CLARIANT

INVESTIGATION REPORT FOR MAERSK OIL

Supply of SCAVTREAT 7103

JULY 31, 2017

CLARIANT OIL SERVICES SCANDINAVIA AS
Bergen, Norway

CONFIDENTIAL

Contents

Executive Summary

SCAVTREAT 7103 is a Clariant hydrogen sulfide scavenger of a type commonly deployed in Oil & Gas production operations. The material is manufactured on behalf of Clariant by Synthite Ltd.

An unauthorised modification was made to the formulation of SCAVTREAT 7103 during 2005, in an attempt to meet a customer's technical specification for the material. The modified formulation had 0.1% active benzyl-C12-16-alkyldimethyl, chlorides ("BAC") added as 0.2% of a 50% solution ("BAC 50").

This modification was not internally approved and was not communicated to any customer. Consequently, HOCNF documentation was not prepared as required and environmental testing was not undertaken. This resulted in the inclusion of a minor component that was classified as "red" to a material that was otherwise classified as "yellow".

Clariant became aware of the situation when one customer using SCAVTREAT 7103 detected some quaternary amine in the condensed water from a gas treatment system. The customer asked Clariant to confirm whether SCAVTREAT 7103 contained quaternary amine. Following consultation with Synthite, it was discovered that SCAVTREAT 7103 contained low levels of BAC.

Immediate action was taken to inform stakeholders, as well as concurrently eliminating BAC from produced material. Clariant conducted detailed technical evaluations to validate alternative solutions including the elimination of BAC from SCAVTREAT 7103.

A transition to supply of material free of undisclosed components to Maersk Oil was completed by 12th June 2017.

Clariant conducted extensive root cause analysis, including review of historic technical records and correspondence, on site visits and discussions with Synthite and review of contract documentation.

It was identified that there were two primary causes for this occurrence:

- Unauthorised instruction was given by an individual to a third party toll manufacturer. This bypassed established control systems which requires approval from four individuals for any product formulation changes.
- Lack of a contractual requirement with this manufacturer that documents changes to product formulations and respective communications to toll manufacturers according to Clariant's standard format, including multiple levels of approval.

Preventive actions have been formulated and are being rapidly implemented to eliminate the risk of a similar occurrence in the future. Full details of the change in Management Systems to ensure no re-occurrence takes place can be found in the Preventive Actions chapter on page 10.

Introduction and Background

The product SCAVTREAT 7103 is manufactured for Clariant Oil Services Scandinavia by a 3rd party toll manufacturer in the UK, Synthite. Between 2003 – 2017 the product has been supplied to customers in Scandinavia.

SCAVTREAT 7103 is a hydrogen sulfide (H_2S) scavenger, supplied as an aqueous solution of 30-60% 2,2',2"-(hexahydro-1,3,5-triazine-1,3,5-triyl)triethanol, commonly referred to as "MEA triazine". It is applied in oil and gas production systems to reduce levels of H_2S, which is a highly toxic and corrosive gas that is naturally present in some oil and gas reservoirs, and thereby contributes to safety and environment protection.

On 22nd March 2017, Clariant Oil Services Scandinavia was contacted by a customer regarding the SCAVTREAT 7103 composition. It was questioned whether the product contained a surfactant, because the customer had detected some quaternary amine in the condensed water from a gas treatment system. The immediate response from Clariant sales personnel was that the product only contained MEA triazine, based on the records in Clariant's product database and Harmonised Offshore Chemical Notification Format (HOCNF) documentation.

The customer asked Clariant to re-check the composition, and come back with a written confirmation. Clariant contacted Synthite by e-mail, and asked whether there was any quaternary amine added to SCAVTREAT 7103. Synthite informed Clariant that SCAVTREAT 7103 contains 0.1% active quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides (commonly known as benzalkonium chloride or "BAC") added as 0.2% of a 50% solution ("BAC 50"). This was confirmed by Synthite to Clariant in writing on 28th March 2017.

Synthite further informed Clariant that BAC had been added to SCAVTREAT 7103 in an attempt to meet technical specifications from Maersk Oil, namely to control the levels of formic acid and N-(2-hydroxyethyl) formamide ("NHEF") generated during normal product application when SCAVTREAT 7103 is exposed to temperatures in Maersk Oil process systems which can be up to 170°C.

Synthite explained that they had received instructions from an individual at Clariant Oil Services Scandinavia in 2005 to add BAC to SCAVTREAT 7103. The individual also requested that this information must not be disclosed to Maersk Oil, ostensibly to protect Clariant proprietary formulation information.

In a meeting on 31st March 2017 with the customer who informed Clariant about the quaternary amine, Clariant was informed that the customer had performed an analysis of SCAVTREAT 7103 that confirmed that the product contained quaternary amine.

The level of addition of BAC was so low that, even if Clariant has been aware of its presence, Clariant would have not been required to show this component on the material Safety Data Sheet (SDS). However, as the component was deliberately added it should have been declared in the HOCNF, which did not happen.

As the addition of BAC to SCAVTREAT 7103 was unauthorised in Clariant's management systems, investigations and internal discussions were initiated to determine:

- The history of when the addition of BAC began and the technical justification for this addition
- The reason that this addition was not properly approved in Clariant's systems
- Possible solutions for continued supply to Clariant customers, including understanding the technical consequences of removing BAC from SCAVTREAT 7103

Supply of SCAVTREAT 7103

SCAVTREAT 7103 is an aqueous solution of 30-60% 2,2',2"-(hexahydro-1,3,5-triazine-1,3,5-triyl) triethanol, commonly referred to as "MEA triazine". This product is an industry standard treatment used globally in the Oil & Gas industry to remove hydrogen sulfide ("H_2S") from process streams. H_2S is a highly toxic and corrosive gas that is naturally occurring in some oil and gas reservoirs. The sequestration of hydrogen sulfide by scavengers, such as SCAVTREAT 7103, greatly improves the safety of oil and gas operations and helps to protect the environment.

Supply of SCAVTREAT 7103 to Maersk Oil began in October 2003. Subsequent discussions with Maersk Oil indicated that NHEF levels were higher than desired. On the instruction of the same individual that later gave direction to change the formulation of SCAVTREAT 7103, testing of samples from Synthite was conducted by the Danish Technological Institute (DTI). This testing evaluated modifications to SCAVTREAT 7103 which would lower levels of formic acid and NHEF generated from SCAVTREAT 7103 under field temperatures. BAC was identified as helping to address this issue. No other Clariant employees received test results.

Beginning in January 2005, BAC was added to the SCAVTREAT 7103 formulation at a rate of 0.1%. Since 12th June 2017 SCAVTREAT 7103 is free of BAC.

Corrective Actions

When we learned of the unauthorised addition of BAC, Clariant identified that SCAVTREAT 7103 was being supplied to three customers in Scandinavia. Based on historic information provided by Synthite, it was also noted that the addition of BAC to SCAVTREAT 7103 was initiated for technical reasons related to application of the product by Maersk Oil, which required detailed discussion and investigation.

Following an internal discussion on 6th April 2017 between Clariant specialists from Commercial, Technical, Product Stewardship, Procurement and Environmental functions, a number of actions were initiated. These included the following:

- Requested samples of SCAVTREAT 7103 with and without BAC to be mobilised from Synthite to Clariant labs in Aberdeen, UK and Bergen, Norway, for the required testing
- Requested samples of SCAVTREAT 7103 with and without BAC to be mobilised from Synthite to DTI in Denmark for testing of formic acid and NHEF levels according to Maersk Oil specifications
- Determined availability of alternative manufacturing sources of SCAVTREAT 7103, in case Synthite produced material without BAC added would not meet customer technical specifications
- Investigated manufacture of material by Synthite via a different process to determine effect on levels of formic acid and NHEF in SCAVTREAT 7103
- Reviewed contract documentation between Clariant, Maersk Oil and Synthite to fully understand responsibilities

Initial discussions within Clariant focussed on finding a technically acceptable product to propose to Maersk Oil, based on their specific requirements and specifications, as the addition of BAC to SCAVTREAT 7103 had originally been carried out to address these specifications. It became clear that this was likely to be a complex task, and that detailed technical discussions with Maersk Oil were the best way to expedite a solution.

Maersk Oil in Denmark were informed by Clariant on 11th April 2017; a copy of this letter is included in Appendix A. On 18th April 2017, Clariant received a reply from Maersk Oil informing Clariant that Maersk Oil would inform the Danish authorities, and advising Clariant to also engage with the authorities. On 20th April 2017, Clariant informed the Danish Product Registry about the undocumented addition of BAC to SCAVTREAT 7103. A copy of this letter is included in Appendix A.

In order to seek a solution to address Maersk Oil's technical requirements and provide a product which was compliant with the applicable regulations, Clariant mobilised the airfreight of the necessary product samples and initiated testing at an independent laboratory, the Danish Technological Institute (DTI).

This testing evaluated the generation of formic acid and NHEF under Maersk Oil's field temperatures by:

- SCAVTREAT 7103; manufactured by Synthite; containing BAC (benchmark)
- SCAVTREAT 7103; manufactured by Synthite; without BAC

- SCAVTREAT 7103; manufactured by Synthite by alternative manufacturing method; without BAC
- SCACTREAT 7103; manufactured by an alternative supplier, without BAC

Results were compared to Maersk Oil specification, and are summarised in the table below. Results highlighted in green are within the specification limits defined by Maersk Oil. Results highlighted in blue are outside of the specification limits and deemed unacceptable by Maersk Oil.

	Specification (%)	Method	Measured content (%)			
			SCAVTREAT 7103 with BAC, batch: 17D025	SCAVTREAT 7103 without BAC, batch: 17D037	SCAVTREAT 7103 alternative production method; without BAC. Batch RD282	SCAVTREAT 7103 alternative supplier; without BAC
Formic acid						
%w/w Formic acid (as received)	0.1 max	Dionex Column	0.014	0.011	0.17	0.0067
%w/w Formic acid (reflux at 140°C for 10 min)	0.2 max	Dionex Column	0.046	0.1	0.23	0.14
%w/w Formic acid (aerated for 7 days)	0.1 max	Dionex Column	0.017	0.021	0.19	0.0076
%w/w Formic acid (aerated for 7 days, then reflux at 140°c for 10 min.)	0.2 max	Dionex Column	0.084	0.036	0.23	0.15
N-(2-hydroxyethyl) formamide ('NHEF')						
%w/w NHEF (as received)	0.25 max	GC-MS	<0.1	<0.1	0.12	<0.1
%w/w NHEF (reflux at 140°C for 10 min)	2.00 max	GC-MS	0.36	1.2	2.0	0.53
%w/w NHEF (aerated for 7 days)	1.00 max	GC-MS	<0.1	<0.1	0.13	<0.1
%w/w NHEF (aerated for 7 days, then reflux at 140°c for 10 min.)	2.00 max	GC-MS	1.7	2.0	2.0	1.6

The full test results reported by DTI are included in Appendix C.

These results confirm that:

- SCAVTREAT 7103 manufactured by Synthite meets the Maersk Oil specification, with and without the addition of BAC
- SCAVTREAT 7103 produced by an alternative manufacturing process (without the addition of BAC) does not meet the Maersk Oil specification
- SCAVTREAT 7103 produced by an alternative manufacturer (without the addition of BAC) does meet the Maersk Oil specification

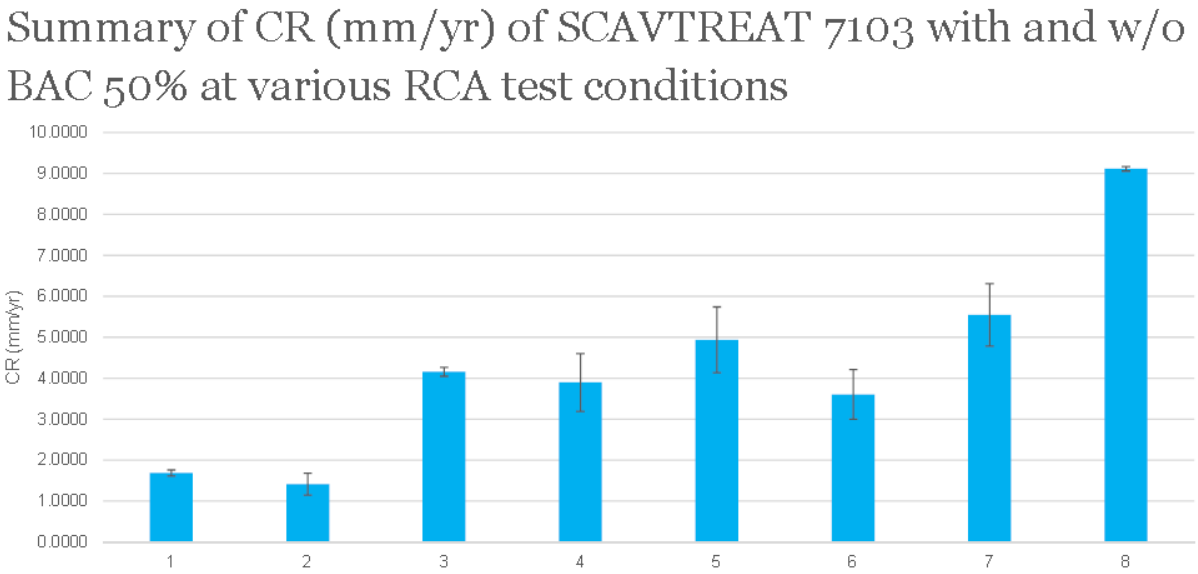
These results give a positive indication that removal of BAC from SCAVTREAT 7103 is a viable solution for continued supply to Maersk Oil, and that an additional source of compliant material is available.

Clariant conducted further testing to evaluate the corrosivity of SCAVTREAT 7103 with and without BAC. This testing was requested by Maersk Oil. Maersk Oil's technical specifications for formic acid and NHEF are designed to reduce the risk of corrosion during application of the product in field process systems, and this testing was requested as an additional confirmation of the technical suitability of SCAVTREAT 7103 without BAC.

