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TSCA Confidential Business Information Center (7407M)
EPA East - Room 6428 Attn: Section 8(e)
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

88110000229

Attention: TSCA 8(e) Coordinator

RE: Information Acquired on the Toxicity of 4,4'-methylenedianiline (MDA) (CASRN 101-77-9) to Aquatic Invertebrates

Dear TSCA 8(e) Coordinator:

CONTAINS NO CBI

The American Chemistry Council's Diisocyanates Panel (Panel) on behalf of its members,[1] is submitting the attached report to EPA pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA). The draft report was prepared by the Ecotoxicology Laboratory of BASF SE, Ludwigshafen, Germany, under the direction of the International Isocyanates Institute and its membership. This report describes new findings related to acute toxicity (immobilization) of 4,4'-methylenedianiline (MDA) to the freshwater invertebrate *Daphnia magna*. Existing guidance is not sufficient to determine whether this information is reportable under TSCA Section 8(e); therefore no determination has been made as to whether a significant risk of injury to health or the environment is actually presented by the findings. A brief summary of the report is provided below.

The attached aquatic toxicity study for 4,4'-MDA (CASRN 101-77-9) describes potential new findings on acute toxicity to aquatic invertebrates. The potential acute toxicity of this substance to aquatic invertebrates (Cladocera) has been assessed previously using a 24 hour static exposure with *Moina macropopa* (EC_{50} = 2.3 mg/L), and the potential effect of chronic exposure on reproduction was evaluated with the same species over 15 days (NOEC = 0.15 mg/L). Similarly, and as communicated in a previous submission under TSCA 8(e) dated 15 May 2008, the acute (48 hr. EC_{50} = 2.5 mg/L) and chronic effects (21 d NOEC reproduction = 0.2 mg/L) of the substance to *Daphnia magna* were reported by the Yokohama Laboratories, Mitsubishi Chemical Safety Institute Ltd, under the direction of the Japan Ministry of Environment. The attached draft report has determined both 24- (1.7 mg/L) and 48- hour (0.4 mg/L) acute EC_{50} values for immobilization of *Daphnia magna*, according to OECD TG 202.

[1] The members of the Panel are BASF Corporation, Bayer MaterialScience, The Dow Chemical Company, and Huntsman Corporation.

Should these results and/or interpretation of this study change upon finalization of the attached draft report, the Agency will be promptly notified of such pertinent changes.

While being submitted in accordance with TSCA 8(e), the Panel has made no determination as to whether a substantial risk of injury to health or the environment is actually presented by these findings. However, the Panel is aware that EPA could consider this information to constitute a substantial risk: therefore, it is being submitted to EPA under 8(e) out of an abundance of caution. This submission should therefore discharge any 8(e) responsibilities that might exist, and should be processed in accordance with EPA's "substantial risk" procedures.

If you have any questions, please contact me, the Diisocyanates Panel Manager, at 202-249-6721, or at sahar_osman-sypher@americanchemistry.com.

Sincerely,

Sahar Osman-Sypher

Sahar Osman-Sypher
Manager, Diisocyanates Panel

Attachment:
4,4'-MDA –Acute toxicity (immobilisation) study in the water flea Daphnia magna STRAUS (Draft Report of Study No. 08E004)

CONTAINS NO CBI

CONTAINS NO CBI

STUDY TITLE

Report

4,4'-MDA
Acute toxicity (immobilisation) study in the water flea
Daphnia magna STRAUS

DATA REQUIREMENT

Commission Regulation 2008/440/EC, C.2
OECD 202

AUTHOR

Dr. phil. E. Salinas (Study Director)

STUDY COMPLETED ON

Draft DD Mon YYYY

TEST FACILITY

Experimental Toxicology and Ecology
BASF SE
67056 Ludwigshafen, Germany

TEST FACILITY PROJECT IDENTIFICATION

Project No.: 50E0290/08E004

SPONSOR

INTERNATIONAL ISOCYANATE INSTITUTE, INC.
Parsippany, NJ 07054, USA

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GLP COMPLIANCE STATEMENT

This study was conducted in accordance with the OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act), which meet the United States Environmental Protection Agency Good Laboratory Practice Standards [40 CFR Part 160 (FIFRA) and Part 792 (TSCA)], with the exception that recognized differences exist between the GLP Principles/Standards of OECD and the Principles/Standards of FIFRA and TSCA.

Study Director ....
Dr. phil. E. Salinas Date

To be filled for USA EPA submission only:

Sponsor	.....	..... Date
Submitter	.....	..... Date

The following statement is for US EPA submissions only:

FLAGGING CRITERIA

The flagging criteria of 40 CFR 158.34 are not applicable for this study type

SIGNATURE PAGE

Study Director:

.....

Dr. phil. E. Salinas

CONTRIBUTORS TO THE STUDY / SUPERVISORY LABORATORY PERSONNEL

Head of Experimental Toxicology and Ecology:	Dr. rer. nat. B. van Ravenzwaay
Study director:	Dr. phil. E. Salinas
Quality Assurance Unit (QAU):	J. Hajok
Coordination QAU:	E. Zachmann
Ecotoxicology:	D. Zenker

STATEMENT OF THE QUALITY ASSURANCE UNIT

The Quality Assurance Unit (QAU) inspected the study and reported any inspection results to the Study Director and to Management.

The final report reflects the raw data.

Alternativ:
The Quality Assurance Unit (QAU) performed the inspections given below, and reported any inspection results to the Study Director and to Management. The conduct of this short-term study was not inspected; the processes of the laboratory and of the study involved are inspected in regular intervals.

The final report reflects the raw data.

Phase of study	**Date of inspection [mm-dd-yyyy]**	**Report to Study Director and to Management [mm-dd-yyyy]**
Study Plan:		
Conduct of study:		
Report:		

Ludwigshafen,

.....
E. Zachmann
(Quality Assurance Unit)

GLP CERTIFICATE (FROM THE COMPETENT AUTHORITY)

RheinlandPfalz

Gute Laborpraxis / Good Laboratory Practice

GLP-Bescheinigung / Statement of GLP Compliance

(gem. /according to § 19 Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung und der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at::

Prüfeinrichtung / Test facility

BASF SE
Experimentelle Toxikologie und Ökologie
67056 Ludwigshafen

BASF SE
Experimental Toxicology and Ecology
67056 Ludwigshafen, Germany

Prüfung nach Kategorien / Areas of Expertise
(gem. / according ChemVwV-GLP Nr. 5.3/OECD guidance)
1,2,3,4,5,8,9
Kat. 9 – Biochemische und pathologische Untersuchungen zu Wirkmechanismen / Biochemical and pathological examinations concerning mode of action

Datum der Inspektion / Date of Inspection
(Tag.Monat Jahr / day.month year)
19.05.2009 & 06. bis 08.07.2009

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.
Eine erneute behördliche Überprüfung der Einhaltung der GLP-Grundsätze durch die Prüfeinrichtung ist so rechtzeitig zu beantragen, dass die Folgeinspektion spätestens vier Jahre nach dem Beginn der o.g. Inspektion stattfinden kann Ohne diesen Antrag wird die Prüfeinrichtung nach Ablauf der Frist aus dem deutschen GLP-Überwachungsprogrann genommen und diese GLP-Bescheinigung verliert ihre Gültigkeit:

Based on the inspection report it can be confirmed, that the test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Verification of the compliance of the test facility with the Priciples of the GLP has to be applied for in time to allow for a follow-up inspection to take place within four years after commencing the above mentioned inspection. Elapsing this term, the test facility will be taken out of the German GLP-Monitoring Programme and this GLP Certificate becomes invalid.

