

EUROFINS Umwelt Ost GmbH · Löbstedter Straße 78 · D-07749 Jena

**Twin Oxide Int. BV
De Maas 28**

**NL-5684 Best
NIEDERLANDE**

Title: **Test report to order 61202279**
Test report: **No. 6007680007**

Project: **No. 6007680**
Title of project: **Product testing**
Number of samples: **1 sample**
Sample type: **product (Twin Oxide Components)**
Sampler: **client**
Receipt of samples: **2012-01-10**
Test period: **2012-01-10 - 2012-02-24**

The test results refer solely to the analysed test specimen. Unless the sampling was done by our laboratory or in our sub-order the responsibility for the correctness of the sampling is disclaimed. This test report is only valid with signature and may only be further published completely and unchanged. Extracts or changes require the authorisation of the EUROFINS UMWELT in each individual case.

Our actual General Terms of Sales GTS (Allgemeine Verkaufsbedingungen AVB) are applicable, if nothing else is agreed. The GTS will be submitted in German on request.

Accredited test laboratory according to DIN EN ISO/IEC 17025 notification under the DAkkS German Accreditation System for Testing. The accreditation shall apply for the tests listed in the certificate.

Jena, 2012-02-24

Dr. A. Herschel
Analytical Service Manager
Tel.: 03641 / 46 49 - 81



EUROFINS Umwelt Ost GmbH
Löbstedter Straße 78
D-07749 Jena

Tel. +49 (0) 3641 4649-0
Fax +49 (0) 3641 4649-19
www.eurofins-umwelt-ost.de
info_jena@eurofins.de

Amtsgericht Jena
HRB 202596
USt.-ID.Nr. DE 151 28 1997

Geschäftsführer:
Dr. Ulrich Erler,
Dr. Benno Schneider

Bankverbindung: NORD LB
BLZ 250 500 00, Kto 150 334 779
IBAN DE91 250 500 00 0150 334 779
BIC/SWIFT NOLA DE 2HXXX

	Sample designation	chlorine dioxide solution from Twin Oxide components (Batch 11WN 16.111)
	Lab-ID	612012376
Parameter	Method	

Determination of the acute lethal toxicity of substances to zebrafish eggs [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidea)]	in according to DIN EN ISO 15088; Fish Embryo Toxicity (FET) Test (Draft OECD Test Guideline)	see below and following pages
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Summary of Results for all Endpoints at the End of Exposure Period: Critical effect and threshold concentration as observed at end of experimental time; EC: Effective concentration for xx% reduction; 95%-CL: 95% Confidence limits; LOEC: Lowest observed effect concentration; NOEC: No observed effect concentration			
		Unit	Results
EC20	48 h	mg/l	1,898
	lower 95%-CL	mg/l	1,626
	upper 95%-CL	mg/l	2,116
EC50	48 h	mg/l	2,563
	lower 95%-CL	mg/l	2,315
	upper 95%-CL	mg/l	2,869
Mortality	LOEC	mg/l	2,75
	NOEC	mg/l	2,063

Median Effective Concentration (EC50): Statistically derived concentration of a substance in an environmental medium expected to produce a certain effect in 50% of test organisms in a given population under a defined set of conditions.

20% Effective Concentration (EC20): Statistically derived concentration of a substance in an environmental medium expected to produce a certain effect in 20% of test organisms in a given population under a defined set of conditions.

Lowest Observed Effect Concentration (LOEC) is the lowest tested concentration of a test substance at which the substance is observed to have a significant effect (at a p-value of <0.05) when compared with the control. However, all test concentrations above the LOEC should have a harmful effect equal to, or greater, than those observed at the LOEC.

No Observed Effect Concentration (NOEC) is the test concentration immediately below the LOEC.

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Assay principle: The test intends to define lethal effects of chemicals on embryonic stages of fish and constitute an alternative test method to the acute toxicity tests with juvenile and adult fish. Zebrafish embryos are individually exposed in 24-well microtiter plates to a range of concentrations of the test substance. The test is initiated immediately after fertilization and is continued for 48 hours. Lethal effects, as described by four apical endpoints, are determined by comparison with controls to identify the LC50, NOEC and LOEC-values. The test method is based on using a minimum of five test concentrations as well as appropriate controls, with ten or more individual embryos per exposure concentration.

