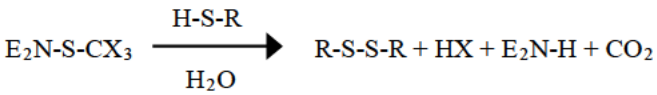
Section A5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)			Official use only
5.1	Function (IIA5.1)	In antifouling paints (product type 21 of the EU biocidal product directive), dichlofluanid is used for the protective treatment of ship vessels in service Generally dichlofluanid has been examined for efficacy in a wide range of applications from controlling grey mould strawberries to incorporation in fungicidal wood preservatives and the use in antifouling paints.	X
5.2	Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)		
5.2.1	Organism(s) to be controlled (IIA5.2)	With regard to PT 21 Dichlofluanid is effective against algae, diatoms and other fouling organisms, which are widespread in the marine and freshwater environment of the European Community. It is less active against barnacles. See also study summary in section 5.3.1 (Effects on target organisms). Generally dichlofluanid has a broad action spectrum which includes moulds, fungi that discolour wood, and yeasts.	X
5.2.2	Products, organisms or objects to be protected (IIA5.2)	Protection of ships vessels against fouling organisms.	
5.3	Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)		
5.3.1	Effects on target organisms (IIA5.3)	See study summary for section 5.3.1 (Effects on target organisms).	
5.3.2	Likely concentra-tions at which the A.S. will be used (IIA5.3)		
	PT21	Active in antifouling paints between 1 and up to > 5 % dichlofluanid in antifouling paints. The lowest concentrations are used in combination products mostly with copper compounds which act as a co-active.	
	PTn	e.g. PT 8 (wood preservatives): up to 0.7 % in solvent based products.	X

Section A5 Effectiveness against target organisms and intended uses

5.4	**Mode of action (including time delay) (IIA5.4)**		
5.4.1	**Mode of action**	The biocidal activity of N-haloalkylthio compounds like dichlofluanid is based on the ability of the N-S bond to open and react with nucleophilic entities within the cell such as SH groups of enzymes. Such reactions proceed by way of several steps and lead to disulphides. The course of the reaction can be summarised as shown below:  $E_2N\text{-}S\text{-}CX_3 \xrightarrow[H_2O]{H\text{-}S\text{-}R} R\text{-}S\text{-}S\text{-}R + HX + E_2N\text{-}H + CO_2$ E = electronegative group X = halogen	
5.4.2	**Time delay**	Time delay is not relevant with regard to the protection of the ship vessels. However, the slow release of the active from the antifouling paints provides the long term protection of the ship hull against fouling organisms.	
5.5	**Field of use envisaged (IIA5.5)**	In addition to product type 21 (antifouling products) dichlofluanid has also been notified as a biocidal active in product type 7 (film preservatives), 8 (wood preservatives) and 10 (masonry preservatives).	
		Dichlofluanid is used both as an active and co-active in antifouling paints mainly for amateur painting of pleasure craft boots by brushing and rolling. It can also be used for brushing and rolling and airless spraying of commercial ships. Dichlofluanid has a broad action spectrum which includes algae, diatoms and other fouling organisms.	**X**
	MG01: Disinfectants, general biocidal products		
	MG02: Preservatives MG03: Pest control	Product types 07, 08 and 10	
	MG04: Other biocidal products Further specification	Product type 21 (antifouling products)	
5.6	**User (IIA5.6)**		
	Industrial	Not applicable	
	Professional	See also Document II B of the PT 21 dossier.	
	i) Open system ii) Closed system	Professional brushing and rolling, airless spraying Not applicable	
	General public	Brushing and rolling of ship vessels, see also Document II-B of the PT 21 dossier	
5.7	**Information on the occurrence or possible occurrence of the development**		