Clariant mobilised the transatlantic airfreight of samples of SCAVTREAT 7103 with and without BAC to our Global Technology Centre at the business unit headquarters in The Woodlands, near Houston, Texas. Testing in the corrosion lab was prioritised over all other work in progress, and all of the necessary resources dedicated to rapid completion of this testing.

The testing conducted was a Rotating Cylinder Autoclave (RCA) test. In order to provide a high degree of accuracy and confidence in the test results, individual tests were conducted in triplicate. Testing was conducted under different temperatures and gas compositions, to reflect field conditions in different parts of Maersk Oil's field operations. Workscopes and methodology were agreed with Maersk Oil prior to testing.

Results are shown in the graph below:



				Gas Composition					
Sample	BAC 50/No BAC 50	Temperature (Deg C)	Agitation (rpm)	N2 (Bar)	CO2 (ppm)	H2S (ppm)	CR Average	Std. Dev	RSD (%)
1	BAC 50	105	600	40	N/A	N/A	1.6849	0.0756	4.5%
2	No BAC 50	105	600	40	N/A	N/A	1.4124	0.2688	19.0%
3	BAC 50	105	600	40	20,000	5	4.1572	0.1099	2.6%
4	No BAC 50	105	600	40	20,000	5	3.8956	0.7037	18.1%
5	BAC 50	170	600	40	N/A	N/A	4.9367	0.8035	16.3%
6	No BAC 50	170	600	40	N/A	N/A	3.6033	0.6051	16.8%
7	BAC 50	170	600	40	2,000	140	5.5463	0.7617	13.7%
8	No BAC 50	170	600	40	2,000	140	9.1132	0.0538	0.6%

These results confirm that the addition of BAC to SCAVTREAT 7103 does not have a significant impact on the corrosivity of SCAVTREAT 7103 under Maersk Oil field conditions. There is not a consistent increase or decrease in corrosion rates caused by the addition of BAC to the product.

The test results detailed above, showing the impact of BAC on formic acid and NHEF levels, as well as the corrosivity of SCAVTREAT 7103 without BAC, satisfied Maersk Oil that BAC could be removed from SCAVTREAT 7103 without undue risk to their process or operations.

Following agreement with Maersk Oil, supply of SCAVTREAT 7103 to Maersk Oil in Denmark has been free of BAC since 12th June 2017.

Ecotoxicology Testing

As Clariant was unaware of the addition of BAC to SCAVTREAT 7103, and had hence not submitted a HOCNF showing this component, the ecotoxicology data was incorrect. Clariant initiated ecotoxicology testing of BAC in order that this data could be retrospectively updated and submitted to Clariant's customers and the concerned regulatory authorities.

The evaluation of the partition coefficient, log Pow, was determined by testing in the Clariant laboratories in Frankfurt, Germany. The other ecotoxicology testing was conducted by an external specialist company, Chemex Environmental International in Cambridge, UK. Samples of BAC 50 were mobilised by Clariant to both Frankfurt and Cambridge to facilitate this testing.

The complete test reports are available in Appendix B.

The mandatory ecotoxicological tests performed on a sample of BAC 50, as specified in the OSPAR* HMCS (Harmonised Mandatory Control System), and subsequently evaluated using the OSPAR Recommendation 2016/4 Harmonised Pre-screening Scheme, confirmed the expected classification of this substance as a candidate for substitution ("red" in Denmark).

* Note: "OSPAR" (stands for Oslo and Paris) refers to The Convention for the Protection of the Marine Environment of the North-East Atlantic (the 'OSPAR Convention'). More details are available at www.ospar.org

Root Cause Analysis

An investigation team was formed to conduct an investigation into the root cause of the occurrence. This was led by Clariant's QHSE Manager for Scandinavia, and included Clariant specialists from QHSE, Procurement, Technical, Regulatory and Commercial functions.

A full document review was conducted, as well as a visit to the Synthite plant in the UK.

A root cause analysis based on the known information was performed to determine why SCAVTREAT 7103 contained an unauthorised and undocumented component.

The investigation reveals:

- That the decision to add BAC at a low concentration to SCAVTREAT 7103 was made based on data indicating that this would control levels of formic acid and NHEF generated by SCAVTREAT 7103 during product application.
- This decision to add BAC to SCAVTREAT 7103 was made by one individual.
- An unauthorised change requirement was communicated to Synthite.
- There was no formal requirement for Synthite to confirm process changes to Clariant.
- The change to the formulation of SCAVTREAT 7103 was not recorded in Clariant's systems. In doing so Clariant's internal approval process was bypassed. Consequently, a HOCNF was not prepared and environmental testing of BAC was not undertaken.
- The customers were not informed of the new formulation with BAC.
- Synthite Ltd were audited on three occasions by Clariant Oil Services Scandinavia AS (formerly Clariant (Norge) AS). On all occasions the audit team included the individual who had requested the addition of BAC to SCAVTREAT 7103.
- The level of BAC present in SCAVTREAT 7103 was too low to be detectable by standard quality control (QC) tests designed to verify compliance with product specifications agreed with customers.

In summary, this occurrence was an isolated incident, as four highly exceptional things came together. The change to the product was (1) unauthorized, (2) undocumented, (3) implemented by a single individual and (4) so slight that it could not be detected by the usual quality control tests.

Preventive Actions

The following measures will be implemented to eliminate the possibility of a single individual instructing a third party toll manufacturer to make unauthorised changes to a formulation.

- All recipes and manufacturing instructions to third party toll manufacturers must be supplied in writing by Clariant on an approved Specification and Manufacturing Instruction Sheet. This requires separate approval from Technical, Commercial, Manufacturing and Management functions.
- Any changes to recipes or manufacturing instructions for existing products must be communicated on an updated Specification and Manufacturing Instruction Sheet (approved as above). Only changes detailed on the updated sheet will be implemented by the third party. Upon receipt of an updated instruction, the Third Party must confirm in writing to Clariant that they have received and understood the required change(s), along with the implementation date.
- Third party manufacturers will be required to periodically confirm that product supplied has been manufactured in line with the Manufacturing Instructions provided by Clariant.
- The above points are to be included in any contract with a Third Party manufacturer.
- These records are to be retained by Clariant for the duration of the contract plus a suitable period (to be defined in consultation with Legal Services).
- The frequency of auditing Third Party manufacturers is to be increased to every two years.
- Audits of the Third Party manufacturers must not be done solely by the Clariant Business Unit organisation. They must involve local, regional or global corporate support functions responsible for Quality, Health, Safety and Environmental (QHSE) affairs.

APPENDIX A

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FAO: Mr. Peter Christensen, Production Chemistry Lead

11.04.2017

SCAVTREAT 7103

Dear Peter,

Clariant Oil Services offers with SCAVTREAT products a suite of water-soluble and oil-soluble scavengers for removing sulfide species from both gases and liquids, as well as a range of batch and continuously injected products designed to remove FeS or treat the cause of its formation. SCAVTREAT 7103 is an aqueous solution of 30-60% 2,2',2"-(hexahydro-1,3,5-triazine-1,3,5-triyl)triethanol, which is currently used by Maersk in Denmark. Clariant discloses in our safety data sheet information on our products that correspond to the present state of our knowledge. This knowledge is well documented in our SAP EH&S system, which is the base of all our Product Stewardship relevant information, including all product compositions.

Very recently it came to our knowledge that SCAVTREAT 7103 contains between 0.06 to 0.12% (typically < 0.1%) quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides, EC 939-350-2. The result of the evaluation of this modified composition is as follows: no change in Classification, Labelling and Packaging (CLP) and thus no impact on workers due to the presence of quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides.

As we were not aware of this ingredient we failed to meet the requirements of the Harmonised Offshore Chemicals Notification Format (HOCNF) for this component. This is very much to our regret. We decided to immediately take care that this component will be removed from our preparation, and we will work closely with Maersk to support the transition and field application of the product following the removal of this component.

We will conduct the necessary testing under Good Laboratory Practice in order to generate the data to confirm the environmental classification of SCAVTREAT 7103 containing the additional component. Based on our current knowledge, we expect that the classification will be “red”.

In addition we routinely updated our Safety Data Sheet to align with the classification of 2,2',2"-(hexahydro-1,3,5-triazine-1,3,5-triyl)triethanol as disseminated by ECHA:

From:

- Acute Tox 2 – Inhalation
- Acute Tox 4 – Oral
- Skin Sens 1
- STOT 1 – Resp.

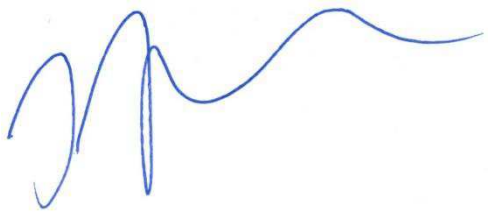
To:

- Acute Tox 2 – Inhalation
- Acute Tox 4 – Oral
- Skin Sens 1
- STOT 1 – Resp.
- Eye Irr 2

The changes to the Safety Data Sheet are not related to the discovery of the additional component in the product.

Both myself and our senior management are committed to addressing this incident promptly and fully to the satisfaction of Maersk. We are happy to attend meetings with Maersk s staff as required and to fully discuss and resolve this issue.

Yours sincerely,



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FAO: Mr. Peter Christensen, Production Chemistry Lead

20.04.2017

SCAVTREAT 7103

Dear Peter,

Further to your letter of 18.04.2017, we would like to update you on the actions taken by Clariant as requested in your letter.

Clariant has today informed in writing the Danish Product Registry of our recent discovery that SCAVTREAT 7103 contains between 0.06 to 0.12% (typically < 0.1%) Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides, EC 939-350-2.

As this occurrence was a unique set of circumstances relating to supply of a material from a third party, we do not believe that there is a significant risk of similar situations with other products supplied by Clariant. However, we are reviewing all products supplied to the Danish oil industry to ensure that there are no additional components added to any product that we are not currently aware of. As most products are manufactured by Clariant, we have full control of all components added to the products, but where necessary we will seek assurances from third party suppliers. We do not expect any changes, but will if required update HOCNF accordingly.

We are also reviewing all MSDS for material supplied to the Danish oil industry. Again, we do not expect any changes to be required but are doing this to ensure full transparency and compliance.

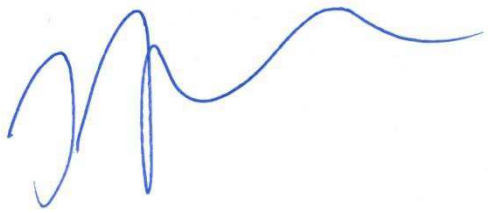
As we have previously explained, we are conducting the necessary 3rd party lab testing to determine the environmental rating of SCAVTREAT 7103 based on the revised understanding of the composition. Once this work is completed, which is expected to take 10 – 12 weeks, we will submit a revised HOCNF to the Danish Environmental Protection Agency based on the updated environmental data.

We are also conducting test work to determine the impact of the removal of the Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides component from the SCAVTREAT 7103 on the specification of the product, in particular the levels of formic acid and formamide. We

will communicate with you further on this subject once the testing is completed. We anticipate that this will take approximately 2 weeks.

As previously expressed, both myself and our senior management are committed to addressing this incident promptly and fully to the satisfaction of Maersk Olie og Gas. We are happy to attend meetings with Maersk staff as required and to fully discuss and resolve this issue.

Yours sincerely,



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03.05.2017

SCAVTREAT 7103

Dear Peter,

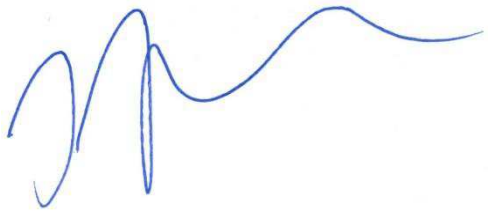
Further to our telephone meeting on 28th April 2017, we would like to provide you with an update regarding the actions we have already taken plus the action plan going forward to resolve the issues related to the supply of our triazine based product SCAVTREAT 7103.

- The Danish Product Registry has been advised of the new knowledge that has emerged which affects the HOCNF for SCAVTREAT 7103. The Product Registry has acknowledged receipt of our letter.
- We have sent a sample of SCAVTREAT 7103 for environmental testing to obtain the correct data to support the HOCNF application for this product.
- Samples of SCAVTREAT 7103 with and without the BAC 50 have been sent to the DTI to assess the formamide and formic acid content.
- We believe that the BAC 50 component was included in the product to reduce the formamide and formic acid levels which to meet the specification requirements for Maersk.
- The formamide and formic acid components which are in the product are a result of the specific raw materials used in the triazine manufacturing process. We are working with our existing supplier to evaluate an alternative triazine manufacturing method which does not result in high levels of formamide and formic acid. Samples of the finished product from this method have been sent to the DTI to determine the formamide and formic acid content.
- We have also identified an alternative source of material from a second reputable supplier, and based on earlier testing at DTI we believe that this will offer an alternative with acceptable levels of formamide and formic acid. In the short term however, we do not believe that this supplier can meet 100% of the volume needs for Maersk's requirements, but may form part of a future supply solution.
- Once all of the results from the DTI are available we will be able compare with the Maersk specifications and advise the best material going forward.
- An investigation team has been brought together from different parts of our organisation and they will review all of the circumstances which has led to the current situation. We are committed to ensure any measures identified to prevent reoccurrence are implemented.

- Our suppliers have been contacted to confirm that no additional components have been added to any of the other products supplied to Maersk.
- We are in the process of commercialising a replacement product called SCAVTREAT 15211 and we will work with Maersk to ensure all product qualification requirements are captured and approved by Maersk prior to supply.

I trust the activities described above meet with your satisfaction and reiterate our commitment to resolve this issue in a timely manner. We will be able to provide a regular updates on progress and attend meetings as required by Maersk.

