B3 OCF 20% MCCP (GWMB3)

Fresh water algal growth inhibition test with *Desmodesmus subspicatus*

SCREENING TEST

Report

BMG study no. A10-00857

July 2010

Labors: Analytik, Ökotoxikologie,
Verfahrenstechnik

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1 Preface

1.1 General

Title	B3 OCF 20% MCCP (GWMB3) Fresh water algal growth inhibition test with *Desmodesmus subspicatus*, Screening Test
Sponsor	FEICA - Association of European Adhesives & Sealants Manufacturers Avenue E. van Nieuwenhuyse, 6 B-1160 Brussels
Study Monitor	[redacted] Henkel AG & Co KGaA Henkelstrasse 67 D-40191 Düsseldorf
Test Facility	BMG Engineering Ltd. Ifangstrasse 11 CH-8952 Schlieren

1.2 Responsibilities

Study Director	[redacted]
Quality Assurance	[redacted]

1.3 Schedule

Experimental starting date	28 June 2010
Experimental completion date	5 July 2010

1.4 Archiving

BMG Engineering Ltd (CH-8952 Schlieren) will retain the study plan, raw data, sample of test item(s) and specimens (as long as the quality permits evaluation) and the final report of the present study for a minimum of ten years. A report amendment has to be written if the archived items are transferred to another facility.

No data will be discarded without the Sponsor's written consent.

1.5 Distribution

This report was distributed as follows:

- Archives of BMG Engineering Ltd, i.e. study file (1 original)
- Sponsor (1 copy)

1.6 Signature

Study Director:

1.7 Quality Assurance Statement

BMG Engineering Ltd.
Ifangstrasse 11
CH-8952 Schlieren

BMG study no.	A10-00857
Test substance	B3 OCF 20% MCCP (GWMB3)
Study Director	[redacted]
Title	B3 OCF 20% MCCP (GWMB3) Fresh water algal growth inhibition test with *Desmodesmus subspicatus*

The laboratory facilities and activities are inspected periodically and the results are reported to the study director and the management.

The study plan, the study procedures and this report were audited by the Quality Assurance. The dates are given below.

Dates and types of study-based QA inspections	Dates of reports to the Study Director and the Management
17 June 2010 (study plan)	17 June 2010
28 June 2010 (application of foam, weighing of test material)	28 June 2010
15 July 2010 (draft report)	15 July 2010

This statement confirms that this final report reflects the raw data. It is also confirmed that the final report was inspected.

Quality Assurance: [redacted]

Date:

1.8 Statement of Compliance

BMG Engineering Ltd.
Ifangstrasse 11
CH-8952 Schlieren

BMG study no.	A10-00857
Test substance	B3 OCF 20% MCCP (GWMB3)
Study Director	[redacted]
Title	B3 OCF 20% MCCP (GWMB3) Fresh water algal growth inhibition test with *Desmodesmus subspicatus*

This study was performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted May 18th, 2005 [RS 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted by the OECD Council on 26th November, 1997 [C(97)186/Final].

Study Director: [redacted]

1.9 Test Guidelines

The study procedures meet the requirements of the following test guidelines:

- Organisation for Economic Cooperation and Development. OECD Guidelines for the Testing of Chemicals - Freshwater Alga and Cyanobacteria, Growth Inhibition Test, TG 201, adopted 23th March 2006.
- European Union. C.3. Algal Inhibition Test. COMMISSION REGULATION (EC) No 761/2009 of 23 July 2009 amending, for the purpose of its adaptation to technical progress, Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

1.10 Classification Guidelines

- Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (O.J. L 225, 21.8.2001).
- Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (O.J. L 353, 31.12.2008).

1.11 Amendment and Deviation Procedures

1.11.1 Study plan amendment

None

1.11.2 Deviation from the study plan

None

2 Summary

The median growth inhibiting concentration (E_rC_{50}/E_yC_{50}) and the low-effect concentrations (E_rC_{10}/E_yC_{10},E_rC_{20}/E_yC_{20}) based on the average specific growth rate and the yield (algal biomass production) of B3 OCF 20% MCCP (GWMB3) to the green alga *Desmodesmus subspicatus* were investigated in a screening test over a period of 72 h.

The nominal concentrations of B3 OCF 20% MCCP (GWMB3) were 100, 316 and 1000 mg/l, respectively. Due to the limited water solubility of the test substance, only the water accommodated fractions were tested.

