

# **LAS and Nonylphenols**

Gro Fremmersvik and Nis Hansen

Eurofins Danmark A/S



## Acknowledgement

This work has been carried out with financial support from the following EU Member States:  
UK, Germany, France, Italy, Spain, Nordic countries, Netherlands, Denmark, Austria, EU DG  
XI and JRC, Ispra.

## CONTENTS

1.	INTRODUCTION	7
2.	EXISTING STANDARDS OR DRAFT STANDARDS	9
3.	EVALUATION OF DRAFTING A HORIZONTAL STANDARD	10
3.1	Pretreatment	11
3.2	LAS	11
3.2.1	Extraction	12
3.2.2	Clean-up	13
3.2.3	Derivatization	13
3.2.4	Chromatographic analysis	14
3.2.5	Identification and quantification	15
3.3	Nonylphenols	16
3.3.1	Extraction	16
3.3.2	Clean-up	18
3.3.3	Derivatization	18
3.3.4	Chromatographic analysis	18
3.3.5	Identification and quantification	19
3.4	Nonylphenolpolyethoxylates	20
4.	CRITICAL POINTS AND RECOMMENDATIONS	21
4.1	Pretreatment	21
4.2	LAS	21
4.3	Nonylphenols	22
	REFERENCES	25

## LIST OF TABLES

Table 1	LAS - selection of detector for LC analysis of different matrices.....	15
Table 2	Nonylphenols - Extraction techniques for different matrices.....	17
Table 3	LAS - Main issues for further work.....	22
Table 4	Nonylphenols - Main issues for further work.....	23

## SUMMARY

First, the need has been identified for horizontal standards for LAS and nonylphenols in sludge and soil samples. If LAS and nonylphenols are found relevant for treated biowaste and sediments, the standards shall include these matrices, if possible.

The two parameters LAS and nonylphenols have been placed in category C on the basis of the terms of HORIZONTAL. This implies that no standards (or draft standards) exist as a basis for the development of horizontal standards for LAS and NP, and that pre-normative research will have to be conducted before horizontal standards can be drafted.

The analytical procedures used for LAS and NP are described. For each analytical step the current knowledge has been described and discussed, and the basis for the preparation of horizontal standards has been evaluated. This indicates that a good basis exists for the preparation of harmonized and horizontal standards for LAS and nonylphenols.

The main issues for the pre-normative research have been presented and discussed.

It is recommended that the horizontal standard for LAS should be a method based on:

1. Drying of the sample – either by freeze-drying or by conventional drying.
2. Extraction of the dry test sample with methanol or 0.5M NaOH in methanol.
3. A clean-up of the extract – for some matrices. The clean-up is carried out on a SPE column.
4. Analysis by liquid chromatography (LC) using UV, fluorescence or MS detectors. The selection of detector depends on the matrix.
5. Calculation based on internal standard procedure.

A number of issues need to be addressed in the pre-normative research on LAS. These are: The range of matrices, storage conditions, drying procedure, extraction technique and extraction solvent, procedure for clean-up, selection of LC detector and internal standard.

For NP it is recommended that the horizontal standard should be a method based on:

1. Analysis performed on a dry or wet test sample.
2. Extraction of the test sample with an organic solvent or solvent mixture using shaking or Soxhlet.
3. A clean-up of the extract – for some matrices. The clean-up is carried out on a SPE column of silica, alumina or florisil.
4. A derivatization - only if necessary!
5. Analysis by gas chromatography using mass spectrometric detector (GC-MS). When no derivatization is used ion 135 is used as the target ion and the ions 107 and 149 as diagnostic ions for confirmation of the identity.
6. Calculation based on internal standard procedure. Labelled 4-n-nonylphenol is used as internal standard for NP.

A number of issues need to be addressed in the pre-normative research on NP. These are: The inclusion of the mono- and diethoxylates in the standard, the range of matrices, storage conditions, use of wet or dry samples and the drying procedure (if used), extraction technique and extraction solvent, procedure for clean-up, and procedure for derivatization (if necessary).

The nonionic detergents NPPE (nonylphenol polyethoxylates) are briefly mentioned since they are the presursors of NP and by their degradation may make a contribution to the load of nonylphenols in the environment. This will be further addressed in the continuation of project HORIZONTAL.

## 1. INTRODUCTION

The objective of the project is to develop horizontal and harmonised European standards in the fields of sludge, soil, and treated biowaste to facilitate regulation of these major streams in the multiple decisions related to different uses and disposal governed by EU Directives.

The working document on revision of the Sewage Sludge Directive (86/278/EEC; 3<sup>rd</sup> draft April 2000), the working document on biowaste (draft February 2001) and the Soil Monitoring Directive call for standards on sampling, on hygienic and biological parameters, on methods for inorganic and organic contaminants, and for mechanical properties of these materials. This project considers the development and implementation of horizontal standards to be used for sludge, soil, and biowaste. When materials can not be utilised, landfilling becomes important, in which case leaching becomes an issue as stipulated by the Council Directive 1999/31/EC on the landfill of waste.

In the 3<sup>rd</sup> draft of the Sewage Sludge Directive limit values are presented for many organic contaminants in sewage sludge; among these are Linear Alkylbenzene Sulphonates (LAS) and nonylphenols (NP) for which the limit values are 2600 mg/kg dry matter and 50 mg/kg dry matter respectively. In the national regulations, limits for LAS are set only in Denmark (1300 mg/kg dry matter), and limits for NP are set in Denmark (10 mg/kg dry matter) and in Sweden as an informal agreement (50 mg/kg dry matter).

Nonylphenols are included in most lists of hazardous substances such as OSPAR and HELCOM. The compounds are included in the EC priority list in the Water Framework Directive by the name of “nonylphenols”, and by the 26th amendment to the directive 76/769/EEC nonylphenol and nonylphenol ethoxylate is expected to be added to Annex I in the directive.

