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11/18/92	04/04/01	8E
Submitting Organization	CONFIDENTIAL	
Contractor	RCC NOTOX	
Document Title	INITIAL SUBMISSION: 96-HOUR ACUTE TOXICITY STUDY IN THE CARP WITH [], SUBSTITUTED PHENYL-AZO-ALKYL-PHENOL, (PRELIMINARY RESULTS), WITH COVER LETTER DATED 3/27/2001 (SANITIZED)	
Chemical Category	SUBSTITUTED PHENYL-AZO-ALKYL PHENOL (CONFIDENTIAL)	

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8EHP-01-14897

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8EHP-0401-14897

March 27, 2001

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Document Processing Center (TS-790)
Attention: Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

COMPANY SANITIZED

Dear Coordinator:

[] submits this notice in accordance with Section 8(e) of the Toxic Substances Control Act.

This letter transmits preliminary results of a acute static toxicity test producing mortality to carp for a chemical substance described in P-88-605, generic name = Substituted-phenyl-azo-alkyl phenol, and EPA Accession No.: 121303.

Ten fish per concentration were exposed for a maximum of 96 hours in a static system to a range of water accommodated fractions (WAF's) of 10, 18, 32, 56 and 100 mg/L. After 96 hours exposure, 70% mortality was recorded in the WAF prepared at 100 mg/L. A 90% mortality was found in the WAF prepared at 56 mg/L. In the WAF prepared at 32 mg/L a total of 30% mortality was found. Other effects observed were discoloring, hypoactivity, loss of equilibrium and immobility.

Analysis of samples taken at start (t=0) of the final test revealed the actual concentration of Mortrace SB conc. In the exposure solutions were just above or below the detection level of 10 µg/L. The LC50 was between 32 and 56 mg/L with a corresponding concentration approximating the minimum detection limit level of 10 µg/L.

[] considers the exact identity of this chemical to be Confidential Business Information (CBI). The substantiation is included as Attachment I.

If you have any questions concerning this submittal, my telephone number is [].

Sincerely,

[_____]
[] []
[] []

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Attachment I

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March 27, 2001

Document processing Center (TS-790)
(Attention: Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

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Dear Sir:

Re: Substantiation of Confidentiality Claims Made in Submittal of March 27, 2001 Under Section 8(e) of TSCA for substituted-phenyl-azo-alkyl phenol (EPA Accession No.: 121303).

In accordance with EPA's guide document, "Support Information for Confidentiality Claims," we provide the answers to the fourteen questions asked to support the claim of confidentiality for chemical identity.

1. Is your company asserting this confidential business information (CBI) claim on its own behalf? If the answer is no, please provide company name, address and telephone number of the entity asserting claim.

ANSWER: We are submitting this claim on behalf of ourselves.

2. For what period do you assert your claim(s) of confidentiality? If the claim is to extend until a certain event or point in time, please indicate that event or time period. Explain why such information should remain confidential until such point.

ANSWER: Confidentiality of the chemical identity should be maintained indefinitely. It is impossible to estimate the time span over which this specific chemical technology might be utilized. Knowledge of the chemical identity with the link to the company could enable competitors to identify the type of chemistry under consideration and thus provide an unfair competitive advantage to us.

3. Has the information that you are claiming as confidential been disclosed to any other governmental agency, or to this Agency at any other time? Identify the Agency to which the information was disclosed and provide the date and circumstances of the same.

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Was the disclosure accompanied by a claim of confidentiality? If yes, attach a copy of said document reflecting the confidentiality agreement.

ANSWER : This connection between the company and the specific chemical identity has not been disclosed.

4. Briefly describe any physical or procedural restrictions within your company relating to the use and storage of the information you are claiming CBI.

ANSWER: Information on the chemical identity and other data for this substance are held "COMPANY CONFIDENTIAL" which means it may not be disclosed outside the company. Accessibility to "COMPANY CONFIDENTIAL" documents is limited to people within the company who have a real need-to-know. Documents so classified are clearly stamped, may not be reproduced without permission, and are filed in security-locked cabinets. Each of our products are assigned a coded name designation so that in normal business and operational activities the chemical identities are not identified, and there is no link between the chemical identity and the coded designation. Most persons within the company that have access to confidential information are under contract which states that intellectual property may not be disclosed upon leaving the organization.