3 parallel test vessels were used for each concentration of the test substance.

No chemical analysis of the test media was conducted.

With respect to algal growth rate inhibition the following effects as compared to the untreated controls were observed:

1000 mg/l (<10%), 316 mg/l (<10%) and 100 mg/l (<10%). No significant effects (determined by Dunnett's Test) were observed at 1000 mg/l.

Based on these data the nominal median effect concentration with respect to growth rate (E_rC_{50}/0-3 days) of B3 OCF 20% MCCP (GWMB3) to *Desmodesmus subspicatus* was estimated to be >1000 mg/l with respect to the loading rate.

The low effect concentrations for the average specific growth rate, i.e. E_rC_{10} and E_rC_{20}, were estimated to be >1000 mg/l, respectively. The no-observed-effect concentration with respect to growth rate (NOE_rC) was ≥1000 mg/l.

For the endpoint yield (biomass production) the following effects as compared to the untreated controls were observed at the respective nominal test concentrations:

1000 mg/l (<10%), 316 mg/l (<10%) and 100 mg/l (<10%). No significant effects (determined by Dunnett's Test) were observed at 1000 mg/l.

Based on these data the nominal median effect concentration with respect to yield (E_yC_{50}/0-3 days) of B3 OCF 20% MCCP (GWMB3) to *Desmodesmus subspicatus* was estimated to be >1000 mg/l with respect to the loading rate.

The low effect concentrations with respect to yield, i.e. E_yC_{10} and E_yC_{20}, were estimated to be >1000 mg/l, respectively. The no-observed-effect concentration with respect to yield (NOE_yC) was ≥1000 mg/l.

3 Purpose

The objective of this study was to determine the effects (E_rC_{10}, E_rC_{20}, E_rC_{50} and E_yC_{10}, E_yC_{20}, E_yC_{50}) of B3 OCF 20% MCCP (GWMB3) on the green alga *Desmodesmus subspicatus* over a period of 72 hours of exposure.

4 Materials and methods

4.1 Culture system

Test organism	Axenic slope culture of *Desmodesmus subspicatus* 86.81 SAG (Collection of Algal Cultures, Institute of Freshwater Ecology, University of Göttingen, D-37073 Göttingen, Germany
Culture	100 ml flask containing 50 ml of sterile OECD medium (Table 1) inoculated with cell material from axenic slope culture
Illumination	Continuous (6000–12000 lux) from high pressure sodium lamp (Eye Sunlux Ace, Eye, Tokio, Japan)
Temperature	22 ± 2 °C, thermo-controlled room
Control of sensitivity	Yearly re-purchase of the culture

4.2 Test substance

Identification	B3 OCF 20 % MCCP (GWMB3)
Batch no.	6710-6544-6459
BMG sample no.	M1005-03832-01
Physical form	liquid / gas
Water solubility	insoluble
Composition / purity	C14-17 chlorinated paraffins, 20% solution in MDI-prepolymer, rest unknown; treated as 100% pure; will be excluded from the statement of compliance
CAS no.	85535-85-9 (C14-17 chlorinated paraffins)
EINECS no.	287-477-0 (C14-17 chlorinated paraffins)
Test substance storage	room temperature
Stability	Stable under storage conditions
Expiry date	February 2011

4.3 Conditions

Test vessel	250 ml flasks, all-glass, with 100 ml of test medium, shaken (125 rpm)
Test medium	Recommended OECD medium (Table 1)
Preculture	Exponentially growing liquid culture of *Desmodesmus subspicatus*
Illumination	Continuous (6000–12000 lux) from high pressure sodium lamp (Eye Sunlux Ace, Eye, Tokio, Japan)

Temperature	22 ± 2°C, thermo-controlled room
Test type	Static exposure conditions over a period of 72 h
Starting cell density	0.5–0.85 μg dry weight / ml (i.e. OD_{680} of about 0.010)

4.4 Handling

A screening test with nominal concentrations of 100, 316 and 1000 mg/l, respectively, was performed. Due to the limited water solubility of the test substance, only the water accommodated fractions were tested. These water accommodated fractions were prepared as follows:

- application of foam in a glass beaker
- vigorously stirring the foam for about 15 s with a glass stick to remove at least part of the propellant; the foam should consequently collapse
- weighing of the respective amounts of the collapsed foam onto microscope slides
- addition of the microscope slides to respective amounts of Alga medium (Table 1) in glass bottles
- the test solutions were moderately stirred for about 24 h; the collapsed foam particles should be in full contact with the Alga medium
- filtration of the solutions (sterile filter, 0.45 µm)

The resulting water accommodated fractions were used in the test. The pH of the OECD medium was adjusted before the test (pH 8.0 ± 0.2). Blanks of test medium without test substance served as controls.

