

DRAFT DETERGENTS REGULATION: BIODEGRADATION and ENSURING PROTECTION OF HUMAN HEALTH AND THE ENVIRONMENT

The Commission proposal for a Regulation on Detergents introduces “ultimate” biodegradability requirements in order to ensure a high level of environmental protection from surfactants in detergents. The biodegradability test methods contained in Annex III of the 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 specially validated by the Commission for use with detergent surfactants. The appropriateness of these biodegradability methods and the 60% threshold pass level has been endorsed in the context of the new detergent legislation both by Member States’ technical experts and, independently, by the CSTEE in its Opinion adopted at the 12th CSTEE Plenary Meeting of 26 November 1999.

The European Parliament Legislative Resolution on the Proposal for a Regulation on Detergents proposed a modified Introduction to Annex III, Part A, as follows: “Surfactants in detergents shall be considered as biodegradable if the level of biodegradability (mineralisation) measured according to one of the five following tests is at least 60% within twenty-eight days ***and the remainder (maximum 40%) is shown not to contain very persistent and/or very bio-accumulative chemicals and/or chemicals with endocrine disrupting properties, and/or not to contain any chemicals that have these properties***”.

Industry would urge the Council to support the text proposed by the Commission for Annex III as entirely appropriate to ensure the environmental safety of detergent surfactants and to reject the additional requirements proposed by the European Parliament since:

- The fact that a chemical may not reach mineralisation levels close to 100% theoretical within 28 days in laboratory screening tests such as the OECD 301-series does not mean poorly biodegradable/persistent degradation products are formed. This apparent shortfall in mineralisation may be explained by:
 - The production of new bacteria ("biomass") which uses a significant amount of the theoretical carbon balance available from the test substance.
 - The speed of degradation is proportional to the residual concentration of undegraded test material. Hence, the degradation will continue beyond the 28-day period of the laboratory test.
- The ultimate biodegradability screening tests are internationally recognised as very stringent and are not intended to simulate real environmental conditions. Scientists agree that achievement of the 60% mineralisation level in one of the tests specified in Annex III is a satisfactory indication that the test substance will be degraded readily and completely under environmental conditions.

- The biodegradation pathways of all major surfactant groups used in detergents have been comprehensively investigated and the results are published in the peer-reviewed scientific literature. The nature of the biodegradation intermediates is thus well known and the studies did not provide any evidence for the formation of stable or recalcitrant metabolites.
- Specific testing for metabolites in the biodegradation of the major surfactant groups used in household detergents, by industry, gave no indication of the formation of poorly biodegradable intermediates
- For the surfactants which will pass the threshold criterion, the oxidative biodegradation normally produces intermediates which are more polar than the parent compound. This means they have less affinity for fat than the parent surfactant and hence will have lower bioaccumulation potential than the parent surfactant. Since the surfactants themselves have a low bioaccumulation potential, it is possible to discount the possibility that their biodegradation intermediates will have significant bioaccumulation potential.
- All major detergent surfactant families have been independently screened and shown to have no adverse endocrine disruption properties. Structure-activity relationships would predict that the metabolites formed during biodegradation of detergent surfactants will also not have endocrine-disrupting properties.

Detailed technical support documentation is appended.

CONCLUSION

The Commission proposal for Annex III should be supported since it will ensure enhanced protection of the environment and protect human health.

ANNEX: COMMENTS ON THE APPROPRIATENESS OF THE ANNEX III BIODEGRADABILITY PROVISIONS FOR CONTROL OF SURFACTANTS USED IN DETERGENTS.

