Scientific Committee on Health and Environmental Risks

SCHER

Opinion on

Anaerobic Degradation of Surfactants

And

Biodegradation of Non Surfactant Organic Ingredients

The SCHER adopted this opinion at its 26th plenary of 17 November 2008

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCHER

Questions relating to examinations of the toxicity and ecotoxicity of chemicals, biochemicals and biological compounds whose use may have harmful consequences for human health and the environment.

In particular, the Committee addresses questions related to new and existing chemicals, the restriction and marketing of dangerous substances, biocides, waste, environmental contaminants, plastic and other materials used for water pipe work (e.g. new organics substances), drinking water, indoor and ambient air quality. It addresses questions relating to human exposure to mixtures of chemicals, sensitisation and identification of endocrine disrupters.

Scientific Committee members

Contact:

European Commission
Health & Consumer Protection DG
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels

Sanco-Sc8-Secretariat@ec.europa.eu

http://ec.europa.eu/health/ph_risk/risk_en.htm

ACKNOWLEDGMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion:

Prof. P. Calow, Roskilde University, Denmark

Prof. H. Greim, Technical University of Munich, Germany

Dr. J. Linders, (*Rapporteur*), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Prof. M. Nuti, University of Pisa, Italy

Dr. J. Tarazona, Spanish National Institute for Agriculture and Food Research and Technology, Spain

External expert:

Dr. M. T. Garcia Ramon, Spanish Council for Scientific Research, Spain

Keywords:

SCHER, scientific opinion, biodegradation, surfactants, anaerobic degradation

Opinion to be cited as:

SCHER scientific opinion on the anaerobic degradation of surfactants and the biodegradation of non surfactants organic ingredients, 17 November 2008

TABLE OF CONTENTS

1. BACKGROUND

In order to decide whether further legislative action would be justified concerning the anaerobic biodegradation of surfactants (as indicated in Article 16(2) of the Detergents Regulation), the Commission forwarded to SCHER the Fraunhofer study on "Anaerobic biodegradation of detergents surfactants", together with two reports on LAS (HERA 2004 and OECD-2005), for evaluation and for an opinion on certain issues of anaerobic biodegradability.

In the SCHER opinion (adopted in November 2005) some concerns were expressed:

(a) about the terrestrial toxicity of LAS in combination with worst case environmental conditions

(b) about the high measured levels of other – even anaerobically biodegradable surfactants in sewage sludge.

Another issue that the Commission needs to review by April 2009, as also indicated by Article 16(2) of the Detergents Regulation, is the biodegradability of non-surfactant organic detergent ingredients. Following a mandate from DG Enterprise, the SCHER reviewed the RPA report on "Non-surfactant organic ingredients and zeolite-based detergents" and adopted an opinion in June 2006.

While for the rest of the detergents' ingredients SCHER agreed that no health or environmental risk were identified, it more specifically concluded that:

(a) phosphonates: the available information is not sufficient to exclude a potential risk at European level, in particular for terrestrial and aquatic organisms;

(b) polycarboxylates: the information available is not complete; therefore a possible risk cannot be excluded.

2. TERMS OF REFERENCE

DG Enterprise would therefore like to request updated opinions of SCHER based on the following reports:

Question (A): HERA-2007 report on Linear Alkylbenzene Sulphonates-LAS[1]

(1) The SCHER 2005 opinion on anaerobic biodegradation highlighted a concern about the high level of surfactants in sludge, including those that are in fact anaerobically biodegradable (*e.g.* soaps, alcohol ethoxylates-AE). SCHER is requested to clarify this topic, based on recent scientific evidence. In particular, considering the HERA report on AE (HERA 2007a), SCHER is requested to comment whether:

(i) AE usage in laundry cleaners and household cleaning products is safe and does not create concern with regard to consumer health or not,

[1]Related scientific paper & reports for subject (A):

1. Fate of linear alkylbenzene sulfonate (LAS) in activated sludge plants, H. Temmink, B. Klapwijk, Water Research 38 (2004) 903—912.

2. Risk assessment of linear alkylbenzene suiphonates, LAS, in agricultural soil revisited: Robust chronic toxicity tests for Folsomia candida (Collembola), Aporrectodea caliginosa (Oligochaeta) and Enchytraeus crypticus (Enchytraeidae), P.R. Krogh et a!., Chemosphere 69 (2007) 872—87.

3. European risk assessment of LAS in agricultural soil revisited:Species sensitivity distribution and risk estimates, J. Jensen ci al., Chemosphere 69 (2007) 880—892.

4. Probabilistic risk assessment for linear alkylbenzene sulfonate (LAS) in sewage sludge used on agricultural soil, D. Schowanek, Regulatory Toxicology and Pharmacology 49 (2007) 245—259.

5. Anaeobic biodegradation of surfactants-scientific review, J.L Berna et al., Tens.SurfDeterg, (2007), 44, 3 13-347

(ii) AB usage in laundry cleaners and household cleaning products is not a cause for concern for the environment (in particular surface water, sediment, sewage treatment facilities, and soil).

(2) SCHER is requested to review the last HERA report concerning LAS (HERA 2007b) and specifically to comment whether it agrees with its main conclusions that:

(i) the risk characterisation as expressed by the PEC/PNEC ratio was below 1 for all environmental compartments, considering the recently reported PNEC values (~35 mg/kg versus 4.6 mg/kg in previous assessments) as calculated by new soil toxicity studies (*e.g.* Jensen et al. 2007)

(ii) the ecotoxicological parameters of LAS have been adequately and sufficiently characterized and that the ecological risk of LAS is judged to be low.

(iii) the use of LAS in household laundry and cleaning poses no risk to consumer health.

(3) in its opinion of 2005, SCHER concluded that single tests for evaluating anaerobic biodegradation are not sufficient, and that a combination of different testing conditions would be more appropriate. SCHER is requested to review the issue of anaerobic test methodology based on the latest scientific evidence.

(4) SCHER is invited, in the light of the latest scientific evidence, to reconfirm the following statements, which were mentioned in the opinion of 2005:

(i) poor biodegradability under anaerobic conditions is not expected to produce substantial modifications in the risk for freshwater ecosystems as the surfactant removal in the WWTP seems to be regulated by its aerobic biodegradability.

(ii) the requirement for ready and ultimate biodegradability under anaerobic conditions is not by itself regarded as an effective measure for environmental protection.

Question (B): HERA-2007 report on polycarboxylates in detergents

(1) SCHER is requested to review the recent HERA report on polycarboxylates in detergents (HERA 2007c) and to comment whether it agrees with the main conclusions that:

(i) the use of polycarboxylates in detergents results in risk characterization ratios (RCR) less than one, indicating no concern, for all environmental compartments with the exception of the soil local compartment for P-AA/MA (an acrylic and maleic acids copolymer or its sodium salt) as a consequence of the standardized test design,

(ii) the use of polycarboxylates in household laundry products and automatic dishwashing detergents poses no risk to the environment from the use by consumers.

(2) SCHER is requested to review any recent evidence concerning the potential risks of phosphonates to the environment at the EU level.

(3) Considering the recent scientific evidence for polycarboxylates and phosphonates, SCHER is requested to clarify whether a potential move to zeolite-based detergents at EU level would increase (or not) the health and environmental risks from detergents.

