# AISE/CESIO OBSERVATIONS ON “ULTIMATE” BIODEGRADABILITY AND THE EUROPEAN DETERGENTS LEGISLATION

## Background

The existing European legislation relating to surfactants used in detergent products imposes controls based upon the concept of “primary” biodegradability. This concept has been completely successful in delivering the original intention to prevent production of foam in receiving waters caused by the presence of intact parent surfactants; which may be toxic to aquatic organisms. The new detergent legislation in development seeks to achieve a high level of environmental protection, especially of the aquatic environment, by introducing criteria based on the concept of “ultimate” biodegradability. The proposals for assessment of biodegradability in the present Draft Regulation represent over four years of work by technical experts from Member States, the European Commission and Industry.

## “Ultimate” Biodegradability

The biodegradability methods listed in Annex III of the draft Detergents Regulation are derived from international standard methods (OECD 301-series), which are already incorporated into EU law for chemicals control, together with a new Reference Method based on EN ISO Standard 14-593 (1999); which has been specifically validated on behalf of the Commission for use with detergent surfactants.

The operating conditions and threshold pass limits for these well-known “ready” biodegradability tests are particularly stringent. For this reason, the OECD expert group, which established the 301-series of “ready” biodegradability tests, pointed out that a positive result in any one of these tests "may allow the assumption that the chemical will undergo rapid and ultimate biodegradation in the environment". Professional environmental scientists widely agree that the 60% threshold pass level (equivalent to 60% mineralisation of the test material) in 28 days under the “ready” test conditions in methods which measure either oxygen consumption or carbon dioxide production is indicative of a high level of biodegradability in the environment. An explanation of the process of biodegradation, of biodegradability testing and why a threshold-pass level of 60% in the prescribed test methods is adequate as a measure of complete biodegradation is attached (Annex I).

The appropriateness of these “ready” biodegradability methods and control limits has been endorsed in the context of the revised detergent legislation both by Member States’ technical experts and, independently, by the CSTEE, in its Opinion adopted at the 12$^{th}$ CSTEE Plenary Meeting of 25 November 1999.

Examples of actual test results, using methods proposed in Annex III of the draft Detergents Regulation, for a number of reference substances such as glucose, which is widely recognised to undergo complete degradation, and for some typical detergent surfactants are also shown in Annex I.

AISE/CESIO thus believes that the test methods and the threshold pass level proposed in Annex III of the draft Detergents Regulation are entirely appropriate to ensure the desired high level of environmental protection. Re-assurance of the potential for ultimate biodegradability may also be drawn from additional information which may be available such as:

- Removal of dissolved organic carbon from solution,
- Consideration of the measured level of oxygen uptake/carbon dioxide production as a percentage of that observed for the reference substance (e.g. glucose) which is required in each test,
- Field data,
- Surfactant structure-related information, or
- Biochemical information on the degradation pathway.

In the absence to date of internationally approved test methodologies these cannot be considered definitive alternatives. They are however highly indicative and confirm that the 60% mineralisation limit is the correct one for use in the Regulation.