Unterschrift, Datum / Signature, Date

22.10.2009

Dr. Pia Hirsch - stellv. Präsidentin -
(Name und Funktion der verantwortlichen Person / name and function of responsible person)

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht Rheinland-Pfalz
Siegel

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht
Kaiser-Friedrich-Straße 7
55116 Mainz

(Name und Adresse der GLP-Überwachungsbehörde / Name and adress of the GLP Monitoring Authority)

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht

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Statistical evaluation
Certificate of Analysis
Analytical Report (Concentration Control Analysis)
Analytical Results (Concentration Control Analysis)

1. SUMMARY

Sponsor:	INTERNATIONAL ISOCYANATE INSTITUTE, INC., Parsippany, NJ 07054, USA	
Study director:	Dr. phil. E. Salinas	
Monitor:	Dr. ir. B. Bossuyt, Global Product EHS department, Huntsman (Europe), Everslaan 45, B-3078 Everberg, Belgium	
Testing facility:	Experimental Toxicology and Ecology, BASF SE, 67056 Ludwigshafen, Germany	
Location of raw data and final report:	BASF SE, Germany	
Test substance:	4,4'-MDA	
Subject:	Report: **4,4'-MDA** –Acute toxicity (immobilisation) study in the water flea *Daphnia magna* STRAUS	
Guidelines:	Commission Regulation 2008/440/EC, C.2	
	OECD Guideline for Testing of Chemicals, No. 202	
Test groups:	0 (control), 0.10, 0.22, 0.46, 1.0, 2.2, 4.6 mg/L as nominal concentrations based on test substance mass without correction for purity	
Test dates:	Study initiation date:	13 Jan 2011
	Experimental starting date (preparation of the test solutions):	17 Jan 2011
	Start of exposure:	18 Jan 2011
	End of exposure:	20 Jan 2011
	Experimental completion date (draft report):	20 Apr 2011
Exposure period / conditions:	48 hours, static	
Test species / strain:	*Daphnia magna* Straus	
Animal supplier:	The clone of *Daphnia magna* STRAUS 1820 used in this study was originally obtained from the Institut National de Recherche Chimique Appliquée, France, in 1978. From this date on this clone was cultured and bred continuously in the Laboratory of Ecotoxicology of the Experimental Toxicology and Ecology of the BASF SE, Ludwigshafen, Germany.	

Test substance preparation:	The stock solution (4.6 mg/L) was prepared by directly adding 9.23 mg of test substance to 2L of test medium. The mixture was placed in an ultrasonic bath for 10 minutes then stirred for about 1 day at 20 ± 2 °C. After stirring the stock solution was clear and colorless with no visible undissolved test substance. However, by request of the sponsor, the stock solution was centrifuged (approx. 20 min at about 17700 G with a Beckman Coulter™, Avanti J-25) to assure the removal of any undissolved test substance. The lower test concentrations were prepared by dilution of this stock solution. Before the dilution was performed, the stock solution was checked for complete dissolution of the test substance. All test solutions were visibly clear over the exposure period.
Concentration control analysis:	The measured concentrations of the test substance in the test water were 94 – 100% of the nominal concentrations at start of exposure and decreased to 77 – 93 % of the nominal concentrations at the end of exposure. However, all measured concentrations were within ±20% of the initial measured values and the mean measured values determined for the whole exposure period. Thus, the test substance was stable in solution under test conditions and mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period. Since mean measured concentrations were all within ±20% of nominal, the results of this study will be based on the nominal concentrations.
Test results:	The following effect concentrations (mg/L) were obtained based on nominal concentrations: **EC_0 (48 h) = 0.22 mg/L** **EC_{50} (48 h) = 0.40 mg/L** confidence limits 95%: 0.20 – 0.83 mg/L **EC_{100} (48 h) = 2.2 mg/L** Since the analytically determined concentrations of the test substance in the test solutions were within ±20% of the nominal concentrations, the effect concentration can be expressed relative to the nominal concentration.

2. INTRODUCTION

2.1. OBJECTIVES

This study investigated the effect of the test substance on the immobilisation of *Daphnia magna*. Juvenile daphnids (< 24 hour old) were exposed to various concentrations of the test substance and compared to a control. Immobilisation of daphnia is determined after 24 and 48 hours of exposure.

2.2. SELECTION OF CONCENTRATIONS

The concentrations for this study were selected in accordance with the requirements set forth in the test guidelines and based on the results of a preliminary range finding test (experimental conduct in accordance with GLP, without a GLP status).

See also section 3.4.

2.3. TEST GUIDELINES

The study was conducted according to the following test guidelines:

- Commission 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), Part C.2: *Methods for the Determination of Ecotoxicity: Daphnia sp., Acute Immobilisation Test*; Official Journal of the European Union, No. L 142
- OECD (2004), *Test No. 202: Daphnia sp. Acute Immobilisation Test*, OECD Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems, No. 380, OECD Publishing.

This study also incorporates guidance in:

- OECD (2002), *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures*, OECD Series on Testing and Assessment, No. 23, OECD Publishing.

For further information see:

International Standard ISO 6341. Water quality - Determination of the inhibition of the mobility of *Daphnia magna* STRAUS (Cladocera, Crustacea) – Acute toxicity test. Third edition, 1996

Elendt, B.-P.: Untersuchungen zur Ernährung von Daphnien. Ein Beitrag zur Optimierung des verlängerten Toxizitätstests mit *Daphnia magna*. Dissertation, Universität Heidelberg (1990)

Finney, D. J.: Probit analysis. Cambridge University Press, 3rd Edition, 1971

2.4. TIME SCHEDULE

Test dates:	Study initiation date:	13 Jan 2011
	Experimental starting date (preparation of the test solutions):	17 Jan 2011
	Start of exposure:	18 Jan 2011
	End of exposure:	20 Jan 2011
	Experimental completion date (draft report):	20 Apr 2011

The daphnids were exposed to the test substance for approximately 48 hours.

