

TEST REPORT

WV Umweltschutz LEV
Institute for Environmental Analysis and Assessments
Microbiology 1
Laboratory, Dr. Kanne, phone. 65568

Biodegradation of	Unimoll 66
Test substance	5.0..
CAS number	84-61-7
Sum formula	C20 H26 O4
Molecular weight	330 B/Mol
Structural formula	
Theoretical oxygen demand (mg/g)	2376
CSB (mg/g)	---
<u>Reference substance</u>	Anilin
Sum formula	C6 H7 N
Molecular weight	93 g/mol
Structural formula	
Theoretical oxygen demand (mg/g)	---
Investigation period	06/22.-07/20/1989

Client	Dr. Paetz (Balk, Geb. DB)
Method	Miti/test OECD test guideline 301 C. The method was modified according to the EC ring test: Assessment of Biodegradability of chemicals in water by manometric respirometry (DG x 1 283/82 Rev. 5). S.O.P. No. 3.003
Vaccine material	10 L-Laboranlage (Wupperverband)
Test manager	Dr. Kanne
Representative of the study manager	Prof. Dr. Caspers
Executors	Husken/Rauer

Test number: 43A/BB

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Test substance	Unimoll 66
Date of receipt	10/11/1988
Quantity delivered	50 g
Type of packaging	Glass bottle
Storage method	Refrigerator

Weighing of the test and reference substance for the test preparation
refer to S.O.P. No. 3.003 paragraph 5.3.

Type of scale Typ" Mettler A 30

Nominal sample weight of the test substance: 25.0 mg

Actual sample weight	Piston: 5 0.025 g
	Piston: 6 0.025 g
	Piston: 7 0.025 g
	Piston: 8 0.025 g

Type of scale Typ" Mettler A 30

Nominal sample weight of the reference substance: 25.0 mg

Actual sample weight	Piston: 3 0.025 g
	Piston: 4 0.025 g
	Piston: 7 0.025 g

Remarks: ---

INTERNAL

Test number: 43A/BB

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Origin of the vaccine material

Laboratory activated sludge plant with
Wupperverband wastewater

Date of sludge sampling

06/22/1989

For dry weight determination see S.O.P. No. 3.003 paragraph 4.1.:

Centrifuge tube	I	II
Gross weight	13.051 g	13.505 g
Tare weight	13.037 g	13.490 g
Net weight	.014 g	.015 g

Mean value: .014 g

For calculation of the input volume, see S.O.P. No. 3.003 paragraph 5.2.

Calculated input volume: 5.173 ml/250 ml

Actual deployment volume: 5.2 ml/250 ml

Type of pipette used: 10 ml graduated pipette

Remarks: ---

INTERNAL

Weights for solutions of the Miti test
see S.O.P. No. 3.003 paragraph 2

Chemicals	Sum formula	Weighing-in NOMINAL	ACTUAL
Potassium hydrogen phosphate	KH ₂ PO ₄	8.5 g	8.591 g
Dipotassium hydrogen phosphate	K ₂ HPO ₄	21.75 g	21.961 g
Disodium hydrogen phosphate dihydrate	Na ₂ HPO ₄ *2H ₂ O	33.4 g	33.4 g
Ammonium chloride	NH ₄ CL	2.5 g	2.502 g
Magnesium sulfate heptahydrate	MgSO ₄ *7H ₂ O	22.5 g	22.499 g
Calcium chloride	CaCl ₂	27.5 g	20.064 g
Iron(III) chloride hexahydrate	FeCl ₃ *6H ₂ O	0.25 g	0.254 g
Manganese sulfate monohydrate	MnSO ₄ *H ₂ O	302 mg	.301 g
Boric acid	H ₃ BO ₃	572 mg	.572 g
Zinc(II) sulfate heptahydrate	ZnSO ₄ *7H ₂ O	428 mg	.427 g
Ammonium molybdate tetrahydrate	(NH ₄) ₆ Mo ₇ O ₂₄ *4H ₂ O	369 mg	.370 g
Iron chelate	FeCl ₃ *EDTA	1000 mg	1.000 g
Biotin		ca. 2 mg	.002 g
Nicotinic acid		20 mg	.020 g
Thiamine		10 mg	.010 g
P-aminobenzoic acid		10 mg	.010 g