GENERAL ASPECTS OF BIODEGRADABILITY TESTING

60% Threshold Pass Level

In the laboratory test methods to assess “ultimate” biodegradability, such as those specified in Annex III, the test substance is the only source of carbon available. Some of this carbon is used by the micro-organisms to provide the energy necessary to sustain life and the balance is used as building material in the process of cell growth; to generate more micro-organisms. The energy-producing part of the metabolic activity consumes oxygen, resulting in the immediate formation of carbon dioxide, water and mineral salts in a process known as “mineralisation”. [1]

Within the limited time period of a biodegradation test, only part of the available organic carbon will be converted to mineralisation products whilst a significant proportion is transformed into microbial biomass. The growth yield of a substance is a measure of the extent of the biotransformation of its carbon content to cellular material. Growth yields of a variety of fully biodegradable substances of natural origin indicate that 30 to 70% of the carbon is used in biomass growth; implying that the residual 70 to 30% of the carbon is used for energy production, hence consuming oxygen and forming carbon dioxide [2,3].

These observations are consistent with the results of a recent in-depth study into the biodegradation of glucose in the CO₂ evolution test (OECD 301B). The measured CO₂ 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, and the inherent variability of biological systems, that the threshold level of 60% of theoretical carbon dioxide production or oxygen consumption (i.e. mineralisation) was chosen by the OECD Expert Group as indicative of complete and ultimate biodegradation in the standard 28-day ready biodegradation tests.

Kinetic Aspects

For environmental fate assessment, screening data from ready biodegradability tests is treated as a first order process with an initial lag phase. First order kinetics, also termed half life kinetics – is described as the time taken for half the chemical to disappear in time t , half of what remains will disappear at time $2t$ etc. and no significant increase in cells occurs. This could be due to insufficient carbon being available because another nutrient may be lacking; the normal situation in the environment.

The sigmoidal curves obtained typically in ready/screening tests can be used to provide kinetic constants and, by a process of reiteration, “best fit” values are

obtained. This has been shown extensively in studies by Hales et, al, [5] (see figure 1 below).

The first phase (1) represents disappearance of the test material from solution and the growth of the organisms on it, and the second phase (2) represents degradation of the biomass during the first phase. For rapidly degrading materials, (curve A) e.g. sodium benzoate, aniline there is very little or no lag, and biodegradation is complete at the inflection point. For material such as commercial LAS (curve B), biodegradation will continue, possibly even beyond 28 days.

It is also possible to fit the first phase of biodegradation to the Monod Equation for microbial growth to yield μ_{max} , K_s , and initial growth rate μ values. It has been reported that high volume household chemicals (LAS etc) may be present in sewage in mg/L range concentrations and consequently their degradation can be expected to follow Monod kinetic behaviour, i.e. growth of competent degrading microorganisms being proportional to the degradation of the test substance, until point is reached at which the growth becomes suppressed by substrate limitation.[6]

Hence overall, the biodegradation curves reflect growth and then death and subsequent metabolism of competent microorganisms. From this type of data it is possible to make reasonably accurate predictions as to the behaviour of the chemicals in a wastewater treatment plant.

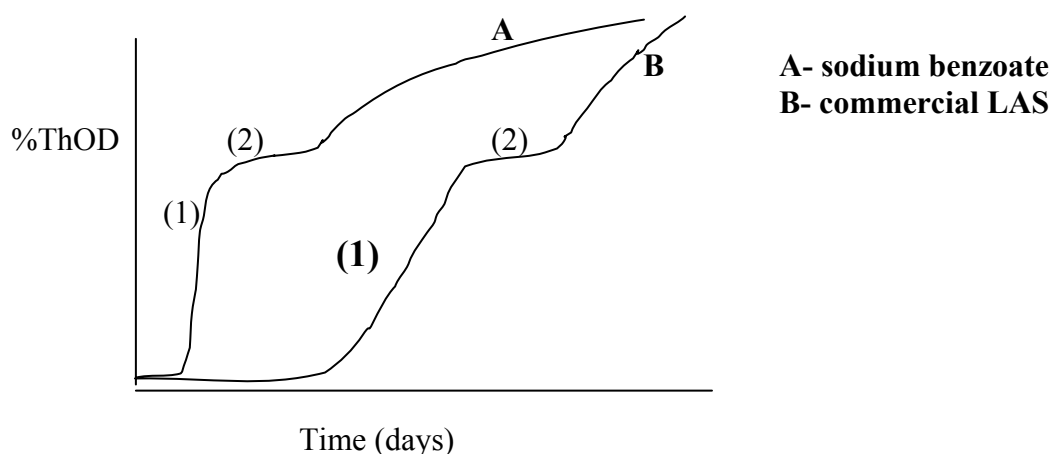


Figure 1- Illustrating typical biodegradation of LAS and sodium benzoate in respirometer-(OECD 301F).

