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Corresponds to study #21 in Attachment A of transmittal memo on CBI
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BASF Aktiengesellschaft

[redacted]

Deutscher Akkreditierungs Rat DAR DAC-P-0030-97-00

FINAL REPORT

Determination of the Inhibition of Oxygen Consumption by Activated Sludge by

Perylimid F

in the Activated Sludge Respiration Inhibition Test

according to **GLP, EN 45001 and ISO 9002**

Project Number
98/0291/08/1

Completion Date: March 1999

Study Director: [redacted]

1 LIST OF CONTENTS

2 QUALITY CRITERIA

This project was performed according to the following quality criteria:

- European Standard EN 45001 "General criteria for the operation of testing laboratories"
- International Standard ISO 9002 "Quality systems - Model for quality assurance in production installation and servicing"
- OECD Principles of Good Laboratory Practice (GLP).

The [redacted] is a part of the [redacted] of BASF AG in [redacted]

- accredited to Deutsche Akkreditierungsstelle Chemie GmbH (DACH) under number DAC-P-0030-97-00 and
- registered for GLP under the name "BASF DUU/O" in Bundesanzeiger (German Federal Gazette) No. 140 page 8633 of 30 July 1996.

Copies of the certificates can be provided by request.

3 SUMMARY

Determination of the Inhibition of Oxygen Consumption by Activated Sludge by **Perylimid F** in the Activated Sludge Respiration Inhibition Test according to Annex of EEC Directive 88/302/EEC, corresponds to OECD Guideline 209 and ISO Standard 8192.

TEST RESULTS

No significant inhibition of respiration was measured up to the highest tested concentration of 1000 mg/l (nominal).

Inoculum:

Activated sludge from laboratory wastewater plants treating municipal and synthetic sewage. Concentration of dry substance 1 g/l.

Remarks:

The EC 20 in the activated sludge respiration inhibition test is >100 mg/l. Disturbances in the biodegradation process of activated sludge are not to be expected if the test substance is correctly introduced into adapted wastewater treatment plants at low concentrations.

4 GLP-STATEMENT OF COMPLIANCE AND SIGNATURES

This study was performed according to the principles of good laboratory practice (GLP) of the OECD, published 4 February 1983 and the German Chemical Law (25 July 1994). This is confirmed by the signature of the study director. All data and the test substances are stored in the [redacted] in Building [redacted] of BASF Aktiengesellschaft in [redacted]).

Date:	March 23, 1999	March 23, 1999	March 24, 1999
Signature:	[redacted]		
Name			
Function	Project Technician	Study Director	Head of Laboratory

5 STATEMENT OF THE QUALITY ASSURANCE UNIT

MSS-Number:	98/346
Test substance:	Perylimid F
Title:	Determination of the Inhibition of Oxygen Consumption by Activated Sludge in the Respiration Inhibition Test

The Quality Assurance Unit inspected the study, audited the final report and reported findings to the study director and to the management.
Laboratories and short term studies are inspected in regular intervals.

Last procedure audit: 27.11.98

Inspection	Date of inspections	Reported at
Study plan	09.12.98	
Study audit		
Draft	17.03.99	
Final report	24.03.99	24.03.99

Ludwigshafen, 24.03.99

[redacted]
(Quality Assurance Unit)

6 DESCRIPTION OF THE TEST METHOD

Test principle of the Activated Sludge Respiration Inhibition Test

This test specifies a method for assessing the inhibitory effect of a test substance on the oxygen consumption rate of aerobic micro-organisms (activated sludge) after short-term exposure (30 or 180 min). The test results are reported as effective concentrations which indicate a respiration inhibition of 20, 50, and 80 % (EC_{20}, EC_{50}, EC_{80}) compared to a control assay without test substance. The EC_{20} is regarded as a limit value for possible toxic effects in wastewater treatment plants. The test concentration in biodegradation tests should not exceed the EC_{20}.
The EC-values are based on added nominal concentrations and not on analytically determined values. The nominal concentrations which indicate a respiration inhibition of 20, 50 and 80 % can be taken from the graph of the inhibition curve.