**Biodegradability Requirements of Annex III**

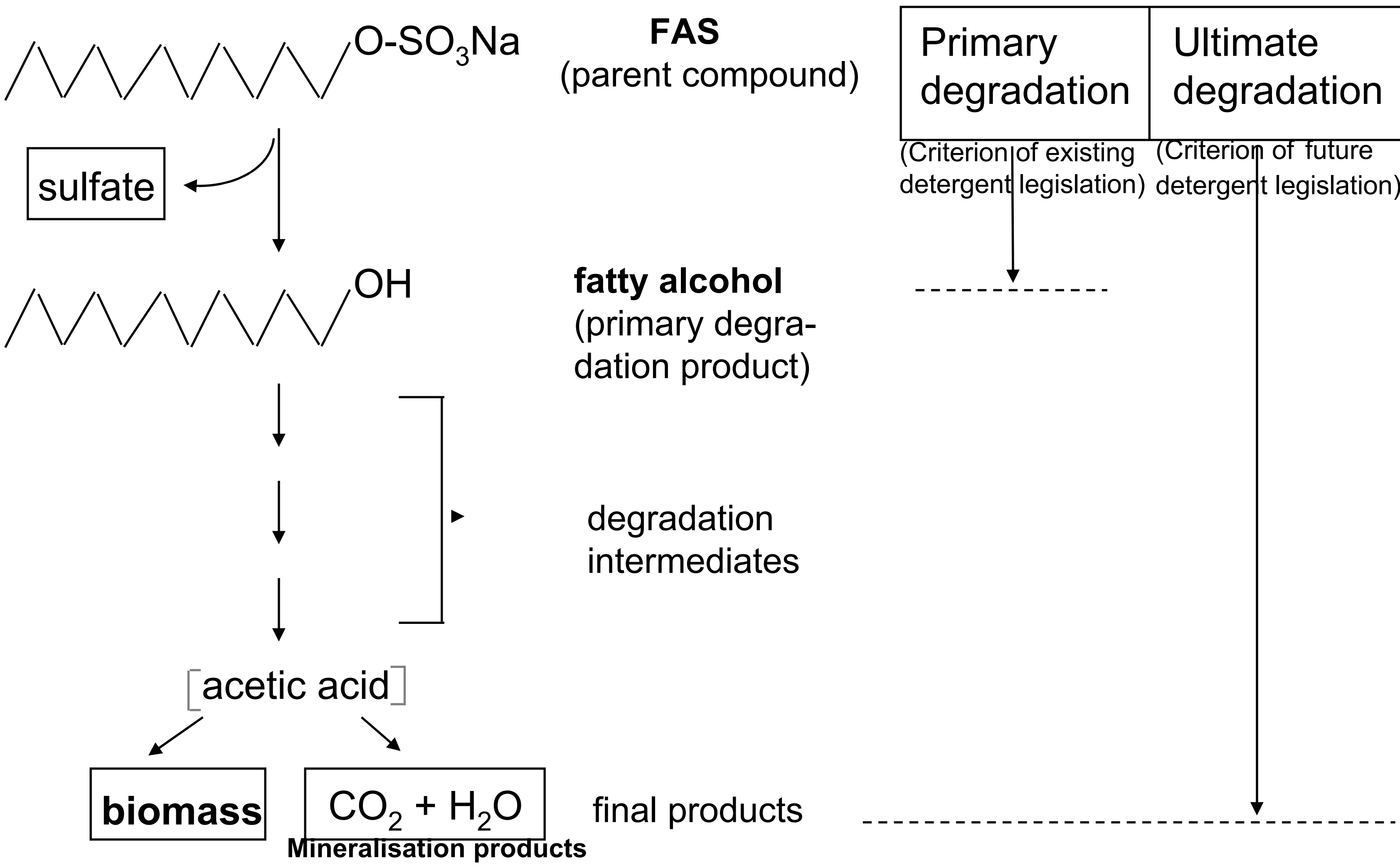
AISE/CESIO notes with concern that the wording of Annex III of the Working Document published for the public consultation exercise may give a misleading impression to those who are not fully aware of the scientific background to the tests.

In particular, the first statement says "The level of biodegradability shall be more than 60%……………..” This does not adequately describe the degree of decomposition actually undergone by the surfactant in this context. The prescribed tests require achievement of a threshold level of **60% of the theoretical oxygen uptake or carbon dioxide production ( ie proof of mineralisation).** Allowing for the inevitable additional use of carbon from the test substance in the formation of new biomass, achievement of the 60% mineralisation threshold corresponds to a much higher level of ultimate biodegradation of the test substance, often approaching complete destruction. This is explained in detail in the attached Annex I. A slight modification to the wording of Annex III to the Working Document is offered in the attached Annex II as an attempt to clarify the situation.