Yours sincerely,



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20.04.2017

SCAVTREAT 7103

Dear Michelle,

Clariant Oil Services UK Ltd. and Clariant Oil Services Scandinavia AS (hereinafter together "Clariant Oil Services") are committed to supporting its customers to ensure compliance with all aspects of global Regulatory compliance, by ensuring that any products that are placed on the market are both sustainable and in regulatory compliance.

However, it has recently came to the knowledge of Clariant Oil Services that SCAVTREAT 7103, as supplied to Maersk, contains between 0.06 to 0.12% (typically < 0.1%) Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides, EC 939-350-2.

Regrettably, as we were not aware of this ingredient in our product, we have failed to meet our obligations under the Harmonised Offshore Notification Format (HOCNF).

Clariant Oil Services has sanctioned the immediate removal of the Quaternary ammonium compound from our product, and aim to fully co-operate with the Danish authorities to fully understand how this situation came to be, and ensure that any remedial actions are promptly implemented.

In order to comply with the regulations governing offshore use, Clariant Oil Services has submitted a sample of the Quaternary ammonium compound to a third party environmental testing facility, Chemex. It is anticipated that the new environmental data will be available within 10 - 12 weeks and upon receipt of the information, an amended HOCNF for SCAVTREAT 7103 will be submitted.

We are working with our customer to find a suitable product replacement.

In the meantime, if you have any queries or require anything further from us then please do not hesitate to contact me in the first instance.

Yours faithfully,

Clariant Production UK Ltd.
On behalf of the selling Clariant group companies

Laura Gordon
Head of Product Stewardship, UK & Ireland

APPENDIX B

Clariant Produkte (Deutschland) GmbH
CLAS R&D Analytics CIC
G860 Physics, Dr. Michael Schopferer
Phone.: 069 - 305 39450

REPORT

Sample:	**Empigen BAC 50**
Project:	logP slow stirring /surf. tens. method
Account:	200318804 - COS - 4504702535/10
Customer:	Phil McWilliams, Clariant Oil Services Scandinavia AS
CLAS Order No.:	17-011734

Summary

The logP was measured with the slow stirring / surface tension method

Apparent logP values at 1 g/L: **1,56**

No corrected for micellisation effects is needed as the cmc is much higher (> 0,25 g/L) as the concentrations in aqueous phase.

Details: see following sheets.

Electronic document valid without signature

Attachments: - -

Distribution: phil.mcwilliams@clariant.com

Clariant Produkte (Deutschland) GmbH
CLAS R&D Analytics CIC
G860 Physics, Dr. Michael Schopferer
Phone.: 069 - 305 39450

LogP WITH SLOW STIRRING / SURFACE TENSION METHOD

Sample: Empigen BAC 50 (analog Dodigen 1611) WS = 50 %

Method

Equilibration

Aqueous solution of surfactant (1 g/L) was equilibrated with octanol under the following conditions:

- 100 mL aq. soln + 100 mL (82g) 1-octanol in a 300 mL conical flask.
- Stirring bar: 4cm long, 7.5 mm diameter
- Stirring speed: 100 ± 7 rpm
- Time: 8h stirring + stand overnight
- Replicates: Experiment carried out in duplicate with the same batch of surfactant solution.

The experiment was performed at room temperature (approx 23 °C).

Determination of surfactant concentration

10 mL aqueous phase was removed and pipetted into a tensiometer glass. It was heated at 130°C for 30 min to remove water and octanol. The residue was redissolved in 50 mL 0.2 M KCl solution. Then the surface tension was measured and the concentration determined by comparison with a calibration curve. Two samples of each experiment were taken.

Surface tension measurements

The surface tension was measured with a Krüss K100 tensiometer using the Pt plate. Due to slow equilibration at these low surfactant concentrations, the measurement was allowed to run for 15 min. The lowest surface tension achieved during the 15 minute period was used for the calculations.

Micellisation effects

This correction is only required if $c_{aq} > cmc_{oct}$ which is not the case:

$c_{aq} < 0.03$ g/L; $cmc_{oct} > 0.25$ g/L

Results

The sample forms a clear solution in both demin. water and 0.2 M KCl at 1 g/L.

A satisfactory calibration curve was obtained (Fig. 1).

Clariant Produkte (Deutschland) GmbH
CLAS R&D Analytics CIC
G860 Physics, Dr. Michael Schopferer
Phone.: 069 - 305 39450

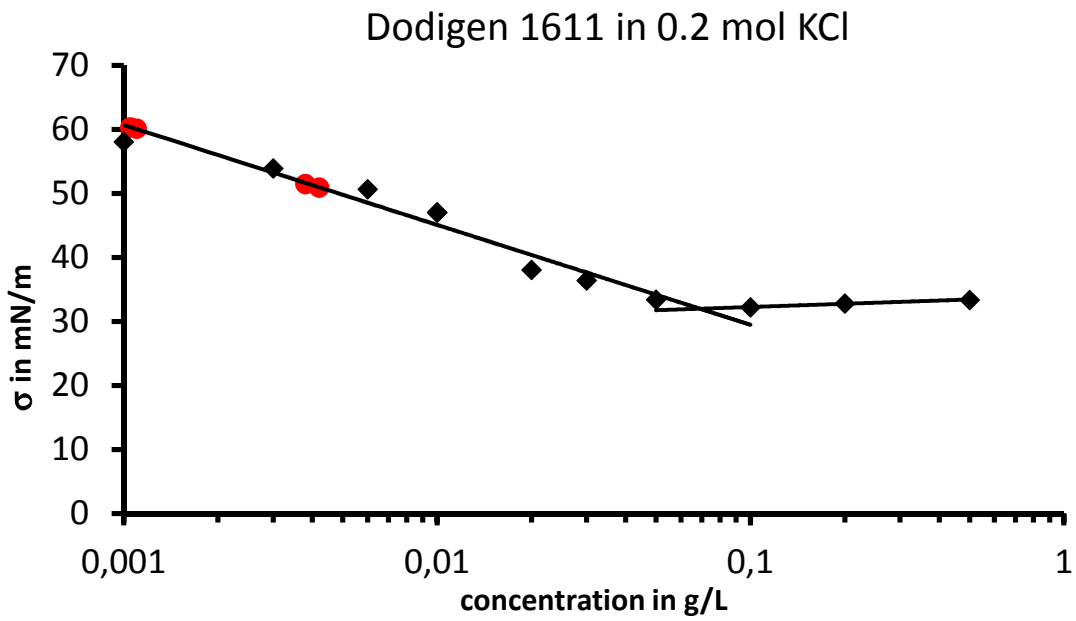

Fig. 1 Calibration curve in 0.2 M KCl

After the evaporation/redissolution procedure, the surface tensions of the diluted aqueous phases were in the steep part of the curve, which is a requirement for satisfactory evaluation. Numerical data are shown in Table 1.

Table 1: results. From each equilibration expt, A and B, four samples of the aq. layer were analysed. The concentration is calculated from the surface tension via the calibration curve. The concentration in the aq. layer is obtained from the sample concentration by multiplying with the dilution factor.

initial concentration 0.5 g/L

sample	dil factor	surf. tens. mN/m	conc. g/L		Apparent logP at 0.5 g/L
			sample	in aq layer	
A3	5	51,15	0,00547	0,0274	1,55
A4	5	50,76	0,00591	0,0296	1,52
B3	5	52,63	0,00399	0,0200	1,69
B4	5	50,38	0,00637	0,0319	1,48
Mean				*0,0272*	*1,56*

The critical micelle concentration in the presence of octanol was determined by diluting stock solution containing 0.5 g/L Empigen BAC 50 and 0.5 g/L octanol. The result of this measurement was used as a rough estimate of the cmc in octanol-saturated solution.

Clariant Produkte (Deutschland) GmbH
CLAS R&D Analytics CIC
G860 Physics, Dr. Michael Schopferer
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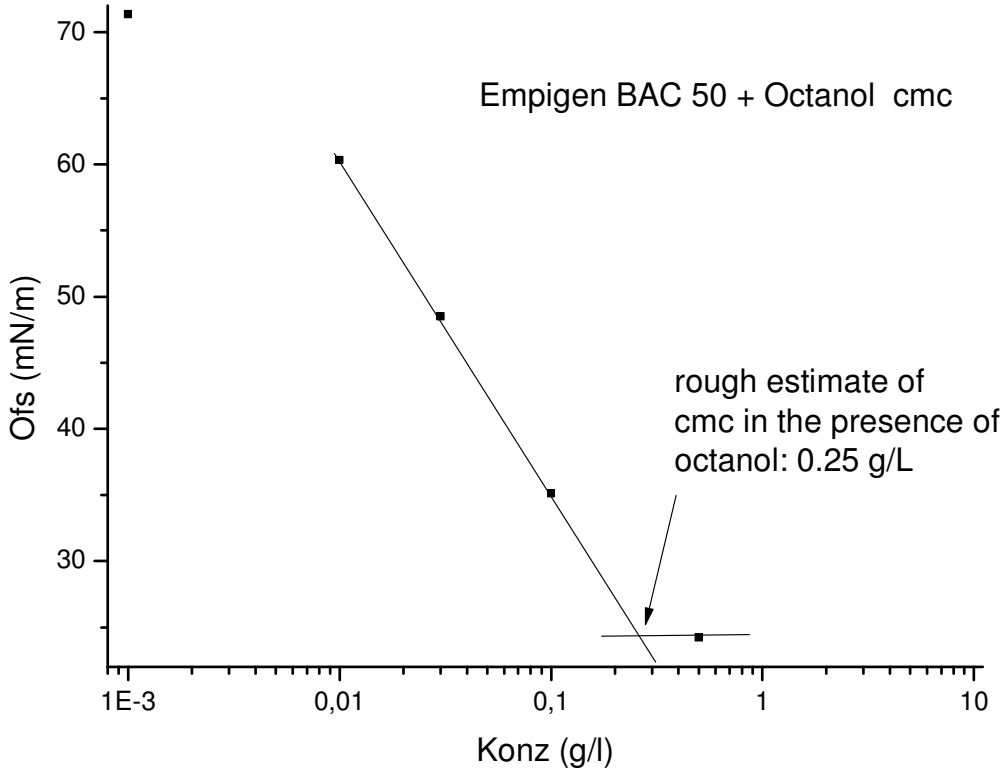


Fig. 1 cmc in the presence of octanol. Concentration axis: surfactant only (i.e. excluding octanol)

The cmc in octanol-saturated solution, $cmc_{oct,} > 0.25$ g/L.

Conclusions

Apparent logP values at 1 g/L:

no corrections for micellisation effects necessary

Empigen BAC 50	1.56

Commercial-in-Confidence

Chemex reference: ENV 11446/ECO 170408

CHEMEX
The Business of Science

The toxicity of Empigen BAC 50 to the marine alga *Phaeodactylum tricornutum* over a 72-hour exposure period

Report for
Clariant Oil Services Scandinavia AS

Report issued by:

Chemex Environmental International Limited
Unit J
Broad Lane Industrial Estate
Cottenham
Cambridge CB24 8SW
UK

Sponsor:

Clariant Oil Services Scandinavia AS
Post box 6054 BS
5892 Bergen
Norway

June 2017

Contents

Page

Compliance with Good Laboratory Practice standards

I, the undersigned, hereby declare that the study described in this report was performed under my supervision, and that the final report fully and accurately reflects the raw data generated during the conduct of the study, in compliance with international codes of Good Laboratory Practice including:

- Section II of Annex 1 to the European Parliament and council Directive 2004/10/EC and Annex 1 to the European Parliament and council Directive 2004/9/EC (Official Journal No. L 50) and embodied within:
- *The UK Good Laboratory Practice Regulations 1999 (The United Kingdom GLP Regulations 1999, Statutory Instrument 3106)* as amended by:
- *The UK Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004 (Statutory Instrument No 994)*

These principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

N. Eggett ..... 30 June 2017 .....

Neil Eggett BSc (Hons) MRSB
Study Director

Date

Key personnel

Other key personnel at Chemex involved in this study were:

Agnes Grabczewska
Monika Zuba-Sosnowska
Sylwia Lojek

Quality Assurance Statement

The Quality Assurance unit inspects the final report to confirm that the methods, procedures and observations are accurately and completely described, and that the reported results accurately and completely reflect the raw data of a regulatory study.

This is achieved by conducting routine annual facility and system inspections at approximately 12 monthly intervals. In addition, an internal process-based audit programme is also adhered to at 3 monthly intervals. Where required, study specific inspections are also conducted. All study plans and amendments are verified by the QA unit to confirm compliance with GLP.

The inspections applicable to this study are detailed below. The dates are given as dd/mm/yy.

Study Number: ENV 11446

Study Title: The toxicity of Empigen BAC 50 to the marine alga *Phaeodactylum tricornutum* over a 72-hour exposure period.

Procedures and Processes	Type	Date of inspection	Date reported to Study Director / Management
Aquatic plant or algal test set up	P	23/05/17	23/05/17
Preparation of growth medium / nutrients / standards	P	06/04/17	15/05/17
Preparation of test solutions/WAF	P	07/04/17	15/05/17
Determination of algal cell density	P	23/05/17	23/05/17
Weighing out test or reference materials	P	07/04/17	15/05/17
Inoculation with algae	P	23/05/17	23/05/17
Organism stock records	P	22/05/17	23/05/17
Equipment calibration	P	23/05/17	23/05/17
Taking and recording readings	P	22/05/17	23/05/17
Labelling and paperwork	P	23/05/17	23/05/17

Key: P- Process-based, S- Study specific, O- other inspection type.

This report has been inspected by the undersigned and, as far as can be reasonably established, the methods, procedures and observations are accurately and completely described and the results incorporated into this report accurately and completely reflect the raw data generated during this study.

Final report and data inspection started: 19/06/17

Final report and data inspection completed: 30/06/17

Signed: .....