On this basis LAS and nonylphenols were included in the project HORIZONTAL.

LAS and nonylphenols (NP) are not single compounds. NP is a mixture of isomers and LAS is a mixture of homologues with many isomers within each homologue group. LAS is used as an important anionic detergent in washing and cleaning agents in households and industry, and the major source of NP is as degradation products from nonylphenol polyethoxylates (NPE or NPPE) used as nonionic detergents. Nonylphenols as such are also used for specific technical purposes, and this use may make a small contribution to the emissions of NP to the environment.

After use LAS is degraded to SPC (sulfophenylcarboxylic acids). These are not included as a analyte in the method for LAS, but is briefly mentioned in the discussions.

NPE are used as nonionic detergents in washing and cleaning agents as well as in shampoos, lotions etc. Most of the polyethoxylates are present in the domestic or industrial wastewater, where they are degraded - mainly by the release of ethoxylate groups. The degradation product nonylphenols (NP) has a much longer half-life time than the predecessors in the degradation chain. In the sewage treatment process, being much more apolar than NPE the NP is adsorbed onto sewage solids and therefore is concentrated in the sewage sludge.

LAS and NP are well known contaminants in sewage sludges, and LAS and NP have been selected as new work items in the Sludge Committee CEN/TC 308. The Waste Committee CEN/TC 292 has resolved that the parameters are not relevant at the moment. The Soil

Committee CEN/TC 345 has started its work only recently and has not yet been able to discuss the relevance of the two parameters in relation to soil.

Sludges may be re-cycled for agricultural purposes and therefore there will be a need for the measurement of LAS and NP in soil samples when sludge is applied onto an agricultural area.

Sediments are handled by the Water Committee CEN/TC 230 but may be included in Project HORIZONTAL.

**In summary it is concluded that there is a need for horizontal standards for LAS and nonylphenols in sludge and soil samples. If LAS and nonylphenols are found relevant for treated biowaste and sediments, the standard shall include these matrices, if possible.**

In the coming Sewage Sludge and Bio-waste Directives, LAS and nonylphenols may be included. Whether this will mean only the NP or in addition some of the ethoxylates is uncertain. Knowledge about the degradation of NPPE in sludges and soils is still lacking, and the intention is to examine the degradation process in the Project HORIZONTAL. However, for NP1EO+NP2EO many analyses indicate that they may make a significant contribution to the total load of nonylphenols in the sludges.

The desk study will include analytical methods for the determination of LAS and nonylphenols (NP), as well as a short description of methods for the polyethoxylates (NPPE) and the mono- and diethoxylates (NP1EO+NP2EO), which will often be determined by other analytical methods.

If a horizontal standard for NP can determine the mono- and diethoxylates, it may therefore make good sense to include these in the horizontal standard.



## 2. EXISTING STANDARDS OR DRAFT STANDARDS

For LAS and nonylphenols very few official methods exist and there are no international standards for any of the parameters.

It has therefore been very important to get information about ongoing work from the CEN and ISO bodies and about existing national methods.

A letter has been sent by the project leader to the CEN and ISO bodies (dated December 12, 2002) to identify, whether activities relevant to the issue at hand are missed. For LAS and nonylphenols the relevant groups are:

CEN/TC 292/WG 5 Waste

CEN/TC 308/WG 1/TG 4 Sludge

CEN/TC 345 Soil (the CEN TC has recently been established)

ISO/TC 190/SC 3 Soil

In these committees no draft standards are being discussed. However, a Draft International Standard (DIS method) exists for analysis of nonylphenols in water:

Water quality – Determination of selected alkylphenols – Part 1: Method for non-filtered samples using liquid extraction and gas chromatography with mass selective detection. ISO/DIS 18857-1 /1/.

The determination of LAS and nonylphenols has been discussed at the meeting of CEN/TC 308/WG 1/TG 4 in Oslo March 2003. For LAS no additional method was presented and for nonylphenols the German representatives presented a draft method from Northrhine Westfalia State Environment Agency dated March 17, 2003 /3/. In resolution 1 from the meeting, members of TG 4 are requested to supply relevant information regarding the desk studies to the work package leader /4/. Until now, no additional documents or information have been received.

The only known national methods are the Danish methods for LAS and nonylphenols /2/. In order to get information about other national methods, a letter has been sent from the project leader to Member States (dated December 12, 2002). The letter inquired about relevant national work on the topics.

No further information has been presented by the Member States, and therefore the methods to be considered are the Danish national method for LAS and nonylphenols and the method for nonylphenols from Northrhine Westfalia State Environment Agency.

### 3. EVALUATION OF DRAFTING A HORIZONTAL STANDARD

The Consortium recognises three types of horizontal and/or harmonised standards:

- Standards (or draft standards) on the same topic already existing in different TCs to be evaluated, preferably as horizontal standards unless this level of horizontal standardisation is not feasible for well-defined reasons (category A).
- Standards (or draft standards) currently existing in one TC but with a potentially wider applicability (category B).
- Pre-normative work leading to a first draft of a standard (category C).

For LAS and nonylphenols it is assessed that they both belong to category C.

The presence of a DIS for nonylphenols in water is not considered sufficient to place this method in a higher category, since the measurement in solid matrices involves analytical problems which do not exist at measurements in water samples.

It is therefore foreseen that for both groups of compounds pre-normative research will have to be conducted. It is the purpose of the desk study to select methods to be investigated and to identify the critical points that should be included in the further research needed in order to prepare a draft horizontal standard.

This chapter describes the different techniques used for the determination of LAS and nonylphenols. The description is based on a limited literature survey and current knowledge about the analysis of LAS and nonylphenols. Since the two parameters are generally analysed separately, the methods are discussed separately.

Sample storage is an important part of the standard and will therefore be covered.

