuous mixing or by the use of a chemical dispersing agent.

These two types of test media and their inherent differences are discussed in greater detail below.

Water accommodated fractions

A WAF is an aqueous medium containing only that fraction of a product which remains in the aqueous phase once any source of mixing energy has been removed and after a period sufficient for phase separation. The product may be present either in true solution or as a stable emulsion. If the dispersed material is removed by centrifugation or filtration, the medium is sometimes referred to as the Water Soluble Fraction (WSF), but this implies that the material is present only in true solution. The term WAF is preferred for those media that may contain part of the oil in an emulsified or dispersed form.

WAFs are prepared by stirring a known amount of test substance with a known volume of test medium. The amount of product per unit volume of medium is referred to as the loading rate. The stirring should be continuous and vigorous enough to ensure that the product equilibrates between the aqueous and non-aqueous phases, but without inducing the production of an emulsion. Equilibrium distribution of the components should be demonstrated by chemical analysis, although practical experience with a number of oil products has shown that a mixing period of 24 hours is generally sufficient.

A series of test media containing varying amounts of a product could be prepared in one of two ways:

- either a single WAF is prepared at a high loading rate and is then used to produce a dilution series.
- or test media are made by preparing a WAF at each of a range of loading rates.

The two methods are not equivalent, particularly when the test material is a mixture of components, each of which has a different solubility in water. At any particular loading rate, the resulting aqueous concentration of each chemical constituent of the mixture is a function of the relative volumes of the two phases, the partition coefficient between the phases, and the maximum
The difference between the two approaches can be illustrated with a hypothetical example, in which a petroleum product is to be tested at a range of concentrations corresponding to loading rates from 1 to 1000 mg/l. If WAFs are prepared individually at each loading rate, the least water soluble components may reach their maximum concentrations in the WAF prepared at the lowest loading rate and their concentration will not change as the loading rate is increased. Components with progressively higher solubilities will reach saturation at progressively higher loading rates. Thus the composition of the media will change both quantitatively and qualitatively as the loading rate changes. This has been clearly demonstrated for a number of crude oils and products \(^{11}\) and for creosote \(^{12}\).

In contrast, if a single WAF is prepared at a high loading rate and then diluted, each member of the dilution series will contain components in the same relative concentrations. Thus, dilution of a WAF prepared at 1000 mg/l by factors of 10, 100 and 1000 will produce a series of media containing each component at concentrations 1/10 th, 1/100 th and 1/1000 th of those present in the original WAF. At the lowest dilution, the concentration of some components, those with the lowest water solubility, may therefore be 1/1000 th that in the corresponding WAF prepared at a loading rate of 1 mg/l.

Thus it can be seen that dilution of a single WAF prepared at a high loading rate generates test media which contain less of the poorly water soluble components than WAFs produced individually at each required loading rate. The situation is of course considerably more complex than this, because the composition of the undissolved product phase will be different at each loading rate, depending on which components have moved into the aqueous phase. This in turn may affect the partition coefficients of each component, and hence its distribution between the aqueous and non-aqueous phase.

From the above discussion it is apparent that WAFs should be prepared at each ratio of product to water that requires testing, because a dilution series derived from a single WAF prepared at high loading ratio may lead to an underestimation of the toxicity of a product. A similar recommendation is also made by GESAMP \(^{13}\) for marine toxicity testing as part of the hazard evaluation of harmful substances carried by ships according to MARPOL 73/78.

The use of WAFs allows the standard test methods developed for pure compounds to be employed with the minimum of modification. However, care must still be taken to avoid problems such as trapping of small organisms (algal cells or invertebrates) in any residual film of oily material on the surface of the test media. This may be present as a result of incomplete separation of the WAF and the remaining product. Problems may also be caused by the adhesion of algal cells to oil emulsified or dispersed in the test medium. The former effects can be at least partially alleviated by discouraging contact between organisms and undissolved material. For example, darkening the area in the vicinity of the test medium surface in daphnia test vessels, by covering the neck of a flask, discourages the daphnia from making contact with any oily material on the surface.

**Dispersions**

A dispersion consists of a quantity of product distributed uniformly in, but not wholly dissolved in, an aqueous phase. Unless the product to be tested has a natural tendency to emulsify, dispersions require either an input of energy or the use of a chemical dispersing agent to maintain the distribution of the solubility in water.
product in the test medium. These two requirements in turn place limitations on the testing methods which can be employed.