3. OPINION

3.1. General comments

Two topics have been identified here:

a) Influence of surfactants on soil (parameters)

Surfactants (chemical- and bio-) are amphiphilic compounds which can reduce surface and interfacial tensions by accumulating at the interface of non-miscible fluids and increase the solubility and mobility of insoluble or hydrophobic organic compounds (Mulligan, 2005). Surfactants, being potentially toxic to certain microorganisms, are exploited in agriculture as biological control agents (Cameotra and Makkar, 2004), or are used in bioremediation to improve degradation of chemical contaminants in soil through their ability to emulsify hydrocarbons, to lower interfacial tensions, or through metal sequestration (Saichek and Reddy, 2005; Cheng et al. 2008). The uses of chemical surfactants and bio-surfactants, in agriculture and the environment are expected to increase (Van Hamme et al., 2006; Singh et al., 2006). Facilitation of desorption of contaminants from soil, aqueous soil washing processes, pesticide-enhancers, growth enhancers in animal feed are receiving increasing attention. Another entry of surfactants into the environment is from industrial applications *e.g.* upstream and downstream processing (production of extracellular and recovery of intracellular products, colloidal gas aphrons in bioprocessing), applied biocatalysis, monophasic organic solvent systems, two-phase systems, micro emulsions for cells encapsulation.

All the above surfactants are entering the environment along with LAS, the major detergent surfactant source, and are routinely deposited on land and into water compartments whether part of an intended process or as industrial or household waste.

Despite the use of surfactants as soil conditioners since the 1960s, information available about the effects of surfactants present in municipal wastewaters on soil properties and on organic chemical transport into soil is limited.

The physical and chemical properties of rhizosphere, *i.e.* bulk soil/plant interface, are affected by surfactants (Dunbabin et al. 2006): phosphorus uptake by the root system is enhanced, whereas soil water content decreases along with phosphate adsorption to soil particles and hydraulic conductivity at any given soil water potential. In other cases, LAS application to soil increases the nitrogen, phosphorus and sodium content of crop plants, whereas calcium and magnesium concentrations reduced (Moreno-Caselles et al., 2006).

The main regulatory factors appear to be total organic matter, dissolved organic carbon, and microbial biomass (Cheng et al., 2008). The functional diversity in soil increases monotonically with soil microbial biomass up to a certain threshold, *i.e.* 1.7% organic carbon (Lynch et al., 2004). Therefore it can be assumed that the higher organic matter content in soil the lower will be the influence of introduced external stressor on the functionality of soil microbes.

The SCHER agrees that, despite the extent of literature coverage on the effects of surfactants on soil biochemical processes (Muller et al., 2007), the information about direct and indirect effects of surfactants, present in municipal wastewaters, on soil physical and chemical traits is scanty. In particular field-based studies taking into account the main soil regulatory factors have been lacking.

b) Availability of OECD 307 (biodegradation in soil) and 308 (biodegradation in aquatic sediment systems).

Both Technical Guidelines contain an anaerobic part.

For the assessment of aerobic biodegradability of organic compounds several screening (OECD 301A-F, 302, 304, 306 and 310) and simulation tests (303, 307, 308 and 309) are available under the OECD Test Guidelines programme. For consideration of the biodegradability of organic compounds under anoxic conditions, potential biodegradability can be assessed in a screening test for anaerobic biodegradability (OECD 311). However, to assess the biodegradation rate in anoxic environmental compartments such as anoxic sediments and soil, simulation tests should be applied. Among the above mentioned simulation tests, TG 307 (transformation in soil) and TG 308 (transformation in aquatic sediment systems) include aerobic as well as anaerobic conditions.

SCHER is of the opinion that the methods described in these two Test Guidelines can provide useful possibilities to determine the biodegradation of the surfactants under consideration here. Guidance is given on the way anaerobic conditions should be maintained, by water logging for the soil system and a N_2 atmosphere in the head space for the water/ sediment system, as well how samples should be taken including the safe treatment, handling and storage of the samples. In addition, provisions for the collection of volatiles, like CO_2 and CH_4 are given.

OECD307 simulates the situation for detergents reasonably well as after the aerobic processes in the WWTP a potential anaerobic degradation phase takes place once the sludge has been deposited on land. Therefore, additional information on the anaerobic degradability may become available for these substances if tests according to these Guidelines are carried out. However, no plants are used in the test systems. This may cause a difference in the fate of the substances, but it is hard to predict whether the degradation would be enhanced or delayed. The o.m. content is very important in this context.

OECD 307 – Aerobic and Anaerobic Transformation in Soil

This is based on existing guidelines developed for pesticides. The method is designed for evaluating aerobic and anaerobic transformation of chemicals in soil. The experiments are performed to determine (i) the rate of transformation of the test substance, and (ii) the nature and rates of formation and decline of transformation products to which plants and soil organisms may be exposed. Such studies are required for chemicals which are directly applied to soil or which are likely to reach the soil environment.

The method is applicable to all chemical substances (non-labelled or radio labelled) for which an analytical method with sufficient accuracy and sensitivity is available. For general chemicals, whose major route of entry into soil is through sewage sludge/farming application, the test substance should be first added to sludge which is then introduced into the soil sample. The chemical should be dosed into the sludge at a concentration that reflects the expected sludge concentration and the amount of sludge added to the soil should reflect normal sludge loading to agricultural soils.

To establish and maintain anaerobic conditions, the soil treated with the test substance and incubated under aerobic conditions for 30 days or one half-life or DT50 is then water-logged and the incubation system flushed with an inert gas.

OECD 308 – Aerobic and Anaerobic Transformation in Aquatic Sediment Systems

This is based on existing Guidelines developed for pesticides. It describes a laboratory test method to assess aerobic and anaerobic transformation of organic chemicals in aquatic sediment systems and allows the measurement of transformation rates of the test substance. Such studies are required for chemicals which can enter shallow or deep surface waters by routes such as direct application, spray drift, run-off, drainage, waste disposal, industrial, domestic or agricultural effluent and atmospheric deposition.

The conditions in natural aquatic sediment systems are often aerobic in the upper water phase. The surface layer of sediment can be either aerobic or anaerobic, whereas the deeper sediment is usually anaerobic. Both aerobic and anaerobic tests are described in this guideline. The aerobic test simulates an aerobic water column over an aerobic sediment layer that is underlain with an anaerobic gradient. The anaerobic test simulates a completely anaerobic water-sediment system.

The method has been applied so far to study the transformation of chemicals in fresh waters and sediments, but in principle can also be applied to estuarine/marine systems. One test concentration of chemical is used. The concentration to be used should be based on predictions from environmental emissions.

Conclusion

SCHER is of the opinion that in the HERA-reports (HERA 2007 a, b, c) many relevant items have been taken into account to perform a reasonably good risk assessment. However, several areas could have been considered that would have taken the risk assessment a step further. The first is the fact that some field-based studies seem to have been overlooked (Cameotra and Makkar, 2004; Saichek and Reddy, 2005; Cheng et al., 2008; Van Hamme et al., 2006; Singh et al.,2006; Dunbabin et al., 2006; Moreno-Caselles et al., 2006; Cheng et al., 2008; Lynch et al., 2004). In particular the importance of the soil organic matter in influencing the functionality of soil microbes in the presence of abiotic external stressors is not considered. Secondly, the possibilities of determining the anaerobic degradability of surfactants have not been explored sufficiently as several methodologies are available (OECD 307 and 308).