5. If anyone outside your company has access to any information claimed CBI, are they restricted by confidentiality agreement(s)? If so, explain the content of the agreement(s).

ANSWER: The chemical identity of this substance and the internal designation have not been disclosed outside of the company in a manner linking the two. The substance designation was used without a link to the exact chemical identity. No additional disclosure of the confidential information is anticipated.

6. Does the information claimed as confidential appear or is it referred to in any of the following:

a) advertising or promotional material for the chemical substance or the resulting product;

b) Material safety data sheets or other similar materials (such as technical data sheets) for the substance or resulting product (include copies of this information as it appears when accompanying the substance and/or product the time of transfer or sale);

c) Professional or trade publications; or

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d) Any other media or publications available to the public or to your competitors.

If you answered yes to any of the above, indicate where the information appears, include copies, and explain why it should nonetheless be treated as confidential.

ANSWER: The chemical identity of the subject material has not been disclosed in any of the documents listed in the question. The chemical identity of this substance and the internal designation have not been disclosed outside of the company in a manner linking the two.

7. Has EPA, another federal agency, or court made any confidentiality determination regarding information associated with this substance? If so, provide copies of such determinations.

ANSWER: No

8. Describe the substantial harmful effects that would result to your competitive position if the CBI information is made available to the public? In your answer, explain the causal relationship between disclosure and any resulting substantial harmful effects. Consider in your answer such constraints as capital and marketing cost, specialized technical expertise, or unusual processes and your competitors access to your customers. Address each piece of information claimed CBI separately.

ANSWER: We do assert that disclosure of the chemical identity and the company name would be likely to result in substantial harm to our competitive position. The exact chemical identity is a trade secret known only to certain persons within the company having a need to know. Disclosure of the composition connected to the company would enable competitors to avoid substantial research and development costs related to new chemical development. This would enable them to compete in the chemical field without incurring the invention, discovery, and research costs, thus doing significant potential economic harm to the company.

9. Has this substance been patented in the U.S. or elsewhere? Is a patent for the substance currently pending?

ANSWER: This substance has been patented in the U.S.

10. Is this substance/product commercially available and if so, for how long has it been available on the commercial market?

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a) If on the commercial market, are your competitors aware that the substance is commercially available in the U.S.?

b) If not already commercially available, describe what stage of research and development (R&D) the substance is in, and estimate how soon a market will be established?

c) What is the substance used for and what type of product does it appear in?

ANSWER: The chemistry subject to this notice has been in commerce since December, 1991. The exact chemical identity is a trade secret known only to certain persons within the company having a need to know. This substance is for a specialized use.

11. Describe whether a competitor could employ reverse engineering to identically recreate the substance?

ANSWER: The chemical identity is not being claimed as confidential.

12. Do you assert that disclosure of this information you are claiming CBI would reveal:

a) confidential processes used in manufacturing the substance;

b) if a mixture, the actual portions of the substance in the mixture; or

c) information unrelated to the effects of the substance on human health or the environment?

ANSWER: Disclosure would reveal information unrelated to the effects of the substance on human health or the environment. The chemical identity and the company has not been disclosed outside of the company in a manner linking the two.

13. Provide the Chemical Abstract Service Registry Number for the product, if known. Is your company applying for a CAS number now or in the near future? If you have applied for a CAS number, include a copy of the contract with CAS.

ANSWER: Accession number 121303

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REPORT

Substituted phenylazoalkylphenol
Accession # 121303

96-HOUR ACUTE TOXICITY STUDY IN THE CARP

WITH

[11/18/92]

RCC NOTOX Project 080819.
RCC NOTOX Substance 25641
RCC Project 327925.