For tests with relatively large and robust organisms such as fish and large shrimp, an exposure system designed by Blackman et al. \(^\text{14}\) has been used to produce uniform dispersions. The vessel allows the product to be continuously dispersed throughout the aqueous medium by isolating the test organism from the stirrer. However, the constant stirring precludes the use of small fragile test organisms such as daphnia and the presence of undissolved material introduces the potential for physical fouling effects.

The use of a chemical dispersant may allow standard toxicity test methods to be used without modification, although dispersants may present the test material in a different form from that encountered following accidental release to the environment. Care has to be exercised in the choice of dispersant because of the possibility that it may have a direct effect on the test organisms or may increase the apparent toxicity of the test substance by enhancing uptake. It should be appreciated that while appropriate dispersant controls must be run in parallel to the test media, they will detect only direct effects of the dispersant and not any interaction between the dispersant and the product.

Volatile materials

To assess the intrinsic toxicity of highly volatile products such as gasoline in aqueous test media, it is essential to ensure that loss of test material from the test medium to air is prevented during preparation of the medium and throughout the duration of the test. This may be achieved by using sealed vessels with minimum air space and renewing the test medium either at regular intervals or continuously with the aid of a flow through system. When using sealed test vessels it is important to ensure that the medium is renewed frequently enough to ensure that the test organisms are not stressed by oxygen depletion.

Although it could be argued that the use of an open test vessel better reflects a real spill in the environment and that an enclosed test system may result in an overestimate of a product's toxicity, it has to be recognised that these tests are designed to measure the intrinsic toxicity of the product. Consequently it is important that the concentrations of the product constituents in the test media are kept relatively constant during the period of the test.

Reporting procedures

When reporting the results of tests with either WAFs or dispersions, it is essential that the conditions under which the test medium was prepared and the details of how the test was carried out are fully and accurately described. This is necessary to allow others both to assess the validity of the data and to repeat the experiment. Any report should therefore detail the following:

- description of material tested
- test organism, including age and strain
- type of medium prepared (WAF or Dispersion)
- type of mixing system
- mass of test material and medium
- geometry of mixing vessel
- sealed or open mixing vessel
- volume of headspace
- method of mixing, including rotor speed, etc.
- duration of mixing and settling/separation time
- method of phase separation in WAF preparation
- type of chemical dispersant (if used)
- water quality - pH, hardness, salinity, temperature, at start and end of test
- oxygen concentration, at start and end of test
- analytical procedures and results

When testing chemical products for regulatory purposes there may be a requirement for quantitative chemical analysis of the test media. This is to confirm or determine dosing in the test medium, to monitor stability of the material during the test period and to characterise the product constituents present. With complex mixtures such as petroleum products such a requirement is impractical, because GC/MS analysis would be required to identify the large number of chemical substances present in the medium.

Total organic carbon analysis of the aqueous phase and gas-liquid chromatography (total peak area) of a solvent extract have been used to demonstrate the achievement of a steady state concentration of components of a petroleum product in an aqueous test medium and to monitor the stability of the medium. Infra-red spectrophotometric analysis of a solvent extract can also be used in this way. The choice of technique is likely to depend on the level of sensitivity which is required to assess the concentration of material in the medium. For the majority of aquatic toxicity tests, one of these techniques should be sufficient to demonstrate the validity of the experiment, without the need for full, compositional analysis. In other cases it may be advisable to analyse the media for specific components known to be of environmental concern.

**Reporting results**

For poorly water soluble complex mixtures, such as petroleum products, it is misleading to express the aquatic toxicity in terms of the measured amount in the aqueous phase. In order to provide a toxicity value comparable with an LC50 for a single chemical substance, the results should be expressed in terms of the loading rate, i.e. as the ratio of test product added to aqueous medium during preparation of the test medium. The same recommendation is made by GESAMP.

This point can best be illustrated with an example. Adema investigated the toxicity of White Spirit to Chaetogammarus marinus. The 96h LC50 was 3.5 mg/l based on loading rate, and 0.54 mg/l based on measured concentrations. Which figure best defines the toxicity of the product? If the purpose is to compare White Spirit with other chemical and oil products, the loading rate is the most meaningful figure because it allows comparisons of products to be made on the basis of the amount of product that is required to produce an effect.