3.2. Reply to question (A): HERA-2007 report on Linear Alkylbenzene Sulphonates-LAS (+ related scientific papers as listed in Annex)

(1) The SCHER 2005 opinion on anaerobic biodegradation highlighted a concern about the high level of surfactants in sludge, including those that are in fact anaerobically biodegradable (*e.g.* soaps, alcohol ethoxylates-AE). SCHER is requested to clarify this topic, based on recent scientific evidence. In particular, considering the HERA report on AE (HERA 2007a), SCHER is requested to comment whether:

(i) AE usage in laundry cleaners and household cleaning products is safe and does not create concern with regard to consumer health or not,

The HERA 2007a report has evaluated the existing literature on human health. The careful evaluation demonstrates that alcohol ethoxylates (AEs) have no skin sensitizing potential, whereas the neat solutions are irritating to the skin and eyes. There is no evidence for genotoxicity or carcinogenicity or adverse reproductive or developmental effects. The majority of available toxicity studies revealed NOAELs in excess of 100 mg/kg bw/d but the lowest NOAEL for an individual AE was established to be 50 mg/kg bw/d. This value was subsequently considered as a conservative, representative value in the risk assessment of AE. There was practically no difference in the NOAEL in oral studies of 90-day or 2-years of duration in rats. The effects were restricted to changes in organ weights with no histopathological organ changes with the exception of liver hypertrophy.

For consumer exposure assessment the following scenarios have been considered:

Direct skin contact with neat (*e.g.*, laundry pre-treatment) or diluted consumer product (*e.g.*, hand-washed laundry, hand dishwashing, surface cleaning); indirect skin contact via release from clothes fibres to skin, inhalation of detergent dust during washing process or aerosols generated by spray cleaners, oral ingestion of residues deposited on dishes, and accidental or intentional overexposure. From this an aggregate daily worst case exposure of 6.48 µg/kg bw/d has been estimated. SCHER agrees with the exposure assessment as carried out in the HERA-report for direct exposure.

The comparison of the aggregate consumer exposure of 6.48 µg/kg bw/d and the systemic NOAEL of 50 mg/kg bw/d results in a MOE of 7,716. The SCHER concludes that the use of AEs in laundry cleaners and household cleaning products is safe and does not create concern with consumer health.

(ii) AE usage in laundry cleaners and household cleaning products is not a cause for concern for the environment (in particular surface water, sediment, sewage treatment facilities, and soil).

Exposure assessment

The assessment of the environmental impact of AE usage in laundry cleaner and household cleaning products has been carried out using the available literature on environmental aspects and filling potential gaps in the data sets by interpolation of available data for specific homologues of the AEs (HERA, 2007a). The methods outlined in the TGD (2003) have been followed as much as possible. SCHER is of the opinion that this way of working was appropriate for establishing a risk assessment for the environment for AEs. The group of AEs has been split into substances with different carbon (C) number and different ethylene oxide (EO) chain length. The C-number varied from 8 to 18 and the EO-chain length from 0 to 22. This provided sufficient coverage of all potential AEs

SCHER agrees that volatilisation and abiotic degradation do not need to be further considered.

For indirect exposure through the food chain and bioaccumulation in aquatic organisms the HERA-report (2007a) contains data on the estimated octanol / water partition coefficients (K_{ow}) and some experimental data on established bioconcentration factors (BCFs) of several AE-homologues. Although the estimated log K_{ow}s and the extrapolated values using QSARs vary from 0.97 – 8.4 none of the experimentally determined BCF-values was higher than 400. Environment Canada and Health Canada (2006) had available one BCF-value of c. 800. The HERA-report and Environment Canada conclude that AE-homologues are not bioconcentrating compounds. This is in agreement with the information on toxicokinetics in mammals presented in the HERA report, indicating rapid metabolism and excretion.

To finally establish estimated concentrations for the different environmental compartments ($PEClocal_{dissolved}$, $PEClocal_{sediment}$, PEC_{stp} and $PECsoil_{local(30days)}$) for all the AE-homologues SCHER has some comments on the methods used and outcomes provided.

1. Generally, QSARs should not be considered as higher tier evaluation methods. However, the application of QSARs to all the individual AE-homologues may be considered as a higher tier evaluation tool as it gives a better estimation than if applied to the group of AEs.
2. For the estimation of the K_d-values of the individual AEs the proposals of van Compernolle et al. (2006) were applied. Some experimental values used for the extrapolations with the QSARs are questionable; e.g. in Table 4.1, the EO-number 3, C-number 12, 13 and EO-number 9 with EO-number 14, 15 do not fit in the series.
3. The results calculated in Table 4.2 could not be checked as different results were achieved using the QSAR mentioned under equation 4.2. The range of the values might be considered correct and so the risk characterisation coefficients will not be affected too much. But these inconsistencies undermine confidence in the conclusions.
4. Efforts to determine the sorption characteristics for AE-homologues have not been carried out using OECD 106 (Adsorption – Desorption using a Batch Equilibrium Method).
5. In Table 4.5 the half-lives of AE-homologues are estimated based on information on a few experimentally determined values. SCHER supports the approach, although the choice of a more conservative approach for AEs with higher C-number of 14 and higher EO-number of 11 does not seem to be realistic as the trend found in the extrapolation of the half-lives is not followed at higher C-number or higher EO-number.

SCHER is of the opinion that in general the calculations are giving the right order of magnitude for exposures but the exact values could not be reproduced by SCHER. This uncertainty should not significantly influence the exposure assessment in the report as the differences are small compared to the order of the parameter values.

The potential of anaerobic degradation of the AE-homologues has not been considered. However, it is the opinion of the SCHER that whenever AEs are in aerobic conditions after having been stored for some time in anoxic environments the aerobic degradation will resume and remove the substances from the environment with sufficient rates so that no additional environmental risk should be expected.

Effect assessment

The effect assessment has been conducted using an innovative approach. Basically, the proposed method, QSARs based on the K_{ow} and the combination of toxicity (and risk) by the added toxicity approach assuming a common mechanism of action is sound. There are some assumptions requiring additional information for a proper validation. These include: the transformation of EC20 QSAR data into EC10/NOEC values; the assessment factors selected; that the probabilistic approach (e.g. SSD) includes additional uncertainty as the distributions are based on the extrapolation of QSARs from one species to another within the taxonomic group, and that some chronic values are for survival rather than for sublethal endpoints.

A weak element in the assessment is the method selected for using the mesocosm data. Mesocosms should be considered higher tier assays that require an in-depth assessment case-by-case in terms of the relevance of the observed effects. The simplification of the results to a single NOEC and the development of a QSAR approach without a clear presentation of the direct and indirect effects observed in each system are not appropriate. In fact, the mesocosm NOECs are about one order of magnitude below the predicted EC10 for the most sensitive group. This should be given further consideration.

