B3 OCF 20% MCCP (GWMB3)

48-hour Acute Toxicity to *Daphnia magna*

SCREENING TEST

Report

BMG study no. A10-00856

July 2010

Labors: Analytik, Ökotoxikologie,
Verfahrenstechnik

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1.1 General

Title	B3 OCF 20% MCCP (GWMB3) 48-hour acute toxicity to *Daphnia magna*, 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]

1.3 Schedule

Experimental starting date	28 June 2010
Experimental completion date	8 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: [redacted]

1.7 Quality assurance statement

BMG Engineering Ltd.
Ifangstrasse 11
CH-8952 Schlieren

BMG study no.	A10-00000
Test substance	B3 OCF 20% MCCP (GWMB3)
Study Director	[redacted]
Title	B3 OCF 20% MCCP (GWMB3) 48-hour acute toxicity to *Daphnia magna*, Screening Test

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
17 June 2010 (study plan)	17 June 2010
28 June 2010 (1st test series: details on test protocols, application of foam)	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: [redacted]

1.8 Statement of Compliance

BMG Engineering Ltd.
Ifangstrasse 11
CH-8952 Schlieren

BMG study no.	A10-00856
Test substance	B3 OCF 20% MCCP (GWMB3)
Study Director	[redacted]
Title	B3 OCF 20% MCCP (GWMB3) 48-hour Acute Toxicity to *Daphnia magna*

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]

Date:

1.9 Test Guidelines

The study procedures described in this study plan meet the requirements of the following test guidelines:

- Organisation for Economic Cooperation and Development. OECD Guidelines for the Testing of Chemicals, "Daphnia sp., Acute Immobilisation Test", Test Guideline 202, adopted: 13 April 2004.
- European Union. C.2. Acute Toxicity for *Daphnia*. Council Regulation (EC) No 440/2008 of 30 May 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) (O.J. L 142 of 31.5.2008).

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

In a 1st test series >10% immobilisation was observed in the blank controls after 48 h of exposure. Therefore, the test had to be repeated (i.e. 2nd test series).

2 Summary

The acute toxicity of B3 OCF 20% MCCP (GWMB3) to *Daphnia magna* was investigated under static exposure conditions over a period of 48 h. A screening test with nominal concentrations of 100, 316 and 1000 mg/l, respectively, was performed.

40 individual *Daphnia* divided in 2 test vessels were exposed to each concentration of the test substance. Due to the limited water solubility of the test substance, only the water accommodated fractions will be tested.

No analytical verification of the test material was conducted. The evaluation of the test was based on the nominal concentrations of the test material, assuming the test compound to be stable in water over 48 h.

No significant effects (≤10% immobilization) were observed at all nominal concentrations after 24 and 48 h of exposure, respectively. No significant effects were also observed in the control after 24 and 48 h of exposure.

Based on these results the 24 and 48 h EC_{50} values of B3 OCF 20% MCCP (GWMB3) are estimated to be >1000 mg/l with respect to the loading rate.

The no-observed-effect concentration (EC_0, NOEC) for 24 and 48 h of incubation is ≥1000 mg/l with respect to the loading rate.

100% immobilization was not detected.

3 Purpose

The objective of this study was to determine the effects of B3 OCF 20% MCCP (GWMB3) to *Daphnia magna* over a period of 24 and 48 h of exposure, respectively.

4 Materials and methods

4.1 Test system and breeding conditions

Test organism	*Daphnia magna* (Straus, 1820)
Breeding	Parental and young *Daphnia* will be held in 40 l glass aquaria at 20 ± 2 °C.
Illumination	16 h per day
Medium	Continuously aerated Elendt M4 medium (Tables 1 and 2); prepared with deionised water (conductivity <1.5 µS/cm).
Feed	15 g sera micropan (sera GmbH, D-52525 Heinsberg) homogenized in 1 l deionised water

Frequency of feeding	Once a day, except weekends.
Control of sensitivity	Periodically conducted acute reference test with potassium dichromate.