Since LAS and NPPE are used in many washing agents, there is a risk that the use of washing agents in the laboratory can result in high blank values in the analysis of LAS and NP, especially at the analysis of matrices where a low LOD is needed. It must therefore be part of the horizontal standards, that each laboratory must make its own check analysis to ensure that the laboratory background is acceptable and does not influence the result.

For both parameters the analytical procedure may be separated into the following steps:

- Pretreatment of the sample
- Extraction
- Clean-up
- Derivatization (if necessary)
- Chromatographic analysis
- Identification and quantification

Procedures for pretreatment will only be mentioned briefly since it is not considered part of the horizontal standard. Nevertheless the standard must describe which pretreatment shall be used for the different kinds of samples.

The purpose of the literature search has been to identify some important references as basis for the development of horizontal standards.

For LAS, the references comprise the Danish method for sludge analysis /2/ and 12 references from the scientific literature /5/-/16/.

For nonylphenols the references comprise the ISO/DIS for water analysis /1/, the Danish method for sludge analysis /2/, the Northrhine Westfalia draft method for sludge /3/ and 7 references from the scientific literature /17/-/23/.

### 3.1 Pretreatment

Although procedures for pretreatment are not part of the horizontal standard, it is necessary to define what sample materials will be relevant by the analysis of LAS or nonylphenols. The following matrices are included:

- Sewage sludge
- Industrial sludge
- Sediment
- Compost (stabilized)
- Biowaste (not stabilized) with organic matter of mainly natural origin
- Soils with different structure (clay, sand etc.)

Other waste materials are not included for the moment, since no interest has been expressed so far.

There are two main problems in the pretreatment step of the analysis: (i) to obtain a representative and homogeneous sub sample (test sample) from the total sample (laboratory sample), and (ii) to decide whether to make the extraction on a wet sample or a dried (often freeze dried) test sample. For soil samples several ISO documents on pretreatment exist:

ISO 11465 – Pretreatment of samples for physico-chemical analyses (1994)

ISO/FDIS 14507 Guideline – Guidance for sample pretreatment for determination of organic contamination in soil

ISO/CD 16720 – Pretreatment of samples by freeze drying for subsequent analysis

The selection of procedures for pretreatment highly depends on the extraction steps used, and it is therefore treated in the chapters about extraction of LAS and nonylphenols.

Often, conditions of storing samples of sludges, wastes and soils are not described in detail. For most matrices the sample storage is expected to be freezing. This is the case for both parameters. Practical conditions like sample containers and special sample materials may call for the use of alternative methods.

### 3.2 LAS

The most important quality parameters are the limit of detection (LOD) and the selectivity.

The relevant LOD will vary from one matrix to the other. In sewage sludges an LOD of 50-100 mg/kg dry matter will probably be sufficient, whereas in sediments the limit of detection must be below 1 mg/kg dry matter, maybe even 0.1 mg/kg dry matter.

The demand for a high selectivity is important for the selection of the analytical technique, e.g. selection of GC or LC detector. Some detectors like the mass spectrometer (MS) have a high selectivity and other detectors which are more general detectors, have a lower selectivity (e.g. UV detector for HPLC). The selection of the detector therefore also implies whether a clean-up step is necessary or not.

The description of the methods for LAS analysis will be separated in the analytical steps:

- Extraction
- Clean-up
- Derivatization (if necessary)
- Chromatographic analysis
- Identification and quantification

### 3.2.1 Extraction

Being an anionic detergent LAS has a hydrophobic and a hydrophilic end of the molecule. This implies that although LAS is very water soluble, it can nevertheless be extracted from water by an organic solvent or by solid phase extraction (SPE). This also means that SPE may be used as a clean-up procedure for LAS.

The selection of extraction technique and extraction solvent depends on the matrix. For the matrices described in chapter 3.1 LAS will be adsorbed to the organic part of the sludge or soil particles, etc., since LAS has a nonpolar end of the molecule. Due to the polar sulphonate group in LAS, the binding to the particles will be less than for the classical nonpolar contaminants like PAH and PCB. This also implies that LAS is more easily extractable even from clayrich soil – samples where the nonpolar contaminants may be bound inside the aggregates and therefore difficult to extract from the solid materials.

As the polar end of the LAS molecule is a sulphonic acid, the addition of bases like sodium hydroxide may in some cases facilitate the extraction with an organic solvent.

#### *Extraction techniques*

Almost all references have used extraction of a dried sample. Only a few have extracted a wet sample /16/, and several extractions were necessary to obtain a good recovery.

Drying of the sample can best be done by freeze-drying or by conventional drying. The Danish national method for sludge /2/ apply overnight drying at 60°C.

The extraction techniques used for LAS in solid samples are mainly shaking, reflux, Soxhlet, and PLE (Pressurized Liquid Extraction).

When extraction is performed on a dry sample, satisfactory recoveries have been found with all the techniques in question: shaking /2/, reflux /5/, /7/, Soxhlet /9/ and PLE /10/.

Shaking, reflux and Soxhlet have the advantage that no expensive equipment is needed, as is the case for PLE.

#### *Extraction solvent*

Extraction of LAS is done by methanol with or without the addition of sodium hydroxide (0.5M in methanol). The Danish national method for sludge /2/ applies basic methanol (0.5M NaOH in methanol).

The addition of base produces the effect that LAS will be present in the ionized form and therefore theoretically easier to extract from the dry matter. However, many references have obtained good recoveries from sludge and sediment with and without the addition of base. It is therefore doubtful if the base is necessary in order to obtain a good recovery for LAS when extracting dry samples.

Extraction technique as well as extraction solvent must be further investigated, since no unequivocal conclusions can be drawn. Shaking, reflux or Soxhlet are the techniques to be evaluated further, and the extraction solvent is methanol or basic methanol.