The loading rate required to give a particular effect, e.g. death of 50% of the test organisms, can be expressed in a manner analogous to that used for water soluble products. Thus:

\[
\text{LL}_{50} \text{ value} = \text{Lethal Loading Rate giving 50% mortality.}
\]

\[
\text{EL}_{50} \text{ value} = \text{Effective Loading Rate giving 50% effect.}
\]

Similarly the NOEC (No Observable Effect Concentration) becomes the NOEL (No Observable Effect Loading Rate).
Selection of test methodology

The choice of methodology to be employed in ecotoxicity testing is dependent on the purpose to which the results will be put. For petroleum products tests may be required for (i) compliance with regulatory requirements for classification and labelling and (ii) the assessment of the environmental hazards that may result from releases of petroleum products to the environment.

Regulatory requirements for classification and labelling, such as those contained in the 12th Adaptation to Technical Progress of the EC Dangerous Substances Directive, cover all types of chemicals, including petroleum products. The purpose of such schemes is to identify those products which, on the basis of their intrinsic toxicity, persistence and physico-chemical properties, are considered to present an environmental hazard. It is important that the standard test protocols do not discriminate between different types of product, such as single compounds and complex mixtures. Tests on single chemicals are carried out up to, but not exceeding, their water solubility. A similar criterion applied to petroleum products argues in favour of WAFs rather than dispersions, at least for tests carried out at the Tier 1 level.

Hazard assessments of oil products may, depending on the results of the Tier 1 tests, require testing at the Tier 2 level or above. The methodology employed should be appropriate to the product and to the exposure conditions in the environment. In these circumstances dispersions may be preferred to WAFs, because of the possibility that products could emulsify or disperse in the environment. A decision on which is the most appropriate methodology has to be made on a case by case basis, taking the properties of the product into account.

Conclusions

It is technically feasible to adapt the standard (e.g. OECD) guideline methods to evaluate the aquatic toxicity of petroleum products. Depending on the purpose of the test, either water accommodated fractions or dispersions, prepared by direct dosing under standard conditions of mixing, can be employed. For testing of volatile products, sealed vessels should be used to prepare test media and sealed or flow through systems should be used to ensure that test organisms are exposed to a constant concentration during the test period.

Tests should be conducted with a series of exposure media prepared at loading rates appropriate for the product to be tested. The results should be expressed in terms of the loading rate because this allows a more meaningful comparison of the toxicity of different products to be made. For most purposes, analysis of the test medium is only necessary to confirm dosing and exposure.
4. BIOACCUMULATION

The extent to which a chemical will bioaccumulate in aquatic organisms is expressed as the bioconcentration factor (BCF), the ratio of the concentration of the chemical in the organism at equilibrium to the concentration of the chemical in water. The BCF has been shown to be strongly correlated with the partition coefficient between octanol and water \(^{18,19}\), and this parameter, normally expressed as the logarithm \(\log \text{Pow}\) is normally measured as an indicator of bioaccumulation \(^8\).

However, the BCF is also dependent on a large number of other factors, including molecular size and shape, and the rates of physiological and biochemical processes such as membrane transport, metabolism, and excretion \(^{20,21}\). As a result, the experimental BCF may be substantially less than that predicted from the \(\log \text{Pow}\).

Log Pow can be determined by the classical "shake flask" method (OECD 107) \(^8\) or by the HPLC method (OECD 117). \(^{22}\) This latter method also introduces the requirement to calculate the \(\log \text{Pow}\) of a compound from its structure, e.g., by using the fragment addition method of Hansch and Leo \(^{23,33}\). These methods were developed primarily for chemical products consisting entirely or largely of single defined chemicals. Their application to oil products presents some technical and interpretive problems, because of the large number of components, each of which may have a different \(\log \text{Pow}\) value. The "shake flask" method cannot be used because it would necessitate measuring the concentration of each individual component in both the water and the octanol phases. However, an estimate can usually be made of the range of \(\log \text{Pow}\) values using the HPLC technique, although this may be difficult in the case of compounds that lack chromophores.