The use of the equilibrium partitioning methods for sediments and soils, compared with experimental data when available, is considered appropriate. However, as the approach depends on the $PNEC_{aquatic}$ organisms, the uncertainty described above also affects to these compartments.

Risk characterization

The information available to the SCHER does not allow an assessment of the proposed PNEC-values. However, the PEC/PNEC-ratios are sufficiently below one that it is unlikely that accounting for the remaining uncertainty would modify this conclusion. The PEC/PNEC values are well below 1 (surface water: 0.041, sediment: 0.316, sewage treatment plant: 0.007 and soil: 0.103) and so it would seem unlikely that accounting for the remaining uncertainty would modify the conclusion that risks to the environment are unlikely. However that cannot be finally substantiated until the PNECs are clarified.

The weakest element in the assessment is the consideration of potential effects on soil microbial communities. Although the assessment of sewage treatment plants does not indicate a potential concern, it should be noted that this assessment is based on assays with timings and endpoints very different from those relevant for the soil/pore water and groundwater environments. The equilibrium partitioning method is based on the aquatic toxicity data, which do not include effects on microbial populations.

(2) SCHER is requested to review the last HERA report (2007b) concerning LAS and specifically to comment whether it agrees with its main conclusions that:

(i) the risk characterisation as expressed by the PEC/PNEC ratio was below 1 for all environmental compartments, considering the recently reported PNEC values (~35 mg/kg versus 4.6 mg/kg in previous assessments) as calculated by new soil toxicity studies (e.g. Jensen et al., 2007; Krogh et al., 2007).

The revised HERA report (2007b) suggests increasing the PNEC soil from 4.6 mg/kg to 35 mg/kg based on the refinement of soil effect assessment, using some additional data an new estimations based on the SSD approach. The relevance of microbial toxicity data is weakly described in the HERA-LAS report, so SCHER considered the discussion published by Jensen et al. (2007). Here the EC10s for seven out of the ten measured endpoints were lower than the proposed endpoints. The relevance of the endpoints is described below.

Microbial iron reduction

According to Jensen et al. (2007) this is the most sensitive parameter. Iron oxides serve as electron acceptors in anaerobic bacterial respiration processes and are a preferential sorption substrate for LAS in agricultural soil (Kristiansen et al., 2003). The argument for disregarding this parameter in the effect assessment is that "in practice, however, LAS enters agricultural soil in sewage sludge, a stronger sorbent of LAS than soil components, and ploughing following amendment ensures adequate soil aeration, making anaerobic bacterial processes unlikely to be disrupted following LAS exposure". This argument is not supported by the SCHER as the PEC soil are estimated for the situation expected 30 days after application. The information provided for other endpoints is too scarce for a proper assessment; and the maximum tested concentrations in the higher tier studies are well below the PNEC derived from the plant and invertebrates SSD. Hence these studies cannot be used to confirm that the effects on microbial soil activity are covered by the proposed PNEC.

Therefore, the SCHER disagrees with the argument that soil microbial functions are covered by the proposed PNEC, and considers that a proper evaluation of the relevance of LAS effects on microbial activity is essential for a proper $PNEC_{soil}$ derivation.

Toxicity data on plants

The HERA-LAS report (HERA 2007b) presents ranges for EC50 and EC10 values on plants. However, the EC50 values are not discussed and only the EC10 values are used. The lowest value reported for the EC50 is 16 mg/kg soil, while the lowest reported EC10 is 52 mg/kg. Hence if the figure of 16 is reliable, the EC10 range does not cover the observed interspecies variability. The HERA report presents the following argument for disregarding the EC50 range "The figures presented in Table 15 are indicative of acute effects. They were not directly used in the present risk assessment, as higher tier data are available". However, the rationale cannot be accepted as in principle, the EC50 and EC10 presented for plants are in reality obtained from the same experiment and endpoint, and do not represent higher tier data in terms of ecological relevance. It is obvious that if the EC50 value of 15 mg/kg is reliable, an EC10 for this species lower than 15 should be expected, assuming a factor of at least 3 between the EC50 and the EC10, the expected EC10 would be one order of magnitude lower than the lowest value reported in the study.

Thus the SCHER considers that the EC50 values should be properly presented and assessed in terms of relevance and interspecies variability.

Regarding soil fauna, the EC50 values are slightly lower than the reported EC10, and therefore it is essential to identify if the EC10 values cover the most sensitive species observed in the acute test.

In conclusion, the SCHER considers that the information provided is not sufficient for justifying the newly proposed PNEC value of 35 mg/kg.

(ii) the ecotoxicological parameters of LAS have been adequately and sufficiently characterized and that the ecological risk of LAS is judged to be low.

The SCHER agrees with the proposed PNEC values for aquatic organisms and sediments. But as stated above, the proposed PNEC for soil is not substantiated with sufficient evidence, and unless additional justification can be provided, SCHER suggests maintaining the previous PNEC soil of 4.6 mg/kg. Moreover SCHER is of the view that the concept of PNEC is not appropriate for the sludge based on extrapolation from the soil scenario. In the information submitted to SCHER measured values in soil and sediment were recorded above the recommended PNECs; in addition, based on the proposed scenario, measured concentrations in some sludge samples if applied to agricultural soil would exceed the PNEC soil value in some cases. Thus SCHER considers that the potential risks of LAS to soil and sediment dwelling organisms should be further investigated. New techniques, such as probabilistic risk assessment, could be helpful for identifying the overall risk at the EU level and the circumstances that represent the highest concern.

(iii) the use of LAS in household laundry and cleaning poses no risk to consumer health.

The substantial data for mammalian toxicity of the LAS have been evaluated within the OECD HPV program. LAS are primarily used as cleaning agents in many laundry detergents and cleaners at concentrations up to 25%.

Acute dermal and oral toxicity is low and there is no eye and skin irritation of solutions up to 0.5-2.5%, moderate irritation occurred at 5% and more severely at higher concentrations up to 50%. Rinsing with water diminished eye irritation after 30 seconds and was slight with rinsing after 4 seconds of exposure. Accidental eye exposures during manufacturing and use of products containing LAS and other surfactants have been moderate, transient and reversible. Therefore labelling of consumer products containing LAS include warnings of the potential for eye irritation and first aid instructions to rinse with water.

Repeated oral and dermal applications to rats, mice, and monkeys resulted in NOAELs between 40 and 250 mg/kg bw/d, the corresponding LOAELs were 115 and 750 mg/kg bw/d. Effects observed are dose-dependent and include reduced body weight gain, diarrhoea, increases in relative liver weight, difference in enzymatic and serum-biochemical parameters and mild effects on the tubular epithelium in the kidneys.

The four long-term carcinogenicity studies in rats were negative, and there is no indication for genotoxicity, sensitization or adverse effects on reproduction.

The lowest NOAEL of 85 mg/kg bw/d from a rat drinking water study is seen as the most appropriate as the reference dose to evaluate the risk of human exposure.

The greatest potential of LAS consumer exposure is from pre-treatment of laundry, due to direct hand and forearm contact with neat product formulations, and from residual product on laundry clothing. The aggregation of dermal exposure was accomplished by adding the modelled exposures within a product category during hand-washing, neat pre-treatment, and residual on clothing. These exposure evaluations included a conservative default assumption of 100% absorption (vs. a measured value of 1%) and resulted in an aggregate daily exposure between 0.02 and 0.15 mg/kg bw/d. Taking into account the NOEL of 85 mg/kg bw/d the MOE is 4250-700, the indirect environmental exposure of LAS was considered negligible. The SCHER concludes that the use of LAS in household laundry and cleaning poses no risk to human health.