Section A5 Effectiveness against target organisms and intended uses

	of resistance and appropriate management strategies (IIA5.7)	
5.7.1	**Development of resistance**	1) Due to the unspecific mode of action a development of resistance is neither to be expected nor has been ever observed. In addition a literature search regarded to resistance with respect to dichlofluanid and wood preservation was negative. The Fungicide Resistance Action Committee (FRAC) lists recently (2003-06-02) dichlofluanid in group M5 (= multi site contact activity / sulphamides) together with the comment "generally considered a low risk group with no signs of resistance developing to the majority of fungicides / No cross resistance between the group members". 2) In addition both the rapid degradation of the active in seawater and freshwater after its release from the paints and the mobility of the treated ship vessels do not provide conditions which would support developing resistances.
5.7.2	**Management strategies**	Not relevant due to point 5.7.1
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	Confidential: See entries in the IUCLID database

Section A5 Effectiveness against target organisms and intended uses

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	7/10/2014
Materials and Methods	N/A
Conclusion	N/A
Reliability	N/A
Acceptability	The Applicant's version is considered acceptable in support of approval.
Remarks	**5.1, 5.2.1, 5.3.2 and 5.5** Only use of the active substance in PT 21 is considered in this evaluation.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

STUDY SUMMARY 1		1 REFERENCE	Official use only
1.1	**Reference**	Klijnstra, J.W. and Head, R.M. (2001): Antifouling efficacy of dichlofluanid. TNO-Report No. CA01.9036, Project-No. 007.60468, date: 2001-12-11. Klijnstra, J.W. (2006): Addendum to TNO Efficacy Reports with dichlofluanid and tolylfluanid. Letter from TNO addressed to Mr. Kugler, LANXESS Deutschland GmbH, Germany, date: 2006-03-16.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LANXESS Deutschland GmbH	
1.2.2	Companies with Letter of Access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.	
1.3	**Guideline study**	Internal test method developed by TNO (test facilities)	
1.4	**Deviations**	–	
		2 METHOD	
2.1	**Test Substance**	Dichlofluanid, [redacted]	
2.1.1	Purity	[redacted]	
2.1.2	Physical state and nature	It is not soluble in water and not stable in water due to hydrolysis	**X**
2.1.3	Method of analysis	Not performed	
2.2	**Reference substance**	DMSO was used as reference substance as well as solvent control for determining the relative settlement	**X**
2.3	**Testing procedure**		
2.3.1	Test population / inoculum / test organism	Target organisms investigated were: Barnacle larvae (*Balanus amphitrite*), zoospores of macro-algae (*Enteromorpha* sp.) and marine diatoms (i.e. slime, *Skeletonema costatum*). For more details on tests organisms *cf.* Table A5_3_1-1a to Table A5_3_1-1c.	
2.3.2	Test system	Barnacle test: Mass reared cyprid larvae of the barnacle species *Balanus amphitrite* were used in the test. Settlement tests were carried out in four replicates in polystyrene multi-well (6 x 4) plates from Greiner. Between 20 and 66 cyprid larvae were injected (using a Finn pipette) in each well containing either 1 mL of a test suspension or 1 mL of a DMSO control. Multiwell plates were incubated for 48 hours at a temperature of 27 ± 2 °C under 15:9 h light:dark cycle. Macro-algae: The macro-algae settlement inhibition test is carried out with zoospores of *Enteromorpha* sp.. The cover slips were placed into wells of a 25 well polystyrene plate. A pipette was used to slowly introduce 2 mL of the test compound or the DMSO/seawater control to partially fill the well. The plates were incubated for 5 days in the algal growth cabinet at TNO (18°C and constant light). Each concentration was tested in threefold.	