**Conclusion**

AISE/CESIO wishes to emphasise that the achievement by a surfactant of the 60% mineralisation pass level is indicative of an ultimate biodegradation extent in the region of 90% and to a primary biodegradation extent far above the 90% pass level stipulated in the existing Detergents Directive. Hence, meeting the 60% threshold pass level proposed in the Draft Regulation is far more demanding than the fulfilment of the 90% primary biodegradation requirement of the existing detergents legislation.

**Figure 2:**
**Surfactant biodegradation scheme**
(Example: Fatty alcohol sulfate)



**Annex 1: ERASM* comments on:**

## <u>Threshold-pass Level for Ultimate Biodegradability as measured by Carbon Dioxide Production or Oxygen Consumption.</u>

The test methods for 'ultimate biodegradability' in Annex III of the draft Detergents Regulation refer to a threshold-pass level of 60% in 28 days. These reference methods are based on EN ISO Standard 14-593 (1999) headspace carbon dioxide and the Annex V (Method Council Directive 67/548/EEC) ready biodegradability test methods measuring carbon dioxide production or oxygen consumption. It has been suggested that the threshold-pass level needs to be much higher to ensure a high level of environmental protection.

In this context it is important to realize that, in the process of biodegradation of organic substances, typically 50-90 % of the carbon content is utilised for energy production, i.e. to support the energy-requiring processes of maintenance and growth of living cells. The balance of the carbon content of the substance is used as building material for the cell constituents (proteins, lipids, carbohydrates etc.) of the biomass (Painter, 1992). Only the energy-producing parts of the metabolic processes result in the immediate production of carbon dioxide and consumption of oxygen. Eventually, of course, the cell biomass will decompose ultimately; also ending up in the mineralisation products carbon dioxide, water and mineral salts. The contribution of this process to the outcome of a 28-day ultimate biodegradability test depends upon a number of factors influenced by the test substance properties and the test method characteristics.

### What happens in a Ready Biodegradability test?

Biodegradability, reflecting a combination of substance properties, physical conditions and biological adaptation of microbes in the environment can be measured in many ways. In a standard ready biodegradability test, the test chemical is incubated in a mineral salts medium with a small amount of microbial organisms generally originating from a municipal wastewater sewage plant. The test chemical is the sole available source of carbon and energy for the microorganisms while inorganic nutrients (e.g. N, P) are present in excess. A relatively high test-chemical concentration is used to ensure that the carbon dioxide produced, the oxygen consumed, or the soluble organic carbon removed during the biodegradation of the test chemical can be analysed adequately.

Biodegradation takes place if the test material is used as a source of carbon and energy (growth-linked biodegradation) by microorganisms introduced with the inoculum. Growth-linked biodegradation results in removal of the test substance,

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increase in the numbers of the organism responsible for degrading the substance and, ultimately, in the formation of carbon dioxide and consumption of oxygen. The most direct measurements of ultimate biodegradation describe mineralisation, i.e. the consumption of oxygen and the production of carbon dioxide.

**Why doesn't the level of $CO_2$ produced or $O_2$ consumed reach 100% of Theoretical?**

From the general description of the biodegradation process of a chemical it is obvious that within the limited time period of a biodegradation test only part of the organic carbon of the chemical will be converted to mineralisation products while a significant moiety is transformed to microbial biomass. The growth yield of a substance is a measure of the extent of the biotransformation of the chemical's carbon to cell material. Growth yields of some fully biodegradable substances of natural origin are shown in Table 1, suggesting that 30 to 70% of the carbon is used in biomass growth. This implies that the residual 70 to 30% of the carbon is used for energy production, hence, forming carbon dioxide (Stanier et al. 1976).