7 TEST METHODS

Activated Sludge Respiration Inhibition Test
Annex to EEC Directive 88/302/EEC November 18th, 1987

This method corresponds to:

OECD Guidelines for Testing of Chemicals
Activated Sludge, Respiration Inhibition Test 209; Paris 1993

International Standard ISO 8192-1986 (E) (Method B)
Water Quality - Test for inhibition of oxygen consumption by activated sludge

8 ORDER INFORMATION

Sponsor : [redacted] Telephone : [redacted]

Department code and building : [redacted] Date of order : April, 30, 1998

9 SPECIFICATION OF THE TEST SUBSTANCE BY THE SPONSOR

Name of test substance	:	Perylimid F
Chemical name	:	Perylene-3,4,9,10-Tetracarboxydiimide
Batch number	:	Partie 18
Date of production	:	2. Quartal 96
Product number	:	073209
CAS number	:	81-33-4
Molecular formula	:	$C_{24}H_{10}N_2O_4$
Molecular weight	:	390,36
Aggregate state	:	solid
Density	:	no data available
Water-solubility	:	2800 mg/l
Colour	:	violet/red
Purity of the test substance	:	98,9 %[3]
Impurities	:	no data available
Homogeneity	:	homogeneous
Stability	:	stable
Remarks for storage	:	no special means, storage at room temperature
Further remarks	:	no remark

Origin of data of purity and homogeneity:

[1]) Observation of the [redacted]
[2]) Report of the Analytical Laboratory No: -
[3]) Report of the sponsor No: analysis from June, 06, 1996

10 PRELIMINARY INVESTIGATIONS

none

11 TEST INFORMATION

Preparation of the stock solution of the test substance: -

Preparation of the inoculum:

The inoculum was washed, brought to a concentration of 5 g/l dry substance and aerated for 24 hours. 50 ml were added to a total volume of 250 ml to obtain a concentration of 1 g/l dry substance in the test.

Begin of the study	: Dec, 09, 1998
Experimental phase	: Dec, 10, 1998
Incubation time	: 30 min
Test temperature	: 20 ± 2 °C
Test vessels	: Erlenmeyer-vessel (nominal volume 250 ml)
Test volume	: 250 ml
synthetic medium	: 8 ml/vessel 100-fold concentrated OECD Medium
Oxygen concentration during aeration	: > 2,5 mg/l
Oxygen concentration immidiately before measurement	: > 6,5 mg/l
Duration of the measurement of oxygen consumption	: 6 - 10 min.