Jane Hawkins MRQA
Quality Assurance

Date: 30/06/17

Summary

This section summarises aquatic toxicity test results obtained by Chemex Environmental International Limited on a sample as detailed below:

Test commissioned by:	Clariant Oil Services Scandinavia AS
Substance under test:	Empigen BAC 50
Chemex reference:	Sample: ECO170408 Study: ENV 11446
Test species:	*Phaeodactylum tricornutum,* strain CCAP 1052/1A.
Test type:	Acute toxicity: 72-hour $EC_{50.}$
Registration guideline:	Static test conditions according to SOP E209 based on ISO 10253:2006 "Water quality – Marine Algal Growth Inhibition Test with *Skeletonema costatum* and *Phaeodactylum tricornutum*".
Experimental period:	Range finding test: 11 to 15 May 2017 Definitive test 1: 23 to 26 May 2017 Definitive test 2: 30 May to 02 June 2017
Test concentrations:	0 (control), 0.27, 0.35, 0.46, 0.59, 0.77, 1.0mg/l
Test performed at:	Chemex Environmental International Limited Unit J, Broad Lane Industrial Estate Cottenham Cambridge CB24 8SW UK

Results: All study validity criteria were met. The results are summarised in the table below.

Exposure period (hours)	**$EC(r)_x$ and NOEC results (95% confidence limits)**	
	$EC(r)_{10}$	**$EC(r)_{50}$**
0 to 48	0.305mg/l (0.249 – 0.338mg/l)	0.420mg/l (0.392 – 0.448mg/l)
0 to 72	0.384mg/l (0.327 – 0.414mg/l)	0.485mg/l (0.462 – 0.511mg/l)
NOEC(r) (0-72h)	0.27mg/l (Determined by Bonferroni t Test (1-Tail, 0.05)[$]	

Statistical methods used in ToxCalc v5.0: Maximum Likelihood-Logit
[$]Following Shapiro-Wilk's Test for normality of distribution and Bartlett's Test which indicated equal variances
All concentrations of the test substance are reported as nominal as received.

1. Introduction

This report contains a description of the methods used and the results obtained during a study to investigate the growth inhibition of Empigen BAC 50 to the marine algae *Phaeodactylum tricornutum*. The objective of this study was to determine the 72-hour effective concentration of Empigen BAC 50 and the NOEC based on specific growth rate relative to the controls. Specifically, the results to be determined if possible are the 72-h $EC(r)_{10}$, 72-h $EC(r)_{50}$ and the 72-h NOEC(r) at 20 ± 2°C. The guideline followed was "Water quality - Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*" (ISO 10253:2006).

2. Materials and methods

Unless otherwise specified all methods mentioned in this report are according to Chemex Environmental International Limited standard procedures.

All records of measurements and observations made during this test will be collated and held in the Chemex Environmental International Limited archives at Unit J, Broad Lane Industrial Estate, Cottenham, Cambridge CB24 8SW, UK.

2.1 Test material

Identification:	Empigen BAC 50
Name as supplied:	Empigen BAC 50
Batch number:	120417SAM1
Type of Substance:	Multi-Constituent Substance
Activity:	50% Active in water
Purity / Composition:	Quaternary ammonium compounds, benzyl-C12-14(even numbered)-alkyldimethyl, chlorides 30-60% (MSDS)
Chemex reference:	ECO 170408
Density (specified):	0.99g/cm^3
Density (observed):	0.9878g/cm^3
Appearance (specified):	Straw Coloured Liquid
Appearance (observed):	Straw Coloured Liquid
Solubility (specified):	Soluble in water
Homogeneity (specified):	Homogeneous
Homogeneity (observed):	Homogeneous
Stability in container:	Stable
Stability in water:	Not known Aquatic Half Life: Freshwater 365 days (MSDS)
Required storage conditions:	Ambient. No Protection from light
Actual storage conditions:	Ambient 15 ± 10°C
Source of supply:	Huntsman Holland BV Merseyweg 10 3197 KG Botlek-Rotterdam The Netherlands
Expiry date:	27 April 2018

All quoted information is taken from TSDS (Test Substance Data Sheet) unless otherwise stated.

2.2 Test organism

Species:	*Phaeodactylum tricornutum* strain: CCAP 1052/1A received 10 January 2017
Source:	Culture Collection of Algae and Protozoa SAMS Research Services Ltd Scottish Marine Institute OBAN Argyll PA37 1QA Scotland, United Kingdom
Culture conditions:	Temperature: 20.9 – 21.6ºC. Illumination: 6290 – 7270 lux continuous white light. Orbiting: set to 200 rpm.
Culture media:	Natural seawater with added nutrients according to the ISO 10253 standard (see Appendix 2).

2.3 Dilution water

The stocks of algal culture were maintained, and the tests performed, in nutrient growth medium (ISO10253) prepared from natural seawater collected from the CEFAS laboratories at Lowestoft. The seawater was collected via a pipeline directly from the estuary. The sand acts as the first stage of filtration. After collection, the water was stored in the dark at approximately 15 ± 2°C within the testing facility. It was then filtered and sterilised by autoclaving at 120°C for 15 minutes.

To make nutrient growth medium, appropriate amounts of nutrient stocks (Appendix 2) were added to sterilised seawater at a pH of 8.14.

2.4 Preparation of test solutions

Information provided by the Sponsor indicated that the sample was soluble in water.

Stock solutions were prepared for both the range-finding and definitive tests by addition of the test sample directly to nutrient growth media in a volumetric flask of adequate volume. Calculated volumes of the stock solution were added to nutrient growth media and made to volume to prepare the test concentrations and provide enough solution for testing and subsequent water quality measurements.

Two definitive tests were performed, the first initial definitive test performed at 0.0032, 0.01, 0.032, 0.1, 0.32 and 1.0mg/l did not provide sufficient data to calculate an EC50 with 100% inhibition at 1.0mg/l with no intermediate response seen from 0.32 to 0.0032mg/l. The test was repeated at concentrations 0.27, 0.35, 0.46, 0.59, 0.77 and 1.0 mg/l and has been reported in full.

2.5 Test methods and conditions

Static test conditions according to "Water quality - Marine algal growth inhibition test with *Skeletonema costatum* or *Phaeodactylum tricornutum*" (ISO 10253:2006).

Chemex SOP reference:	E209 “Marine Algal Growth Inhibition test with *Skeletonema costatum* or *Phaeodactylum tricornutum*”
Preliminary test method:	A preliminary (range-finding) test was conducted at concentrations of 0 (Control), 1, 10, 100 and 1000mg/l. The duration of the preliminary study was 72 ± 2 hours. There was a single replicate at each concentration.
Preliminary test results:	Data from the preliminary test identified the 72 hour EC_{50} as being under 1.0mg/l (by growth rate)

Nominal concentration (mg/l)	Percent inhibition by growth rate
	0 - 72 hours
1	100
10	100
100	100
1000	100

Note: Negative numbers indicate an increase in growth compared to control data.

All concentrations of the test substance are reported as nominal as received.

Definitive test 1 results:

Nominal concentration (mg/l)	Percent inhibition by growth rate
	0 - 72 hours
0.0032	3
0.0100	4
0.0320	4
0.1000	2
0.3200	10
1.0000	100

Note: Negative numbers indicate an increase in growth compared to control data.

All concentrations of the test substance are reported as nominal as received.

Definitive Test Method:	
Test period:	30 May to 02 June 2017
Test duration:	72 ± 2 hours
Test volume:	200ml
Test vessel:	250ml conical flask
Number of replicates:	Six control flasks, three replicates at each test concentration.
Test concentrations:	0 (control), 0.27, 0.35, 0.46, 0.59, 0.77 and 1.0 mg/l
Composition of test medium:	Nutrient medium prepared according to the ISO 10253 (see Appendix 2) in natural seawater (Section 2.3). The final salinity of the medium used was 33‰.
Algal test inoculum:	From a pre-culture growing under conditions described in 2.2 above. Inoculum level adjusted to give an initial cell density of 1 x 10^4 cells/ml.
Test conditions:	Initial pH of at 0 hours: 8.14 (Required: 8.0±0.2) pH range in control and test concentrations throughout test: 7.96 – 8.60 Temperature range within incubator throughout test: 20.1 – 21.6°C (Required: 20±1°C) Illumination range within incubator throughout test: 6240 - 7480 Lux (Required: 6x10^3-10x10^3 Lux) Orbital shaking: 200rpm (Required: 200-250rpm)
Water quality measurements:	The temperature (to 0.1°C) and light intensity (lux) within the incubator was recorded at the beginning of the study, after 24, 48 hours and at the end of the 72-hour test period. The pH (to 0.01) and temperature (to 0.1°C) were recorded for each test and control solution at the beginning of the test and on the pooled replicates at the end of the 72-hour test period.
Observations/frequency:	Cell densities were measured microscopically by direct cell counts on each test and control replicate in triplicate at 24, 48 and 72 hours (±2h). The cell counts were made using a haemocytometer and microscope.
Analysis of test substance:	The Sponsor did not request analytical confirmation of exposure concentrations. All effect concentrations have, therefore, been calculated from nominal concentrations.
Calculation of results:	The average specific growth rate was calculated for each test and control culture using equation 1: (1) $\mu = (\ln N_L - \ln N_0)/(t_L - t_o)$ where:

t_0 = time of test start
t_L = time of test termination or the time of the last measurement within the exponential growth period in the control
N_0 = the nominal initial cell density
N_L = the measured cell density at time t_L
The percentage inhibition for each individual test flask was calculated from equation 2:
(2)
$I_{\mu i} = ((\bar{\mu}_c - \mu_i)/ \bar{\mu}_c) * 100$
where:
$I_{\mu i}$ = percentage inhibition (growth rate) for flask *i*
μ_i = growth rate for flask I
μ_c = mean growth rate for the control
Growth curves for each test concentration were plotted as the logarithm of the mean cell density against time. Where possible, the $EC(r)_{10}$ and $EC(r)_{50}$ values with 95% confidence limits and NOEC(r) were calculated according to the statistical methods of ToxCalc™ Version 5.0 “Comprehensive Toxicity Data Analysis and Database Software”, copyright 1994-1996.

Reference substance:

A separate reference study (ENV11398) was run from 20 to 23 January 2017 using 3,5-Dichlorophenol as a means of checking the test procedure and sensitivity of the test species. The 72-hour $EC(r)_{50}$ was 2.6mg/l, which was within the acceptable range (2.3 to 3.1mg/l) quoted in ISO 10253:2006 guideline. In addition, at 2.7mg/l a percentage inhibition, for growth rate at 72-hours, of 56% relative to the controls was seen. This was within the range (20 to 80%) required for OSPAR compliance (OSPAR Guidelines 2005).

2.6 Study Plan Deviations

1.) The study plan stated (Section 9.4) vessels are placed in a temperature controlled incubator set at 20±1°C and temperature of incubation should not vary by more than 2°C. The temperatures recorded during the study were within this 2°C variability. However the temperatures recorded at test initiation and the maximum temperature recorded at the 24 hour time point were 21.3°C and 21.6°C (taken from manual temperature records of incubator) respectively. The temperature returned to within 20±1°C for remainder of the study.

It was considered that this slight deviation outside of the permissible range stated in the study plan would not have affected the integrity of the study. The growth parameters of the algae in the control vessels were achieved and no abnormalities were recorded for cell observations.

2.) The study plan (Section 9.2) stated that the dilution water is stored at 15±2°C within the facility until use when it would be filtered and sterilised. During storage and before use on the study the temperature exceeded the range specified in the study plan on two occasions, the maximum temperature recorded for each occasion respectively was 26.3°C and 17.1°C. On both occasions the temperature exceeded the range for a period of 24 hours and then returned to within acceptable limits.

The principle behind storing under cool conditions is to limit any biological activity from organisms that may be present in the water should there be a need to use unsterilised water. However in this instance whereby all water was sterilised prior to use any increase in biological activity that may have been caused by the higher storage temperatures would have been negated due to the sterilisation process. Therefore this deviation was considered not to have affected the integrity of the study.

3. Results

Cell densities were measured microscopically by direct cell counts on each test and control replicate in triplicate. Average results of the cell density determinations are given in 3.1. All results in this study are calculated from the measured cell densities. All concentrations of the test substance are reported as nominal as received.

3.1 Cell density measurements

Mean initial cell density: Approximately 1 x 10^4 cells/ml based upon inoculation volume, not counted microscopically.

Nominal Concentration (mg/l)	Replicate	Cell density measurements (cells/ml x 10^4)		
		24 hours	48 hours	72 hours
0 (control)	1	6.0	17.7	86.3
	2	2.0	14.3	78.0
	3	7.0	24.3	106.7
	4	2.7	18.0	115.0
	5	1.3	16.7	71.3
	6	5.3	20.3	90.7
	Mean	**4.1**	**18.6**	**91.3**
0.27	1	2.0	10.3	52.0
	2	1.3	14.3	59.3
	3	1.7	16.0	68.3
	Mean	**1.7**	**13.5**	**59.9**
0.35	1	1.7	7.0	34.7
	2	2.3	10.7	41.0
	3	1.7	11.0	36.3
	Mean	**1.9**	**9.6**	**37.3**
0.46	1	1.0	2.7	19.7
	2	2.0	4.0	23.3
	3	2.0	3.0	22.3
	Mean	**1.7**	**3.2**	**21.8**
0.59	1	1.3	0.7	0.0
	2	2.0	0.3	2.7
	3	1.0	0.7	1.0
	Mean	**1.4**	**0.6**	**1.2**
0.77	1	0.3	0.3	0.0
	2	1.3	0.3	0.0
	3	0.3	0.0	0.0
	Mean	**0.6**	**0.2**	**0.0**
1.0	1	0.0	0.0	0.0
	2	0.0	0.0	0.0
	3	0.0	0.0	0.0
	Mean	**0.0**	**0.0**	**0.0**

Growth curves of logarithm of cell density against time are shown in Graph 1.

3.2 Test observations

The algal cells were examined microscopically during the determination of the cell density, all cells within the control and all test concentrations where applicable appeared normal, no morphological abnormalities were observed. Solution appearance in the control progressed from clear colourless to very little brown colouration to brown by 72 hours. Test concentrations 0.27 to 0.46mg/l were clear colourless at 0 and 24 hours, had very little brown colouration at 48 hours to a little brown colouration at 72 hours. 0.59 to 1.0mg/l appeared clear colourless throughout.