Study of the actual bioaccumulation of an oil product in fish also presents considerable difficulties. Each constituent chemical will bioaccumulate to the extent determined by its physico-chemical properties, and each component would need to be measured independently. The analytical problems are further complicated by the similarity of many of these constituents with compounds naturally present in fish. In fact, many oil constituents enter the central metabolic pathways of the fish leading to energy or new biomass production. Such processes cannot be regarded as bioaccumulation in the normally accepted sense of the term.

Chemicals with a \(\log \text{Pow}\) below 3 are generally regarded as having a low or negligible potential to bioaccumulate \(^{24}\). Above 3 the presumption is that the chemical will bioaccumulate unless there is experimental evidence to show that it does not. However, there is scientific uncertainty about how to interpret \(\log \text{Pow}\) values > 6 (see discussion in ref. 20), which would apply whether the values were derived by the HPLC method or by calculation. Determination of such high \(\log \text{Pow}\) values by the "shake flask" method is technically very difficult, and there are insufficient data either to extend the correlation of \(\log \text{Pow}\) with BCF, or to validate the HPLC method, above a \(\log \text{Pow}\) value of 6. These constraints have been recognised in the relevant OECD guidelines \(^{22}\) and by a recent EC Workshop on Environmental Hazard and Risk Assessment \(^{25}\). In addition, chemicals with a \(\log \text{Pow}\) > 6 have extremely low water solubilities. The exposure concentration and rate of uptake from solution may both be so low that harmful concentrations are not reached within the lifetime of the organism.
Conclusions

The logPow of a petroleum product can be estimated by the OECD HPLC method or by calculation. In either case the results would normally be expressed as a range of logPow values for the constituents of the product. A product must be considered to have the potential to bioaccumulate if it contains a significant fraction of components with logPow exceeding 3, unless there is experimental evidence to show that it does not bioaccumulate in practice.
5. BIODEGRADATION

Biodegradation is only one possible fate of a substance in the environment. For petroleum products, many of the components are volatile and will quickly evaporate to air when released to the environment where their ultimate fate is photooxidation. Aquatic photodegradation also plays a role in the removal of less volatile aromatic components from the aquatic environment. The low water solubility and high adsorption coefficients of the larger hydrocarbon components cause them to partition into sediments. The transport and degradation (both biotic and abiotic) properties of a substance need to be taken into account when assessing its potential to persist in the aquatic environment.

Tests for biodegradability were initially developed for water soluble materials, such as detergents, that are discharged to sewage treatment systems. The classification of the tiered tests - "ready", "inherent", "simulation" - relates therefore to the behaviour of products in rivers and sewage treatment plants and the relevance of such tests to many oil products, which are not generally discharged to sewers, may legitimately be questioned. This argument carries some weight in the case of simulation tests which are modelled on sewage treatment processes, and it is developed further below. However, the tests for ready biodegradability have become widely accepted as providing a first indication of whether a product will degrade in aerobic environments and they form part of the criteria used in the classification of products as Dangerous for the Environment. It is clearly desirable in this case that all products are tested in comparable tests in order to ensure consistency of classification.

As with toxicity testing, biodegradation tests may be carried out on the portion of the test material that is soluble in the water phase or on the entire product. Both approaches have been used by various laboratories. When assessing the hazard a product may present to the aquatic environment, it may be appropriate to assess the biodegradability of only that portion of the product that is dissolved or dispersed in the water. However, in order to assess the persistence of the product itself in the environment it is necessary to test the complete product, although it must be recognised that the less water soluble components are generally slower to biodegrade.

Following the tier testing approach, a product is first tested in Tier 1 tests to determine whether it is readily biodegradable. Products failing to meet the agreed criteria may subsequently be tested in Tier 2 or 3 tests depending on the toxicity of the product and the resultant need to estimate the environmental concentration as part of a hazard assessment of the product.