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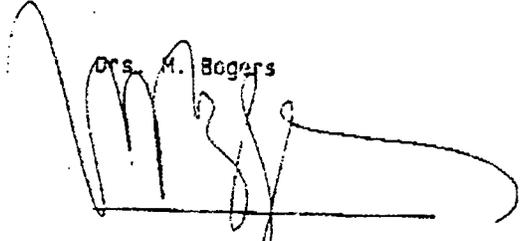
RCC NUTOX 060819

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REPORT APPROVAL

STUDY DIRECTOR:

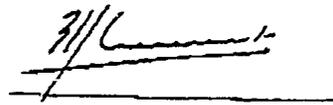
Drs. M. Bogers



Date: 12 November, 1992

MANAGEMENT:

Ing. E.J. van de Waart
(Section Head, Genetic &
Ecotoxicology)



Date: 18/11/1992

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SUMMARY

96-hour Acute Toxicity Study in the Carp with []

Analysis of samples from a vessel containing a WAF (Water Accommodated Fraction) prepared at 1000 mg/l at the start of the range-finding test revealed an actual [] concentration of 0.14-0.17 mg/l (see the appended Analytical Report).

Ten fish per concentration were exposed for a maximum of 96 hours in a static system to a range of WAF's prepared at 10, 18, 32, 56 and 100 mg/l. Weighed amounts of test substance were spread out on microscope slides. Each slide with test substance was put in 3 liters of ISO-medium. Subsequently, the solutions were stirred for ca. 66 hours until the start of the exposure. After stirring, the microscope slides were removed. The colour of the solutions was a light brownish yellow and the intensity of this colour increased with increasing nominal concentration.

After 96 hours of exposure 70% mortality was recorded in the WAF prepared at 100 mg/l, whereas 90% mortality was found in the WAF prepared at 56 mg/l. In the WAF prepared at 32 mg/l a total of 30% mortality was found. Other effects observed were discolouring, hypoactivity, swimming at the bottom, loss of equilibrium and immobility. No mortality or other effects were recorded in the fish exposed to the WAF's prepared at 10 and 18 mg/l.

Analysis of the samples taken at the start ($t=0$) of the final test revealed that the actual concentrations of [] in the exposure solutions were just above or below the detection level of 10 $\mu\text{g/l}$. Hence, there was no further relevance for analyses of samples to be taken at the end of the test.

The LC50 was between the WAF prepared at 32 mg/l and the WAF prepared at 56 mg/l. This corresponded with a concentration approximating the minimum detection level of 10 $\mu\text{g/l}$.

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PREFACE

GENERAL

Title 96-hour Acute Toxicity Study in the Carp with

Sponsor []

Study Monitor Mr. C.G. Oger

Testing Facility RCC NOTOX B.V.
Hambakenwatering 3
5231 DD 's-Hertogenbosch
The Netherlands

RCC NOTOX Project 080819

(RCC Project 327925)

Test Substance []

Test System Carp (Cyprinus carpio)

PROJECT STAFF

Aquatic Toxicology:
Study Director Drs. M. Begers (RCC NOTOX B.V.)
Technical Head Ing. J.J.C. van der Pol (RCC NOTOX B.V.)

Analytical Chemistry:
Principal Scientist Dr. Ir. M.M. Gladdines (RCC NOTOX B.V.)

SCHEDULE

Start of the study September 4, 1992

Completion of the study October 8, 1992

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QUALITY ASSURANCE STATEMENT

RCC NOTOX B.V.
5231 DD 's-Hertogenbosch / The Netherlands

RCC NOTOX Project 080819

Test Substance []

Study Director Drs. M. Rogers

Title 96-hour Acute Toxicity Study in the Carp with []

Study procedures were subjected to periodic inspections.

This report was audited by the Quality Assurance Unit and, as far as can be reasonably established, the methods and results accurately reflect the raw data.

Dates of QAU Inspections / Audits	Reporting Date
28-07-1992	28-07-1992
24-09-1992	24-09-1992
09-11-1992	09-11-1992

General non study specific processes are also inspected at least once every 3 months and results reported to management.

Manager, Quality Assurance Unit

C.J. Mitchell B. Sc.
i.a.