		Diatoms: The diatom bioassay determined biocidal activity by assessing the inhibition of microalgal growth (*Skeletonema costatum*) caused by treatment with the test substance. Tests were carried out in multiwell plates each containing an oven sterilised glass cover slip; a 1 mL aliquot of EFSW (silicate enriched F2 growth media) or EFSW + test compound was added to the wells in a Latin Square experimental design. An equal volume of cell suspension was then added to each well giving a final volume of 2 mL per well. After 24 hours cover slips were removed from the wells, dip rinsed in sterile seawater and mounted on to slides for counting. Details are additionally listed in Table A5_3_1-3.
2.3.3	Application of TS	The test compound was reported to hydrolyse rapidly in an alkaline environment such as natural seawater (the pH usually fluctuates between 7.4 and 8.1). Therefore, the time interval between preparation of test suspensions and start of the tests was minimised to prevent premature degradation of the compounds and ensure maximum exposure to designate concentrations. Due to the low solubility in water, dichlofluanid was pre-dissolved in dimethylsulfoxide (DMSO) and subsequently in sterile filtered natural seawater (pH 7.4) at a concentration of 1000 µg/mL (ppm). DMSO concentration in the stock solution was 0.3 %. Serial dilution from the stock suspension resulted in a series of test suspensions of the test compound with concentration ranging from 0.01 to 100 ppm. Details are presented in Table A5_3_1-2.
2.3.4	Test conditions	For an overview on test conditions see Table A5_3_1-4.
2.3.5	Duration of the test / Exposure time	48 hours (barnacle larvae), 5 days (zoospores of macro-algae) and 24 hours (marine diatoms).
2.3.6	Number of replicates performed	4 replicates (barnacle larvae) and 3 replicates (zoospores of macro-algae and marine diatoms) per test concentration.
2.3.7	Controls	The control used was DMSO in sterile filtered natural seawater at the highest concentration used with the test compound.
2.4	**Examination**	
2.4.1	Effect investigated	Barnacle test: Relative settlement (number of settled and non-settled larvae at test termination). Settlement in the DMSO control was determined and used as an absolute standard to which settlement responses in the various concentrations of the test compound could be compared. In this way a relative settlement response is obtained for each concentration of the compound. A response of 100 % relative settlement means that larvae settle equally well as in the control. Subsequently, a settlement response curve in relation to substance concentration was drawn and an EC_{50} value, the concentration at which the settlement response is reduced to 50 %, calculated for the test compound. Macro-algae: Toxicity signs on the surviving cells (lack of healthy green cells). Two distinctions were made for counting: white unhealthy or lysed cells and green germinating cells. Diatoms: Toxicity signs on the surviving cells (lack of pigmentation or cell

		numbers). Cell numbers on the replicate cover slips are compared with seawater controls.	
2.4.2	Method for recording / scoring of the effect	Barnacle test: The test was terminated by the addition of one drop of 20 % formaldehyde and the numbers of settled and non-settled larvae were counted using a stereo microscope. The number of settled larvae was expressed as a percentage of the total number of larvae per well. Average percentage settlement of four replicates was calculated. Macro-algae: After incubation the cover slip was removed, rinsed twice with seawater and scored for germination using brightfield microscopy, 400x magnification. Settled spores are counted in at least 20 fields. Two distinctions were made for counting: white unhealthy or lysed cells and green germinating cells. All the green cells appeared to be in good condition. Diatoms: Bright field microscopy at 400x magnification was used for counting of cell numbers. A minimum of 300 cells or 20 fields of view (fov) were counted.	
2.4.3	Intervals of examination	Only at test termination	
2.4.4	Statistics	Data from the barnacle settlement assay, *Enteromorpha* germination assay and the diatom assay were used to estimate EC_{50} values as effective concentrations for dichlofluanid using a standard model (Graphpad Prims Software). The EC_{50} is the effective concentration at which the measured function (settlement or survival) of the population is reduced by 50%.	
2.4.5	Post monitoring of the test organism	No	X
		3 RESULTS	
3.1	**Efficacy**		
3.1.1	Dose/Efficacy curve	It is available in the original report (page 7-9)	X
3.1.2	Begin and duration of effects	Barnacle test: Inhibition of settlement starts around 5 µg/mL and is almost complete at 25 µg/mL. **EC_{50} was estimated to be 12.4 µg/mL.** Macro-algae: Healthy green cells were not found at 10 and 100 ppm. White lysed cells occurred at all surfaces with concentrations higher than 0.01 µg/mL. There was no evidence that dichlofluanid deterred the process of settlement at the levels tested, however, there was a clear toxicity effect. In the 5 days germination studies the populations in the DMSO/seawater controls had over 98 % of cells germinating successfully, ungerminated zoospores never accounted for more than 5 % of the total green zoospore counts. In the compound tests no zoospores survived in the wells with starting concentrations of 10 µg/mL or more. According to information from the sponsor the compounds would be largely and possibly completely hydrolysed to non-toxic end products within 5 days. **EC_{50} was estimated to be 1.72 µg/mL.** Diatoms: During counting diatom cells were observed to be both pigmented and healthy in appearance or showed no pigmentation and were assumed to be dead. No cells were observed to survive at concentrations above 1 µg/mL but at and below this level the cells remained pigmented and	X