### 3.2.2 Clean-up

Generally, a clean-up step shall only be used when necessary to obtain a good selectivity of the method. In a horizontal standard for LAS the need for a clean-up step depends on the matrix, the limit of detection (LOD), and the analytical method to be used.

The Danish method for sewage sludge /2/ uses no clean-up, and the detection limit of the method is 50 mg/kg dry matter. However, for a considerable part of the sludge samples the LOD cannot be obtained; this may amount to as much as 20-30% of the samples. The measurement is done by HPLC-UV.

So with this very high LOD of 50 mg/kg dry matter, a complex matrix like sewage sludge will need a clean-up step if the measurement is done by HPLC-UV. The use of other detectors will improve the selectivity and make it possible to exclude the clean-up step. This is further discussed in chapter 3.2.4.

For a less complex matrix like sediment, the relevant limit of detection may imply the use of a clean-up step. However this highly depends on the selection of detector – with a more selective detector like the MS a clean-up step will not be necessary /6/.

However it is certain that a horizontal standard will include a clean-up step for some of the matrices in question.

The methods used for clean-up in LAS analysis are all based on SPE-columns. The most commonly used columns are the nonpolar columns RP C<sub>2</sub>, C<sub>8</sub> or C<sub>18</sub> /5/-/7/, /12/-/14/, /16/. Also strong anion exchange (SAX) columns are used /9/, /14/. A more special column is the Graphitized Carbon Black (GCB) /8/, /13/ acting as a reversed phase and anionic exchange at the same time.

In some cases the columns are used in combinations, like a SAX followed by a C<sub>8</sub> column or a C<sub>8</sub> followed by a SAX column.

The clean-up step will have to be examined in the further work. If the measurement of LAS is done by HPLC on an RP column, a clean-up using a RP column will be of limited value, since it is based on the same principles and only partly removes the interfering compounds. The SAX column and the GCB column may be a better choice. The advantages of the GCB column are the simultaneous reversed phase and ion exchange effect, and in addition the column is easy to use. A GCB column may therefore replace the combined use of two columns.

### 3.2.3 Derivatization

Since most measurements of LAS have been performed by HPLC rather than GC, not much work has been done on the derivatization of LAS.

Yet several derivatization methods have been described, like desulphonation of LAS in boiling phosphoric acid or conversion of the sulphonates to sulphonyl chlorides, methyl esters or trifluoroethyl esters /9/. Ding et al /10/ have used a more sophisticated ion-pair derivatization with tetrabutyl ammonium hydrogen sulphate taking place in the GC injection port.

In the analysis of LAS, derivatization is only needed if GC is to be used as the final analysis. Since HPLC has proved to be a good and robust method for LAS determination (see chapter 3.2.4) and since a derivatization is troublesome and time-consuming, derivatization will not be recommended as part of the horizontal standard for LAS.

### 3.2.4 Chromatographic analysis

The classical analysis of anionic detergents is the determination of methylene blue active substance (MBAS), a colorimetric procedure which determines LAS together with other anionic surface active compounds.

For a specific determination of LAS, a chromatographic procedure must be used. Other methods like capillary electrophoresis have been used, but have no practical importance.

Today GC as well as LC is used for LAS determination. However most analyses are carried out by LC and only few by GC. The reason is that LAS is not directly amenable to GC analysis due to the low volatility and the anionic form of LAS. As a consequence, derivatization is necessary if GC is to be used.

On the other hand LC methods for LAS have been developed during the past two decades, and they have shown great potential for separating and quantifying the different isomers and homologues of LAS.

The technical LAS products consist of a mixture of homologues with alkyl lengths from C<sub>10</sub> to C<sub>14</sub>, where C<sub>11</sub> - C<sub>13</sub> are the dominant homologues. Many isomers are present for each homologue, and LAS is therefore a very complex mixture.

By LC analysis a complete separation of the homologues and the isomers can almost be obtained applying a long C<sub>18</sub> column with small particles and optimizing the elution gradient. However, in a standard method this is not practical or relevant, and a less complete separation is used.

By the selection of column and eluent it is possible to elute all the isomers of every homologue in a single peak, which may be an advantage at the quantitative determination, when the concentration of every homologue is determined. The use of other columns and eluents makes it possible to make a partial separation of the isomers of every homologue, which is an advantage in the identification by fingerprinting.

By LC it is also possible to determine the carboxylated degradation products SPC; however an alternative analysis will probably be necessary.

With LC, using different detectors is possible. For LAS the relevant detectors are UV, fluorescence and mass spectrometric detectors. The detector used for the horizontal standard will depend on the selectivity which is necessary to obtain the desired LOD in the actual matrix.

The UV detector is the most widespread detector but also the least specific detector. LAS is measured at the wavelength of 225 nm /2/, /7/ or 230 nm /5/. The fluorescence detector is more specific; LAS is typically measured at the wavelengths of 225 nm/295 nm (excitation/emission). The MS detector is the most specific detector for LAS. Using electrospray ionization in negative

mode, the target ions are the deprotonized molecular ions (M-1), i.e. 297, 311, 325, 339 and 353 for C<sub>10</sub>, C<sub>11</sub>, C<sub>12</sub>, C<sub>13</sub> and C<sub>14</sub> LAS, respectively.

As mentioned in chapter 3.2.2, UV detection is used in the Danish method for sewage sludge, and without clean-up of the extract the selectivity is not sufficient. The use of fluorescence will improve the selectivity, and for sewage sludge it will be sufficient for most samples. However, with a clean-up step UV detection is probably sufficient for sewage sludge.

For a less complex matrix such as sediment the relevant limit of detection is much lower and will imply the use of a clean-up step if UV or fluorescence is used. However, with the more selective MS detector a clean-up step will not be necessary /6/.

Other sludges may also be analysed by HPLC-UV, yet better by HPLC-fluorescence. The other matrices will probably require the use of fluorescence or MS.

The required LODs are not known at present. In table 1, estimations of LODs are presented together with selected detectors for the different matrices in question.