Date: 23 November, 1992

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RCC NOTOX 080819 **SANITIZED**

STATEMENT OF GLP COMPLIANCE

RCC NOTOX Project 080819
Test Substance []
Study Director Drs. M. Rogers
Title 96-hour Acute Toxicity Study in the Carp with []

To the best of my knowledge and belief, the study described in this report was conducted in compliance with the most recent edition of:

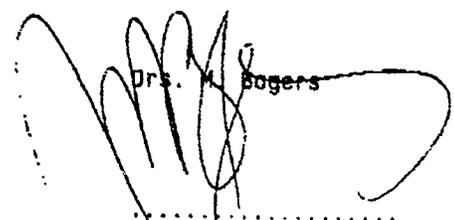
OECD Principles of Good Laboratory Practice.

United States Environmental Protection Agency, (FIFRA). Title 40 Code of Federal Regulations Part 160.

United States Environmental Protection Agency, (TSCA). Title 40 Code of Federal Regulations Part 792.

United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

Study Director


Drs. M. Rogers

Date: 12 November, 1992

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GUIDELINES

The study procedures described in this report were based on the following guidelines:

Organization for Economic Co-operation and Development (OECD), OECD guidelines for Testing of Chemicals, guideline No. 203: "Fish Acute Toxicity Test", Adopted April 4, 1984.

European Economic Community (EEC), EEC directive 84/449, Methods for the determination of ecotoxicity, Publication No. L251, C-1: "Acute Toxicity for Fish", adopted September, 1984 (with the exception of the length of the fish).

SUMMARY OF PROTOCOL AMENDMENTS

1. The procedure for preparation of the exposure solutions was as follows: weighed amounts of test substance were spread out on microscope slides. These amounts corresponded to the nominal concentrations to be tested. To be able to handle the substance, it is was melted in a water-bath at a temperature of ca. 60°C. Each slide with test substance was put in the required volume of ISO-medium, which was subsequently stirred for ca. 72 hours. After stirring, the microscope slides were removed. Then the resulting solutions (= water accommodated fractions (WAF's)) were tested.
2. Sampling for analysis of actual exposure concentrations at the end of the test was cancelled, because no detectable amounts of [] were found in the samples taken at the start of the test.

ARCHIVING

RCC NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test substance reference sample, all specimens and raw data.

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OBJECTIVE**PURPOSE**

The purpose of the study was to evaluate the test substance for its ability to generate acute toxic effects in Cyprinus carpio during an exposure period of 96 hours and, if possible, to determine the LC50 at all observation times.

DEFINITIONS

Fish were considered to be dead when no reaction was observed after touching the caudal peduncle and visible breathing movements were absent.

The LC50 is the concentration killing 50% of the fish after a defined period of exposure.

The Water Accommodated Fraction (WAF) is defined as the soluble fraction of a defined amount of test substance after a certain period of exposure to water. A weighed amount of test substance is spread out on an inert surface (e.g. a microscope slide) to optimize the surface ratio between the substance and the test medium. The period of exposure of the substance to water should be long enough to reach an equilibrium between the concentration in water (WAF) and the amount of substance present on the inert surface.

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MATERIALS AND METHODS

TEST SYSTEM

Species	Carp (<u>Cyprinus carpio</u> , Teleostei, Cyprinidae) (Linnaeus, 1758)
Source	Zodiac, proefacc, "De Haar Vissen", L.U. Wageningen, the Netherlands.
Mean length	2.0 ± 0.16 cm
Mean weight	0.24 ± 0.05 g
Characteristics	Pathogen-free F1 from a single parent-pair.
Reason for selection	This system has been selected as an internationally accepted species.
Total fish used	80

HOLDING

Acclimation period	At least 14 days after delivery.
Measurements	Oxygen concentration, pH, nitrate and nitrite concentration, ammonia concentration and hardness of the water: once a week. Temperature: every working day.
Feeding	Daily with Trouvit or Artemia.
Control of sensitivity	A reference test with pentachlorophenol (PCP, SIGMA) is carried out within a period of 3 months. The results of the most recent test are appended to this report.
Validity of batch	Following a 48-hour settling-in period the fish were allowed to acclimatise to the test medium without test substance for at least seven days. In the batch of fish used for the test, mortality during the seven days prior to the start of the test was less than 5%.