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 Test conditions

Test vessel	100 ml flasks, all-glass, with 50 ml of test medium
Test medium	Continuously aerated Elendt M4 medium (Tables 1 and 2); prepared with deionised water (conductivity <1.5 μS/cm)
Number of Daphnia	40 individuals per test concentration, 20 per vessel
Size	<2 mm
Light	16 h photoperiod a day, supplied by overhead white fluorescent tubes
Temperature	20 ± 2 °C (temperature-controlled room)
Test type	Static exposure conditions
Test duration	48 h

4.4 Handling

Prior to the test, a batch of Elendt M4 medium (Tables 1 and 2) was aerated over a period of 2 h.

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 aerated *Daphnia* medium (Tables 1 and 2) in glass bottles
- the test solutions were moderately stirred for about 24 h; the collapsed foam particles was in full contact with the *Daphnia* medium
- filtration of the water accommodated fractions (MILLIPORE AP15 glass fibre filter)
- the resulting water accommodated fractions were used in the test.

100 ml of the filtered test solutions were filled in one of the two replicate test vessels. The solution was aerated by stirring for about 0.5 h. O_2 concentration and pH were measured. The pH was adjusted if necessary. Afterwards, half of the solution (50 ml) was filled in the 2nd replicate test vessel.

Daphnia, which were selected in size, were acclimatised to the test medium for about 1 h before introduction into the formulated test media.

For each test series the following number of test flasks was set up:

- Test suspension (T_n); containing test medium, test substance, two replicate samples with 20 *Daphnia* each
- Blank control (B_n); containing test medium, three replicate samples with 20 *Daphnia* each

4.5 Observations and measurements

- Observations

  Observations of immobile *Daphnia* were made after 24 and 48 h of exposure.

- Measurements

Temperature	determined in a control flask at the start and at the end of the test
Oxygen, pH	determined in all vessels prior to the introduction of *Daphnia* to the test medium and at the end of the test

4.6 Chemical analyses

No analyses of test concentrations were conducted. For the evaluation of the test the nominal concentration of the test substance in the test media was used.

4.7 *Daphnia* medium

Daphnia magna is cultivated in Elendt M4 medium.

Separate stock solutions (I) of individual trace elements were first prepared in deionised water. From these different stock solutions (I) a second single stock solution (II) was prepared, which contains all trace elements (combined solution), as shown in Tables 1 and 2.

5 Evaluation of the test results

5.1 Definitions

The median effective concentration (EC_{50} value) is the concentration estimated to immobilize 50% of the *Daphnia* after 24 or 48 h of exposure. Those individuals not able to swim within 15 s after gentle agitation of the test vessel are considered to be immobile.

The EC_0, EC_{50} and EC_{100} values (including 95% confidence limits and regression data) were estimated.

5.2 Determination of no observed effect concentrations

The no observed effect concentration (NOEC, EC_0) is the highest concentration for which the observed effect is not significantly different from the controls. The limit of statistical significance was set to a mortality >10%.

5.3 Validity of the test

The test was considered valid, since not more than 10% of the *Daphnia* in the control have been immobilized or trapped at the surface of the water. The dissolved oxygen concentration at the end of the test was ≥3 mg O_2/l.

Table 1 Composition of the stock solutions for the Elendt M4 *Daphnia* medium

Stock solution(s) I (single substance)	Amount added to water (mg/l)	Concentration	To prepare the combined stock solution II, the following amount of stock solution I is added to water (ml/l)
H_3BO_3	57'190	20'000-fold	20
$MnCl_2 \times 4H_2O$	14'420	40'000-fold	10
LiCl	12'240	40'000-fold	10
RbCl	2'840	40'000-fold	10
$SrCl_2 \times 6H_2O$	6'080	40'000-fold	10
NaBr	640	40'000-fold	10
$Na_2MoO_4 \times 2H_2O$	2'460	40'000-fold	10
$CuCl_2$	528	40'000-fold	10
$ZnCl_2$	520	40'000-fold	10
$CoCl_2 \times 6H_2O$	400	40'000-fold	10
KI	130	40'000-fold	10
Na_2SeO_3	87.6	40'000-fold	10
NH_4VO_3	23	40'000-fold	10
$Na_2EDTA \times 2H_2O$	100'000	40'000-fold	-
$FeSO_4 \times 7H_2O$	39'820	40'000-fold	-
Both Na_2EDTA and $FeSO_4$ solutions are prepared separately, poured together and autoclaved immediately. This gives:			
2 l Fe-EDTA solution		10'000-fold	20