Table 1 LAS - selection of detector for LC analysis of different matrices

Matrix	LOD mg/kg dm	UV detection	Fluorescence	MS detection
Sewage sludge	50-100	+ *	+	+
Industrial sludge	50-100	(+) *	+	+
Sediment	0.1-1		(+) *	+
Compost	2-10		+ *	+
Biowaste	5-20		+ *	+
Soil	1-5		(+)	+

\* including a clean-up step.

Due to the many problems with UV detection, it is recommended, that UV should not be used as the only detector, but only in combination with fluorescence or MS.

### 3.2.5 Identification and quantification

As mentioned earlier LAS is a complex mixture. It consists of homologues with alkyl chains from C<sub>10</sub> to C<sub>14</sub> with many isomers for each homologue.

The LC chromatogram of LAS therefore shows many peaks with C<sub>10</sub> coming out first in the chromatogram and C<sub>14</sub> coming out as the last peak. For all detectors the identification of LAS is based on the retention times and the chromatographic fingerprint. By LC-MS this is supplemented with the relative intensities of the diagnostic ions.

The calibration is performed by a LAS standard of high purity with a known content of the 5 homologues of alkylbenzene sulphonate. The result is calculated as concentrations of the 5 homologues, which are subsequently summed to the concentration of LAS.

Since the chromatogram can be very complex it may be difficult to obtain a good integration of the peaks without a contribution from interfering compounds. This is a serious problem with UV detection and less serious with fluorescence detection. The MS detection is much more

selective and has the advantage of using several diagnostic ions and relative peak intensities, and the interferences are therefore small.

The calculation is best done by internal standard method. The internal standard most often used is C<sub>8</sub> LAS. This is added to the sample before extraction and helps to correct for the losses during the analysis. It has been described that the column clean-up may show lower recoveries for C<sub>8</sub> LAS than for the higher C<sub>10</sub> - C<sub>14</sub> LAS due to less adsorption of C<sub>8</sub> LAS. To compensate for this, MacAvoy et al /14/ used a mixture of C<sub>9</sub> and C<sub>15</sub> LAS as internal standards.

### 3.3 Nonylphenols

The relevant limits of detection will vary from one matrix to the other. In sewage sludges a detection limit of 50-100 mg/kg dry matter will probably be sufficient, whereas in sediments the limit of detection must be below 1 mg/kg dry matter, maybe even 0.1 mg/kg dry matter.

By the analysis for NP the demand for a high selectivity is met by the selection of the MS detector /see chapter 3.3.4). This again influences the decision whether a clean-up is necessary or not.

The description of the methods for LAS analysis will be separated in the analytical steps:

- Extraction
- Clean-up
- Derivatization (if necessary)
- Chromatographic analysis
- Identification and quantification

#### 3.3.1 Extraction

The choice of extraction technique and extraction solvent depends on the sample matrix. For the soil matrices described in chapter 3.2.1 the NP are adsorbed to the organic part of the sludge or soil particles, etc. – as is the case for all nonpolar organic compounds with  $\log P_{ow} > 3-4$ . It is expected that NP – like other nonpolar compounds - may be bound inside the aggregates and therefore difficult to extract from the solid materials. This is most critical for old contaminations and for soil samples with a high clay content.

##### *Extraction techniques*

The extraction techniques used for NP in solid samples are the same as for other nonpolar contaminants – mainly shaking and/or ultrasonification, soxhlet, PLE (Pressurized Liquid Extraction) and SFE (Supercritical Fluid Extraction).

The mechanical techniques shaking and/or ultrasonification is necessary at the extraction of the more critical samples like clayrich soil, some sludges, and maybe sediments. The extraction of such sample matrices can be facilitated by the use of chemicals that will help disintegrate the aggregates and thereby increase the extraction efficiency. Examples of these chemicals are acetone and tetrasodium pyrophosphate (0.05 M in water).

The mechanical techniques may be applied on all sample materials, and these can be extracted after drying or as wet samples. The other techniques – Soxhlet, PLE and SFE - can best be applied on dry samples and not on samples like clayrich soils. This is presented in table 2.



Table 2 Nonylphenols - Extraction techniques for different matrices

Matrix	Dry/wet sample	Shaking/ ultrasonic	Soxhlet	PLE	SFE
Sewage sludge	Dry	+	+	+	+
	Wet	+	(+)	(+)	(+)
Industrial sludge	Dry	+	+	+	+
	Wet	+	(+)	(+)	(+)
Sediment	Dry	+	(+)	(+)	(+)
	Wet	+	(+)	(+)	(+)
Compost	Dry	+	+	+	+
	Wet	+	(+)	(+)	(+)
Biowaste	Dry	+	+	+	+
	Wet	+	(+)	(+)	(+)
Soil – clay	Dry	+			
	Wet	+			
Soil – other	Dry	+	+	+	+
	Wet	+	(+)	(+)	(+)

#### *Extraction solvent*

Extraction of nonylphenols has been performed with different solvents. The ISO/DIS method for water /1/ applies toluene as solvent, the Danish national method for sludge /2/ applies dichloromethane and the German method for sludge /3/ a mixture of acetone:hexane (1:1).

Many other solvents have been described in the literature, and a systematic investigation of the extraction of sewage sludge has been described by Meesters et al /18/, who concentrated on the examination of soxhlet and PLE. They found disappointing results for many solvents, also for some solvents which in other works are described as good extractants. This was the case for ethyl acetate:dichloromethane (1:1) and acetone:dichloromethane (1:1) by soxhlet and for many combinations of ethyl acetate, methanol, acetone and dichloromethane by PLE. A few solvents showed very promising extraction efficiency: dichloromethane:methanol (2:1) and toluene by soxhlet and ethyl acetate:formic acid (9:1) by PLE.

