

**CERTIFICATE FOR**  
**QC ENDOCRINE DISRUPTORS IN MUNICIPAL SLUDGE**  
**ORGANIC MATERIAL**

**BATCH:** VKI-27-1-0297

**INSTRUCTION FOR USE OF THE REFERENCE MATERIAL**

**Description, Quantity and Preservation**

This reference material consists of dried and homogenised sludge in containers of approx. 100 g each. The reference material is certified for endocrine disruptors (compounds which exhibit estrogenic properties):

DEHP (di(2-ethylhexyl)phthalate)  
NPE (4-nonylphenols and 4-nonylphenol mono- and diethoxylates)

The certified values are calculated both for individual components and for the sums of components in accordance with the Order on the Use of Waste for Agricultural Purposes of the Danish Ministry of Environment /3/. The reference material is preserved by drying and homogenisation. If kept dry, the content of dry matter is at least 97%.

**Use**

The reference sample is intended for quality control, i.e. measurement and control of the trueness and precision of analyses. The reference is typically intended for analyses of the above-mentioned organic parameters in sludge, including analyses of sludge intended for agricultural use /3/. It is important that the batch numbers of the reference material and on the certificate are identical.

**Analysis**

For quality control the reference sample is analysed at the same time and in the same manner as other samples.

**Storage and Durability**

Store the reference material in the sample container, tightly sealed. The certificate is valid until **1st of October 2019** provided the material is stored under the recommended conditions.

After opening, the sample may be affected by microbial pollution and humidity.

**PRODUCTION OF THE REFERENCE MATERIAL AND DOCUMENTATION**

**Production**

The reference material consists of municipal sludge from Køge Egnens Rensningsanlæg (a Danish municipal wastewater treatment plant), and has been dried in a sludge drying plant where the sludge is heated to a temperature of approx. 80°C. It has been milled to a particle size of < 63 µm, homogenised and filled in metal

containers. The material has been produced in accordance with the quality management procedures of VKI (now Eurofins Miljø A/S), with the aim to obtain the intended quality of the material.

## Documentation of Content

### *Internal control*

The analytical quality of VKI (now Eurofins A/S) has been documented and found satisfactory by regular participation in proficiency tests conducted by NERI /4, 5/, Denmark.

### Homogeneity:

The homogeneity of the reference material has been investigated as part of two interlaboratory comparisons. The test for homogeneity has been performed by testing the standard deviation between container units in this certification against the standard deviation of duplicate determinations of individual containers (F-test, 95%). The samples were found homogenous.

### Stability:

The stability is being followed by regular analyses of all individual certified compounds stored at 5°C, room temperature and 37°C. No signs of instability were observed at the date of this certificate.

### *External documentation*

The external laboratory documentation was performed by Danish laboratories. The laboratories were requested to analyse the reference material in two containers in the same analytical series, where one was analysed in duplicate. For control of the analytical quality of the laboratory, the laboratories were requested to analyse two control samples consisting of a known amount of DEHP and NPE. The external control values have been calculated by using the statistical procedures of the international standard ISO Guide 35 /1/. The following calculations have been made on the basis of the analytical results submitted by the laboratories:

$\bar{Y}$  : average calculated in accordance with ISO Guide 35, 10.5.2.

$s_L$  : standard deviation between the laboratories calculated in accordance with ISO Guide 35, 10.5.2:

$$\frac{1}{p-1} \sqrt{\sum (y_i - \bar{Y})^2}$$

The 95% confidence interval of the true mean value of analytical results is:

$$\bar{Y} \pm t_{0,025}(v) \cdot \frac{s_L}{\sqrt{p}}$$

where

p: number of laboratories included in the calculations

v: p-1, degrees of freedom

$t_{0,025}(v)$ : t-value 0,025-level at v degrees of freedom.

Selection of results for certification is based on the following:

- the laboratory being experienced, i.e. having analysed minimum five analytical series using the analytical methods applied in this certification
- the results of the laboratory from its participation in one or both proficiency test(s) for these components in sludge conducted by NERI in 1997 and 1998 /4, 5/. The requirements for each individual parameter are that the laboratory's results do not deviate by more than  $\pm 1$  times the standard deviation or  $\pm 40\%$  from the median value
- the recovery of the results of the analyses of the above-mentioned control samples lies within  $\pm 40\%$  of the nominal value.

Finally, results are excluded if they are Grubbs or Cochran outliers /6/, or if a discussion with the laboratory indicates that the analytical quality is not optimal. The statistical parameters are listed in the table overleaf. The laboratories selected for certification and the data included in this certification are listed in the annexes of this certificate.