An exponentially growing preculture of algae was used to inoculate sterile test flasks. For each test series the following number of test flasks will be set up:

- Test suspension (T_n); containing test medium, test substance and algae (three replicate samples)
- Blank control (B_n); containing test medium and algae (five replicate samples)

Test flasks are placed on an orbital shaker at a nominal shaking speed of about 130 revolutions per min.

4.5 Observations and measurements

pH	Determined in the medium batch which is used for the preparation and dilution of the stock solution.
Observations	Cell concentrations are determined every 24 h using a spectrophotometer (680 nm wavelength). Aliquots of 5 ml are removed from each test flask under sterile conditions. Cell concentrations at time 0 h are only determined in the control vessels.

4.6 Chemical analysis

No analyses of the test concentrations were conducted. For calculations the nominal concentrations of the test substance in the test media were used (calculations based on assumption that test material consists of 100% a.i.).

Table 1 OECD algal medium for the culturing of *Desmodesmus subspicatus*.

Nutrient	Final concentration in test solution (mg/l)	Remarks
NH_4Cl	15	pH-value after equilibration with air:
$MgCl_2 \times 6\ H_2O$	12	8.0 ± 0.2
$CaCl_2 \times 2\ H_2O$	18	
$MgSO_4 \times 7\ H_2O$	15	
KH_2PO_4	1.6	
$FeCl_3 \times 2\ H_2O$	0.08	
Na_2-EDTA $\times 2\ H_2O$	0.1	
H_3BO_3	0.185	
$MnCl_2 \times 4\ H_2O$	0.415	
$ZnCl_2$	3×10^{-3}	
$CoCl_2 \times 6\ H_2O^3$	1.5×10^{-3}	
$CuCl_2 \times 2\ H_2O$	10^{-5}	
$Na_2MoO_4 \times 2\ H_2O$	7×10^{-3}	
$NaHCO_3$	50	

5 Evaluation of the test results

5.1 Calculation of average growth rate inhibition

The median effective concentration (E_rC_{50}) is the concentration of test substance which causes a 50% reduction in growth rate. The average specific growth rate (μ) in each test flask is calculated as the logarithmic increase in the biomass during a given number of days according to equation 1:

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i} \quad (1)$$

where:

μ_{i-j} is the average specific growth rate from time *i* to *j*;
X_i is the biomass at time *i*;
X_j is the biomass at time *j*.

The percentage growth rate inhibition in each sample (I_r) was calculated as the difference between the mean growth rate of the controls (μ_C) and the growth rate at each test substance concentration (μ_T) as given in equation 2:

$$\%I_r = \frac{\mu_C - \mu_T}{\mu_C} \times 100 \quad (2)$$

where:

$\%I_r$ percent inhibition in average specific growth rate;
μ_C mean value for average specific growth rate (μ) in the control group;
μ_T average specific growth rate for the treatment replicate.

5.2 Calculation of yield inhibition

The median effective concentration (E_yC_{50}) is the concentration of test substance which causes a 50% reduction in yield (algal biomass production). The average yield in each test flask was calculated as the biomass at the end of the test minus the starting biomass for each single vessel of controls and treatments. The percent inhibition in yield ($\%I_y$) was calculated as given in equation 3:

$$\%I_y = \frac{Y_C - Y_T}{Y_C} \times 100 \quad (3)$$

where:

$\%I_y$ percent inhibition of yield;
Y_C mean value for yield in control group;
Y_T value for yield for the treatment replicate.

5.3 Calculation of E_rC_x and E_yC_x

The E_rC_x (x= 50, 20, 10) and E_yC_x (x= 50, 20, 10) values were calculated with ToxRat® Standard Version 2.10 (ToxRat® Solutions GmbH, Alsdorf, Germany). For the calculation of confidence intervals values below 0% inhibition was set to 0.1% inhibition.

5.4 Determination of no observed effect concentrations (NOE_rC and NOE_yC)

The no observed effect concentration (NOE_rC or NOE_yC) is the highest concentration for which the observed effect is not significantly different from the controls. The statistical significance of differences between effects observed for control and test substance treatments were determined by Dunnett's test using the ToxRat® Standard Version 2.10 (ToxRat® Solutions GmbH, Alsdorf, Germany).