2.5. RETENTION OF RECORDS

GLP-relevant records and materials are stored at BASF SE for at least the period of time specified in the GLP principles. Details concerning responsibilities or locations of archiving can be seen from the respective SOPs and from the raw data.

3. MATERIALS AND METHODS

3.1. TEST ITEM

The analyses of the test item (= test substance) was used with the given specification of the supplier (SIGMA-ALDRICH, Switzerland).

Name of test substance:	4,4'-MDA
Test substance No.:	08/0290-3
Batch identification:	1435367
CAS No.:	101-77-9
Purity:	98.0 area-% (see Certificate of Analysis in the appendix)
Homogeneity:	Homogeneous
Storage stability:	The stability under storage conditions over the exposure period was guaranteed by the sponsor, and the sponsor holds this responsibility.

ADDITIONAL TEST SUBSTANCE INFORMATION

Chemical Name:	4,4-Methylenedianiline
Synonyms:	4,4-Methylenedianiline, 4,4-Diaminodiphenylmethan, MDA, MMDA
Date of production:	Unknown
Expiry date:	16 Aug 2013
Physical state/ Appearance:	Solid/ yellowish
Water solubility:	soluble
Storage conditions:	The test substance was stored at ambient temperature.

3.1.1. Reference substance

Name of reference substance:	potassium dichromate ($K_2Cr_2O_7$)
Reference substance No.:	04/0063-1
Batch identification:	Lot 20980
CAS No.:	7778-50-9
Expiry date:	Feb 2014

3.2. TEST SYSTEM

Test species :	*Daphnia magna* STRAUS
Reason for selection of the test species:	Recommended species in the test guidelines.
Origin :	The clone of *Daphnia magna* STRAUS 1820 used was supplied by the *Institut National de Recherche Chimique Appliquée*, France, in 1978. From this date on this clone was cultured and bred continuously in the Ecotoxicology Laboratory of Experimental Toxicology and Ecology, BASF SE, Ludwigshafen Germany.
Culture conditions:	*Daphnia* brood stock are kept in mass cultures consisting of approx. 20 – 30 individuals for a maximum of 4 weeks and fed live unicellular algae (*Desmodesmus subspicatus*), cultured continuously at the test facility. All individuals in each mass culture originate from a single female and are thus genetic clones. After approximately 14 days the adults have produced at least 3 broods and the young can be used in tests. Offspring are removed from the mass cultures at least once daily during the normal work week to ensure that young daphnia are <24-h old (first instar) at test initiation. Detailed records are kept (in test facility archives) to monitor the health of *Daphnia* brood stock cultures including observations of young production, mortality, ephippia, and measurement of water chemistry parameters. Only young from healthy cultures without signs of stress are used for testing.
Acclimatization:	The *Daphnia* are cultured under the identical conditions as the test including test media (Elendt M4), water quality, and temperature (20 ±1°C).
Age at test initiation:	< 24 hours (at least 3rd brood progeny)
Reference substance testing:	In order to verify that the *Daphnia magna* culture is responding normally to toxic stress, tests with a reference substance, potassium dichromate ($K_2Cr_2O_7$), are conducted monthly. Reference substance tests are conducted according to OECD 202 guidelines and in accordance with GLP, but without a GLP status. The EC_{50}(24h) of the reference substance potassium dichromate was **1.04** mg/L (experiment date: 09 Dec 2010, project number: 50E0063/043039). This result is within the range of 0.6 – 2.1 mg/L (ISO 6341) and indicates that the culture of *Daphnia magna* used in this study is responding normally to toxic stress.

3.3. HOUSING AND DIET

Test medium:	A synthetic fresh water (Elendt M4) is used as media for culture and test purposes. For the composition of this M4 medium see OECD 202. The general properties of this medium are as follows. Total hardness: 2.20 – 3.20 mmol/L Acid capacity up to pH 4.3: 0.80 – 1.00 mmol/L Molar ratio Ca:Mg: about 4 : 1 pH value: 7.5 – 8.5 Conductivity: 550 - 650 µS/cm Dissolved oxygen: Must remain ≥ 3mg/L during the test. To assure optimal dissolved oxygen levels, the M4 medium is aerated for approximately 24 hours prior to use. The measured values of these parameters during the experiment are provided in chapter 4.3. Water quality.
Test vessels :	Glass beakers (nominal volume 100 mL)
Test volume:	50 mL
Test solution renewal:	Static exposure via test water.
Test temperature:	19 °C (continuous monitoring)
Biological loading:	5 animals / test vessel (0.1 animals/mL)
Light intensity / Photo period:	About 20 – 850 lux at a wave length of 400 -750 nm; 16 hours light : 8 hours darknes
Aeration	none
Diet:	No feeding during the exposure period.

3.4. TEST GROUPS AND CONCENTRATIONS

Test groups :	0 (control), 0.10, 0.22, 0.46, 1.0, 2.2, 4.6 mg/L as nominal concentrations based on test substance mass without correction for purity
Replicates / test group:	4 replicates with test animals (5 animals per replicate)
Daphnids / test group:	20
Reason for the selection of the test concentrations :	According to the test guidelines, at least 5 concentrations in a geometric series with a separation factor of ≤2.2 should be used, preferably encompassing the range from no effect to 100% immobilization. In a preliminary range finding test (experimental conduct in accordance with GLP but without a GLP status) the 48 hour EC_{50} was between 0.1 and 1 mg/L. However, a 48-h EC_{50} = 2.47 mg/L was provided by the sponsor, therefore this study was performed with 6 concentrations to cover a potentially broader range of effect. The raw data of the range finding test are archived together with the raw data of this study.

3.5. TEST SUBSTANCE PREPARATIONS

The stock solution (4.6 mg/L) was prepared by directly adding 9.23 mg of test substance to 2L of test medium. The mixture was placed in an ultrasonic bath for 10 minutes then stirred for about 1 day at 20 ± 2 °C. After stirring the stock solution was clear and colorless with no visible undissolved test substance. However, by request of the sponsor, the stock solution was centrifuged (approx. 20 min at about 17700 G with a Beckman Coulter™, Avanti J-25) to assure the removal of any undissolved test substance. The lower test concentrations were prepared by dilution of this stock solution. Before the dilution was performed, the stock solution was checked for complete dissolution of the test substance.

By dilution of this stock solution with M4 medium the test concentrations were prepared.