Chemicals	Sum formula	Weighing-in	
		NOMINAL	ACTUAL
Pantothenic acid		10 mg	.010 g
Pyridoxamin		50 mg	.050 g
Cyanocobalamin		20 mg	.020 g
Folic acid		50 mg	.050 g
Mercury chloride	HGCL2	1.0 g	1.006 g
Potassium hydroxide	KOH	20.0 g	20.105 g

Type of scale used: Mettler A 30

Exception: disodium hydrogen phosphate dihydrate: Mettler PC 4000

Date of manufacture of the solutions: 06/12/1989

Exception: phosphate solution on 06/21/1989, ferric chloride 6-hydrate solution and KOH solution on 06/22/1989

Remarks: S.H. annex page 6A

Annex to the weighing

Potassium dihydrogen phosphate is 99% after conversion of the corresponding weight. Likewise the following substances:

potassium hydrogen phosphate 99% and calcium chloride 98%.

For the contents of the piston, see S.O.P. No. 3.003 paragraph 5.3.

Piston	Test substance	MG	Mercury solution	ML	Medium
		Anilin		Inoculum	
1	0	0	.00	5.20	250
2	0	0	.00	5.20	250
3	0	25	.00	5.20	250
4	0	25	.00	5.20	250
5	25	0	.00	5.20	250
6	25	0	.00	5.20	250
7	25	25	.00	5.20	250
8	25	0	.25	.00	250

Remarks: ---

Miti (degradation) test according to Dr. Painter

Test laboratory: WV-LE UWS/AWALU Microbiology Dr, Kanne

Test substance: Unimoll 66

Input quantities:

Test substance 100 mg/l

Theoretical oxygen demand 2.376 mg O₂/mg Substanz

Theoretical TOC ---

Test period: 06/22.-07/20/1989

Time	Oxygen consumption mg/l			Biodegradation %	
Day	Test substance	Anilin	Blank value	Test substance	Anilin
2	-	-	-	-	-
4	17	6	11	3	0
6	20	6	13	3	0
8	21	43	13	3	12
10	-	-	-	-	-
12	29	179	13	7	69
14	75	188	13	26	73
16	-	-	-	-	-
18	145	190	13	56	73
20	155	190	13	60	73
22	162	190	13	63	73
24	-	-	-	-	-
26	169	190	13	66	73
28	172	190	13	67	73

Remarks: S.H. annex page 8A and 8B

Annex to page 8

- Cell 2 defective, values were not included in the evaluation
- Values of cell 6 not plausible, were not included in the evaluation. Based on the previous checks, cell 5 was evaluated.

Test number: 43A/BB

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Biodegradability - Miti test
Test substance: 43 A/88 Unimoll 66

Day

Test substance: + Anilin

% degradation

INTERNAL

Consumption of the test substance and whereabouts of the residual amount

Quantity delivered	50 g
Consumed were	approx. 0.3 g
Residual amount	approx. 49.7 g

Residual amount returned: 07/31/1989 (DE Backer-March Geb. W15)
date, recipient

Remarks: ---

Execution of the test

Husken

Job title

Biology lab technician

08/07/1989
Date

((Signature))
Signature

Head of the work unit

Husken

Job title

Biology lab technician

08/07/1989
Date

((Signature))
Signature

Test nanager

Dr. Kanne

Job title

Graduate biologist

08/07/1989
Date

((Signature))
Signature

Quality Assurance

Dr. Stottmeister

Job title

Graduate biologist

08/07/1989
Date

((Signature))
Signature