5.5 Validity of the test

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72 h test period.
- The average specific growth rate of the controls must exceed 0.92 day-1.
- The coefficient of variation of the average specific growth rates during the whole test period in replicate control cultures must not exceed 7%.
- The mean of the section by section (days 0–1, 1–2, and 2–3) specific growth rates must not exceed 35% in the control samples.

6 Data compilation

The following data were recorded on data sheets and transcribed for compilation and analysis: amount of test material applied, dilution of stock solutions, cell concentrations using a spectrophotometer (680 nm wavelength) at 0 h (only control replicates) and after 24, 48 and 72 h, respectively.

Statistical analysis was performed with respect to 95% confidence limits and regression data.

7 Results and discussion

7.1 Range finding test

A screening test with nominal concentrations of B3 OCF 20% MCCP (GWMB3) of 100, 316 and 1000 mg/l, respectively, was performed. Therefore, no range finding test was conducted.

7.2 Definitive test

Cell concentrations (A_{680}) are presented in Table 2.

The average specific growth rates over 3 days and the respective inhibition percentages are presented in Table 3. The section by section (days 0-1, 1-2, and 2-3) specific growth rates are presented in Table 4.

The average yield over 3 days and the respective inhibition percentages are presented in Table 5.

Growth curves for the evaluation of the validity of the test are plotted in Figure 1.

7.2.1 Determination of growth rate inhibition

With respect to algal growth rate inhibition the following effects as compared to the untreated controls were observed:

1000 mg/l (<10%), 316 mg/l (<10%) and 100 mg/l (<10%). No significant effects (determined by Dunnett's Test) were observed at 1000 mg/l.

Based on these data the nominal median effect concentration with respect to growth rate (E_rC_{50}/0-3 days) of B3 OCF 20% MCCP (GWMB3) to *Desmodesmus subspicatus* was estimated to be >1000 mg/l with respect to the loading rate.

The low effect concentrations for the average specific growth rate, i.e. E_rC_{10} and E_rC_{20}, were estimated to be >1000 mg/l, respectively. The no-observed-effect concentration with respect to growth rate (NOE_rC) was ≥1000 mg/l.

7.2.2 Determination of algal yield (biomass production) inhibition

For the endpoint yield (biomass production) the following effects as compared to the untreated controls were observed at the respective nominal test concentrations:

1000 mg/l (<10%), 316 mg/l (<10%) and 100 mg/l (<10%). No significant effects (determined by Dunnett's Test) were observed at 1000 mg/l.

Based on these data the nominal median effect concentration with respect to yield (E_yC_{50}/0-3 days) of B3 OCF 20% MCCP (GWMB3) to *Desmodesmus subspicatus* was estimated to be >1000 mg/l with respect to the loading rate.

The low effect concentrations with respect to yield, i.e. E_yC_{10} and E_yC_{20}, were estimated to be >1000 mg/l, respectively. The no-observed-effect concentration with respect to yield (NOE_yC) was ≥1000 mg/l.

7.2.3 Validity of the definitive test

To evaluate the growth in the control samples, growth curves of the definitive test are plotted in Figure 1 using the logarithm of the average biomass at a given time x.

Since the control samples showed nearly a straight line from days 0 to 3, it can be concluded that the growth in the control samples was exponential for that time period. Also all other validity criteria as specified in section 5.5 were met (especially the biomass increase, attained in the control cultures over 72 h, was higher than the requested minimum factor of 16).

Table 2 Cell concentration (A_{680}) in individual test cultures after 24, 48 and 72 h of exposure to the test material.

		Cell concentration (A_{680}) [a]			
Nominal concentration (mg/l)	Code	0 h [b]	24 h	48 h	72 h
Control	A	0.013	0.039	0.154	0.507
	B	0.013	0.045	0.142	0.529
	C	0.013	0.040	0.160	0.503
	D	0.013	0.041	0.201	0.526
	E	0.013	0.044	0.230	0.631
100	A	0.013	0.045	0.161	0.569
	B	0.013	0.044	0.164	0.522
	C	0.013	0.043	0.218	0.581
316	A	0.013	0.047	0.251	0.609
	B	0.013	0.043	0.186	0.536
	C	0.013	0.040	0.154	0.548
1000	A	0.013	0.039	0.175	0.558
	B	0.013	0.040	0.182	0.513
	C	0.013	0.043	0.141	0.482

[a] Initial biomass: 0.5–0.85 μg dry weight / ml

[b] Mean absorbance (A_{680}) of blank controls

Table 3 Average specific growth rates between 0 and 72 h.