3.3 Percent inhibition

Nominal Concentration (mg/l)	Percent inhibition by specific growth rate	
	48 hours	72 hours
0.27	11	9
0.35	23	20
0.46	60	32
0.59	100	96
0.77	100	100
1.0	100	100

3.4 $EC(r)_{10}$, $EC(r)_{50}$ values and NOEC(r)

Exposure period (hours)	$EC(r)_x$ and NOEC results (95% confidence limits)	
	$EC(r)_{10}$	$EC(r)_{50}$
0 to 48	0.305mg/l (0.249 – 0.338mg/l)	0.420mg/l (0.392 – 0.448mg/l)
0 to 72	0.384mg/l (0.327 – 0.414mg/l)	0.485mg/l (0.462 – 0.511mg/l)
NOEC(r) (0-72h)	0.27mg/l (Determined by Bonferroni t Test (1-Tail, 0.05)[$]	

Statistical methods used in ToxCalc v5.0: Maximum Likelihood-Logit
[$]Following Shapiro-Wilk's Test for normality of distribution and Bartlett's Test which indicated equal variances
All concentrations of the test substance are reported as nominal as received.

3.5 Test validity criteria

The control cell density should increase by a factor of more than 16 in 72 hours, corresponding to a specific growth rate of 0.9 d^{-1}. The measured control cell density increase was recorded as 91.3, corresponding to a specific growth rate of 1.505 d^{-1}.

The pH of the control media should not increase by more than 1.0 during the test. The pH was 8.14 at the beginning of the test and 8.60 at the end of the test.

The coefficient of variation of the control specific growth rate should not exceed 7%. The table below shows the calculation for variation coefficient of the control replicates for this study.

Control growth rate data coefficient of variation calculations.

Control replicate no.	Cell density (1.0×10^4 cells/ml)				0-72 hr average specific growth rates
	0 hr	24 hr	48 hr	72 hr	
1	1.0	6.0	17.7	86.3	1.49
2	1.0	2.0	14.3	78.0	1.45
3	1.0	7.0	24.3	106.7	1.56
4	1.0	2.7	18.0	115.0	1.58
5	1.0	1.3	16.7	71.3	1.42
6	1.0	5.3	20.3	90.7	1.50
				Mean:	1.50
				Standard deviation:	0.0616
				Coefficient of variation:	4.11

Note: 0-hour cell density based upon inoculation volume and not counted microscopically.

4. Discussion

The definitive test conducted from 30 May to 02 June 2017 was performed according to the ISO 10253:2006 guideline.

The growth curves illustrated in Graph 1 demonstrate that the algae in the control were in logarithmic growth for the duration of the study.

The 48-hour $EC(r)_{50}$ and 72-hour $EC(r)_{50}$ of Empigen BAC 50 to *Phaeodactylum tricornutum* were 0.420mg/l (determined by Maximum Likelihood-Logit) and 0.485mg/l (determined by Maximum Likelihood-Logit) respectively. Graphical representations of the 0 to 72 hours $EC(r)_x$ values and NOEC(r) and 0-72 hour dose-response plot are given in Graphs 2 and 3, respectively.

The 0 to 72-hour NOEC(r) and LOEC(r) were 0.27mg/l and 0.35mg/l respectively (determined by Bonferroni t Test (1-Tail, 0.05).

The algal cells were examined microscopically during the determination of the cell density, all cells within the control and all test concentrations where applicable appeared normal, no morphological abnormalities were observed. Solution appearance in the control progressed from clear colourless to very little brown colouration to brown by 72 hours. Test concentrations 0.27 to 0.46mg/l were clear colourless at 0 and 24 hours, had very little brown colouration at 48 hours to a little brown colouration at 72 hours. 0.59 to 1.0mg/l appeared clear colourless throughout.(see section 3.2).

All validity criteria for the definitive test were met (see section 3.5).

No analytical confirmation of the test concentrations was performed. The dissolved concentrations were likely to be as stated in this report as Empigen BAC 50 was soluble in water.

The water quality measurements and incubation conditions are given in the summary tables in Appendix 1 and were within accepted limits.

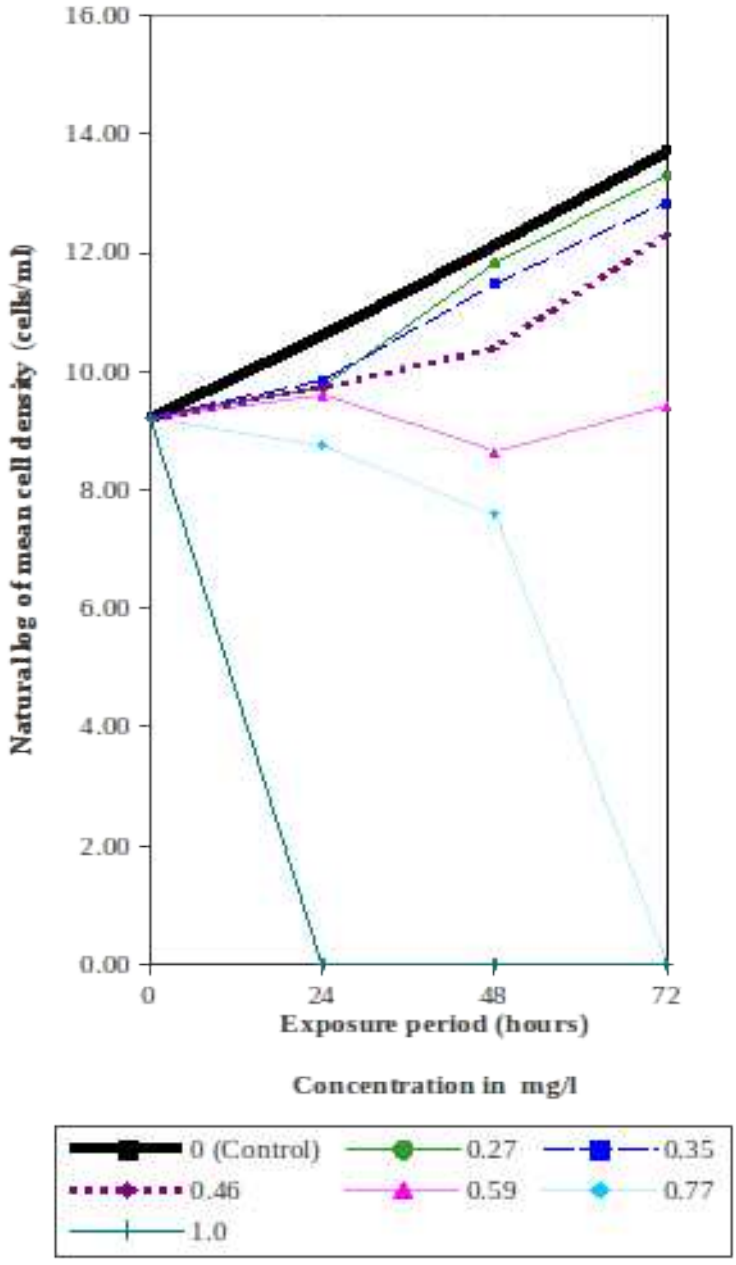
5. References

1. ISO 10253:2006 “Water quality – Marine Algal Growth Inhibition Test with *Skeletonema costatum* and *Phaeodactylum tricornutum*”.

2. OSPAR – Guidelines for Toxicity Testing of Substances and Preparations Used and Discharged Offshore (Reference number: 2005-12)

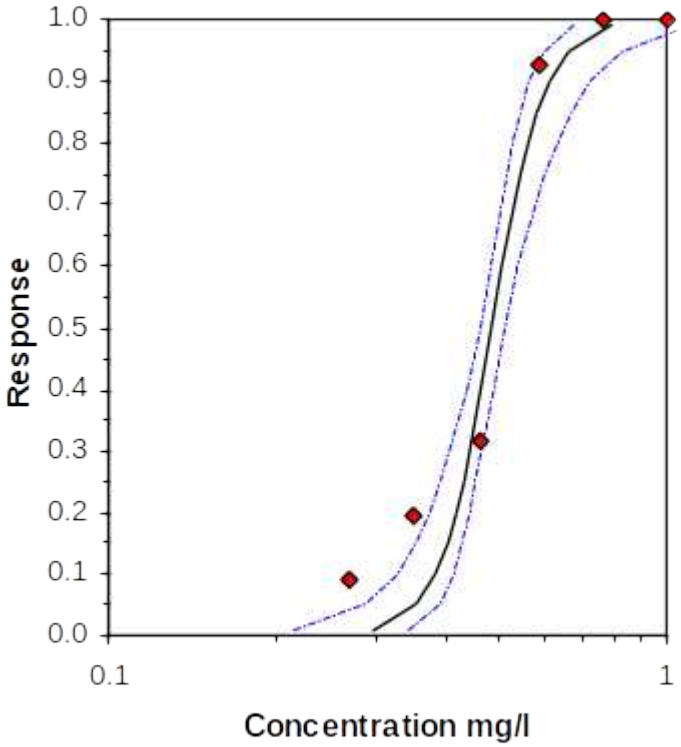
3. ToxCalc™ Version 5.0 “Comprehensive Toxicity Data Analysis and Database Software”, copyright 1994-1996.

Graph 1 Growth curves for control and test concentrations



Graph 2

Determination of 0 to 72 hours $EC(r)_x$ values and NOEC(r)



– - - - - Indicates 95% confidence limits ◆——◆ Indicates actual data plot

Statistical data

$EC(r)_{10}$ value = 0.384mg/l (0.327 – 0.414mg/l)

$EC(r)_{50}$ value = 0.485mg/l (0.462 – 0.511mg/l)

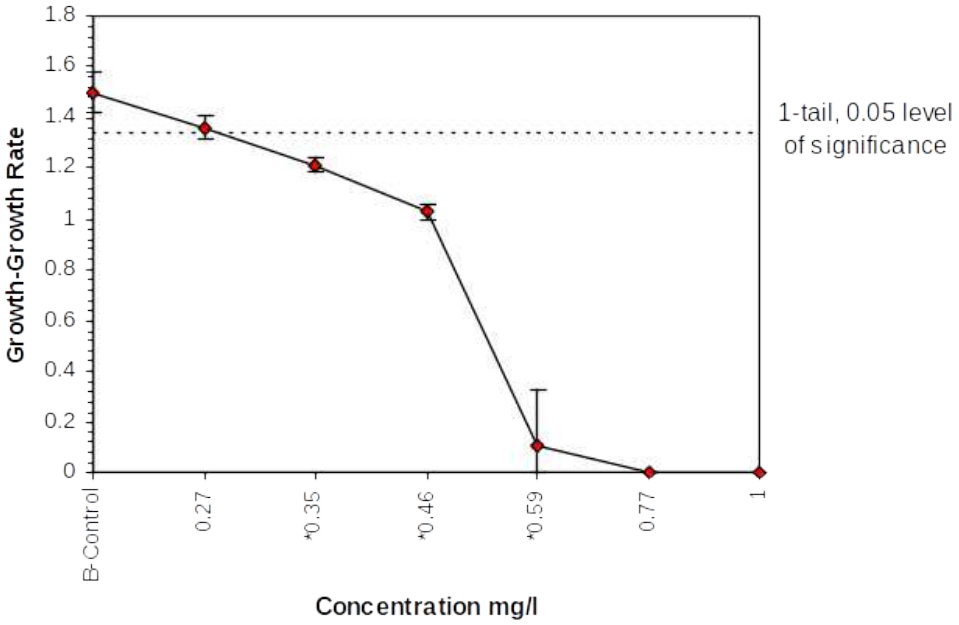
Determined by Maximum Likelihood-Logit (P = 0.58), using ToxCalc v5.0.

NOEC(r) = 0.27mg/l
LOEC(r) = 0.35mg/l

Determined by Bonferroni t Test (1-Tail, 0.05), following tests for normality of distribution and equal variances, using ToxCalc v5.0.

Graph 3

0 to 72 hours dose-response plot



* = location of statistically significant difference (1-tail, P=0.05)

Appendix 1 – Test Conditions

pH values and temperatures of the test solutions:

Concentration (mg/l)	0 hours		72 hours	
	pH	Temp (°C)	pH	Temp (°C)
0 (Control)	8.14	21.9	8.60	20.2
0.27	7.96	21.9	8.40	20.5
0.35	8.04	21.7	8.33	20.1
0.46	8.08	21.7	8.21	20.3
0.59	8.10	21.6	8.05	19.9
0.77	8.13	21.7	8.03	20.0
1.0	8.12	21.8	8.01	20.5

Note: Water quality was determined on pooled replicates for the test and control solution at the end of the 72-hour test period and on excess solution at the start of the study.

Temperature and light intensity measurements recorded in test incubator:

Within incubator	Temperature (°C)			
	0 hours	24 hours	48 hours	72 hours
Current	21.3	20.6	20.8	20.8
Minimum		20.3	20.3	20.1
Maximum		21.6	21.0	21.0

Within incubator	Light intensity (lux)			
	0 hours	24 hours	48 hours	72 hours
Minimum	6370	6300	6240	6330
Maximum	7270	7480	7320	7350

Appendix 2

Culture media

Culture medium: Prepared in natural seawater according to the ISO 10253 standard.

Seawater: Filtered, and sterilised by autoclaving at 120°C for 15 minutes.

Method of preparation: Sterile nutrient stock solutions were prepared and added to the seawater to obtain the culture medium. The pH was 8.14 at test initiation.