Environmental relevance of a positive result in a screening test for ultimate biodegradability (OECD 301)

Passing the threshold level of 60% mineralisation (CO₂ or BOD) in an ultimate biodegradability screening test of the OECD 301 type is the decisive criterion for the evaluation of a chemical as "readily biodegradable". Acknowledging that the stringency of the test conditions in these generic biodegradability tests does not

simulate the real environmental situation [7], the OECD Expert Group on Degradation and Accumulation concluded [8] that a positive test result will allow the assumption that the chemical will undergo rapid and ultimate biodegradation in the environment and that no further work on the biodegradability of the chemical or on the possible environmental effects of biodegradation products should normally be required. The validity of these conclusions has been substantiated for many chemicals in comprehensive studies which have included a number of surfactants. Hence, the fact that a chemical meets the ready biodegradability criteria is not only a warranty of a very fast and complete removal of the parent compound in environmental conditions but also implies a high probability that the degradation intermediates formed reflect transient stages of the ultimate biodegradation process, i.e. are not persistent.

SURFACTANT-SPECIFIC ARGUMENTS

Biodegradation pathways and metabolites of detergent-relevant surfactant groups

Detergent surfactants represent one of the best investigated groups of chemicals in terms of their environmental fate and effects. Based on a number of published comprehensive studies there exists a sound view of the basic processes involved in the rapid and, ultimately, complete microbial degradation of these chemicals exhibiting common structural characteristics. While the hydrophilic moiety of anionic, non-ionic, cationic and amphoteric surfactants is different, their hydrophobic part generally consists of a single linear or 1-methyl-branched alkyl chain (C8-18). Consequently, the common principle of the microbial degradation of surfactants is either an enzymatic cleavage of the two surfactant molecule moieties (forming a fatty alcohol /acid and a hydrophilic organic or inorganic degradation product) or is a terminal oxidation and subsequent stepwise degradation of the alkyl chain (leaving again a hydrophilic degradation product). Both mechanisms lead to products exhibiting a less complex structure and more hydrophilic properties than the parent compound. Hence, the two structural moieties of every surfactant are well known and can be assessed in terms of their accessibility to biodegradation. This is in line with the fact that there are no indications of poorly biodegradable intermediates of surfactants which have themselves been proved to be readily biodegradable.

Comprehensive studies into the biodegradation pathways and metabolite formation have been published for many major surfactant groups used in detergents. Such information is available for anionic surfactants (LAS, alcohol ether sulfates, alcohol sulfates, α -methyl estersulfonates, secondary alkane sulfonates/ α -olefin sulfonates), non-ionic surfactants (alcohol ethoxylates) and several cationic surfactants (quaternary ammonium salts) [9].

Test for detecting recalcitrant metabolites

The "Test for detecting recalcitrant metabolites" (Metabolite test) was developed [10] to evaluate chemical substances in terms of their potential to form degradation intermediates which are poorly biodegradable. This test is experimentally based on the Coupled Units Test (OECD 303A), i.e. a sewage treatment plant simulation model. In contrast to the latter, the effluents of both the test and the control units are

re-used each day as fresh influent; after addition of a concentrate of nutrients (control unit) and the test substance. Thus, a continuous recycling of the test liquor is achieved which gives the degrading organisms an extended chance to cope with the test compound and its degradation products. Since the metabolite test is run for several weeks, corresponding up to 100 cycles, even small amounts of recalcitrant metabolites would accumulate and could be analytically detected. Based on this elaborated test method, no evidence for the presence of any recalcitrant metabolites was obtained for all readily biodegradable surfactant groups used in detergents, like LAS, alcohol ether sulfates, alcohol sulfates, α -methyl estersulfonates, secondary alkane sulfonates, alcohol ethoxylates, several alcohol alkoxyates (EO/PO) [11], amine oxides, alkyl polyglucosides, fatty acid monoethanolamides and some cationic and amphoteric surfactants [12].