(3) in its opinion of 2005, SCHER concluded that single tests for evaluating anaerobic biodegradation are not sufficient, and that a combination of different testing conditions would be more appropriate. SCHER is requested to review the issue of anaerobic test methodology based on latest scientific evidence.

Review of anaerobic test methodology

Test methods to determine the ultimate anaerobic biodegradability of organic compounds at screening and simulation level are available. Screening tests are characterised primarily by a high test substance to biomass ratio, while simulation tests aim to reach realistic concentration ranges of the chemical and the bacterial biomass. In addition, screening tests usually have a relatively simple test design making them suitable for routine testing, whereas simulation tests require the use of ^{14}C labelled materials or specific analytical methods.

The OECD 311 describes a screening test designed to assess the ultimate anaerobic biodegradability of organic chemicals in heated digesters for anaerobic sludge treatment. The high concentration of test substance may inhibit the ultimate biodegradation of toxic chemicals. The OECD has published recently the first test for assessing inhibition (OECD 224) of a test substance on gas production in anaerobic digesters.

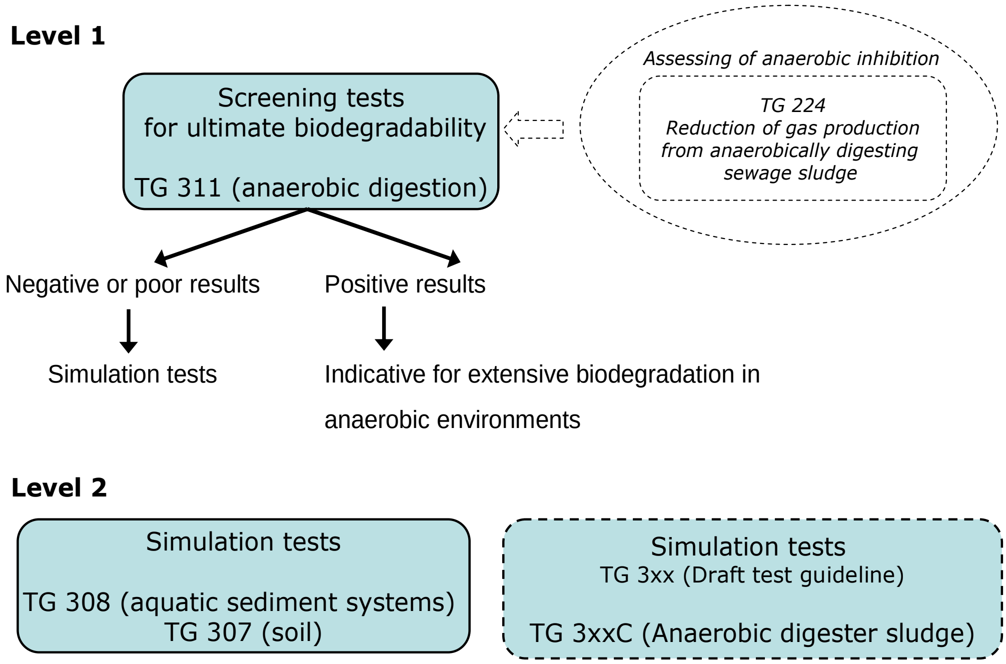
Simulation tests are specific for different anaerobic environments. Although different simulation systems have been described, only the Anaerobic Transformation test in Aquatic Sediment Systems (OECD 308) has been accepted for international standardization. The OECD Guideline 308 describes a laboratory test method to assess aerobic and anaerobic transformation of organic chemicals in aquatic sediment systems and allows the measurement of transformation rates of the test substance. This method is based on existing Guidelines developed for pesticides and has been applied so far to study the transformation of chemicals in fresh waters and sediments, but can also be applied to estuarine/marine systems. However, experimental data for surfactants based on these simulation tests are not available.

Currently, a new proposal for an OECD Guideline consists of five simulation methods to assess the primary and ultimate biodegradability of chemicals discharged to wastewater. This is under revision (OECD 3xx, draft test guideline). One of the simulation tests included is 3xxC "Biodegradation in Anaerobic Digester Sludge Test". The purpose of this test is to evaluate biodegradation during anaerobic sludge digestion. Given the simulation of in-situ conditions and the realistic ratio of the test substance/biomass the system can generate relevant kinetic data.

In recent years, the OECD has revised and adopted different anaerobic tests to fill the gap on anaerobic biodegradability testing. The obvious need, especially concerning chemicals which are insoluble and/ or are adsorbed onto sludge and sediments, for a screening method for assessing the anaerobic biodegradability in anaerobic digesters and for a test to determine inhibition of biogas production by chemicals have been covered by OECD 311 and OECD 224 methods adopted in 2006 and 2007, respectively. In addition, a standardized simulation test for assessing anaerobic degradation in aquatic sediment systems is available (TG 308, 2002) whereas a simulation test method for measuring biodegradation in sludge anaerobic digester is currently under revision (TG 3XX).

SCHER believes that the existing OECD methods for anaerobic biodegradation together with the simulation test currently under revision provide an appropriate methodology for the assessment of the anaerobic biodegradability of organic compounds.

OECD Standard methods for testing anaerobic biodegradability



Industry criticisms to the anaerobic methodology for testing surfactants

A recent review has been published on the issue of the anaerobic biodegradation of surfactants (Berna et al., 2007). This document was commissioned by ERASM (Environmental Risk Assessment and Management- a detergent industry group) and represents a recent compilation of data, interpretation and assessment of the relevance of anaerobic biodegradation of surfactants. Authors emphasized that available screening test methods to assess the anaerobic biodegradation do not simulate the real conditions prevailing in these anaerobic compartments but rather reflect more stringent conditions, due to the high test substance/biomass ratio, possibility of inhibitory effects and limited possibility for adaptation.

TEGEWA, a trade association of the German chemical industry, has recently carried out a study on the feasibility of OECD 311 standard method to study anaerobic biodegradability of surfactants (Schwarz et al., 2008). They emphasize the limitations of the screening methodology for assessing anaerobic biodegradability and its low reproducibility for testing surfactants. In the same line with respect to standard screening methods, Willing has recently investigated (2008) an alternative test for determining ultimate anaerobic biodegradation of surfactants based on the method DIN 38414, part 8 (1985). Willing (2008) reported on the apparent suitability of this test to determine the degradation rates of surfactants. In addition, he concluded that the resulting degradation data can be considered relevant for the assessment of the environmental compatibility of surfactants used in household detergents due to the fact that degradation is determined under real conditions.

The general approach for the evaluation of the biodegradability of organic compounds consists of tests varying in complexity, environmental realism and cost. This strategy allows preliminary screening of chemicals, using relatively simple and economic tests of ultimate biodegradability, with the identification of those compounds for which more detailed, and hence more costly, studies are required.