Composition of culture medium:

Nutrient	**Final concentration in test solution ± 5%**
$FeCl_3$	149µg/l (Fe)
$MnCl_2$	605µg/l (Mn)
$ZnSO_4.7H_2O$	150µg/l (Zn)
$CuSO_4.5H_2O$	0.6µg/l(Cu)
$CoCl_2.6H_2O$	1.5µg/l (Co)
H_3BO_3	3.0mg/l (B)
Na_2 EDTA.$2H_2O$	15mg/l (as Na_2 EDTA)
Thiamin hydrochloride	25µg/l
Biotin	0.005µg/l
B_{12} (cyanocobalamin)	0.05µg/l
K_3 PO_4 H_2O	0.438mg/l (P)
$NaNO_3$	8.24mg/l (N)
$Na_2SiO_3.9H_2O$	1.97mg/l (Si)

Commercial-in-Confidence

Chemex reference: ENV 11447 /ECO 170408

CHEMEX
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The acute toxicity of Empigen BAC 50 to *Acartia tonsa* over a 48-hour exposure period

Report for Clariant Oil Services Scandinavia AS

Report issued by:

Chemex Environmental International Limited
Unit J
Broad Lane Industrial Estate
Cottenham
Cambridge CB24 8SW
UK

Sponsor:

Clariant Oil Services Scandinavia AS
Post box 6054 BS
5892 Bergen
Norway

July 2017

Contents

Page

Compliance with Good Laboratory Practice Standards

I, the undersigned, hereby declare that the study described in this report was performed under my supervision, and that the final report fully and accurately reflects the raw data generated during the conduct of the study, in compliance with international codes of Good Laboratory Practice based on:

- Section II of Annex 1 to the European Parliament and council Directive 2004/10/EC and Annex 1 to the European Parliament and council Directive 2004/9/EC (Official Journal No. L 50) and embodied within:
- *The UK Good Laboratory Practice Regulations 1999 (The United Kingdom GLP Regulations 1999, Statutory Instrument 3106)* as amended by:
- *The UK Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004 (Statutory Instrument No 994)*

These principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

N. Eggett ..... 11 JULY 2017 .....

Neil Eggett BSc (Hons) MRSB
Study Director

Date

Key personnel

Other key personnel at Chemex involved in this study were:

Agnes Grabczewska
Monika Zuba-Sosnowska
Sylwia Lojek

Quality Assurance Statement

The Quality Assurance unit inspects the final report to confirm that the methods, procedures and observations are accurately and completely described, and that the reported results accurately and completely reflect the raw data of a regulatory study.

This is achieved by conducting routine annual facility and system inspections at approximately 12 monthly intervals. In addition, an internal process-based audit programme is also adhered to at 3 monthly intervals. Where required, study specific inspections are also conducted. All study plans and amendments are verified by the QA unit to confirm compliance with GLP.

The inspections applicable to this study are detailed below. The dates are given as dd/mm/yy.

Study Number: ENV 11447
Study Title: The acute toxicity of Empigen BAC 50 to *Acartia tonsa* over a 48-hour exposure period.

Procedures and Processes	Type	Date of inspection	Date reported to Study Director / Management
Aquatic crustacean test set up	P	22/05/17	23/05/17
Weighing out test or reference materials	P	22/03/17	03/05/17
Preparation of growth medium / nutrients / standards	P	21/03/17	03/05/17
Preparation of solution / WAF	P	27/03/17	07/04/17
Equipment calibration	P	22/05/17	23/05/17
Labelling and paperwork	P	22/05/17	23/05/17
Organism stock records	P	22/05/17	23/05/17
Taking and recording readings	P	22/05/17	23/05/17

Key: P- Process-based, S- Study specific, O- other inspection type.

This report has been inspected by the undersigned and, as far as can be reasonably established, the methods, procedures and observations are accurately and completely described and the results incorporated into this report accurately and completely reflect the raw data generated during this study.

Final report and data inspection started: 29/06/17

Final report and data inspection completed: 11/07/17

Signed: .....

Kim Utting MRQA
Quality Assurance

Date: 11 July 2017

Summary

This section summarises aquatic toxicity test results obtained by Chemex Environmental International Limited on a sample as detailed below:

Test commissioned by:	Clariant Oil Services Scandinavia AS
Substance under test:	Empigen BAC 50
Chemex reference:	Sample: ECO 170408 Study: ENV 11447
Test species:	*Acartia tonsa*
Test type:	Acute toxicity: 48-hour EC_{50}
Registration guideline:	ISO 14669:1999 "Water quality - Determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea)"
Experimental period:	Range finding test 1: 16 to 18 May 2017 Range finding test 2: 17 to 19 May 2017 Definitive test 1: 23 to 25 May 2017 Definitive test 2: 15 to 17 June 2017
Test concentrations:	0 (control), 0.63, 1.25, 2.5, 5 and 10 mg/l
Test performed at:	Chemex Environmental International Limited Unit J, Broad Lane Industrial Estate Cottenham Cambridge CB24 8SW UK

Results: All study validity criteria were met. The results are summarised in the table below.

Exposure Period (hours)	**LC_x and NOEC values mg/l (95% confidence limits)**	
	LC_{10}	**LC_{50}**
24	*	2.775mg/l (2.371 – 3.247mg/l)
48	1.09mg/l (0.4473 – 1.519mg/l)	1.989mg/l (1.373 – 2.520mg/l)
NOEC (48 hours)	1.25 mg/l determined by Dunnett's Test (1-tail , 0.05)[$]	

Statistical methods used in ToxCalc v5.0: Trimmed Spearman Karber 0% Trim (24 hours), Maximum Likelihood Probit (48 hours).
* Not possible to determine with Trimmed Spearman Karber
[$] Following Shapiro-Wilk's Test indicating normal distribution (equality of variance could not be determined)
All concentrations of the test substance are reported as nominal as received.

1. Introduction

This report contains a description of the methods used and the results obtained during a study to investigate the acute toxicity of Empigen BAC 50 to *Acartia tonsa*. The objective of this study was to determine the 48-hour lethal concentrations of Empigen BAC 50 specifically, the 24 and 48-hour LC_{50} and the NOEC according to ISO 14669:1999.

2. Materials and methods

Unless otherwise specified, all methods mentioned in this report are according to Chemex Environmental International Limited standard procedures.

All records of measurements and observations made during this test will be collated and held in the Chemex Environmental International Limited archives at Unit J, Broad Lane Industrial Estate, Cottenham, Cambridge CB24 8SW, UK.

2.1 Test material

All quoted information is taken from TSDS (Test Substance Data Sheet) unless otherwise stated.

Identification:	Empigen BAC 50
Name as supplied:	Empigen BAC 50
Batch number:	120417SAM1
Type of Substance:	Multi-Constituent Substance
Activity:	50% Active in water
Purity / Composition:	Quaternary ammonium compounds, benzyl-C12-14(even numbered)-alkyldimethyl, chlorides 30-60% (MSDS)
Chemex reference:	ECO 170408
Density (specified):	0.99g/cm^3 (SDS)
Density (observed):	0.9878g/cm^3
Appearance (specified):	Straw Coloured Liquid
Appearance (observed):	Straw Coloured Liquid
Solubility (specified):	Soluble in water
Homogeneity (specified):	Homogeneous
Homogeneity (observed):	Homogeneous
Stability in container:	Stable
Stability in water:	Not Known Aquatic Half Life: Freshwater 365 days (MSDS)
Required storage conditions:	Ambient. No Protection from light
Actual storage conditions:	Ambient 15 ± 10°C
Source of supply:	Huntsman Holland BV Merseyweg 10 3197 KG Botlek-Rotterdam The Netherlands
Expiry date:	27 April 2018

2.2 Test organism

Species:	*Acartia tonsa*
Source:	Guernsey Sea Farms
Date *Acartia* delivered to laboratory:	15 June 2017
Age of copepods at start of test:	12-14 days old
Life stage:	Adult

2.3 Dilution water

The test organisms were maintained, and tests performed in natural seawater collected from CEFAS Laboratories, Lowestoft. The seawater was collected via pipeline directly from the estuary. The sand acting as the first stage of filtration. After collection, the water was stored in the dark at approximately 15 ± 2°C within the testing facility. Prior to use it was filtered through a Whatman 54 filter and sterilised by autoclaving at 120°C for 15 minutes.

The pH of dilution water was 8.17.

2.4 Preparation of test solutions

Information provided by the Sponsor indicated that the sample was soluble in water.

Both the preliminary range-finding test and the definitive test were prepared by direct addition of weighed amounts of test substance to dilution water made to volume.

For the preliminary (range-finding) tests initially a 1000mg/l stock (±5% by weight) of test substance was prepared. 0.5000g was dissolved in 500ml of dilution water. Preparation of the remaining concentrations were by dilution of this 1000mg/l test concentration. 0.25, 2.5 and 25ml were made to 250ml volume with dilution water to prepare 1, 10 and 100mg/l respectively.

The second preliminary (range-finding) was performed between 0.001 and 1 mg/l. A 20mg/l stock solution was prepared by weighing 0.0201g of test substance and making to 1000ml volume. 1mg/l was prepared by the addition of 12.5 ml of this stock solution and made to 250 ml volume with dilution water. The remaining test concentrations were prepared by serial dilution.

Two definitive tests were performed, the first failed the control validity criteria with 20% mortality recorded after 48 hours. The second successful definitive test has been reported in full.

The definitive test concentrations were prepared by weighing 0.0200g (±5% by weight) and dissolving in 2000ml of dilution water to prepare a 10mg/l stock solution. This stock solution was used to prepare the nominal test concentrations. 15.75, 31.25, 62.5 and 125 ml of the 10 mg/l stock were diluted individually to 250ml with dilution water to prepare 0.63, 1.25, 2.5 and 5 mg/l respectively. Enough solution was prepared for testing and subsequent water quality measurements.

2.5 Test methods and conditions

Static test conditions according "Water quality - Determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea)" ISO 14669:1999.

Chemex SOP reference:	E207 "Testing of the marine copepod, *Acartia tonsa*"
Preliminary test method:	Preliminary (range-finding) tests were conducted at concentrations of 0 (Control), 1, 10, 100 and 1000mg/l and 0.001, 0.01, 0.1 and 1mg/l. The duration of the preliminary tests were 48 ± 1 hour. There was a single replicate at each concentration with five animals per replicate.
Preliminary test results:	Data from the preliminary tests identified the 48-hour LC_{50} as likely being between 1 and 10mg/l. 1.) Control (0%), 1mg/l (20%), 10mg/l (100%), 100mg/l (100%) and 1000mg/l (100%). 2.) Control (0%), 0.001mg/l (0%), 0.01mg/l (40%), 0.1mg/l (40%) and 1.0mg/l (40%).
1st Definitive test results:	The first definitive test was repeated as the control validity criteria were not achieved, the mortality results of this test were as follows: Control (20%), 0.63mg/l (30%), 1.25mg/l (50%), 2.5mg/l (80%) 5mg/l (100%) and 10mg/l (100%).
Definitive Test Method:	
Test period:	15 to 17 June 2017
Test duration:	48 ± 1 hours
Test volume:	25ml
Test vessel:	40ml glass dishes.
Number of replicates:	Four replicates at each concentration.
Test concentrations:	0 (control), 0.63, 1.25, 2.5, 5 and 10mg/l
Test Media:	Natural Seawater (Batch Number: ECO170308). Salinity at start of exposure: 34ppt
Test organism:	Five *Acartia* (Batch No: A22b) were transferred to each control and test concentration vessel.
Replacement regime:	Static
Test conditions:	Initial pH at 0 Hours: 7.97 (Required: 8±0.3) pH range in control and test concentrations throughout test: 7.95 – 8.15 Initial Dissolved Oxygen at 0 Hours: 98.6% ASV (equivalent to approx. 7.13mg/l) DO range in control and test concentrations

	throughout test: 7.01 – 7.59mg/l (Required: ≥4mg/l) Temperature range within incubator throughout test: 18.9 – 19.7°C (Required: 20±2°C) Illumination: 16 Hours Light, 8 Hours Dark
Water quality measurements:	The temperature (to 0.1°C) within the incubator was recorded at the beginning of the study, after 24 hours and at the end of the 48-hour test period. The pH (to 0.01), temperature (to 0.1°C), dissolved oxygen (%ASV 0.1 and mg/l to 0.01) and salinity (to 1‰) were recorded for each test and control solution at the start of the test and on the pooled replicates at the end of the 48-hour test period.
Observations/frequency:	The number of dead and alive *Acartia* was recorded after 24 and 48-hour (±1h) exposure periods.
Analysis of test substance:	The Sponsor did not request analytical confirmation of exposure concentrations. All effect concentrations have, therefore, been calculated from nominal loading rates.
Calculation of results:	Where possible the LC_{50} values are shown graphically and calculated with 95% confidence limits following Abbott's correction for background response if required. LOEC and NOEC are also calculated, following tests to determine normality of distribution and equality of variance, using ToxCalc™ Version 5.0 "Comprehensive Toxicity Data Analysis and Database Software", copyright 1994-1996.
Reference Substance:	A separate reference study (ENV11397) was conducted from 17 to19 January 2017 using 3,5 Dichlorophenol as a means of checking the test procedure. The 48-hour LC_{50} was 0.72mg/l which is within the acceptable range (0.5 - 1.5mg/l) quoted in ISO 14669:1999 guideline.

2.6 Study Plan Deviation

1.) The Study Plan stated that the seawater is stored in the dark at approximately 15±2°C within the testing facility. The seawater batch used in the study deviated from the required temperature on one occasion during storage. The temperature recorded was 26.3°C and returned to within the required range the following day and for the remainder of the time it was stored prior to it being sterilised.

This deviation in temperature was for a short period of time and its effects were considered to be minimal. Any effects the high temperature may have had on biological activity in the water would have been reduced once sterilised prior to use therefore this deviation has not impacted the integrity of the study.

3. Results

3.1 Cumulative mortalities

Nominal concentration (mg/l)	Number dead		% mortality	
	24 hours	48 hours	24 hours	48 hours
Control	0	2	0	10
0.63	0	0	0	0
1.25	1	6	5	30
2.5	6	13	30	65
5	20	20	100	100
10	20	20	100	100

Graph 1 illustrates the estimation of the 48-h LC_x values and Graph 2 shows the dose-response curve at 48-hours.