Elendt M4 medium is prepared using stock solution II, the macro-nutrients and vitamin as follows (Table 2):

Table 2 Final preparation of the Elendt M4 *Daphnia* medium

	Amount added to water (mg/l)	Concentration	Amount of stock solution II added to prepare medium (ml/l)
Stock solution II (combined trace elements)		400-fold	2.5
Macro nutrient stock solutions (single substance)			
$CaCl_2 \times 2H_2O$	117'500	400-fold	2.5
$MgSO_4 \times 7H_2O$	49'300	400-fold	2.5
KCl	2'320	400-fold	2.5
$NaHCO_3$	25'920	400-fold	2.5
Na_2SiO_3	1'718	400-fold	2.5
$NaNO_3$	109.6	400-fold	2.5
KH_2PO_4	57.2	400-fold	2.5
$K_2HPO_4 \times 3H_2O$	96.6	400-fold	2.5
Combined vitamin stock	-	10'000-fold	0.1
The combined vitamin stock solution is prepared by adding the 3 vitamins to 1 litre of water, as shown below:			
Thiamine hydrochloride	750	10'000-fold	
Cyanocobalamine (B12)	10	10'000-fold	
Biotine	7.5	10'000-fold	

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, immobile *Daphnia* after 24 and 48 h of exposure, pH and O_2 determinations.

7 Results and discussion

7.1 Effects on mobility

This study represents a screening test. Therefore, no range finding test was conducted.

Immobility data are presented in Table 3.

No significant effects (≤10% immobilization) were observed at all nominal concentrations after 24 and 48 h of exposure, respectively. No significant effects were also observed in the control after 24 and 48 h of exposure.

7.2 Estimation of EC_{50}, EC_0 and EC_{100}

Based on these results the 24 and 48 h EC_{50} values of B3 OCF 20% MCCP (GWMB3) are estimated to be >1000 mg/l with respect to the loading rate.

The no-observed-effect concentration (EC_0, NOEC) for 24 and 48 h of incubation is ≥1000 mg/l with respect to the loading rate.

100% immobilization was not detected.

7.3 pH-values, oxygen concentrations and temperature

pH-values and oxygen concentrations during the definitive test are presented in Table 4. The temperature during the whole test period was 20 ± 2 °C. All values were within an acceptable range.

Table 3 Immobilization of *Daphnia magna* after 24 and 48 h of exposure to the test material.

Nominal concentration (mg/l)	Code	Number of individuals per test vessel	Immobile *Daphnia* (No./vessel) 24 h	Immobile *Daphnia* (No./vessel) 48 h	% immobile *Daphnia* after 24 h of exposure	% immobile *Daphnia* after 48 h of exposure
Control	A	20	0	0	0	0
0	B	20	0	0	0	0
	C	20	0	0	0	0
100	A	20	0	0	0	0
	B	20	0	0	0	0
316	A	20	0	0	0	0
	B	20	0	0	0	0
1000	A	20	0	0	0	0
	B	20	0	0	0	0

Table 4 pH-values and oxygen concentrations at the start and at the end of the test.

Nominal concentration (mg/l)	Code	pH-values 0 h	pH-values 48 h	Oxygen concentration (mg/l), 0 h	Oxygen concentration (mg/l), 48 h
Control	A				
0	B	7.4	7.7	5.9	5.9
	C				
100	A	7.2	7.8	5.7	5.9
	B				
316	A	7.3	7.8	5.6	5.8
	B				
1000	A	7.3	7.8	5.4	5.9
	B				

Values were determined in the combined fractions only.

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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