Dilution scheme:

Nominal concentration (mg/L)	Volume of stock solution (mL)	Final Volume of test media (mL)
0 (control)	-	1000
0.10	22	1000
0.22	48	1000
0.46	100	1000
1.0	217	1000
2.2	478	1000
4.6	1000	-

All test solutions were colorless and clear.

3.6. ANALYSES

The analyses were carried out as a separate study at the test facility Competence Center Analytics, BASF SE, Ludwigshafen, Germany under the responsibility of the Study Director of this test facility. The study was carried out in compliance with the Principles of Good Laboratory Practice.

Detailed information on the analytical method is provided in the Appendix.

By request of the study monitor, additional analytical investigations of the test substance preparations were carried out at the Biodegradation Laboratory of Experimental Toxicology and Ecology of BASF SE, Ludwigshafen, Germany by determination of total organic carbon, as a part of this study. Only the frozen retained samples were used for these analyses.

3.6.1. Stability

The stability of the test substance as a solution in test media and under testing conditions was determined by concentration control analysis.

3.6.2. Concentration control analysis

At the start of the exposure (0 h) samples from vessels without daphnids and at the end of the exposure (48 h) samples from vessels with daphnids were analyzed.

For each test group, two retained samples were collected at each time point and stored frozen. These samples were sent for analysis only as needed and were otherwise discarded upon finalization of this study.

The samples were transported to the Analytical Laboratory on the day of sampling. For the dates of analysis and storage of samples (if applicable) see the Analytical report in the Appendix.

3.7. EXPERIMENTAL PROCEDURE

The test was initiated by impartially distributing 20 neonate (< 24 hours old) *Daphnia magna* into each of the 4 test vessels per test group. The neonate daphnids all originated from the same mass culture and were thus genetic clones (see chapter 3.2). The *Daphnia* were transferred into the test vessels with little to no culture water by capturing each daphnid in a pipette then gently expelling it onto a Teflon mesh (70µm) held by forceps. Each daphnid was then immediately transferred from the mesh into the corresponding test vessel from lowest to highest concentration. The test vessels were maintained as described in chapter 3.3.

Each test vessel was visually checked for immobilized daphnids after 0, 24 and 48 hours. In addition any abnormal behavior or appearance was documented.

Throughout the test, the appearance of the test solutions and dissolution behavior of the test substance was observed and recorded daily. The chemical and physical parameters of the test medium (total hardness, acid capacity, pH and conductivity) were determined after aeration and prior to use in the test. Dissolved oxygen, pH and temperature were measured at the start of the exposure and at the end of the exposure in replicate 1 of all test concentrations and the control. In addition, temperature was measured continuously during the whole exposure period in a separate vessel filled with water proximal to the test vessels.

3.8. DATA EVALUATION

Tables and/or figures of measured parameters presented in this report were produced using relevant computing software. All reported data values are rounded to the appropriate significant figures based on the precision of the analytical method and/or consistent with the requirements in the pertinent test guideline(s).

For the statistical evaluation of the data and calculation of the EC_{50} the probit method (Finney, 1971) was used and performed with the commercial software "TOXRAT Professional 2.10" (ToxRat Solutions GmbH, Alsdorf, Germany).

3.9. VALIDITY CRITERIA

This test was fully compliant with all the following validity criteria required by the corresponding test guidelines and is considered valid.

- $\leq$ 10% immobilisation in the control
- O_2 concentration > 3 mg/L in control and test vessels

4. RESULTS

4.1. ANALYTICAL CONCENTRATION CONTROL

Method of analysis: High performance liquid chromatography with UV detection

Analytically measured concentrations of the test substance in the test solutions:

Nominal Concentration	Analytically measured concentration				Mean Measured Concentration	
	0 hour test solution		48 hour test solution			
[mg/L]	[mg/L]	[% of nominal]	[mg/L]	[% of nominal]	[mg/L]	[% of nominal]
0	< 0.02	—	< 0.02	—	< 0.02	—
0.10	0.10	100%	0.08	80%	0.09	90%
0.22	0.21	95%	0.17	77%	0.19	86%
0.46	0.44	96%	0.37	80%	0.41	88%
1.0	0.95	95%	0.86	86%	0.91	91%
2.2	2.07	94%	1.96	89%	2.0	92%
4.6	4.35	95%	4.29	93%	4.3	94%

for details see analytical report in the Appendix.

The measured concentrations of the test substance in the test water were 94 – 100% of the nominal concentrations at start of exposure and decreased to 77 – 93 % of the nominal concentrations at the end of exposure. However, all measured concentrations were within ±20% of the initial measured values and of the mean measured values determined for the whole exposure period. Thus, the test substance was stable in solution under test conditions and mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period. Since mean measured concentrations were all within ±20% of nominal, the results of this study will be based on the nominal concentrations.

By request of the sponsor, a secondary concentration control analysis was performed on retained samples from all test groups using a sum parameter method, determination of total organic carbon. The results of these analyses are as follows:

Nominal Concentration	Analytically measured concentration				Mean Measured Concentration	
	0 hour test solution		48 hour test solution			
[mg/L]	[mg/L][a]	[% of nominal]	[mg/L][a]	[% of nominal]	[mg/L]	[% of nominal]
0	<LOQ	—	<LOQ	—	—	—
0.10	<LOQ	—	<LOQ	—	—	—
0.22	<LOQ	—	<LOQ	—	—	—
0.46	<LOQ	—	<LOQ	—	—	—
1.0	<LOQ	—	<LOQ	—	—	—
2.2	1.9	86%	1.85	84%	1.9	85%
4.6	4.2	91%	4.3	93%	4.3	92%

a = mean measured value from two retained samples

LOQ= 1.1 – 1 5 mg/L; for details see analytical results in the Appendix

Test concentrations could only be measured in the two highest test groups with total organic carbon. The measured concentrations of the test substance in the test water were 86 - 91% of the nominal concentrations at start of exposure and 84 - 93 % of the nominal concentrations at the end of exposure.