Test solutions appeared clear and colourless throughout the study.

3.2 LC_{10}, LC_{50} and NOEC values

Exposure Period (hours)	LC_x and NOEC values mg/l (95% confidence limits)	
	LC_{10}	LC_{50}
24	*	2.775mg/l (2.371 – 3.247mg/l)
48	1.09mg/l (0.4473 – 1.519mg/l)	1.989mg/l (1.373 – 2.520mg/l)
NOEC (48 hours)	1.25 mg/l determined by Dunnett's Test (1-tail , 0.05)[$]	

Statistical methods used in ToxCalc v5.0: Trimmed Spearman Karber 0% Trim (24 hours), Maximum Likelihood Probit (48 hours).
* Not possible to determine with Trimmed Spearman Karber
[$] Following Shapiro-Wilk's Test indicating normal distribution (equality of variance could not be determined)
All concentrations of the test substance are reported as nominal as received.

4. Discussion

The definitive test conducted from 15 to 17 June 2017 was performed according to the ISO 14669:1999 guideline and met all validity criteria.

The 24-hour and 48-hour LC_{50} of Empigen BAC 50 to *Acartia tonsa* were determined to be 2.77mg/l and 1.989mg/l respectively (determined by Trimmed Spearman Karber and Maximum Likelihood Probit). Graphical representations of the 48-h LC_x values and the 48-hour dose-response curve are given in Graphs 1 and 2.

The 48-hour NOEC and LOEC were 1.25mg/l and 2.5mg/l respectively (Determined by Dunnett's, 1-tail, P=0.05).

The highest concentration tested (10mg/l) resulted in 100% mortality. No mortalities were observed at the lowest concentration tested (0.63mg/l).

Test solutions appeared clear and colourless throughout the study.

Two (10%) of the twenty control copepods died during the study. This indicates a satisfactory level of "health" of organisms maintained under test conditions according to the test guideline allowing for 10% mortality within control vessels.

The dissolved oxygen concentration remained ≥7.01mg/l throughout the test. The toxicity of the reference chemical was within the range specified in the guideline (see point 2.5).

No analytical confirmation of the test concentrations was performed. The dissolved concentrations were likely to be similar to those stated in this report as Empigen BAC 50 was soluble in water.

The water quality measurements of the test solutions and incubation conditions are given in Appendix 1.

5. References

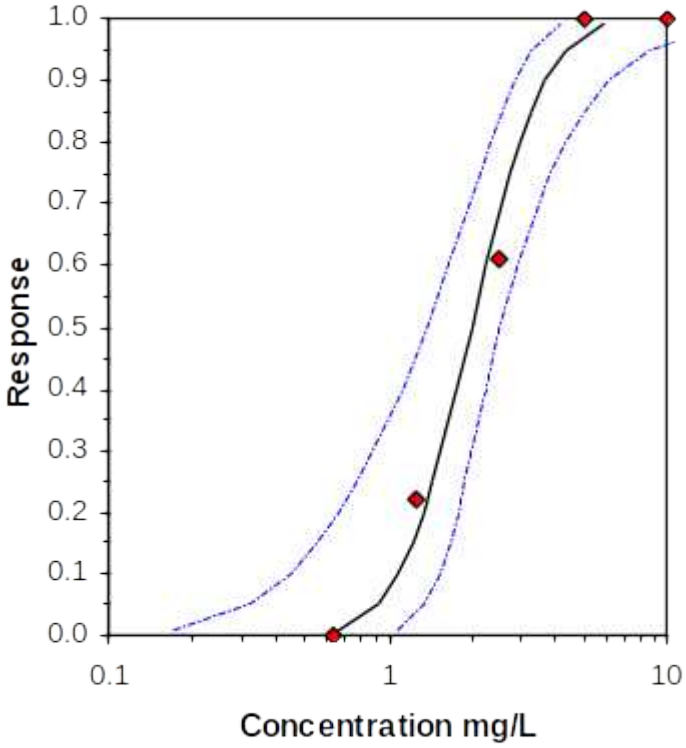
(1) ISO 14669:1999 "Water quality - Determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea)".

(2) OSPAR Guidelines for Toxicity Testing of Substances and Preparations Used and Discharged Offshore (Ref 2005-12).

(3) ToxCalc™ Version 5.0 "Comprehensive Toxicity Data Analysis and Database Software", copyright 1994 – 1996.

Graph 1

Determination of 48-hour LC_x values



---------- Indicates 95% Confidence Limits ◆——◆ Indicates actual data plot

Number of *Acartia tonsa* per test concentration = 20

Statistical data

LC_{10} value(1) = 1.090mg/l (0.4473 – 1.519mg/l)
LC_{50} value(1) = 1.989mg/l (1.373 – 2.520mg/l)
LC_{90} value(1) = 3.631mg/l (2.830 – 6.224mg/l)

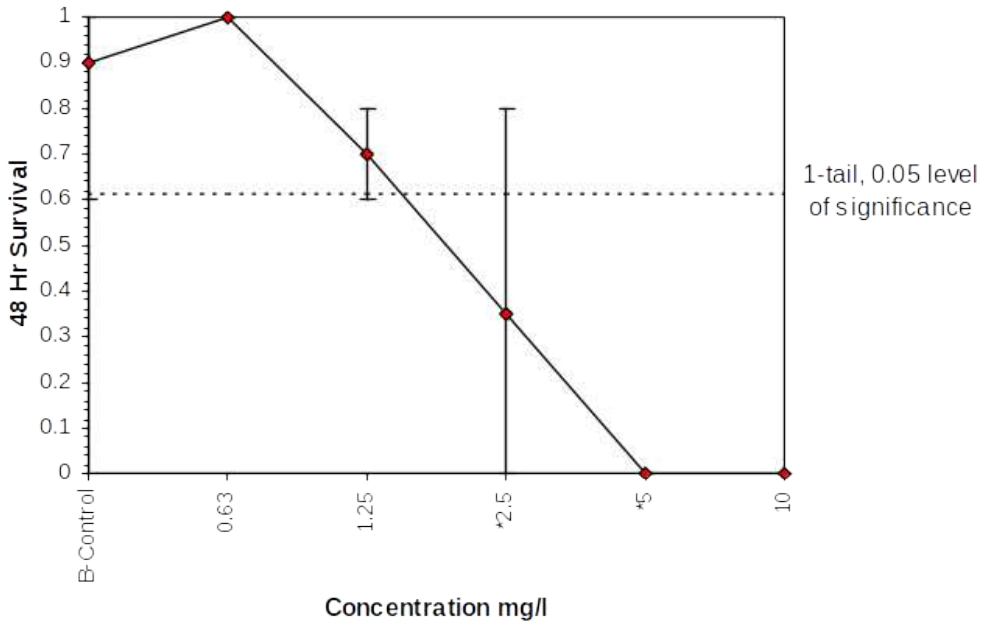
Determined by Maximum Likelihood Probit using ToxCalc v5.0.

NOEC = 1.25mg/l
LOEC = 2.5mg/l

Determined by Dunnett's Test, 1-tail, P=0.05, following tests for normality (equality of variance could not be determined), using ToxCalc v5.0.

Graph 2

Dose-response plot for 48-hours exposure



Appendix 1

Water qualities and incubation conditions

Concentration (mg/l)	0 hours				48 hours			
	pH	Temp (°C)	DO %ASV	Salinity ‰	pH	Temp (°C)	DO (mg/l)	Salinity ‰
Control	7.97	21.6	98.6 7.13 mg/l	34	7.95	20.9	7.14	35
0.63	8.08	21.6	97.1 7.01 mg/l	34	8.04	21.1	7.48	35
1.25	8.13	21.6	99.0 7.16 mg/l	34	8.05	21.3	7.36	35
2.5	8.11	21.6	98.9 7.15 mg/l	34	8.08	21.4	7.52	35
5	8.14	21.6	98.1 7.09 mg/l	34	8.11	21.6	7.59	35
10	8.15	21.6	99.5 7.19 mg/l	34	8.09	21.2	7.39	35

Note: Water quality was determined on pooled replicates for the test and control solution at the end of the 48-hour test period and on excess solution at the start of the study.

Summary of temperature values (°C) within incubator

Within incubator	Temperature (°C)		
	0 hours	24 hours	48 hours
Current	19.5	19.0	19.0
Minimum		19.0	18.9
Maximum		19.7	19.6

Commercial-in-Confidence

Chemex reference: ENV 11450/ECO 170408

CHEMEX
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The toxicity of Empigen BAC 50 to *Corophium* sp over a 10-day exposure period.

Report for
Clariant Oil Services Scandinavia AS

Report issued by:

Chemex Environmental International Limited
Unit J
Broad Lane Industrial Estate
Cottenham
Cambridge, CB24 8SW
UK

Sponsor:

Clariant Oil Services Scandinavia AS
Post box 6054 BS
5892 Bergen
Norway

June 2017

Contents

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Compliance with Good Laboratory Practice standards

I, the undersigned, hereby declare that the study described in this report was performed under my supervision, and that the final report fully and accurately reflects the raw data generated during the conduct of the study, in compliance with international codes of Good Laboratory Practice including:

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- *The UK Good Laboratory Practice Regulations 1999 (The United Kingdom GLP Regulations 1999, Statutory Instrument 3106)* as amended by:

- *The UK Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004 (Statutory Instrument No 994)*

These principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

N. Eggett ..... 30 June 2017 .....

Neil Eggett BSc (Hons) MRSB
Study Director

Date

Key personnel

Other key personnel at Chemex involved in this study were:

Agnes Grabczewska
Monika Zuba-Sosnowska
Sylwia Lojek

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The inspections applicable to this study are detailed below. The dates are given as dd/mm/yy.

Study Number: ENV 11450
Study Title: The toxicity of Empigen BAC 50 to *Corophium* sp over a 10-day exposure period.

Procedures and Processes	Type	Date of inspection	Date reported to Study Director / Management
Aquatic crustacean test set up	P	14/02/17	14/02/17
Organism stock records	P	14/02/17	14/02/17
Preparation of sediment/soil	P	01/03/17	07/03/17
Weighing out test or reference materials	P	27/03/17	07/04/17
Preparation of solution/WAF	P	27/03/17	07/04/17
Taking and recording readings	P	27/03/17	07/04/17
Labelling and paperwork	P	29/03/17	05/04/17
Equipment calibration	P	29/03/17	05/04/17
Behavioural Observations	P	03/04/17	05/04/17
Determination of dry matter and % water	P	03/04/17	05/04/17

Key: P- Process-based, S- Study specific, O- other inspection type.

This report has been inspected by the undersigned and, as far as can be reasonably established, the methods, procedures and observations are accurately and completely described and the results incorporated into this report accurately and completely reflect the raw data generated during this study.

Final report and data inspection started: 30/05/17

Final report and data inspection completed: 30/06/17

Signed: .....
Jane Hawkins MRQA
Quality Assurance

Date: 30/06/17

Summary

This section summarises aquatic toxicity test results obtained by Chemex Environmental International Limited on a sample as detailed below:

Test commissioned by:	Clariant Oil Services Scandinavia AS
Substance under test:	Empigen BAC 50
Chemex reference:	Sample: ECO 170408 Study: ENV 11450
Test species:	*Corophium volutator*
Test type:	Toxicity: 10-day LC_{50}
Registration guideline:	Static test conditions according to SOP E211 based on OSPAR / PARCOM Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry 2006 Part A: A sediment bioassay using an amphipod *Corophium* sp.
Experimental period:	Definitive test: 10 to 22 May 2017
Test conditions range:	Temperature: 14.1 – 14.7°C Dissolved Oxygen: >85.3% ASV pH value 8.16 – 8.36 Salinity: 35-36ppt Photoperiod: 16 hours light, 8 hours dark.
Test performed at:	Chemex Environmental International Limited Unit J, Broad Lane Industrial Estate Cottenham Cambridge CB24 8SW UK

All study validity criteria were met. The results are summarised below.

Result:	10-day LC_{50}[(1)]	=	306.5 mg/kg dry sediment
	95% C.L.	=	232.9 – 403.4 mg/kg dry sediment
	NOEC[(2)]	=	125mg/kg dry sediment
	LOEC[(2)]	=	401mg/kg dry sediment

[(1)]calculated by Trimmed Spearman-Karber (Trim Level 5.3%) using ToxCalc v5.0.

[(2)]calculated by Fisher's Exact Test using ToxCalc v5.0.

1. Introduction

This report contains a description of the methods used and the results obtained during a study to investigate the toxicity of Empigen BAC 50 to *Corophium volutator*. The objective of this study was to determine the 10-day LC_{50}, which is defined as the concentration that kills 50% of the test animals after a 10-day exposure period according to OSPAR/PARCOM 2006 Protocol[(1)].

2. Materials and Methods

Unless otherwise specified, all methods mentioned in this report have been carried out according to Chemex Environmental International Limited standard operating procedures.

All records of measurements and observations made during this test will be collated and held in the Chemex Environmental International Limited archives at Unit J, Broad Lane Industrial Estate, Cottenham, Cambridge CB24 8SW, UK.

2.1 Test substance

Identification:	Empigen BAC 50
Name as supplied:	Empigen BAC 50
Batch number:	120417SAM1
Type of Substance:	Multi-Constituent Substance
Activity:	50% active in water
Purity / Composition:	Quaternary ammonium compounds, benzyl-C12-14(even numbered)-alkyldimethyl, chlorides 30-60% (MSDS)
Chemex reference:	ECO 170408
Density (specified):	0.99g/cm^3
Density (observed):	0.9878g/cm^3
Appearance (specified):	Straw Coloured Liquid
Appearance (observed):	Straw Coloured Liquid
Solubility (specified):	Soluble in water
Homogeneity (specified):	Homogeneous
Homogeneity (observed):	Homogeneous

Stability in container	Stable
Stability in water:	Not Known Aquatic Half Life: Freshwater 365 days (MSDS)
Required storage conditions:	Ambient. No Protection from light
Actual storage conditions:	Ambient 15 ± 10°C
Source of supply:	Huntsman Holland BV Merseyweg 10 3197 KG Botlek-Rotterdam The Netherlands
Expiry date:	27 April 2018

All quoted information is taken from TSDS (Test Substance Data Sheet) provided by the sponsor unless otherwise stated.