The potential biodegradability under anoxic conditions can be examined in a screening test for anaerobic biodegradability. Standardised screening tests determine the anaerobic mineralization by measurement of the methane and carbon dioxide production. It has been reported that the high surfactant/biomass ratio used in these tests can inhibit the anaerobic activity and lead to failure of the biodegradability assays. A positive result in an anaerobic screening test can be considered as highly predictive for extensive biodegradation in anaerobic environments. On the other hand, a poor biodegradation result is not necessarily a proof of recalcitrance in the real environment, but implies that further investigation could be necessary.

SCHER agrees that the use of high surfactant / biomass ratios in screening anaerobic assays may have little semblance to environmentally realistic concentrations and depending on the chemical can be inhibitory of the methanogenic activity and result in incorrect evaluations of biodegradation potential. But on the other hand, the use of low substrate concentrations can lead to end products not distinguishable from background microbial metabolism. No systematic effort to design widely applicable and routine biodegradation screening test procedures employing more realistic substrate concentrations has been made.

There is a new approach for the assessment of the anaerobic biodegradation proposed by Willing et al. 2008. The main differences regarding the standard OECD 311 method are the use of undiluted sludge as test medium and the presence of an external source of carbon. SCHER notes that there are currently limited data from this and believes that further work should be done in order to validate this test method.

New insights on surfactant anaerobic biodegradation

Lara-Martin et al. (2007) have reported for the first time the degradation of LAS in coastal marine sediments under anaerobic conditions, together with the presence of metabolites and the identification of microorganisms involved in this process. These authors concluded that the persistence of LAS in anoxic compartments, such as marine sediments, should be reconsidered when evaluating its environmental risks.

Despite most of the biodegradation studies show that LAS is poorly biodegradable under the anaerobic conditions of the laboratory test methods or in anaerobic digesters of sewage sludge, some findings suggest that partial anaerobic biodegradation of LAS is at least feasible and the environmental data reported by Lara-Martin et al. (2007) seem to indicate that LAS has at least a potential for degradation under anaerobic conditions.

However, further investigation is needed to confirm these results.

(4) SCHER is invited, in the light of the latest scientific evidence, to reconfirm the following statements, which were mentioned in the opinion of 2005:

(i) poor biodegradability under anaerobic conditions is not expected to produce substantial modifications in the risk for freshwater ecosystems as the surfactant removal in the WWTP seems to be regulated by its aerobic biodegradability.

The HERA-report (2004) contained no recent publications which affected the conclusion of SCHER in its opinion of 2005 (SCHER, 2005). Similarly recent publication, later than 2004, Garcia et al., 2005; Garcia et al. 2006a and b; all references cited in HERA, 2007b), did not give grounds for any change of that opinion.

(ii) the requirement for ready and ultimate biodegradability under anaerobic conditions is not by itself regarded as an effective measure for environmental protection.

Also there has not been any new scientific evidence for a change of this opinion.

3.3. Reply to question (B) HERA-2007c report on polycarboxylates in detergents

(1) SCHER is requested to review the recent HERA report on polycarboxylates in detergents (HERA, 2007c) and to comment whether it agrees with the main conclusions that:

(i) the use of polycarboxylates in detergents results in risk characterization ratios (RCR) less than one, indicating no concern, for all environmental compartments with the exception of the soil local compartment for P AA/MA (an acrylic and maleic acids copolymer or its sodium salt) as a consequence of the standardized test design

Exposure assessment

There are two general caveats. First, most fate and exposure data have been unpublished in industry reports that could not be checked. Second, HERA does not present sufficient information on test conditions and results for a proper assessment. Thus the following comments are made taking the data and reliability index presented in the HERA report as given.

P-AA: Several biodegradation tests have been carried out with P-AA, mostly CO_2-evolution tests and OECD-301, -302, and -303-tests. Generally, a degradation rate was determined around 20 to 25%, with some deviation into the lower range, 5 – 6%, and in the higher range, up to even 99%. The conclusion is that the P-AA substances should be considered as not readily biodegradable. SCHER is in agreement with this assessment. Also under anaerobic conditions P-AA showed no biodegradability. Most of the substances were adsorbed to soil and sediment, about 80%, however no K_d- or K_{oc}-values were given. SCHER is of the opinion that a better exposure assessment could have been carried out if in addition K_d-values would have been determined.

P-AA/MA: The available biodegradation and elimination data for several P-AA/MA compounds show the same overall picture as for the P-AA-substances. Generally, the values are somewhat higher for aerobic conditions. For anaerobic conditions, no degradation could be observed. SCHER agrees with these findings.

Effect assessment

Again most ecotoxicity data are in unpublished industry reports and the HERA report does not present sufficient information on test conditions and results for a proper assessment. Thus the following comments are made taking the data and reliability index presented in the HERA report as given.

P-AA: Despite its very large molecular weight, acute and chronic effects on aquatic organisms have been observed. In general, the information is scarce and a significant variability in the toxicity results for the same species and endpoint is observed even for samples with the same molecular weight (*i.e.*, chronic NOEC on *Daphnia magna* ranging from 5.6 to 450 for MW of 4500). The PNEC derivation for aquatic organisms properly follows the TGD although the inter-sample variability should be further investigated. For the derivation of a $PNEC_{sediment}$, the information is very limited; thus, in addition to the experimental data, the equilibrium partitioning method should be applied and the lowest value (derivation from experimental data or derivation through the partitioning method) selected as PNEC sediment. No information on soil microbial functions is available; thus only a tentative $PNEC_{soil}$ can be derived.

P-AA/MA: Despite its very large molecular weight, chronic effects on aquatic organisms have been observed. In general, the information is scarce and a significant variability in the toxicity results for the same species and endpoint is observed even for samples with the same molecular weight (*i.e.*, chronic NOEC on *Daphnia magna* ranging from 3.75 to 350 for MW of 70000). The PNEC derivation for aquatic organisms cannot be accepted. as the lowest NOEC observed for daphnids in a reliable (level 1) assay is not used under the justification that effects are of limited relevance; this argument is not sufficiently justified. For these large molecular weight substances gill uptake is expected to be limited but other routes including the oral uptake can be of significant relevance and cannot be disregarded as unrealistic. The test conditions are environmentally relevant, and, therefore, the effects descried as secondary uptake of the precipitates should also expected to occur under natural conditions. In addition, the longest study on fish is reported as OECD 205, which corresponds to an avian toxicity guideline, and no report on the measured endpoint is presented. In the absence of higher tier tests, that could indicate a low relevance, the PNEC should be derived from the lowest reliable value, 3.75 mg/l, using an application factor of 50, that could be reduced to 10 if the endpoint measured in the fish test could be consider as a sub-lethal chronic endpoint. Inter sample variability should be further investigated. The proposed $PNEC_{sediment}$ cannot be accepted as it is obtained from the non acceptable $PNEC_{aquaticorganisms}$ using the equilibrium partitioning method. Both PNECs should be at least one order of magnitude lower than those proposed (pending confirmation that fish are properly covered and that therefore an application factor of 10 is acceptable). No information on soil microbial functions is available; thus only a tentative $PNEC_{soil}$ can be derived.