Bioaccumulation potential of biodegradation intermediates

The potential to bioconcentrate has been investigated for a number of surfactants. The available data are most extensive for the major surfactants, LAS and AE. The bioconcentration factors (BCF) have been measured for a number of isomers found in these surfactants. The BCF value ranged between 2 and 987 for LAS isomers/homologues and < 5 and 387 for AE homologues [13 and references therein]. The individual structural differences between these homologues/isomers, which influence their hydrophobicity, are the main reason behind the observed variation in BCF values. All homologues/isomers for both LAS and AE were observed to be rapidly metabolised; with an uptake/elimination steady state being reached in a few days. The elimination rates of LAS and AE were high due to the rapid formation of water-soluble metabolites. In addition, there was no relationship between the bioaccumulation of LAS and AE and the lipid content of the fish, which is in direct contrast to what is observed for metabolically stable hydrophobic substances.

The surfactant bioconcentration data are most comprehensive for LAS and AE. However, there are sufficient data available to show that surfactants in general are amenable to biotransformation. The metabolism of the surfactant alkyl chain through a combination of ω - and β -oxidation has been demonstrated for vertebrates as well as some invertebrates. The formation of small, low molecular weight, water-soluble metabolites has been shown for a wide range of materials including alkyl sulphonates, sodium laurate, alkyl sulphates, alkyl ether sulphates, alkyl sulphosuccinates, amine oxides and alkyl trimethyl ammonium halides [13 and references therein].

The above evidence therefore suggests that surfactants must be seen as a group of substances, which possess common structural features that are easily accessible to biotransformation processes. These processes are probably ubiquitous to all organisms; as they have been observed in bacteria, invertebrates, lower vertebrates and mammals. Surfactants therefore have little in common with typical bioaccumulative substances that exhibit high bioconcentration factors and persistence in lipid tissues.

Endocrine disrupting potential of Detergent Surfactants and metabolites.

Metabolites of alkylphenol ethoxylates have been shown to be weakly oestrogenic, which has led to other major surfactant types being tested for potential oestrogenic activity. Investigators have used *in vitro* screens for oestrogenicity to assay a range of surfactants which consist of linear alkylbenzene sulfonates (and the metabolite sulphophenyl carboxylate), alcohol sulphates, alcohol ether sulphates, secondary alkane sulphonates, linear- α -olefin sulphonate, sodium lignosulphonate, alcohol ethoxylates, di(hydrogenated tallow) dimethyl ammonium chloride, benzalkonium chloride, betaines and imidazoline [14, 15]. These studies have shown that representative materials from anionic, cationic and nonionic surfactants do not possess oestrogenic activity (exception APE and metabolites).

It is known that the oestrogen receptor can bind with a variety of non-steroidal phenolic compounds. It has been suggested that the affinity of a substance is related to the hydrogen bonding between the phenolic hydroxyl group and the receptor binding site as well as the compound's hydrophobic and steric properties [16 and references therein]. Both the size of the alkyl chain and the degree of branching are important in determining the oestrogenic potency. Few of the surfactants that are commonly used possess a benzene ring but it is known that the LAS metabolite, sulphophenyl carboxylate (SPC) does not possess oestrogenic activity. The other surfactants with a benzyl group, alkyl benzyldimethylammonium compounds, appear to undergo excision and oxidation of the benzyl group at an early stage in the biodegradation pathway [17]. This is likely to produce a transient metabolite, phenol, which is not oestrogenic *in vivo*. The evidence suggests, therefore, that none of the common surfactants that may be released to the environment or their likely metabolites possess oestrogenic activity.

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