Sample preparation:

Primary dilution: Chlorine dioxide solution generated according to manufacturer's instructions (concentration of stock solution: 3800 mg/L chlorine dioxide). The concentration of primary dilution was 16,5 mg/l ClO₂.

pH adjustment: Yes, at 6.9

Sample concentration:

Test concentration of sample (mg/L)						
1,03	1,38	1,65	2,06	2,75	3,30	4,13

Test organism:

Species: Brachydanio rerio

Source: Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV) Berlin

Test conditions:

Endpoint: (i) coagulation of fertilized eggs,
(ii) lack of somite formation,
(iii) lack of detachment of the tail-bud from the yolk sac, and
(iv) lack of heart-beat.

Exposure time: 48 h

Test temperature: 26 ± 1 °C

Calculation of results: probit analysis (statistical software package: ToxRat Standard 2.10)

Validity criteria:

	<i>critereon fulfilled?</i>
<i>The maximum control mortality is no more than 10,0%.</i>	<i>yes</i>
<i>In a reference assay with 3,7 mg/L 3,4-Dichloroaniline the inhibition of the used batch of fish eggs is more than 10% after a contact time of 48 h.</i>	<i>yes</i>
<i>At the beginning of the test, oxygen saturation should be above 90 % (ca. 7.4 mg/L); during the test, oxygen saturation should be ≥ 50 % (ca. 4.1 mg/L).</i>	<i>yes</i>

Validity of test: The test is valid.

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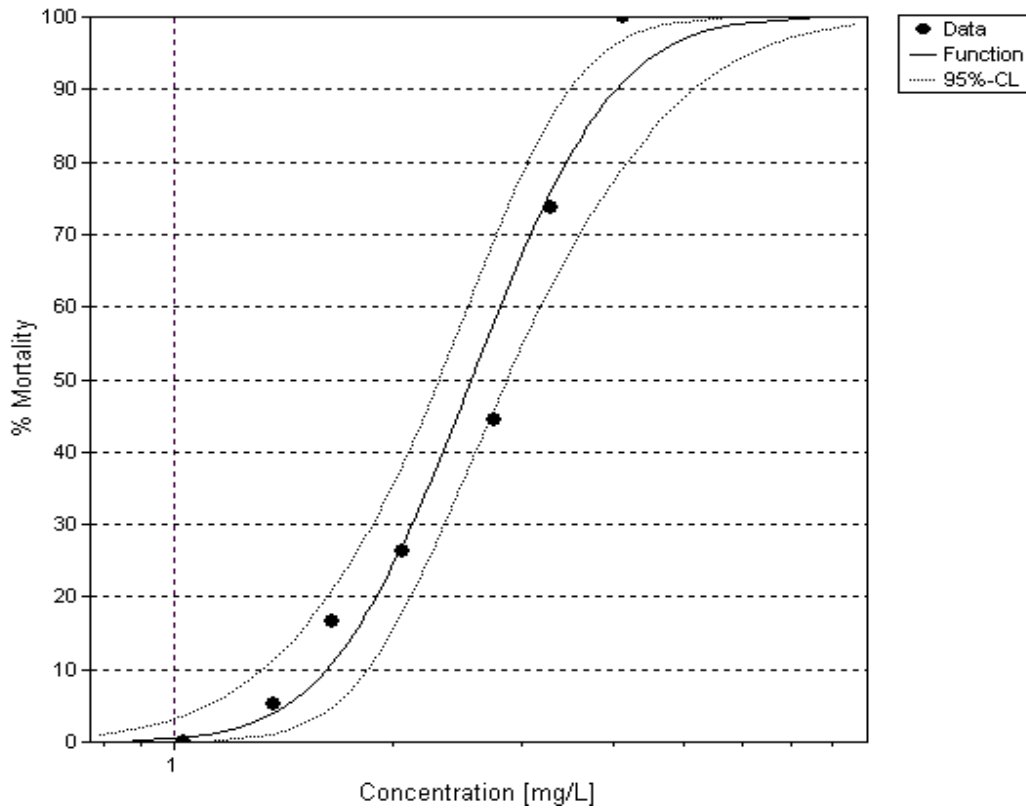
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Overview Mortality: % Mortality caused by the test item at 48 h.

Treatm. [mg/L]	Introduced	Survived	Dead	% Mortality
Control	20	20	0	0
1,031	23	23	0	0
1,375	19	18	1	5,3
1,65	18	15	3	16,7
2,063	19	14	5	26,3
2,75	18	10	8	44,4
3,3	19	5	14	73,7
4,125	15	0	15	100

Concentration-effect curve showing the influence of the test item on mortality of the introduced Danio rerio embryo (eggs) as observed after 48 h.



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