**Table 1 :** Statistical summary of sludge yield values for heterogeneous populations of wastewater origin grown on various carbon sources.

| Carbon Source | Average cell yield, % | Number of determinations | Range |
|---|---|---|---|
| Fructose | 53.0 | 8 | 34-69 |
| Galactose | 51.9 | 24 | 36-76 |
| Glucose | 61.9 | 118 | 36-88 |
| Glycerol | 46.5 | 31 | 31-61 |
| Lactose | 47.1 | 12 | 30-61 |
| Maltose | 51.7 | 7 | 39-86 |
| | | | |
| Acetic acid | 41.2 | 6 | 26-53 |
| Butyric acid | 45.0 | 1 | - |
| Citric acid | 31.0 | 1 | - |
| Lactic acid | 29.0 | 1 | - |
| Pyruvic acid | 68.0 | 1 | - |

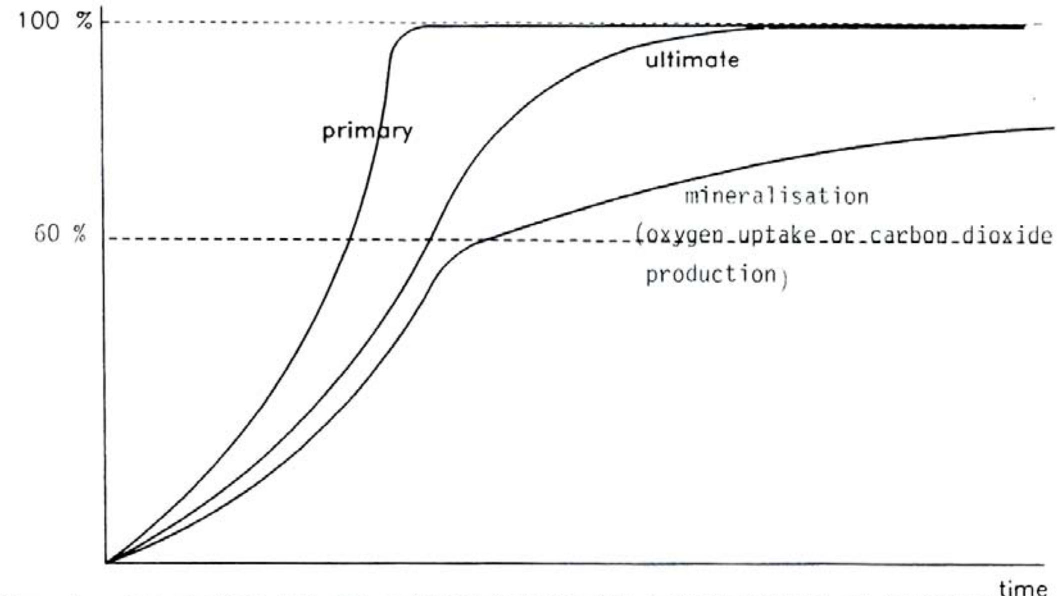
Cell yield is expressed as the percentage of the organic carbon source that is converted to cells, i.e., (dry weight of cells produced/weight of carbon source utilized) x 100.

Reference : Ramanathan and Gaudy, 1972

These observations are in line with the results of a recent in-depth study into the biodegradation of glucose in the $CO_2$ evolution test (OECD 301B). The measured $CO_2$ formation of 55% theoretical corresponded to an ultimate biodegradation extent of 85-98% taking the biomass production into account.

It is in recognition of these facts that the threshold level of 60% of theoretical carbon dioxide production or oxygen consumption was chosen by the OECD Expert Group as the level indicating complete and ultimate biodegradation in the standard 28-day ready biodegradation test

A complete description of the growth and mineralisation process during a ready biodegradability tests can be found in the Guidance document for the interpretation of biodegradability test data (EU Commission Contract # B-3040/93/001114). This document more fully describes the use of the concept of 'ready biodegradability' as a measure for ultimate or complete biodegradability and supports the fact that a threshold-pass level of 60% is adequate as a measure for complete biodegradation in the Annex V (Method Council Directive 67/548/EEC) test methods measuring carbon dioxide production or oxygen consumption. Figure 1, taken from the EU study, clearly illustrates in general terms the correlation between different endpoints for biodegradability. The difference between the "ultimate" and "mineralisation" curves may be considered to reflect the amount of carbon taken up by the biomass.