Ready biodegradability tests

Application of the standard ready biodegradability tests to petroleum products presents a number of technical problems related to their low solubility in water and/or volatility, although these properties are not unique to oil products. The OECD test guidelines on biodegradation, which are the accepted international standards, are currently being revised, and the Table shows the applicability of the revised OECD tests for poorly soluble and volatile products.
Table 1  Applicability of test methods for ready biodegradability (ref. 26)

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<td>poorly volatile</td>
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<td>DOC Die-Away (301 A)</td>
<td>Dissolved organic carbon</td>
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<td>CO₂ Evolution (301 B)</td>
<td>Respirometry: CO₂ evolution</td>
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<td>MITI (l) (301 C)</td>
<td>Respirometry: oxygen consumption</td>
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<td>Closed Bottle (301 D)</td>
<td>Respirometry: dissolved oxygen</td>
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An ECETOC report on biodegradation tests for poorly soluble compounds also identified a number of tests which could be modified for the testing of such products. The report recommended the use of an emulsifier or solvent to aid dispersion of the material, provided that it was non-toxic and not biodegradable. Within the oil industry itself, there is already experience of testing products such as lubricating oils using such an approach. The modified Sturm Test has been the most widely adapted for this purpose.

Volatile materials raise the more fundamental question, viz the need for Tier 1 testing of every product. Highly volatile products would generally not persist long enough in surface water or soil to be subject to any substantial biodegradation and it may be argued that Tier 1 testing of such products is irrelevant. Rather, appropriate tests should be designed in Tier 2 or 3, if necessary, that take into account the volatility of the product and the special circumstances in which the capacity to biodegrade may become important. An example could be to assess the fate of gasoline components leaking from underground tanks, by conducting tests for anaerobic and/or soil biodegradation.

Biodegradability testing at Tier 2 and above

The fact that the majority of the components of petroleum products do biodegrade in the environment is not in doubt, although the rate of biodegradation may be limited by the availability of nitrogen and phosphorus nutrients. This has been well demonstrated by the EPA's bioremediation project following the Exxon Valdez incident. Microorganisms with the capability to degrade hydrocarbons are ubiquitous, although they are often present in such low numbers that there may be a substantial lag period before biodegradation can be observed. The issue in question here is whether any current test can reflect the known behaviour of oil products in the environment, and if not, how such a test may be developed. Such test protocols should be appropriate for the specific product and for the environmental compartments that may be affected.

Standard OECD tests for inherent biodegradability depend upon monitoring the biodegradation of test material by DOC analysis. They are therefore unsuitable for use with petroleum products. One alternative test that has received some attention is the CEC L-33-T-82 test. This tentative test method has been designed to compare the biodegradability of two stroke cycle outboard engine lubricants. The test measures loss of the starting product by estimating the reduction in IR absorbance at a frequency specific to the stretching of C-H bonds in methylene groups. This method can only demonstrate primary degradation, but it is being used extensively with a wide range of lubricants and has been adopted by the "Blue Angel" German Eco-labelling scheme. However, considerable further work is required to demonstrate whether this test may also provide an indication of the inherent biodegradability of oil products.

Conclusions

Biodegradation is an important mechanism for the ultimate degradation of products. Together with solubility, volatility, adsorption to sediment, hydrolysis and photolysis, biodegradation is an important factor in determining the concentration of petroleum products in the aquatic environment.

A number of the standard tests for ready biodegradability have been successfully adapted for use with oil products in Tier 1 tests, although the testing of volatile products still presents some problems. However, the
relevance of such biodegradability data for the more volatile products can be questioned, given that such products would rapidly be lost to air if spilled on soil or water.

There is a clear requirement for tests that can be utilised for assessing the biodegradability of oil products at level 2 or above in the Tier testing scheme. Such tests should provide information on the potential of petroleum products to biodegrade under realistic, environmental conditions. The need for further work to develop such alternative tests for biodegradation has been recognised by CONCAWE and a special task force has been set up to pursue the matter. This topic will be the subject of a future report.
6. FUTURE DIRECTIONS

Much of the discussion in this report has concerned the problems inherent in the acquisition of basic information on the ecotoxicity, bioaccumulation and biodegradation of petroleum products, and for the most part the tests discussed have been at the Tier 1 level. Many of the issues raised in this report may also apply when considering higher tier tests on petroleum products. However, because such tests are tailored to the specific product and its potential environmental hazard, there is greater flexibility in the choice of both test and test protocol. Thus the methodology should be designed to take account of the full range of properties of the product to be tested. Given the increasing need to provide information on the environmental impact of our products, there will be a requirement to develop such test procedures, both to look at longer term fate and effects, and to study a wider range of environmental compartments than those considered in this report.
7. REFERENCES


33. Pomona College Medchem Project's CLOGP program licensed by Daylight Chemical Inform 92713