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TEST SUBSTANCE

Identification	[]
Description	Dark red-brown liquid
Batch	MR 26592 SBC
Purity	Concentrate
Instructions for test substance storage	At room temperature protected from light
Stability under storage conditions	Stable ¹
Expiry date	June 03, 1993
Stable for at least 2 hours in vehicles	Water: no

¹ The original container was placed in a water-bath at 60°C for 3 days, in order to melt the test substance completely. The test substance was then divided over several smaller containers, which were used for the different projects. Chemical analysis (HPLC) of the test substance after melting revealed that this procedure had no effect on the stability of the original compound.

RANGE-FINDING TEST

A range-finding test was performed to provide information about the range of concentrations to be used in the final test. Five fish per concentration were exposed to a range of WAF's prepared at 10, 100 and 1000 mg/l.

TEST PROCEDURE AND CONDITIONS

Test duration	96 hours
Test type	static
Test vessels	8 litres, all-glass.
Test medium	ISO-medium, formulated using Milli-Ro water with the following composition: Ca ²⁺ 80 mg/l Mg ²⁺ 12 mg/l Na ⁺ 15 mg/l K ⁺ 3 mg/l Cl ⁻ 145 mg/l SO ₄ ²⁻ 49 mg/l HCO ₃ ⁻ 47 mg/l This dilution water was aerated until the dissolved oxygen concentration had reached saturation and the pH had stabilized. The hardness was 250 mg CaCO ₃ per litre and the pH 8.0-8.1 after aeration.
Milli-Ro water	Tap-water purified by reverse osmosis (Millipore Corp., Bedford, Mass., USA).
Test concentration	Based on the results of the range-finding test: WAF's prepared at 10, 18, 32, 56 and 100 mg/l.
Control	Test medium without test substance or other additives (0 mg/l).
Number of fish	10 fish per concentration and controls.
Loading	0.8 g fish/litre, i.e. 10 fish per 3 litres of test medium.
Illumination	16 hours photoperiod daily.
Aeration	The test media were aerated continuously during the test.
Feeding	No feeding from 24 hours prior to the test and during the total test period.

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[]

Introduction of fish

Directly after preparation of the test media fish were introduced into the test medium, provided that the temperature of the medium was within the optimal range.

PREPARATION OF TEST MEDIA

Weighed amounts of test substance were spread out on microscope slides. To be able to handle the substance, it was melted in a water-bath at a temperature of ca. 60°C. The actual amounts weighed were 29.2, 52., 100.6, 166.9 and 311.7 mg. Each slide with test substance was put in 3 liters of ISO-medium. Subsequently, the solutions were stirred for ca. 66 hours until the start of the exposure. After stirring, the slides were removed. The final test solutions were all clear without precipitation. Further, the colour of the solutions was a light brownish yellow and the intensity of this colour increased with increasing nominal concentration.

SAMPLING FOR ANALYSIS OF TEST CONCENTRATIONS

Stability of the exposure concentration under test conditions was determined by analyzing samples taken from the WAF prepared at 1000 mg/l during the range-finding test.

Sampling: Frequency	at t=0h, t=24h and t=96h.
Volume	10 ml from the approximate centre of the test vessel.
Storage	Samples were transferred to Analytical Chemistry on the day of sampling.

During the final test duplicate samples were taken from the WAF's prepared at 0, 10, 32 and 100 mg/l.

Sampling: Frequency	at the start of the exposure (t=0h)
Volume	10 ml from the approximate centre of the test vessels.
Storage	Samples were transferred to Analytical Chemistry on the day of sampling.

Additionally, reserve samples of 25 ml were taken from all test solutions. These samples were stored at -20°C for possible analysis, and until delivery of the final report, pending on the decision of the sponsor for additional analysis.

The method of analysis is described in the appended Analytical Report.