Risk characterisation

The proposed changes in the $PNEC_{aquaticorganisms}$ derivation for P-AA/MA have consequences on the risk assessment. Unfortunately the SCHER cannot provide a final answer on the potential environmental risk due to the lack of information on the reliability of fish chronic study. PEC/PNECs ratios would remain at or below one if an application factor of 10 is used in the PNEC derivation, while PEC/PNECs higher than 1 are obtained in the local and regional risk assessments for aquatic organisms including sediment dwellers when a factor of 50 is applied.

In addition SCHER considers that information on soil microbial functions is essential for the assessment of these chemicals.

Therefore SCHER concludes that additional information is required before it can be concluded that these chemicals are of low environmental concern

(ii) the use of polycarboxylates in household laundry products and automatic dishwashing detergents poses no risk to the environment from the use by consumers.

The use of polycarboxylates in household laundry products and automatic dishwashing detergents should be considered as a subset of the general use of polycarboxylates as dealt with under item (i). Therefore, it should be concluded that the environmental exposure to this application is lower than to the P-AA-substances of item (i) itself.

SCHER has been informed that the use of polycarboxylates in household laundry products and automatic dishwashing machines consists of about 95% of all detergent use of polycarboxylates. The HERA risk assessment (HERA 2007c) was therefore only carried out for domestic use. Hence SCHER is of the opinion that risk assessment obtained under item (i) does not need further nuancing for the situation under (ii). Additional information is, therefore, required for answering the question posed under (ii).

(2) SCHER is requested to review any recent evidence concerning the potential risks of phosphonates to the environment at the EU level.

An additional request to the detergent industry did not result in information requested on this topic. Therefore, as no recent evidence concerning the potential risk of phosphonates to the environment at the EU level was brought forward, it is not possible for SCHER to comment on this topic. SCHER suggests the Commission comes back to this question at an appropriate time.

(3) Considering the recent scientific evidence for polycarboxylates and phosphonates, SCHER is requested to clarify whether a potential move to zeolite-based detergents at EU level would increase (or not) the health and environmental risks from detergents.

Polycarboxylates

In its opinion on "Non surfactant organic ingredients and zeolite-based detergents" adopted May 29, 2007 SCHER concluded that P-AA and P-AA/MA have a low acute toxicity after oral and dermal administration.

Some P-AAs were slightly irritating to rabbit eyes. No sensitizing potential has been identified. There is no indication of genotoxic or teratogenic effects. A long-term carcinogenicity study is not available. The NOEL in the 90 days drinking water study is 1000 mg/kg bw/d. Due to the low dermal absorption of about 0.3% the SCHER concluded that oral ingestion is the predominant route of exposure. Since exposure via drinking water is about 0.1 µg/kg bw/d the MOE is 10,000,000. However, it has pointed out that this estimate needs to be confirmed by appropriate data on concentrations in drinking water, food and other possible contributors to human exposure.

Polyphosphonates

In its opinion on "Non surfactant organic ingredients and zeolite-based detergents" adopted May 29, 2007 SCHER described that the phosphonates show no sensitizing, mutagenic, carcinogenic or reproductive effects and the acute oral and dermal toxicity is low. Based on an oral long-term carcinogenicity study in rats a NOEL of 100 mg/kg bw/d has been determined. The direct skin contact from laundry tablets and powder is considered to be insignificant. Exposure via drinking water is 0.0032 µg/kg bw/d. Similarly, inhalation of aerosols and powder is about 0.003 µg/kg bw/d.

Due to the use in carpet cleaners, as machine dishwashing detergent, laundry additives, compact laundry detergents, regular laundry detergents, hand dishwashing detergents the total daily exposure is about 0.5 µg/kg bw/d including residues on eating utensils and dishes.
Taking into account a daily human exposure of 0.5 µg/kg bw/d, a MOE between NOEL and human exposure of 200,000 has been calculated. Consequently, the SCHER has not considered the use levels as a risk to human health. At present SCHER has not found additional information, which questions this conclusion.

4. LIST OF ABBREVIATIONS

AE	Alcohol Ethoxylates
BCF	Bioconcentration Factor

DG	Directorate General
DIN	Deutsches Institut für Normung
DT50	Degradation or dissipation time for 50% of the active substance
ECETOC	European Centre for the Ecotoxicology and Toxicology of Chemicals
ECxx	Effect Concentration for xx % of the population
EO	Ethylene Oxide
ERASM	Environmental Risk Assessment and Management
HERA	Human and Environmental Risk Assessment
HPV	High Production Volume
ISO	International Standardization Organisation
K_d	Adsorption coefficient
K_{oc}	Adsorption coefficient normalised for organic matter
K_{ow}	octanol / water partition coefficients
LAS	Linear Alkylbenzene Sulphonates
MOE	Margin of Exposure
MW	Molecular Weight
NOAEL	No Observed Adverse Effect Level
NOEC	No Effect Concentration
NOEL	No Observed Effect Level
o.m.	organic matter
OECD	Organization for Economic Cooperation and Development
P-AA/MA	Homopolymers of acrylic acid (P-AA) and copolymers of acrylic/ maleic acid (MA)
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
QSAR	Quantitative Structure Activity Relationship
RCR	Risk Characterization Ratio
RPA	Risk and Policy Analysis
SCHER	Scientific Committee on Health and Environmental Risks
SSD	Species Sensitivity Distribution
TEGEWA	Tendering Generating Watching (Trade Association of the German Chemical Industry)
TGD	Technical Guidance Document
TG	Test Guideline
WWTP	Waste Water Treatment Plant

5. REFERENCES

Berna J.L., G. Cassani, D. Hager, N. Rehman, I. López, D. Schowanek, J. Steber, K. Taeger,T. Wind. (2007) Anaerobic Biodegradation of Surfactants- Scientific Review. *Tenside Surfactant Detergents* 44 312-347.

Birch R. R., Biver C., Campagna R., Gledhill W.E., Pagga U., Steber J., Reust H. and Bontinck W.J. (1989) Screening of chemicals for anaerobic biodegradation. *Chemosphere* 19, 1527-1550. *(Also published as ECETOC Technical Report No. 28, June 1988).*

Cameotra S.S., Makkar R.S. (2004) Recent applications of biosurfactants as biological and immunological molecules. Crr.Opin.Microbiol. 7, 262-266.

Cheng K. Y., Lai K. M., Wong J.W.C. (2008) Effect of pig manure compost and nonionic surfactant Tween80 on phenanthrene and pyrene removal from soil vegetated with *Agropyron elongatum*. *Chemosphere*, in the press.

DIN 38414, Part 8 (1985) Sludge and sediments (Group S) – Determination of the amenability to anaerobic digestion (S8). *German standard methods for the examination of water, waste water and sludge.*

Dunbabin V.M., McDermott S., Bengough A.G. (2006) Upscaling from rhizosphere to whole root system: modelling the effects of phospholipid surfactants on water and nutrient uptake. *Plant and Soil* 283, 57-72.

Environment Canada and Health Canada (2006). Response to the ICG Aliphatic Working Group's Proposal Regarding Environment Canada's Preliminary Categorization of Ethoxylated Aliphatic Alcohols. February 2006, Ottawa Canada. 4p.

HERA 2004. Human and environmental risks assessment of Linear Alkylbenzene Sulphonates.

HERA 2007a. Human and environmental risks assessment of Alcohol Ethoxylates.