4.2. ACUTE TOXICITY TEST

4.2.1. Test substance

Number of mobile Daphnids

Nominal concentration [mg/L]	Replicate 1 [h]			Replicate 2 [h]			Replicate 3 [h]			Replicate 4 [h]			Σ	Σ
	0	24	48	0	24	48	0	24	48	0	24	48	24	48
0 (control)	5	5	5	5	5	5	5	5	5	5	5	5	20	20
0.10	5	5	5	5	5	5	5	5	5	5	5	5	20	20
0.22	5	5	5	5	5	5	5	5	5	5	5	5	20	20
0.46	5	5	1	5	5	1	5	5	1	5	5	1	20	4
1.0	5	5	1	5	3	0	5	2	0	5	1	0	11	1
2.2	5	2	0	5	1	0	5	2	5	5	1	0	6	0
4.6	5	1	0	5	2	0	5	1	0	5	1	0	5	0

Number of test animals per replicate: 5
Number of test animals per test group: 20

Effect concentrations after 24 hours and 48 hours of exposure. The EC values based on nominal concentrations were:

Time [h]	**EC_0 [mg/L]**	**EC_{50} [mg/L]**	**Confidence limits 95% [mg/L]**	**EC_{100} [mg/L]**
24	0.46	1.7	1.3 – 2.4	> 4.6
48	0.22	0.40	0.20 – 0.83	2.2

Effect concentrations after 24 hours and 48 hours of exposure. The EC values based on measured concentrations were:

Time [h]	**EC_0 [mg/L]**	**EC_{50} [mg/L]**	**Confidence limits 95% [mg/L]**	**EC_{100} [mg/L]**
24	0.41	1.5	1.2 – 2.2	> 4.3
48	0.19	0.40	0.18 – 0.71	2.0

A graphical presentation concentration effect response on Daphnia immobility after 24 and 48 hours can be found with the statistical evaluation in the appendix.

Since the mean measured concentrations of the test substance in the test solutions were all within ±20% of the nominal concentrations, the effect concentrations can be expressed relative to the nominal concentration.

No additional adverse effects or abnormal behavior were observed in any of the test groups.

4.3. WATER QUALITY

4.3.1. Culture medium

Physical and chemical characteristics of the M4-medium used.

pH value	Conductivity [µS/cm]	Total hardness [mmol/L]	Acid capacity up to pH 4.3 [mmol/L]	Date of measurement
7.9	573	2.45	0.82	14 Jan 2011

4.3.2. pH and dissolved oxygen content

pH- and oxygen values in the test solutions after 0h and 48h

Test Group [mg/L]	pH value after 0 h [a]	pH value after 48 h [a]	O_2 value [mg/L] after 0 h [a]	O_2 value [mg/L] after 48 h [a]
0 (control)	7.9	7.8	8.7	8.8
0.10	7.9	7.8	8.6	8.9
0.22	7.9	7.8	8.7	8.9
0.46	7.9	7.8	8.7	8.9
1.0	7.9	7.8	8.6	8.9
2.2	7.9	7.8	8.6	8.9
4.6	7.9	7.8	8.7	9.0

[a] measured in the replicate 1 of each concentration

[b] measured in the replicate 1 of each concentration

4.4. DISSOLUTION BEHAVIOR

All of the test solutions were visibly clear and colorless after preparation and remained so after 24 and 48 hours of exposure in the test. No undissolved test substance was visible and there were no other remarkable observations.

5. CONCLUSION

In conclusion the 48 hour EC_{50} values for **4,4'-MDA** in this acute toxicity study with *Daphnia magna* were 0.40 mg/L based on the nominal concentration of the test substance and 0.40 mg/L based on the mean measured concentrations.

Since the analytically determined concentrations of the test substance in the test solutions were within ±20% of the nominal concentrations, the effect concentration can be expressed relative to the nominal concentration for the evaluation of the test substance.

The toxicity results presented here are consistent with the results from preliminary tests.

The results in this study are consistent with all validity criteria and the test is valid according to the guidelines of this study. No deviations from test guidelines or other incidents occurred during the course of the reported test which may have influenced the results.

6. LIST OF ABBREVIATIONS AND DEFINITIONS

The following list contains abbreviations and definitions generally used in reports for this study type. This report will not necessarily use all expressions listed below.

Immobilisation: Animals that were unable to swim within 15 seconds following gentle agitation of the test vessel were considered as immobile.

µS/cm: micro Siemens/centimeter

ECx is the experimentally derived concentration of the test substance dissolved in dilution water that is calculated to result in a x % immobilisation of *Daphnia magna* within a stated exposure period.

ELx values are based on loading rateloading concentrationand calculated using the same statistical methods as for ECx.

Loading rate: The mass to volume ratio of test substance to test medium (in mg/L) used to prepare a WAF test solution.

Loading concentration: For poorly soluble chemicals, the nominal mass to volume ratio of test substance to test medium (in mg/L) used to prepare the test solution.

Test item: The test item refers to the item provided by the sponsor and applied in the test system. Test item is generally synonymous with the terms "test article", "test material", "test sample" and "test substance" which may appear in documents provided in the Appendix of this report or in relevant test guidelines.

Water-accommodated fraction (WAF): An aqueous fraction containing the dissolved and/or suspended and/or emulsified fraction of a multi-component substance or a mixture.

UVCB: Substances of unknown or variable composition, complex reaction products or biological materials.

rpm: revolutions per minute

TOC: Total organic carbon

APPENDIX

Statistical evaluation

Certificate of Analysis

Analytical Report (Concentration Control Analysis)

Analytical Results (Concentration Control Analysis)

Daphnia, Acute Immobilisation Test (OECD 202): 50E0290/08E004

General:

Test identification/project no.	50E0290/08E004
Test item	4,4'-MDA
Unit of test item concentration	mg/L
Start of experiment on day	18.1.2011
Date and time of the evaluation	13.04.2011; 08:44:23
Raw data filename:	08E004-toxrat.xls

Test design

Number of treatments (incl. control(s))	7
Duration of the test	48 h
Test system	Daphnia magna

Validity of the test

To be a valid test, a maximum control mortality of 10.0% is allowed.

In the present test 0.0% of the introduced animals died.

Thus the test is valid.

Relation of Daphnia magna Endpoints on Concentration

Effective Concentrations (ECx) for Mobility at 24 h

Probit analysis using linear max. likelihood regression

Tab. 1: Probit analysis using linear max. likelihood regression: Determination of the concentration/response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of organisms; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

Treatm. [mg/L]	Log(x)	% Immobility	n	Emp. Probit	Weight	Reg. Probit
0		0.00	20			excluded
0.100	-1.000	0.00	20	-1.2533	0.276	-3.020
0.220	-0.658	0.00	20	-1.2533	1.917	-2.177
0.460	-0.337	0.00	20	-1.2533	6.122	-1.388
1.000	0.000	45.00	20	-0.1253	11.364	-0.557
2.200	0.342	70.00	20	0.5013	12.358	0.286
4.600	0.663	75.00	20	0.6267	8.263	1.075

excluded· value not in line with the chosen function

Parameters of the probit analysis

Tab. 2: Parameters of the probit analysis: Results of the regression analysis

Parameter	Value
Computation runs:	7
Slope b:	2.46334
Intercept a:	-0.55712
Variance of b:	0.16796
Goodness of Fit	
Chi^2:	7.25780
Degrees of freedom:	4
$p(Chi^2)$:	0.12288
Log EC50:	0.22617
SE Log EC50:	0.06514
g-Criterion:	0.10633
F:	19.912
p(F) (df: 1;4):	0.011

Chi^2 is a goodness of fit measure. If the probability, $p(Chi^2)$, is lower or equal than 0.100, data is much scattering round the computed dose/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).