Nominal concentration (mg/l)	Code	**GRa**[a] $\mu_{3-0}(d^{-1})$ [b]	Coefficient of variation (%)	Inhibition (%)	Mean value (%)
Control	A	1.22	**2.5** [c]	1.6	0.0
	B	1.24		0.4	
	C	1.22		1.8	
	D	1.23		0.6	
	E	1.29		-4.3	
100	A	1.26	**1.5**	-1.5	-1.0
	B	1.23		0.8	
	C	1.27		-2.1	
316	A	1.28	**1.8**	-3.4	-1.3
	B	1.24		0.1	
	C	1.25		-0.5	
1000	A	1.25	**2.0**	-1.0	1.0
	B	1.23		1.2	
	C	1.20		2.9	

[a] average specific growth rate over 3 days

[b] control average must exceed 0.92 day^{-1}

[c] must not exceed 7% in control samples

Table 4 Section by section specific growth rates.

Nominal conc. (mg/l)	Code	GRs[a] $\mu_{1\text{-}0}(d^{-1})$ day 1	GRs[a] $\mu_{2\text{-}1}(d^{-1})$ day 2	GRs[a] $\mu_{3\text{-}2}(d^{-1})$ day 3	Coefficient of variation (%)			
					day 1	day 2	day 3	day1-3
Control	A	1.10	1.37	1.19	5.3	14.0	12.7	**15.7** [b]
	B	1.24	1.15	1.32				
	C	1.12	1.39	1.15				
	D	1.15	1.59	0.96				
	E	1.22	1.65	1.01				
100	A	1.24	1.27	1.26	1.9	13.6	12.6	13.5
	B	1.22	1.32	1.16				
	C	1.20	1.62	0.98				
316	A	1.29	1.68	0.89	6.7	11.1	17.9	18.4
	B	1.20	1.46	1.06				
	C	1.12	1.35	1.27				
1000	A	1.10	1.50	1.16	4.4	13.2	8.6	13.8
	B	1.12	1.52	1.04				
	C	1.20	1.19	1.23				

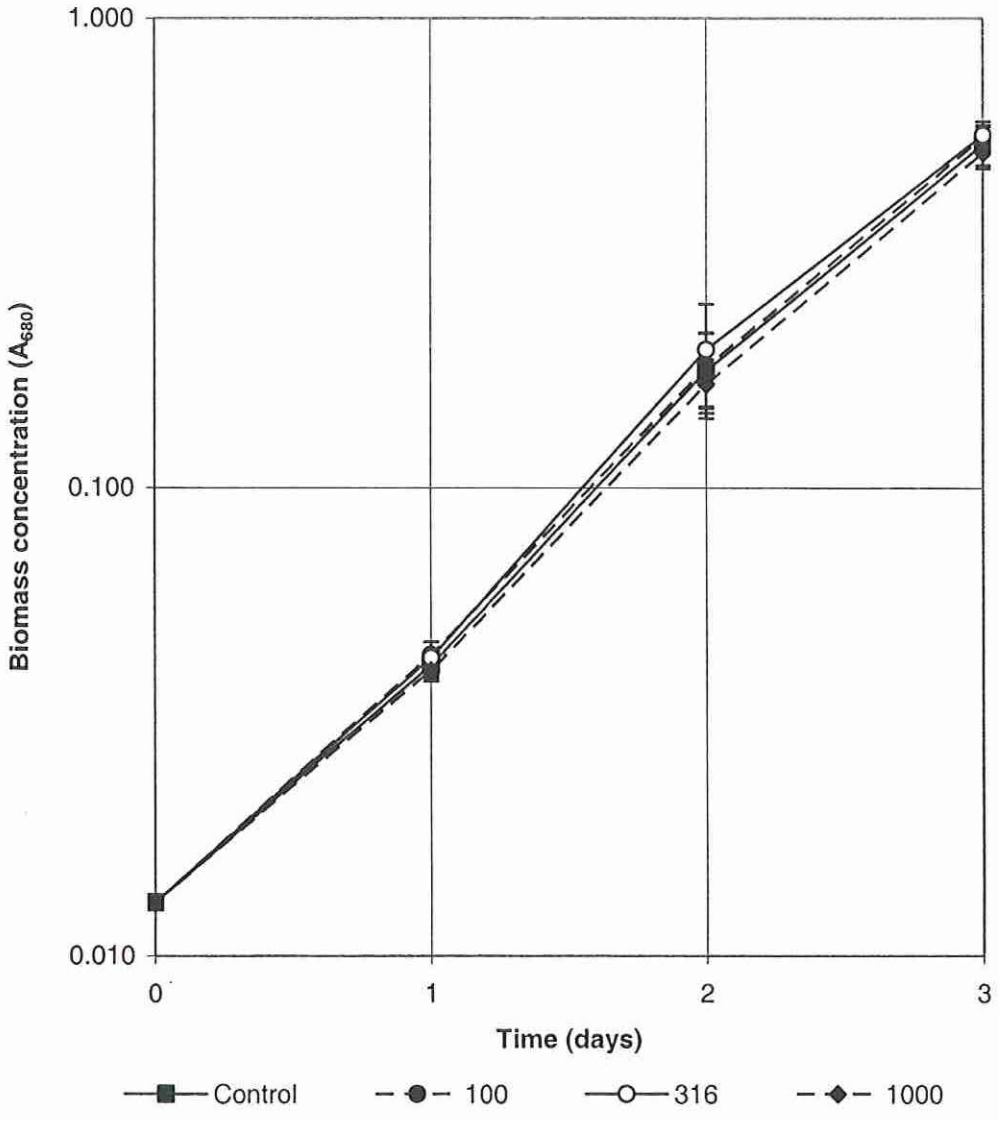
[a] section by section specific growth rate