Fig; 1 : Correlation between primary degradation (disappearance of substance), ultimate degradation and respiration (oxygen uptake).
Source : Commission of the European Communities. Guidance document for the interpretation of biodegradability test data (Contract No. B - 3040/93/001114).

**Why is the cut-off level of 60% mineralisation adequate in the context of the EU detergents legislation?**

Based on the facts presented above, it can be concluded that a 60% threshold level for mineralisation in a 28-day screening test is scientifically justified and sound for the prediction of ultimate biodegradation under environmental conditions.

In the context of surfactants in detergents, the EU Expert Working Group deliberately chose the mineralisation methods and the 60% threshold limit in order to obtain an unequivocally measurable parameter of ultimate biodegradation. For surface active materials, it was recognised that the analytical parameter 'carbon removal' used in some OECD ready biodegradability tests, and which may address the biomass formation, may be influenced by physical-chemical processes like adsorption and, hence, may not purely reflect ultimate biodegradation.

Table 2 provides examples of ready biodegradability test results for selected fully degradable reference substances and for soap and indicative surfactants. The similar mineralisation data sets between the reference substances and surfactants re-emphasize the very high mineralisation potential of those surfactants in the environment.

**Table 2**: Mineralisation in the OECD 301 test

| SUBSTANCE | 28-day mineralization (OECD 301) Oxygen uptake or CO2 incorporation |
|---|---|
| Glucose | 55 - 90% |
| Benzoate | 61 - 95% |
| Na-Acetate | 86 - 90% |
| 2-propanol | 52 - 79% |
| Fatty Acid / Soap (C12 to C20) | 52 - 99% |
| Linear Alkylbenzene Sulphonate | 53 - 98% |
| Alkyl Sulphate (C12 to C18) | 62 - 95% |
| Alkyl ether sulphates (C8 - C18) | 76 - 96% |
| Alcohol ethoxylates (C8 – C18,<20EO) | 72 -88% |
| C13-15-alcohol ethoxylate (7EO) | 50 - 99% |

Reference: AISE /CESIO company data and the Study on the possible problems for the aquatic environment related to surfactants in detergents (WRc Ref: EC 4294, May 1997)

An important feature of Table 2 is the spread of results observed both for the simple reference substances and for the surfactants. Such variability is to be expected in biological systems and the 60% threshold-pass level defined for the “mineralisation” tests takes this into account.

Re-assurance of the potential for ultimate biodegradability may be drawn from DOC removal measured in OECD 301 tests. Similar DOC removal values (in the range of 70% to > 95%) for the reference substances, soap and the indicative surfactants listed in Table 2 are noted in those tests. Degradation pathways of major surfactant groups have been described (WRc: EC 4294, May 1997) and provide more detailed metabolic information on how degradation products are used in living cells for energy production or biomass formation. For example, Figure 2 depicts the biodegradation pathway of fatty alcohol sulfate.

## Conclusion

In conclusion, a substance attaining the 60% mineralisation threshold level would be expected to undergo fast and virtually complete ultimate biodegradation in the aquatic environment. Most importantly, this implies that the biodegradation processes will bring about a high removal of the test substance and its degradation intermediates in sewage treatment plants. Indeed, it has already been shown that there is a good correlation between positive results in ultimate biodegradability screening tests and the extent of carbon removal in sewage plant simulation tests (Gerike & Fischer, 1979). Surfactants represent a group of chemicals very comprehensively investigated in terms of biodegradation behaviour and the processes involved (Karsa & Porter, 1995). The existing information from simulation tests and environmental monitoring data covers virtually all surfactant groups relevant to detergents and shows unequivocally that surfactants meeting the ultimate biodegradation limits of the Draft Detergents Regulation exhibit a high level of removal; in the region of 99%. A number of comprehensive studies using radiolabeled model compounds (e.g. Federle and Itrich 1997) have confirmed that this excellent removal is due to ultimate biodegradation, i.e. to mineralisation and biomass formation.