Results of the probit analysis

Tab. 3: Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller`s theorem).

Parameter	EC50
Value [mg/L]	1.683
lower 95%-cl	1.258
upper 95%-cl	2.346
lower 99%-cl	1.141
upper 99%-cl	2.588

n.d.: not determined due to mathematical reasons or inappropriate data

Slope function after Litchfield and Wilcoxon: 2.547

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

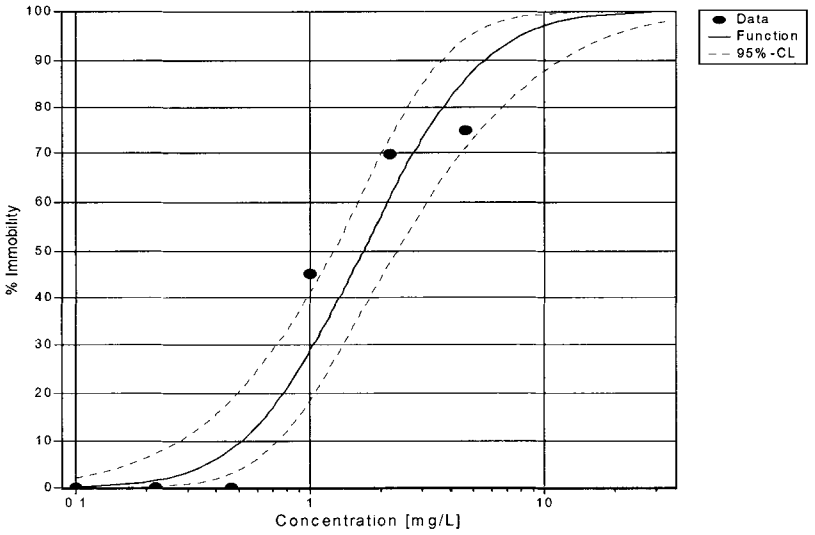


Fig. 1: Concentration-effect curve showing the influence of the test item on mobility of the introduced Daphnia magna as observed after 24 h.

Effective Concentrations (ECx) for Mobility at 48 h

Probit analysis using linear max. likelihood regression

Tab. 4: Probit analysis using linear max. likelihood regression: Determination of the concentration/response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of organisms; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

Treatm. [mg/L]	Log(x)	% Immobility	n	Emp. Probit	Weight	Reg. Probit
0		0.00	20			excluded
0.100	-1.000	0.00	20	-1.2533	0.039	-3.653
0.220	-0.658	0.00	20	-1.2533	4.960	-1.566
0.460	-0.337	80.00	20	0.7520	12.059	0.386
1.000	0.000	95.00	20	1.1280	1.131	2.441
2.200	0.342	100.00	20	1.2533	0.001	4.528
4.600	0.663	100.00	20	1.2533	0.000	6.481

excluded: value not in line with the chosen function

Parameters of the probit analysis

Tab. 5: Parameters of the probit analysis: Results of the regression analysis

Parameter	Value
Computation runs:	9
Slope b:	6.09448
Intercept a:	2.44128
Variance of b:	1.74853
Goodness of Fit	
Chi^2:	8.23661
Degrees of freedom:	4
$p(Chi^2)$:	0.08329
Log EC50:	-0.40057
SE Log EC50:	0.03848
g-Criterion:	0.74701
F:	10.316
p(F) (df: 1;4):	0.033

Chi^2 is a goodness of fit measure. If the probability, $p(Chi^2)$, is lower or equal than 0.100, data is much scattering round the computed dose/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).

Results of the probit analysis

Tab. 6: Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller's theorem).

Parameter	EC50
Value [mg/L]	0.398
lower 95%-cl	0.203
upper 95%-cl	0.827
lower 99%-cl	0.128
upper 99%-cl	1.314

n d not determined due to mathematical reasons or inappropriate data

Slope function after Litchfield and Wilcoxon: 1.459

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

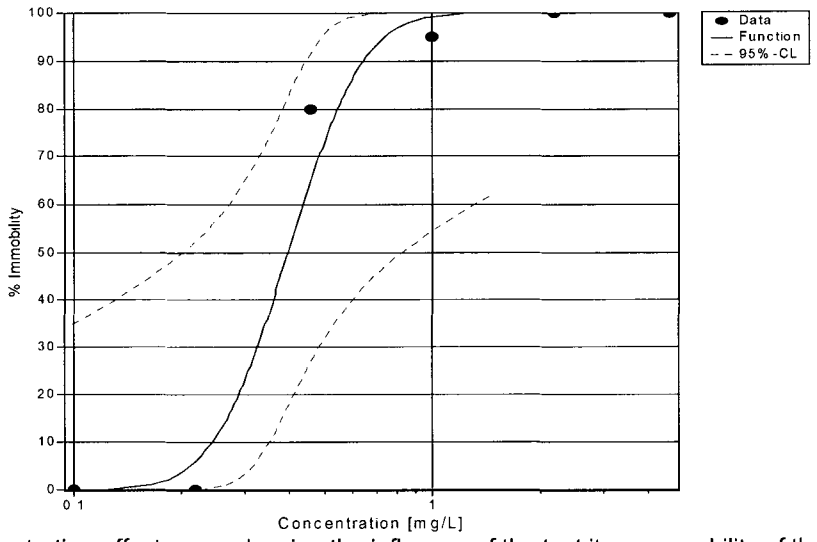


Fig. 2: Concentration-effect curve showing the influence of the test item on mobility of the introduced Daphnia magna as observed after 48 h.