### Certified Values - Individual Components

PARAMETER	UNIT	AVERAGE	STANDARD DEVIATION BETWEEN LABORATORIES	95% CONFIDENCE LIMITS OF THE AVERAGE		NUMBER OF LABORATORIES INCLUDED IN THE CALCULATION	EXCLUDED LABORATORIES
				Lower	Upper		
		$\bar{Y}$	$s_L$	$\bar{Y} \pm t_{0,025}(v) \cdot \frac{s_L}{\sqrt{p}}$		(p)	
di(2-ethylhexyl) phthalate (DEHP)	mg/kg	26,7	5,78	20,7	32,8	6	1
4-nonylphenols	mg/kg	78	27	49	106	6	1
4-nonylphenol-monoethoxylates *	mg/kg	(5,5)	(2,1)	(3,2)	(8,4)	5	1
4-nonylphenol-diethoxylates *	mg/kg	(2,4)	(0,71)	(1,5)	(3,3)	5	1

\*: As fewer than 6 sets of results are included in this certification the data are stated in brackets.

### The sum of components in accordance with the Order of the Danish Ministry of the Environment /3/

PARAMETER	UNIT	AVERAGE	STANDARD DEVIATION BETWEEN LABORATORIES	95% CONFIDENCE LIMITS OF THE AVERAGE		NUMBER OF LABORATORIES INCLUDED IN THE CALCULATION	EXCLUDED LABORATORIES
				Lower	Upper		
		$\bar{Y}$	$s_L$	$\bar{Y} \pm t_{0,025}(v) \cdot \frac{s_L}{\sqrt{p}}$		(p)	
NPE	mg/kg	86	27,7	53	119	5-6	1
DEHP	mg/kg	26,7	5,78	20,7	32,8	6	1

### Methods

NPE, DEHP: Extraction by dichlormethane and analysis by GC-MS with SIM (selected ion monitoring)

### Use of Certified Values

For laboratories with an analytical quality comparable with that of the laboratories that have contributed with the external control data of this certificate, the following applies:

- 1) For single determinations, analytical results will, with a probability of 95%, lie within the interval:

$$\bar{Y} \pm t_{0,025}(v) \cdot s_L$$

### REFERENCES

- /1/ ISO guide 35:2006. Certification of reference materials - General and statistical principles for certification.
- /2/ ISO guide 31:2000. Reference materials - Contents of certificates and labels.
- /3/ Order No. 1650 on the Use of Waste for Agricultural Purposes, 2006, The Ministry of the Environment (In Danish).
- /4/ Organic Pollutants in Sludge. Proficiency Test, NERI (National Environmental Research Institute) October 1997 (In Danish).

- /5/ PAH, PCB, plastisizers, alkylphenols and LAS in sludge. Proficiency Test, November 1998.
- /6/ ISO/DIS 5725-2, 1994, Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

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Certificate revision history: January 2014 (expiry date added); October 2002 (original certificate date)
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# ANNEX TO THE CERTIFICATE ENDOCRINE DISRUPTORS IN MUNICIPAL SLUDGE

## Laboratory Measurements

DEHP		
$m_i$ mg/kg	$s_{ri}$ mg/kg	$n_{ri}$
18,6	0,6	3
25,0	0,6	3
23,5	0,7	3
32,2	2,0	3
26,6	1,5	3
34,3	0,7	3

Nonylphenols		
$m_i$ mg/kg	$s_{ri}$ mg/kg	$n_{ri}$
73	1,7	3
110	2,4	3
46	0,7	3
76	0,8	3
53	0,9	3
108	3,2	3

Nonylphenol- monoethoxylates		
$m_i$ mg/kg	$s_{ri}$ mg/kg	$n_{ri}$
5,11	0,09	3
4,86	0,26	3
5,01	0,24	3
4,53	0,19	3
9,57	0,33	3

Nonylphenol- diethoxylates		
$m_i$ mg/kg	$s_{ri}$ mg/kg	$n_{ri}$
1,68	0,03	3
1,61	0,19	3
2,90	0,44	3
2,85	0,04	3
3,05	0,33	3

$m_i$  : average for laboratory i  
 $s_{ri}$  : standard deviation for laboratory i within an analytical series  
 $n_{ri}$  : number of results for determination of  $s_{ri}$

# ANNEX TO THE CERTIFICATE ENDOCRINE DISRUPTORS IN MUNICIPAL SLUDGE

## Certifying Laboratories

A/S Analycen, Fredericia  
Levnedsmiddelkontrollen, Skovlunde  
Miljø- og Levnedsmiddelcentret, Holbæk  
Miljø-Kemi, Viborg  
MLK-Fyn I/S, Odense  
MLK Vestjylland I/S, Holstebro  
MLK Østjylland I/S, Silkeborg  
Steins Laboratorium, Brørup  
VKI, Hørsholm