The combination of acetone and petroleum ether (or another aliphatic hydrocarbon) has the advantage that it can be used to break down the aggregates in e.g. clayrich soils. Acetone is added first and mechanical technique like shaking is applied. Subsequently petroleum ether is added and a repeated shaking applied so that the extraction is done with a mixture of acetone and petroleum ether. Acetone is then removed by the addition of water, followed by the separation of the petroleum ether phase and the water phase (waste).

This combination of acetone and petroleum ether is used for the extraction of other nonpolar contaminants in solid samples. This may become an advantage if different classes of compounds can be extracted by the same method.

Extraction technique as well as extraction solvent must be further examined, since no clear conclusions can be drawn. It will be the goal of the project to use the same extraction technique and the same solvent(s) for all the matrices in question, but several techniques or solvents may be needed.

### 3.3.2 Clean-up

It is certain that a horizontal standard will include a clean-up step to be used for many or maybe all the sample materials, which will be covered by the standard. A clean-up of the sample extract will for many samples be necessary in order to obtain a good chromatographic analysis with sufficient selectivity.

The methods used for clean-up in NP analysis are all based on column chromatography on a polar column like silica, alumina or florisil. The ISO/DIS method for water /1/ uses a silica clean-up when necessary. The Danish method for sludge /2/ uses no clean-up and the German method for sludge /3/ uses silica clean-up.

Other methods uses alumina /17/, /18/, /22/ or florisil /20/.

The clean-up step will have to be examined in the further work. It seems that the use of silica will be a good choice, since this is already used in the ISO/DIS method for water analysis.

### 3.3.3 Derivatization

It is now uncertain whether the horizontal standard for NP will include a derivatization step. Since a derivatization can be troublesome it will only be used when necessary in order to obtain a good chromatography, a good selectivity, or in the case of NP, in order to include the minor ethoxylates with 1 and 2 ethoxylate groups.

The ISO/DIS method for water /1/ and the Danish method for sludge /2/ do not apply a derivatization step, and the German method for sludge /3/ uses a silylation with MSTFA (followed by GC-MS analysis).

The simplest derivatization method for NP is probably anhydride used by Meesters et al /6/. The method results in GC chromatograms with nice peak shape and a good selectivity. The recovery is not reported. Other derivatization methods are mostly other silylation methods using different silylation reagents /20/, /21/. Some of these methods will include the mono- and diethoxylates NP1EO and NP2EO.

A special method is described by Wahlberg /23/ who uses pentafluorbenzoyl chloride for the formation of benzylesters. For NP the method is hardly better than the other methods, but if the method shall include the minor ethoxylates, this derivatisation may be a possibility – even if the recovery is not described.

### 3.3.4 Chromatographic analysis

Environmental analyses of nonylphenols are mostly performed by gas chromatography and in a few cases by HPLC using e.g. fluorescence detector. Today GC is recommended since it is a more robust method and since GC is cheaper to use in combination with MS.

For a horizontal standard with a broad application, detection by MS is recommended to obtain the necessary selectivity and the necessary sensitivity – i.e. low detection limits. The three methods in /1/ - /3/ all use GC-MS for the final analysis.

The detection is performed by Selected Ion Monitoring (SIM), where only selected ions are monitored in order to obtain a high sensitivity. The target ion is most often 135 and the other diagnostic ions may be 149, 107 or even others.

The analyte NP is a mixture of isomers with different positions of carbons in the branched C<sub>9</sub>H<sub>19</sub>-chain. All the isomers are branched, and the main isomers have two methyl groups at the

C<sub>1</sub> position in the chain, i.e. at the carbon closest to the phenol structure. Ion 135 is the dominant ion in the technical product and it reflects the fragment with two methyl groups in the C<sub>1</sub> position, whereas ion 149 reflects the fragment with one methyl group and one ethyl group in the C<sub>1</sub> position. Ion 107 is the fragment HO-C<sub>6</sub>H<sub>4</sub>-CH<sub>2</sub>. From the GC-MS chromatograms at least 13 isomers can be seen.

### 3.3.5 Identification and quantification

There may be confusion about the identity of nonylphenols, since the CAS numbers are not very precise. CAS has two numbers for nonylphenol: 104-40-5 and 25154-52-3. The CAS number 104-40-5 is described as “4-nonylphenol” or “4-nonylphenol, mixture of isomers”, and the CAS number 25154-52-3 is described as “nonylphenol” or “nonylphenol, mixture of isomers” and may therefore include 2-nonylphenol and 3-nonylphenol.

The analyte is CAS No. 104-40-5, but this number will cover the 4-n-nonylphenol as well as the mixture of 4-nonylphenols. It should therefore be emphasized that the analyte is the mixture of isomers, and that this shall be used for calibration.

The identification of NP is based on the retention times, the chromatographic fingerprint and the relative intensities of the diagnostic ions.

Being a mixture of at least 13 isomers, NP has a chromatographic fingerprint, which can be used for identification purposes. The ion 135 chromatogram shows 7 distinct peaks and 3 small peaks, ion 149 shows 6 peaks and ion 107 show 11 or 12 peaks.

A good selectivity of the method highly depends on the integration of the mixture – only nonylphenols must be included in the integration and calculation. There are several ways to obtain this: by using the fingerprints from several diagnostic ions or by using the relative peak intensities. Since it can be difficult to avoid interferences a combination of fingerprints and relative intensities will probably be used.

The quantification must be based on the sum of nonylphenol peaks.

The use of an internal standard is important since the method shall cover different matrices and the method includes many steps where losses of nonylphenols may occur. The addition of a good internal standard to the sample before extraction makes it possible to correct for the losses during the analysis.

Different internal standards have been used. The ISO/DIS method for water /1/ uses <sup>13</sup>C-labelled 4-n-nonylphenol. The Danish method for sludge /2/ uses phenanthrene-d<sub>10</sub>, and the German method for sludge /3/ mentions 4-n-nonylphenol as an example. Among these the <sup>13</sup>C-labelled 4-n-nonylphenol is recommended, since it behaves like NP during the analysis and the background is zero.