[b] must not exceed 35% in control samples

Table 5 Algal cell growth (yield) between 0 and 72 h.

Nominal concentration (mg/l)	Code	Yield biomass days 0-3	Coefficient of variation (%)	Inhibition (%)	Mean value (%)
Control	A	0.49	10.0	6.1	0.0
	B	0.52		1.9	
	C	0.49		6.9	
	D	0.51		2.5	
	E	0.62		-17.4	
100	A	0.56	5.7	-5.7	-3.4
	B	0.51		3.3	
	C	0.57		-7.9	
316	A	0.60	7.1	-13.3	-4.8
	B	0.52		0.6	
	C	0.54		-1.7	
1000	A	0.55	7.6	-3.6	4.1
	B	0.50		5.0	
	C	0.47		10.9	

Figure 1 Biomass increase over time in the definitive test.



8 Certificate of GLP compliance

The Swiss GLP Monitoring Authorities

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Home Affairs DHA
Federal Office of Public Health FOPH

Federal Department of the Environment, Transport, Energy and Communications DETEC
Federal Office for the Environment FOEN

SWISSmedic
Swiss Agency for Therapeutic Products

Statement of GLP Compliance

According to Article 14 paragraph 3 Ordinance on Good Laboratory Practice [OGLP, SR 813.112.1]

The notification authority for chemicals confirms that the following test facility was inspected with respect to the compliance with the Swiss Ordinance on Good Laboratory Practice, adopted on 18th May 2005 [OGLP, SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted on 26th November 1997 by decision of the OECD Council [C(97)186/Final].

Unequivocal name and address of the test facility:	Areas of expertise according to article 3 paragraph 1 letter d OGLP:
BMG Engineering AG Labors Ifangstrasse 11 8952 Schlieren, Switzerland	3. environmental toxicity studies on aquatic and terrestrial organisms, 4. studies on behaviour in water, soil and air; bioaccumulation, 7. physical-chemical testing.

Inspection authority: Federal Office for the Environment (FOEN)

Date of inspection: 25th and 26th November 2009

Date of decision: 25th January 2010

Based on the above mentioned decision it can be confirmed that the above mentioned test facility is able to conduct studies according to the aforementioned areas of expertise in compliance with the principles of GLP. The above mentioned test facility is listed in the register and GLP list according to the Article 14 OGLP and is inspected on a regular basis according to Article 6 paragraph 2 OGLP.

Swiss Federal Office of Public Health
Consumer protection directorate
Notification authority for chemicals
CH-3003 Bern

[redacted]

The notification authority for chemicals is the coordination and decision authority for the good laboratory practice (GLP) for the FOEN, the FOPH and Swissmedic.

Swiss Federal Office of Public Health, Consumer protection directorate, Notification authority for chemicals, CH-3003 Bern,

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