Final Report

Concentration control of "4,4'-MDA" in "M4-Medium"
Study No. 11L00008 (confidential)

BASF
The Chemical Company

Test item	"4,4'-MDA"
Batch identification	1435367
Date of production (test item)	Unknown
PSN	08/0290-3
Project no.	50E0290/08E004
Vehicle	"M4-Medium"
Sponsor	Dr. Salinas, GV/TC - Z570
Date of receipt of order	Jan 17, 2011
Testing facility	Competence Center Analytics, BASF SE, D-67056 Ludwigshafen
Study director	Mr. Beringer, GKC/C - E210
Storage cond. test item	Ambient temperature
Storage cond. vehicle	Ambient temperature
Storage cond. samples	Ambient temperature
Test period	Jan 18 – 20, 2011
Storage of records	GLP archives, Competence Center Analytics

Determination	"4,4'-MDA"
Method	Reversed phase HPLC with UV detection and evaluation by the method of external standard.
Apparatus	Automated HPLC system equipped with programmable auto-sampler and UV/vis detector and linked to an electronic data processing system.
Reagents	Demineralized water (Milli-Q-station) Disodium hydrogen phosphate dihydrate (Riedel-de Haen) Orthophosphoric acid (85%; Sigma-Aldrich) Acetonitrile (Gradient Grade Far UV, Fisher Scientic) Test item (hypothetical purity 100 %) and vehicle were delivered by the sponsor.
Calibration	About 22 – 39 mg of the test item, exactly weighed to 0.01 mg, were transferred into 100 ml volumetric flasks, dissolved and filled up to the mark with acetonitrile. Further dilutions were done with mobile phase.
Sample preparation	The samples were injected directly.
Test parameters	Column: Purospher RP18e, 125 x 3 mm (Merck) *Mobile phase:* 500 ml of 5 mM Na_2HPO_4, adjusted to pH 6.0 with H_3PO_4 + 500 ml of acetonitrile Flow rate: 0.7 ml/min Injection volume: 10 µl Temperature: 25°C Detection: UV, λ = 245 nm

Results

Sample no.	Date of sample preparation	Date of sampling	Expected concentration (mg/l)	Value found (mg/l)
1	Jan 18, 2011	Jan 18, 2011	0	<0.02[1)]
2	Jan 18, 2011	Jan 18, 2011	0.1	0.10
3	Jan 18, 2011	Jan 18, 2011	0.22	0.21
4	Jan 18, 2011	Jan 18, 2011	0.46	0.44
5	Jan 18, 2011	Jan 18, 2011	1	0.95
6	Jan 18, 2011	Jan 18, 2011	2.2	2.07
7	Jan 18, 2011	Jan 18, 2011	4.6	4.35
11	Jan 18, 2011	Jan 20, 2011	0	<0.02[1)]
12	Jan 18, 2011	Jan 20, 2011	0.1	0.08
13	Jan 18, 2011	Jan 20, 2011	0.22	0.17
14	Jan 18, 2011	Jan 20, 2011	0.46	0.37
15	Jan 18, 2011	Jan 20, 2011	1	0.86
16	Jan 18, 2011	Jan 20, 2011	2.2	1.96
17	Jan 18, 2011	Jan 20, 2011	4.6	4.29

1) The result was verified with standard addition tests.

All values are mean values out of two determinations. Blank value correction was not carried out because no signals were observed.

Discussion of results:
The found values correspond to about 77 - 100 % with the expected concentrations.

Head of laboratory Dr. Euler, GKC/C – E 210

Feb. 02, 2011

Study director
(Mr. Beringer)

Date

GLP Compliance Statement

This study was conducted in accordance with the OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act).

Feb 02, 2011

Study director
(Mr. Beringer)

Date

Statement of the Quality Assurance Unit

The Quality Assurance Unit inspects the laboratories of the department Competence Center Analytics in regular intervals. Besides these general inspections we inspected the following items of this study in accordance with the OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act). Findings are reported to study director and to management.

Verification of study plan: Jan 18, 2011

Inspection of	Date of inspection	Reported to study director and to management
Conduct of study:	Jan 20, 2011	Jan 25, 2011
Raw data:	Feb 01, 2011	Feb 02, 2011
Final report:	Feb 01, 2011	Feb 02, 2011

Ludwigshafen

QAU: Meyer-Schlieker

Feb 02, 2011
Date

Concentration Control Analysis of 4,4'-MDA in M4 Medium

1. PROJECT AND TEST SUBSTANCE INFORMATION

Project No.:	50E0290/08E004
Test substance:	4,4'-MDA
Test substance number:	08/0290-3
Batch No.:	1435367

2. SAMPLE DATA

2.1. CONCENTRATION CONTROL ANALYSIS

Matrix / Medium:	M4 Medium
Storage conditions of the samples until analysis:	Retained samples, stored in a freezer until delivered

3. MATERIALS AND METHODS

3.1. SAMPLE PREPARATION FOR ANALYSIS

Before measurement, all samples were acidified with few drops of hydrochloric acid and the inorganic carbon fraction was stripped using pressurized air. No further dilution or preparation procedures were used prior to sample analysis.

3.2. ANALYTICAL METHOD

Determination of the total organic carbon (TOC)

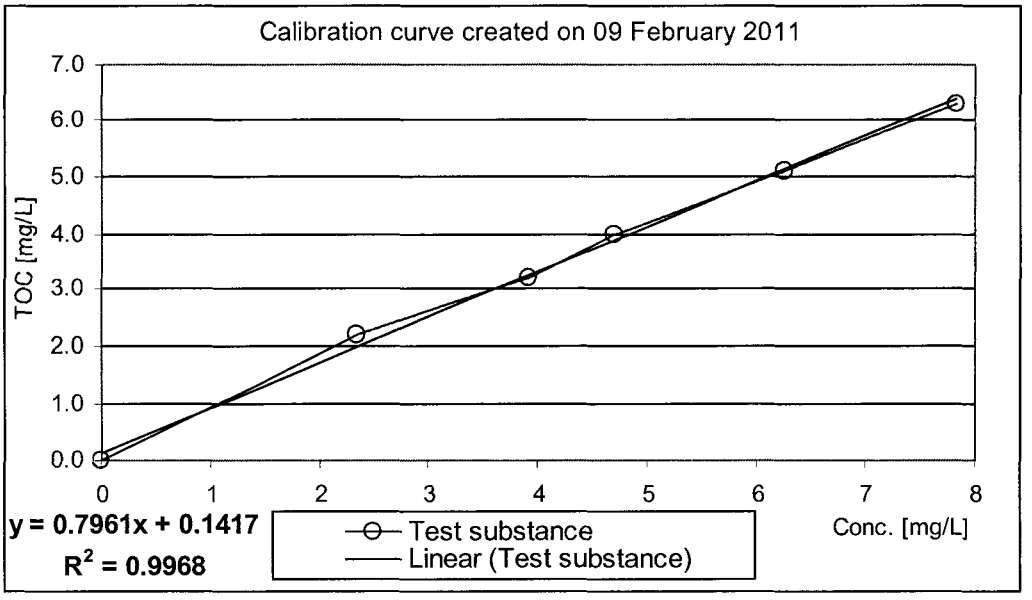
TOC-analyzer:	Shimadzu TOC V_{CSN}
Auto sampler:	ASI V
Detector:	Infrared Gas Analyzer
Injection volumes:	TC: 75 µL; IC: 800 µL
Carrier Gas	Synthetic air

For the quantification of the test substance, a calibration curve based on five concentrations was prepared. The amount of 7.83 mg 4,4'-MDA was weighed to a small glass plate and transferred into a 1000 mL volumetric flask. After addition of about 600 mL test medium the solution was treated in a super sonic bath for about 10 minutes and stirrer over night. On next day the solution was made up to the mark (solution 5). The solution appeared colorless and clear, the substance was completely dissolved. Further stock solution were prepared by dilution. Suitable aliquots were taken from solution 5 and pipetted to 50 mL and 25 mL volumetric flaks respectively and made up to the mark with test medium. The following solutions for the calibration were prepared: solution 4 with 6.26 mg/L test substance, solution 3 with 4.70 mg/L test substance, solution 2 with 2.92 mg/L test substance and solution 1 with 2.35 mg/L test substance.