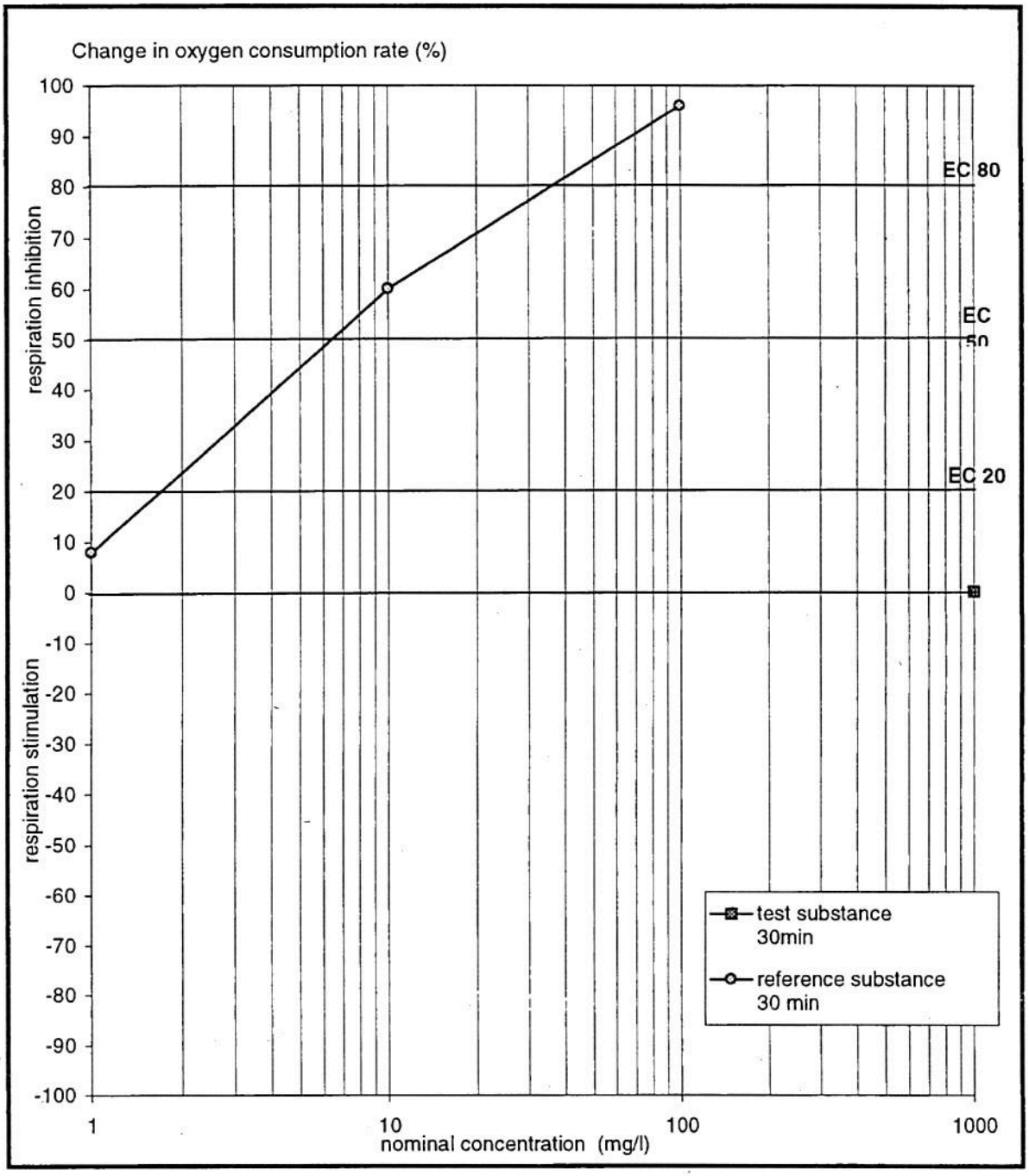
12 VALIDITY CRITERIA

Deviation of blank controls < 15 %	: yes
EC_{50} of 3,5-dichlorophenol in the range of 5-30 mg/l	: yes
Test is valid	**: yes**

13 ACCOUNT OF THE TEST RESULTS

Activated Sludge Respiration Inhibition Test

Test substance: **Perylimid F**
Reference substance: **3,5-dichlorophenol**



Name of test substance: Perylimid F

Name of reference substance: 3,5-dichlorophenol

	Stock solution input (mg)	Stock solution volume (ml)	Direct addition
Test substance	2000,0	1000	-
Reference substance	500,0	1000	-

MEASURED DATA - blank control (BC) and test substance (TS),
mv BC = mean value of blank control

Table 1

Vessel no.:	BC1	BC2	BC3	mv BC	TS1
Concentration of test substance (mg/l)	-	-	-	-	1000
Added volume of stock solution(ml/vessel)	-	-	-	-	125,0
Direct addition (mg/vessel)	-	-	-	-	-
Oxygen concentration start (mg O_2/l)	7,7	7,9	7,8	-	7,7
Oxygen concentration after 6 min (mg O_2/l)	5,3	5,5	5,2	-	5,2
Oxygen consumption rate (mgO_2/l*6 min)	2,4	2,4	2,6	2,5	2,5
Oxygen consumption rate (mgO_2/l*h)	24	24	26	25	25
Specific oxygen consumption rate (mgO_2/g*h)	24	24	26	25	25
Change in oxygen consumption rate compared with the blank control (%)	-	-	-	-	0

pH-VALUES - blank control (BC) and test substance (TS)

Table 2

Vessel no.:	BC1	BC2	BC3		TS1
pH-value before adding the inoculum					
before correction:	6,4	6,4	6,4		6,5
after correction:	7,2	7,3	7,3		7,4
pH-value after an incubation time of 30 min	-	-	-		7,4

MEASURED DATA - reference substance (RS)

Table 3

Test vessel number:	mv BC	RS1	RS2	RS3
Concentration of substance (mg/l)	-	1	10	100
Added volume of stock solution (ml/vessel)	-	0,5	5,0	50
Oxygen concentration start (mg O_2/l)	-	7,6	8,4	9,1
Oxygen concentration after 6 min (mg O_2/l)	-	5,3	7,4	9,0
Oxygen consumption rate (mgO_2/l*6 min)	2,5	2,3	1,0	0,1
Oxygen consumption rate (mgO_2/l*h)	25	23	10	1
Specific oxygen consumption rate (mgO_2/g*h)	25	23	10	1
Change in oxygen consumption rate compared to the blank control (%)	-	8	60	96

pH-VALUES - reference substance (RS)

Table 4

Vessel no.:	RS1	RS2	RS3
pH-value before adding the inoculum			
before correction:	6,4	6,5	6,5
after correction:	7,3	7,4	7,1

Effective concentrations EC values

Table 5

	EC_{20} mg/l	EC_{50} mg/l	EC_{80} mg/l	Highest concentration tested (mg/l)
Test substance	-	-	-	1000
Reference substance	ca. 1,8	ca. 6,5	ca. 36	100