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MEASUREMENTS AND RECORDINGS

Mortality and other effects At 3, 24, 48, 72 and 96 hours following the start of exposure. Dead fish were removed when observed.

Fish length and weight Ten fish of the batch used for the test, were weighed and measured prior to the start of the test.

Dissolved oxygen content and pH Daily in all vessels, beginning at the start of the test.

Temperature of medium Daily in one control vessel, beginning at the start of the test.

Euthanasia At the end of the test the surviving fish were rapidly killed by exposing them to ca. 1.2% ethylene glycol monophenylether in water.

ACCEPTABILITY OF THE TEST

The mortality in the blank and, if relevant, in the control containing the vehicle should not exceed 10%.

DATA HANDLING

The LC50 was determined using the maximum likelihood estimation method with the probits of the percentages of dead fish as function of the logarithms of the corresponding concentrations (Finney, D.J., 1971: Probit analysis, Cambridge University Press, Cambridge, U.K., 3rd edition).

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RESULTS

STABILITY OF [] UNDER TEST CONDITIONS

The results of analysis of samples taken at the start of the range-finding test revealed an actual concentration of [MORTRACE SB CONC.] of 0.14-0.17 mg/l in the WAF prepared at 1000 mg/l. After 24 hours, the actual concentration had decreased to just above the detection level of 41 ug/l (4.1×10^{-2} mg/l, see the appended Analytical report).

RANGE-FINDING TEST

In the range-finding test, all fish exposed to the WAF's prepared at 100 and 1000 mg/l had died within 24 hours of exposure. No mortality was seen in the WAF prepared at 10 mg/l.

FINAL STUDY: MORTALITY AND OTHER EFFECTS

The mortality data are presented in Table 1.

In contrast to the results of the range-finding test, not all fish exposed to the WAF prepared at 100 mg/l died during the 96-hour exposure period. After 96 hours of exposure 70% mortality was recorded in the WAF prepared at 100 mg/l, whereas 90% mortality was found in the WAF prepared at 56 mg/l. In the WAF prepared at 32 mg/l a total of 30% mortality was found. No mortality of fish was observed in the WAF's prepared at 10 and 18 mg/l.

Effects other than mortality, were observed in the fish exposed to the WAF's prepared at and above 32 mg/l. The effects recorded were discolouring, hypoactivity, swimming at the bottom, loss of equilibrium and immobility (see Table 2: Specification of effects). No effects were recorded in the fish exposed to the WAF's prepared at 10 and 18 mg/l.

FINAL STUDY: EXPERIMENTAL CONDITIONS

The results of measurement of pH and oxygen concentrations are presented in Tables 3 and 4, respectively.

The pH ranged from 7.4 to 8.1.
Oxygen concentration in the test media was found to be > 7 mg/l for all measurements performed during the final study.
The temperature of the test medium measured in the blank control ranged from 22 to 23°C.

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FINAL STUDY: ACCEPTABILITY OF THE TEST

In the control no mortality or any other effects were observed. Further, all test conditions remained within the ranges prescribed by the protocol.

FINAL STUDY: ACTUAL VERSUS NOMINAL CONCENTRATIONS

The results of analysis of the samples taken during the study are described in the appended Analytical Report.

Analysis of the samples taken at the start (t=0) of the final test revealed that the actual concentrations of [] in the exposure solutions were just above or below the detection level of 10 µg/l. Hence, there was no relevance for analyses of samples to be taken at the end of the test.

CALCULATION OF LC50-VALUES AND RELATED PARAMETERS

No LC50-values with a 95%-confidence interval could be determined using the Finney model at any of the time points during the exposure period, because each index of regression significance was > 1. The LC50 was between the WAF prepared at 32 mg/l and the WAF prepared at 56 mg/l. This corresponds with a concentration approximating the minimum detection level of 10 µg/l.

CONCLUSION

Under the conditions of the present test the 96h-LC50 for carp exposed to MORTRACE SB CONC. approximated the minimum detection level of MORTRACE SB CONC. in water (10 µg/l).