Test medium was used as the blank control. The TOC-content of the blank control was subtracted from each sample for the calculation of the TOC content. The content of total carbon (TC) was analyzed as a fivefold determination. The inorganic carbon (IC) content was verified with a triplicate determination. The calibration curve was obtained by correlation of the TOC-content of the standard solutions to their corresponding concentration in mg/L.

The samples of the concentration control analysis were evaluated as follows:
The mean values of TIC-measurement were calculated and subtracted from each value of the TC-measurement.

For creation of linear slope of the calibration the mean values of the TC determination of each concentration were calculated and the corresponding TIC value was subtracted.



TOC = ax + b converted: x = (TOC – b) ÷ a

x = (TOC – 0.1417) ÷ 0.7961

TOC = peak area of TOC
x = concentration of the test substance in the samples [mg/L]
a = slope
b = y-axis intercept

Calculation of the recovery rates for calibration curve:

Test substance, nominal [mg/L]	TOC [mg/L]; mean values	Test substance, recovered [mg/L]	recovery rate [%]
Blank control	0.6		
2.35	2.2	2.6	111
3.92	3.2	3.8	97
4.70	4.0	4.8	102
6.26	5.1	6.2	99
7.83	6.3	7.7	98

4. RESULTS

4.1. Measurement of the blank values and calculation of the LOQ (Limit of Quantification) for the individual measurements

Sample measurement	TOC single values of the blank control [mg/L]					Mean value [mg/L]	Standard deviation [mg TOC]	LOQ, TOC [mg/L]	equiv. to test substance [mg/L]
Preparation of the calibration, M4 Medium	0.6	0.6	0.6	0.7	0.7	0.6	± 0.0548	1.1	1.2
Retain samples from start of exposure, sample 1b	0.8	0.9	0.9	0.9	0.9	0.9	± 0.0447	1.3	1.5
Retain samples from end of exposure, sample 11b	1.0	0.9	0.9	0.9	1.0	0.9	± 0.0548	1.4	1.6
Retain samples from start of exposure, sample 1c	0.8	0.8	0.8	0.8	0.9	0.8	± 0.0447	1.2	1.3
Retain samples from end of exposure, sample 11c	1.1	1.1	1.1	1.1	1.1	1.1	± 0.000	1.1	1.2
Verification of the calibration M4 Medium	1.0	1.0	1.0	1.1	1.0	1.0	± 0.0447	1.4	1.6

TOC measurements above are from the study control samples and are used as baseline values to calculate LOQ.

$LOQ = Y_B + 9 \times S_B$

Legend: Y_B = mean value of the blank; S_B = standard deviation

4.2. ANALYTICAL RESULTS

The results obtained for the concentration control analysis of 4,4'-MDA are summarized in the following tables:

Data from the start of exposure (retained samples):

Date of sample preparation:	18.Jan 2011
Date of sampling:	18.Jan 2011
Date of receipt of samples in the biodegradation laboratory:	10 Feb 2011
Date of analytical determination:	10 / 11 Feb 2011

Sample No.	Nominal concentration [mg/L]	Analytical concentrations [mg/L] [a]						% of nominal concentration
		1.	2.	3.	4.	5.	Mean	
1b	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
2b	0.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
3b	0.22	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
4b	0.46	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
5b	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
6b	2.2	1.8	1.8	1.8	1.8	2.0	1.8	82
7b	4.6	4.1	4.1	4.1	4.1	4.2	4.1	89

[a] LOQ = 1.5 mg/L

Sample No.	Nominal concentration [mg/L]	Analytical concentrations [mg/L] [a]						% of nominal concentration
		1.	2.	3.	4.	5.	Mean	
1c	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
2c	0.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
3c	0.22	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
4c	0.46	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
5c	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
6c	2.2	1.8	2.0	2.0	2.0	2.0	2.0	91
7c	4.6	4.3	4.3	4.2	4.2	4.3	4.3	94

[a] LOQ = 1.3 mg/L

Data from end of exposure after 48 hours (retained samples):

Date of sample preparation:	18.Jan 2011
Date of sampling:	20.Jan 2011
Date of receipt of samples in the biodegradation laboratory:	10 Feb 2011
Date of analytical determination:	10 / 11 Feb 2011

Sample No.	Nominal concentration [mg/L]	Analytical concentrations [mg/L] [a]						% of nominal concentration
		1.	2.	3.	4.	5.	Mean	
11b	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
12b	0.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
13b	0.22	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
14b	0.46	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
15b	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
16b	2.2	2.0	2.0	2.0	2.0	2.0	2.0	91
17b	4.6	4.5	4.5	4.3	4.3	4.5	4.4	96

[a] LOQ = 1.6 mg/L

Sample No.	Nominal concentration [mg/L]	Analytical concentrations [mg/L] [a]						% of nominal concentration
		1.	2.	3.	4.	5.	Mean	
11c	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
12c	0.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
13c	0.22	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
14c	0.46	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
15c	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--
16c	2.2	1.7	1.7	1.7	1.8	1.8	1.7	77
17c	4.6	4.2	4.2	4.2	4.1	4.3	4.2	91

[a] LOQ = 1.2 mg/L

Verification of the calibration from 09 February 2011:

Two test substance solutions in test medium for each calibration range were prepared in the same manner as the solutions of the calibration procedure and measured on 10 February 2011 with the retain samples.

The following values were obtained:

Sample No.	Nominal concentration [mg/L]	Analytical concentration [mg/L]						% of nominal concentration
		1.	2.	3.	4.	5.	Mean	
1	4.86	4.2	4.3	4.3	4.2	4.3	4.3	89
2	7.07	6.2	6.4	6.2	6.2	6.5	6.3	89

Recovery rates of 89 % of the nominal concentrations were found.

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