It is a consequence that surfactants failing to reach the 60% threshold pass level within the 28-day period will require additional evidence to prove their environmental safety. Industry has proposed a stepwise process for this purpose that takes account of the concerns about the formation of recalcitrant and potentially toxic degradation intermediates.

## References

Commission of the European Communities. Guidance document for the interpretation of biodegradability test data. Contract No. B-3040/93/001114. BKH Consulting Engineers. Delft, 1994.

Federle T.W. and Itrich N.R. (1997). Comprehensive approach for assessing the kinetics of primary and ultimate biodegradation of chemicals in activated sludge: Application to linear alkylbenzene sulfonate. Environ. Sc. Technol. 31, 4, 1178-1184.

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Stanier, R.Y. , E. A Adelberg, , J.L. Ingraham. *The Microbial World*. 4th Edition. 1976. Prentice Hall, Inc. Englewood Cliffs, New Jersey. pages 283-284.

Water Quality – Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds – Method by analysis of inorganic carbon in sealed vessels (CO2 headspace test), International Organization for Standardization, International Standard ISO/DIS 14593 (1999).

*ANNEX III*

**"ULTIMATE BIODEGRADABILITY" TEST METHODS FOR SURFACTANTS IN DETERGENTS**

The biodegradability of surfactants used in detergents shall be assessed according to the tests indicated below.

**A)-** The reference method for laboratory opinion on ultimate biodegradability shall be based on the EN ISO Standard 14593 (1999) headspace CO2.

Surfactants in detergents shall be considered as very substantially biodegradable (typically around 90%) if passing this reference test or at least one of the five following tests[1] in which the threshold-pass level for mineralisation shall be 60% in 28 days:

1. EN ISO Standard 14593: 1999. Water quality -- Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium -- Method by analysis of inorganic carbon in sealed vessels (CO2 headspace test) *(Ed. 1, 15 p, H)*. Pre-adaptation is not to be used. The 10 days window principle is not applied. (Reference method).

2. Method Council Directive 67/548/EEC Annex V C.4-C (Carbon dioxide (CO2) Evolution Modified Sturm Test): Pre-adaptation is not to be used. The 10 days window principle is not applied.

3. Method Council Directive 67/548/EEC Annex V C.4-E (Closed Bottle): Pre-adaptation is not to be used. The 10 days window principle is not applied.

4. Method Council Directive 67/548/EEC Annex V C.4-D (Manometric Respirometry): Pre-adaptation is not to be used. The 10 days window principle is not applied.

5. Method Council Directive 67/548/EEC Annex V C.4-F (MITI-Ministry of International Trade and Industry-Japan): Pre-adaptation is not to be used. The 10 days window principle is not applied.

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[1] These five tests are identified as the most suitable for surfactants

**B)-** Depending on the physical characteristics of the surfactant, one or other of the methods listed below might be used if appropriately justified[1]. It should be noted that the threshold limit of 70% of these methods are to be considered as equivalent to the threshold limit of 60% referred to in methods listed in point A) above. The adequacy of the choice of the methods listed below should be decided on a case by case confirmation, in accordance with Article 6 of thisRegulation.

1. Method Council Directive 67/548/EEC Annex V C.4-A (Dissolved Organic Carbon DOC Die-Away): Pre-adaptation is not to be used. The 10 days window principle is not applied. The threshold-pass level measured according to the test shall be 70% in 28 days.

2. Method Council Directive 67/548/EEC Annex V C.4-B (Modified OECD Screening-DOC Die-Away): Pre-adaptation is not to be used. The 10 days window principle is not applied. The threshold-pass level measured according to the test shall be 60% in 28 days.

(***Note:*** All the above mentioned methods from Council Directive 67/548/EEC can also be found in the publication: "Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2-Testing Methods" European Commission 1997. ISBN 92-828-0076-8.)

[2] The DOC methods could give results on the removal and not on the ultimate biodegradation. The Manometric Respirometry and the MITI would not be appropriate in some cases because the high initial test concentration could be inhibitory.