The discussion has only dealt with NP. If the method also is to include the mono- and diethoxylates, it will be necessary to calibrate for these compounds as well. These are today commercial available and may therefore be used as calibrants. The Danish method for sludge /2/ includes these compounds, uses calibration by NP, and a correction of the response with the relative molecular masses. This procedure was selected in 1977, since standard compounds of mono- and diethoxylates were not commercial available.

### 3.4 Nonylphenolpolyethoxylates

Although it is not part of the desk study the analysis of the nonionic detergents NPPE shall be briefly mentioned in this chapter.

At the moment, the relevance of including NPPE in the analysis of nonylphenol compounds in sewage sludges and soils is not clearly understood. A significant amount of NPPE in sludges will mean that NPPE makes a contribution to the load of NP in sludge, since NPPE will be degraded to NP. The intention of the project is to study the degradation of NPPE via NP2EO and NP1EO to NP and in addition to acquire better knowledge of the levels of NPPE in sewage sludge by measurements of sewage sludges from different countries.

The determination of NPPE is very complicated because of the very complex mixture of homologues. The method must be able to measure the sum of nonylphenol-polyethoxylates from about 3 and up to as many ethoxy groups as possible. Today, this is done by LC-MS.

It is important to state that NPPE cannot be determined by the same method as NP. It may be possible to extract NPPE and NP by the same procedure, but they cannot be measured by the same analytical method. NPPE is best measured by LC-MS using positive mode electrospray ionization whereas NP is best measured by negative ionization mode. Even the smaller ethoxylates NP1EO and NP2EO is difficult to measure by any of these methods.

These conclusions are very general, since some LC-MS instruments are able to combine some of the measurements. But with the currently used methods, the analysis of NP and the analysis of NPPE are performed by two different methods.

## 4. CRITICAL POINTS AND RECOMMENDATIONS

From the description in chapter 3 it is concluded that draft horizontal standards for LAS and/or nonylphenols cannot yet be prepared. Consequently, no draft standard is included in the desk study report.

The description also indicates that a good basis exists for the preparation of harmonized and horizontal standards for the determination of LAS and nonylphenols in sludges and soils. If it is found relevant to include compost, biowaste, and sediments, this may be possible.

Thus further prenormative research will have to be conducted before horizontal standards for LAS and nonylphenols can be drafted.

In this chapter, the main issues for the further research is presented and discussed.

It is the intention that the continued work will result in 2 horizontal standards for LAS and nonylphenols (NP), respectively (maybe including NP1EO and NP2EO). If NPPE is found relevant – primarily in sludges and soils - a third draft horizontal standard for NPPE may be prepared.

### 4.1 Pretreatment

Procedures for sample pretreatment are not part of the actual horizontal standards. It is recommended that procedures for sample pretreatment is prepared elsewhere, so it can be used as a horizontal reference for the analytical standards.

In the presentation of standards for LAS and NP, the procedures for pretreatment are therefore not covered.

### 4.2 LAS

Based on the description in chapter 3.2 some conclusions can be drawn.

The horizontal standard for LAS will consist of the following steps:

1. Drying of the sample – either by freeze drying or by conventional drying
2. Extraction of the dry test sample with methanol or 0.5M NaOH in methanol
3. A clean-up of the extract – for some matrices. The clean-up is carried out on an SPE column
4. Analysis by liquid chromatography (LC) using UV, fluorescence or MS detectors. The selection of detector depends on the matrix
5. Calculation based on internal standard procedure

Due to the use of LC for the final analysis, a derivatization procedure will not be included in the horizontal standard.

Important parts of the standard have not yet been decided upon due to the present lack of knowledge. The main issues for the further research are summarized in table 3.

Table 3 LAS - Main issues for further work

	<b>Questions to be answered/Decisions to be made</b>
Scope and working area	Range of matrices to be included.
Sample storage and pretreatment	Storage conditions (temperature, materials etc.) for each matrix. Selection of drying procedure.
Principle and procedure	Selection of extraction technique. Selection of extraction solvent. Shall clean-up be mandatory or only used when necessary? Selection of procedure for clean-up, e.g. column. Selection of LC-detector - may be different for each matrix. Selection of internal standard.

It must be decided which matrices shall be included in the horizontal standard. Sludges and soils are definite candidates, whereas the situation is doubtful for treated biowaste and sediments.

Conditions for storage of samples must be decided upon. The conditions may vary from matrix to matrix, but it is expected that the storage procedure will be freezing for all matrices. However this must be further examined.

The drying procedure must be decided upon. The two procedures: freeze drying and conventional drying must be examined.

The three extraction techniques - shaking, reflux and Soxhlet - must be further examined before the extraction technique can be decided upon.

As extraction solvent, either methanol or 0.5M NaOH in methanol shall be used. The influence of base must be examined on the matrices in question.

If the clean-up step is not mandatory but only used when necessary, criteria must be established for the decision when to use clean-up.

The candidate columns for clean-up must be examined and the procedure must be optimized. When it is decided for which matrices the clean-up shall be applied, the clean-up procedure will be tested on the matrices in question.

The whole procedure will be tested on the actual matrices, applying the three detectors: UV, fluorescence and MS. Based on the results the use of detector is decided upon. It is assumed that the final horizontal standard will include the use of two or three detectors.

### 4.3 Nonylphenols

Based on the description in chapter 3.3 the following conclusions can be drawn.