HERA 2007b. Human and environmental risks assessment of Linear Alkylbenzene Sulphonates.

HERA 2007c. Human and environmental risks assessment of Polycarboxylates used in detergents.

ISO 11734 (1995) Water Quality - Evaluation of the ultimate anaerobic biodegradation of organic compounds in digested sludge - Method by measurement of the biogas production. *International Organization for Standardization.*

Jensen J., N. Schraepen, S.R. Smith, P.H. Krogh, D.J. Versteeg, A. Temara (2007) European risk assessment of LAS in agricultural soil revisited: Species sensitivity distribution and risk estimates. *Chemosphere* 69, 880-892.

Kristiansen, I.B., Jonge, H. de., Nornberg, P., Mather-Christensen, O., Elsgaard, L. (2003). Sorption of linear alkylbenzene sulfonate to soil components and effects on microbial iron reduction. *Environ. Toxicol. Chem*. 22, 1221–1228.

Krogh P.H., C.V. Lopez, G. Cassani, J. Jensen, M. Holmstrup, N. Schraepen, E. Jørdensen, Z. Gavor, A.Temara (2007) Risk assessment of LAS in agricultural soil revisited: Robust chronic toxicity tests for *Folsonia candida* (Collembola), *Aporrectodea caliginosa* (Oligochaeta) and *Enchytraeus crypticus*(Enchytraeidae). *Chemosphere* 69, 872-879.

Lara-Martin P.A., Gómez-Parra A., Köchling T., Sanz J.L., Amils R., González-Mazo E. (2007) Anaerobic Degradation of Linear ALkylbenzene Sulfonates in Coastal Marine Sediments. *Environ. Sci. Technol*. 41, 3573-3579.

López I., Prieto C.A., Berna J.L., Cantarero S. (2008) Distribution of LAS and soap in Spanish sewage sludge. *7th World Surfactant Congress CESIO*, Paris, June 2008.

Lynch J.M., Benedetti A., Insam H., Nuti M.P., Smalla K., Torvik V., Nannipieri P. (2004) Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. *Biol. Fertil. Soils* 40, 363-385.

Moreno-Caselles J., Prats D., Moral R., Perez-Murcia M.D.,Perez-Espinosa A., Paredes C., Leon V. (2006) Effects of linear alkylbenzene sulfonates (LAS) in sewage-amended soils on nutrient content of broccoli plants. *Comm. Soil Sci. and Plant Anal.* 37, 2605-2614.

Muller K., Magesan G.N., Bolan N.S. (2007§) A critical review on the influence of effluent irrigation on the fate of pesticides in soil. *Agric. Ecosyst. Env*. 120, 93-116.

Mulligan CN (2005) Environmental applications of biosurfactants. *Environ.Pollut*. 113, 183-198.

Nuck B.A, Federle T.W. (1996). Batch test for assessing the mineralization of ^{14}C radiolabelled compounds under realistic anaerobic conditions. *Environ Sci. Technol* 30 3597-3600.

OECD 224 (2007) Determination of the inhibition of the activity of anaerobic bacteria-reduction of gas production from anaerobically digesting (sewage) sludge. *OECD Guidelines for the Testing of Chemicals.*

OECD 301 (1992). OECD Guideline for Testing of Chemicals; Ready Biodegradability, OECD Paris, 1992, 62 pp.

OECD 302 (1981, 1992, 1981) OECD Guideline for Testing of Chemicals; Inherent Biodegradability: A: Modified SCAS Test, OECD Paris, 1981, pp. 7; B: Zahn-Wellens/ EMPA Test, OECD, Paris, 1992, pp. 8; C: Modified MITI Test (II), OECD, Paris, 1981, 21 pp.

OECD 303 (2001), OECD Guideline for Testing of Chemicals; Simulation Test Aerobic Sewage Treatment, OECD Paris, 2001, 50 pp.

OECD 304 (1981), OECD Guideline for Testing of Chemicals; Inherent Biodegradability in Soil, OECD Paris, 1981, 11 pp.

OECD 307 (2002). OECD Guideline for Testing of Chemicals; Aerobic and Anaerobic Transformation in Soil, OECD Paris, 17 pp.

OECD 308 (2002) Aerobic and Anaerobic Transformation in Aquatic Sediment Systems. *OECD Guidelines for the Testing of Chemicals.*

OECD 308 (2002). OECD Guideline for Testing of Chemicals; Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, OECD Paris, 2002, 19 pp.

OECD 311 (2006) Anaerobic Biodegradability of Organic Compounds in Digested Sludge: By Measurement of Gas Production. *OECD Guidelines for Testing of Chemicals.*

OECD 3xx Draft Test Guideline (2007) Simulation tests to assess the primary and ultimate biodegradability of chemicals discharged to wastewater: Biodegradation in Wastewater, Activated Sludge, Anaerobic Digester Sludge, Mixing Zone for Treated Effluent and Surface water and Mixing Zone for Untreated Wastewater and Surface water. 3xxB Biodegradation in Activated Sludge. *OECD Guidelines for the Testing of Chemicals.*

Saichek S.E., Reddy K.R. (2005) Electrokinetically enhanced remediation of hydrophobic organic compounds in soils: a review. *Crit.Rev.Environ.Sci.Technol*. 35,115-192.

SCHER (2005) SCHER scientific opinion on "The environmental risk assessment of non biodegradable detergent surfactants under anaerobic condition", *25 November 2005*.

Schowanek D., H. David, R. Francaviglia, J. Hall, H Kirchmann, P.H. Krogh, S. Smith, N Schraepen (2007) Probabilistic risk assessment for LAS in sewage sludge used on agricultural soil. *Regulatory Toxicology and Pharmacology* 49, 245-259.

Schwarz K., Schwarz H., Taeger K., Top M., Willing A. (2008) Methodology of Anaerobic Biodegradability of Surfactants. *7thWorld Surfactants Congress CESIO*, Paris, June 2008.

Singh A, Van Hamme JD, Ward OP (2006) Surfactants in microbiology and biotechnology. Part 2. Application aspects. *Biotech.Adv*. 25, 99-121.

Steber J., Wierich P. (1987) The anaerobic degradation of detergent range fatty alcohol ethoxylates. Studies with ^{14}C-labelled model surfactants. *Water Res*., 21, 661-667.

Temmink, H. and B. Klapwijk (2004). Fate of linear alkylbenzene sulfonate (LAS) in activated sludge plants, *Water Res.* 38, 903—912.

TGD (2003), TGD (2003).Technical Guidance Document on Risk Assessment *in support* of Commission Directive 93/67/EEC, Commission Regulation (EC) No. 1488/94 and Directive 98/8/EC of the European Parliament and of the Council. European Commission. EUR 20418 EN/1. 1996 and April 2003.

Van Hamme JD, Singh A, Ward OP (2006) Surfactants in microbiology and biotechnology. Part 1. Physiological aspects. *Biotech.Adv*. 24, 604-620.

Willing A. (2008) A new approach for the assessment of the anaerobic biodegradability of surfactants. *7th World Surfactant Congress CESIO*, Paris, June 2008. (+ Test Report: Anaerobic Degradability under Sewage Plant Simulation Conditions. A. Willing, Cognis GmbH; confidential report).