2.2 Test organism

Test species:	*Corophium volutator*
Source:	Todd Fish Tech Ltd. Dalgety Bay, Fife, Scotland
Date of receipt:	19/04/17
Holding conditions:	Temperature: 13.9 – 14.4°C Dissolved oxygen: 92.4 – 98.9% ASV pH: 7.87 – 8.07 Salinity: 32 - 34‰

The stock of *Corophium volutator* used in this study was received from Todd Fish Tech Ltd of Dalgety Bay, Fife, Scotland. The animals were sieved from the sediment and transported in natural seawater. The animals were then maintained in the laboratory under static conditions in reconstituted seawater, in the presence of a small amount of detrital material, until the start of the test. During holding they were fed with homogenized Aqua Care Tropical® fish food. They were not fed during the test.

The lengths of a sample of the control animals were measured at the end of the test and the mean was 11.6mm with a range of 10 - 13mm.

2.3 Sediment

Sediment from Snettisham Beach, Norfolk collected on 21 March 2017 was used for the test. This site is known to be populated by *Corophium*, and to the best of our knowledge free from significant contamination. The aerobic layer (top 5 to 10cm) of sediment was collected, sieved to 500µm, washed, settled and stored refrigerated (4± 2°C) in the dark until the start of the test.

The sediment was thoroughly homogenised and a small sample dried at 97°C for >24 hours to determine the dry matter weight. From this it was determined that the water content of the sediment was 23.93%.

2.4 Dilution water

The stock of test organisms was maintained, and the tests performed, in artificial seawater. The seawater was prepared using dechlorinated mains water with artificial sea salt (Tropic Marin®) to give a salinity of approximately 34‰. The dilution water was stored in a constant temperature room set at 15 ± 2°C and at low light level within the test facility.

2.5 Test procedure

The toxicity test was carried out according to Chemex SOP E211 and the procedures given below, based on the guidelines produced by the OSPAR / PARCOM Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry 2006 Part A: A sediment bioassay using an amphipod *Corophium* sp.

Information supplied from the Sponsor indicated the test substance was soluble in water.

The test concentrations were therefore prepared by weighing out appropriate quantities of test substance and washing into a 2-litre conical flask containing 500g wet sediment with 300ml of artificial seawater.

The following test concentrations were used: 0 (control), 95, 305, 977, 3125 and 10000mg/kg expressed as the amount of test substance as received per kg wet sediment.

The 2-litre conical flasks containing the test substance/sediment/seawater mixtures were then placed on an orbital shaker and the slurries were mixed at approximately 150rpm for 3 hours. The sediment/seawater slurry was then divided between two replicate 2-litre beakers. These were the test vessels. The beakers were left overnight to allow the sediment to settle before carefully adding seawater to bring the total volume up to the 1400ml graduation mark. A plastic disc was placed above the sediment to avoid disturbance of sediment during this process. This resulted in a total depth of approximately 133 - 136mm and sediment layer depth of 23 - 28mm in each beaker.

After a further period of settling, gentle aeration was applied to each beaker until the dissolved oxygen content reached a minimum of 80% air saturation value (ASV). The pH value (to 0.01), dissolved oxygen (to 0.1% ASV), temperature (to 0.1°C) and salinity (to 1‰) were measured in each test beaker immediately prior to initiating the test, and then at intervals during the test period.

Individual *Corophium* of >5mm length were selected from the stock using a wide bore Pasteur pipette. Batches of 10 animals were transferred to plastic pots containing 50ml seawater. The exposure period was started on addition of 10 *Corophium* to each test vessel.

Records were made of the numbers of animals observed alive and dead on the surface of the sediment as well as any showing abnormal behaviour on days 3, 5 and 10. At the end of the 10-day exposure period, water quality measurements were made, and each of the test and control sediments were sieved to determine the number of animals still alive. As dead animals may decompose or be consumed, any missing animals were counted as dead.

Where possible the 10-day LC_{50} was determined, with 95% confidence limits, together with the highest no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) for mortality, using ToxCalc version 5.0 “Comprehensive Toxicity Data Analysis and Database Software”. The Abbotts correction for control response was applied where necessary.

The 10-day LC_{50}, NOEC and LOEC were determined, where possible, using test concentrations expressed as mg/kg dry sediment.

3. Results

3.1 Mortality after 10-days exposure

Nominal concentration (mg/kg)		Number alive[1]		Total number dead	Percent mortality
Wet sediment	Dry sediment	Replicate 1	Replicate 2		
0 (control)	0 (control)	9	10	1	5
95	125	10	8	2	10
305	401	2	4	14	70
977	1285	0	0	20	100
3125	4107	0	0	20	100
10000	13141	0	0	20	100

[1] Number exposed in each replicate = 10.

Abnormal behaviour among the *Corophium* was recorded on day 5 and 10 at 125mg/kg dry sediment, no abnormal behaviour was recorded for the other other test concentrations for the duration of the study.

3.2 LC_{50}, NOEC and LOEC values after 10-days exposure

10-day LC_{50} value (mg/kg dry sediment)	95% confidence limits (mg/kg dry sediment)	NOEC value (mg/kg dry sediment)	LOEC value (mg/kg dry sediment)
306.5[1]	232.9 – 403.4	125[2]	401[2]

[1]calculated by Trimmed Spearman-Karber (Trim 5.3%) using ToxCalc v5.0.

[2]calculated by Fisher's Exact Test using ToxCalc v5.0.

3.3 Water quality data

Nominal concentration (mg/kg)		Range of values			
Wet sediment	Dry sediment	pH	Dissolved oxygen (% ASV)	Temperature (°C)	Salinity (‰)
0 (control)	0 (control)	8.16 – 8.28	85.4 – 92.6	14.1 – 14.7	35 -36
95	125	8.19 – 8.30	85.3 – 95.1	14.1 – 14.5	35 -36
305	401	8.20 – 8.31	86.0 – 96.4	14.2 – 14.6	35 -36
977	1285	8.20 – 8.34	86.3 – 96.5	14.2 – 14.6	35 -36
3125	4107	8.20 – 8.36	86.2 – 97.8	14.1 – 14.6	35 -36
10000	13141	8.20 – 8.33	85.9 – 98.3	14.2 – 14.7	35 -36

4. Discussion

The 10-day LC_{50} of Empigen BAC 50 to *Corophium volutator* was determined to be 306.5mg/kg dry sediment (232.9 – 403.4 mg/kg dry sediment 95% C.L).

The highest no-observed effect concentration (NOEC) after 10-days exposure was 125mg/kg dry sediment and the lowest observed effect concentration (LOEC) was 401mg/kg dry sediment.

Abnormal behaviour among the *Corophium* was recorded on day 5 and 10 at 125mg/kg dry sediment, no abnormal behaviour was recorded for the other other test concentrations for the duration of the study.

The water quality measurements of the test solutions are given in 3.3 and were within the ranges specified in the test guideline which were for temperature: 13-17°C and within a range of 2°C. The dissolved oxygen was maintained at ≥85.3% ASV. The salinity remained at 35-36‰ throughout the test and this was acceptable.

One (5%) of the twenty control *Corophium* died during the study and this represents an acceptable level of health of the test organisms maintained under test conditions.

5. References

(1) OSPAR / PARCOM Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry 2006 Part A: A sediment bioassay using an amphipod *Corophium* sp.

(2) ToxCalc™ Version 5.0 “Comprehensive Toxicity Data Analysis and Database Software”, copyright 1994 – 1996.

APPENDIX C

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Analysis Report No. 716974

Assignment:	Analysis of MEA-Triazin 51% Purchase order 4504539049
Sampling by:	The client
Sample(s) received:	19 September 2016
Test performed:	19 September – 14 October 2016
Test results:	The results of the analysis and the method(s) used concern only the sample(s) analysed or the subsample(s) selected for analysis.

The Laboratory for Chemistry and Microbiology

Inge Bondgaard Nielsen
Consultant

Ulla Christensen
Team Manager

Sample preparation

The sample of H_2S scavenger was divided into four subsamples:
Subsample I was analysed directly.
Subsample II was heated to 140°C by reflux for 10 minutes and then cooled to room temperature with running water.
Atmospheric air was blown through subsample III for seven days.
Subsample IV was aerated for seven days and then heated to 140°C.
After the treatment, all subsamples were analysed for content of formic acid (HCOOH) and N-(2-hydroxyethyl) formamide (NHEF).

Analytical methods

Formic acid
Formic acid were determined by Ion chromatography (DIONEX AS 14).

N-(2-hydroxyethyl) formamide
N-(2-hydroxyethyl) formamide were determined by extraction with solvent added internal standard and analyses by gas chromatography with mas selective detection (GC-MS)

Results

Subsample	Substance	
	Formic acid %w/v	N-(2-hydroxyethyl)-formamide %w/w
I	0.0067	<0.1
II	0.14	0.53
III	0.0076	<0.1
IV	0.15	1.6
Precision:	± 4% rel.	± 25% rel.

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Analysis Report No. 755621

Assignment:	Analysis of Scavtreat 7103 Batch No. 17D025
Sampling by:	The client
Sample(s) received:	28 April 2017
Test performed:	28 April – 16 May 2017
Test results:	The results of the analysis and the method(s) used concern only the sample(s) analysed or the subsample(s) selected for analysis.

The Laboratory for Chemistry and Microbiology

Peter Nørby
Specialist

Dorthe Kvistgaard
Laboratory Assistant

The sample of H_2S-scavenger was divided into four subsamples:

Subsample I was analysed directly.

Subsample II was heated to 140°C by reflux for 10 minutes and then cooled to room temperature with running water.

Atmospheric air was blown through subsample III for seven days.

Subsample IV was aerated for seven days and then heated to 140°C.

After the treatment, all subsamples were analysed for content of formic acid (HCOOH) and N-(2-hydroxyethyl) formamide (NHEF).

In the calculations, a density of 1.08 g/mL is assumed.

Results (% weight)

Subsample Batch No. 17D025	% Formic acid	% N-(2-hydroxyethyl) formamide
I	0.014	<0.1
II	0.046	0.36
III	0.017	<0.1
IV	0.084	1.7
Precision	± 4% rel.	± 10% rel.

'<' Means less than the detection limit

Analytical methods

Formic acid (HCOOH) was determined by Ion chromatography (DIONEX AS 14).

N-(2-hydroxyethyl) formamide (NHEF) was determined by extraction with solvent added internal standard, and it was analysed by gas chromatography with mass selective detection (GC-MS).

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Analysis Report No. 755623

Assignment:	Analysis of Scavtreat 7103 Batch No. 17D037
Sampling by:	The client
Sample(s) received:	28 April 2017
Test performed:	28 April – 16 May 2017
Test results:	The results of the analysis and the method(s) used concern only the sample(s) analysed or the subsample(s) selected for analysis.

The Laboratory for Chemistry and Microbiology

Peter Nørby
Specialist

Dorthe Kvistgaard
Laboratory Assistant

The sample of H_2S-scavenger was divided into four subsamples:

Subsample I was analysed directly.

Subsample II was heated to 140°C by reflux for 10 minutes and then cooled to room temperature with running water.

Atmospheric air was blown through subsample III for seven days.

Subsample IV was aerated for seven days and then heated to 140°C.

After the treatment, all subsamples were analysed for content of formic acid (HCOOH) and N-(2-hydroxyethyl) formamide (NHEF).

In the calculations, a density of 1.08 g/mL is assumed.

Results (% weight)

Subsample Batch No. 17D037	% Formic acid	% N-(2-hydroxyethyl) formamide
I	0.011	<0.1
II	0.10	1.2
III	0.021	<0.1
IV	0.036	2.0
Precision	± 4% rel.	± 24% rel.

'<' Means less than the detection limit

Analytical methods

Formic acid (HCOOH) was determined by Ion chromatography (DIONEX AS 14).

N-(2-hydroxyethyl) formamide (NHEF) was determined by extraction with solvent added internal standard, and it was analysed by gas chromatography with mass selective detection (GC-MS).

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Analysis Report No. 756864

Assignment:	Analysis of Scavtreat 7103 Batch No. RD 282
Sampling by:	The client
Sample(s) received:	4 May 2017
Test performed:	4 – 16 May 2017
Test results:	The results of the analysis and the method(s) used concern only the sample(s) analysed or the subsample(s) selected for analysis.

This analysis was carried out in accordance with Danish Technological Institute's General Terms and Conditions Regarding Commissioned Work Accepted by Danish Technological Institute. This analysis report may be quoted in extract only if the Laboratory for Chemistry and Microbiology has approved of the extract in writing.

The Laboratory for Chemistry and Microbiology

Peter Nørby
Specialist

Dorthe Kvistgaard
Laboratory Assistant

The sample of H_2S-scavenger was divided into four subsamples:

Subsample I was analysed directly.

Subsample II was heated to 140°C by reflux for 10 minutes and then cooled to room temperature with running water.

Atmospheric air was blown through subsample III for seven days.

Subsample IV was aerated for seven days and then heated to 140°C.

After the treatment, all subsamples were analysed for content of formic acid (HCOOH) and N-(2-hydroxyethyl) formamide (NHEF).

In the calculations a density of 1.08 g/mL is assumed.

Results (% weight)

Subsample Batch No. RD 282	% Formic acid	% N-(2-hydroxyethyl) formamide
I	0.17	0.12
II	0.23	2.0
III	0.19	0.13
IV	0.23	2.0
Precision	± 2% rel.	± 20% rel.

'<' Means less than the detection limit

Analytical methods

Formic acid (HCOOH) was determined by Ion chromatography (DIONEX AS 14).

N-(2-hydroxyethyl) formamide (NHEF) was determined by extraction with solvent added internal standard, and it was analysed by gas chromatography with mass selective detection (GC-MS).