The horizontal standard for nonylphenols will consist of the following steps:

1. Weighing of a test sample of dry or wet sample – drying, possibly freeze–drying, or conventional drying.
2. Extraction of the test sample with an organic solvent or solvent mixture using shaking or Soxhlet.
3. A clean-up of the extract – for some matrices. The clean-up is carried out on a SPE column of silica, alumina or florisil.
4. Derivatization - only if necessary!
5. Analysis by gas chromatography using mass spectrometric detector (GC-MS). When no derivatization is used ion 135 is used as the target ion, and the ions 107 and 149 are used as diagnostic ions for confirmation of the identity.
6. Calculation based on internal standard procedure. Labelled 4-n-nonylphenol is used as internal standard for NP.

Also for NP important parts of the standard have still not been decided upon due to the present lack of knowledge. The main issues for the further research are summarized in table 4.

Table 4 Nonylphenols - Main issues for further work

	<b>Questions to be answered/Decisions to be made</b>
Scope and working area	Shall mono- and diethoxylates (NP1EO and NP2EO) be included? Range of matrices to be included.
Sample storage and pretreatment	Storage conditions (temperature, materials etc.) for each matrix. Selection of procedure for pretreatment for each matrix – shall wet or dry sample be used?
Principle and procedure	Optimization of extraction technique. Selection of extraction solvent. Shall clean-up be mandatory or only used when necessary? Selection of procedure for clean-up. Selection of derivatization procedure – if derivatization is necessary.

It must be decided whether the horizontal standard shall include the mono- and diethoxylates. This may depend on the future EU directives and it has a significant influence on the selection of analytical procedure.

It must be decided which matrices shall be included in the horizontal standard. Sludges and soils are definite candidates whereas the situation is doubtful for treated biowaste and sediments.

Conditions for storage of samples must be decided upon. The conditions may vary from matrix to matrix, but it is expected that the storage procedure will be freezing for all matrices. However this must be further examined.

In some sample matrices the use of wet or dried samples depends on the stability of the sample. The NPPE can be very easily degraded and NP thereby formed in the sample. This has to be examined for the matrices in question. If drying must be used, the drying procedure shall be selected.

The extraction techniques – shaking and Soxhlet – must be must be further examined before the extraction technique can be decided upon.

By the selection of extraction solvent it may be relevant to use solvents applied in the analysis of other contaminants like PAH and PCB. The use of acetone followed by petroleum ether or hexane is a candidate, but it must be examined on the matrices in question.

If the clean-up step is not mandatory but only used when necessary, criteria must be established for the decision when to use clean-up.

The candidate columns for clean-up must be examined and the procedure must be optimized. When it has been decided for which matrices the clean-up shall be applied, the clean-up procedure will be tested on the matrices in question.

The selection of derivatization procedure highly depends on the decision if mono- and diethoxylates shall be included in the horizontal standard. If only NP is included, a derivatization is hardly necessary.



## REFERENCES

1. Draft international Standard ISO/DIS 18857-1. ISO (6 March 2003).
2. Standard method for Determination of environmental contaminants in sludge- PAH, DEHP, NPE and LAS. The Danish Environmental Protection Agency. (1997).
3. Method for nonylphenols in sludge - draft. Northrhein Westfalia State Environment Agency (17 March 2003).
4. CEN/TC 308/WG 1/TG 4: Resolutions of the 7<sup>th</sup> meeting, Oslo, Norway, 27 March 2003.
5. Matthijs, E.; De Henau, H. *Tenside Surf. Det.* 24:4 (1987) 193-199.
6. Folke, J.; Cassani, G; Ferrer, J.; Lopez, I.; Karlsson, M.; Willumsen, B. *Tensides Surf. Det.* 40:1 (2003) 19-24.
7. Comellas, L.; Portillo, J.L.; Vaquero, M.T. *J Chromatogr. A* 657 (1993) 25-31
8. Crescenzi, C.; Di Corcia, A.; Marchiori, E.; Samperi, R.; Marcomini, A. *Wat. Res.* Vol. 30 No. 3 (1996) 722-730.
9. Reiser, R.; Toljander, H. O.; Giger, W. 69 (1997) 4923-4930
10. Ding, W-H.; Fann, J. C. H. *Analytica Chimica Acta* 408 (2000) 291-297
11. Fujita, M.; Ike, M.; Goda, Y.; Fujimoto, S.; Toyoda, Y.; Miyagawa, K.-I. *Environ. Sci. Technol.* 32 (1998) 1143-1146
12. Eichorn, P.; Flavier, M. E.; Paje, M. L.; Knepper, T. P. *The Science of the total Environment* 269 (2001) 75-85
13. González-Mazo, E.; Gómez-Parra, A. *Trends in analytical chemistry*, vol 15, no.8 (1996) 375-380
14. MacAvoy, D.C.; Eckhoff, W.S.; Rapaport, R.A. *Environ. Toxicology and Chemistry* Vol. 12 (1993) 977-987
15. Ding, W-H.; Chen, C.-T. *J Chromatogr. A*, 857 (1999) 359-364
16. Tabor, C-F.; Barber, L.B. *Environ. Sci. Technol.* 30 (1996) 161-171
17. Ding, W-H.; Fann, J.C.H. *J. Chromatogr. A*, 866 (2000) 79-85.
18. Meesters, R.J.W ; Schröder, H.Fr. *Anal. Chem.* 74 (2002) 3566-3574.
19. Kojima, M., Tsunoi, S.; Tanaka, M.. *J. Chromatogr. A*, 984 (2003) 237-243.
20. Li, D.; Park, J.; Oh, J.-R.. *Anal Chem.* 73 (2001) 3089-3095.
21. Rudel, R.A.; Melly, S.J.; Geno, P.W.; Sun, G.; Brody, J.G. *Environ. Sci. Technol.* 32 (1998) 861-869.
22. Valsecchi, S.; Polesell, S.; Cavalli, S.. *J. Chromatogr. A*, 925 (2001) 297-301.
23. Wahlberg, C.; Renberg, L., Wideqvist, U.. *Chemosphere*, 20 (1990) 179-195.