		did not appear to be effected. The distinction between concentrations with 0 and 100 % effect is small, only one order of magnitude (factor 10) difference. **EC_{50} was estimated to be 6.0 µg/mL.**	
3.2	**Tabular and/or graphical presentation of the summarised results**	See Tables A5_3_1-5, A5_3_1-6, and A5_3_1-7. A dose response curve based on the test results is given for each one of the target organisms in the report	**X**
3.3	**Efficacy limiting factors**		
3.3.1	Occurrences of resistances	Efficacy-limiting factors were not reported.	
3.3.2	Other limiting factors	Efficacy-limiting factors were not reported	
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	The applied laboratory method allows high-throughput screening for potential target organisms.	
4.2	**Intended actual scale of biocide application**	The applied amount of active substance is comparable with the intended scale of product to be applied in practice	
4.3	**Relevance compared to field conditions**		
4.3.1	Application method	Not applicable (laboratory tests with active in sea water solution)	
4.3.2	Test organism	Yes, the test organisms are among the intended target organisms.	
4.3.3	Observed effect	Yes, the protective effect was significant.	
4.4	**Relevance for read-across**	Yes	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Laboratory efficacy tests with the biocidal compound dichlofluanid were carried out in order to determine its antifouling activity towards three different groups of marine fouling organisms when exposed to a saltwater solution containing 0.01 to 100 ppm of the test substance. Organisms used were settling barnacle larvae, zoospores of macro-algae and marine diatoms (slime).	
5.2	**Reliability**	1	**X**
5.3	**Assessment of efficacy, data analysis and interpretation**	▪ Standardised tests with three groups of marine fouling organisms have clearly shown antifouling activity of the compound dichlofluanid against these three groups. ▪ For cyprid barnacle larvae, an effective concentration at which 50% settlement inhibition takes place was found to be 12.4 µg/mL. ▪ For zoospores of macro-algae an EC_{50} value for survivorship in a 5-day germination test was found to be 1.72 µg/mL for dichlofluanid. ▪ In a 24 hours settlement test with diatoms a very low fraction of cells was found to survive concentrations higher than 10 µg/mL of the test compound. Below a concentration of 1 µg/mL all cells remained pigmented and did not appear to be effected. In this test	**X**

		an EC_{50} value of 6.0 µg/mL was found.	
5.4	**Conclusion**	Dichlofluanid has an excellent activity against fouling barnacle larvae, zoospores of macro-algae and marine diatoms.	**X**
5.5	**Proposed efficacy specification**	Dichlofluanid provided substantial protection against the fouling action of the following seawater organisms: barnacle larvae (*Balanus amphitrite*), zoospores of macro-algae (*Enteromorpha* sp.) and marine diatoms (slime, *Skeletonema costatum*).	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	7/10/2014
Materials and methods	The UK CA accepts the Applicant's version, with the following comments. **2.1.2** As the active substance was not soluble in seawater, it was pre-dissolved in dimethylsulfoxide (DMSO) and subsequently dissolved in sterile filtered natural seawater. **2.2** No reference substance was used in the study, although DMSO was used as the solvent control. Although no reference substance was used, this, in the UK CA's view, is not required in efficacy testing. The UK CA does not, therefore, consider the absence of a reference substance to be an issue. **2.4.5**. Although no post-monitoring of the test organism was conducted, this, in the UK CA's view, is not required in efficacy testing. The UK CA does not, therefore, consider the absence of such monitoring to be an issue. **5.2** The efficacy template does not require the Applicant to state a number for the reliability indicator. Although the study was not conducted to an internationally recognised test standard, the UK CA considers the methodologies used to be acceptable. The UK CA therefore considers the reliability indicator to be 2 (see below).
Results and discussion	The UK CA accepts the Applicant's version, with the following comments. **3.1.1, 3.1.2, 3.2 & 5.3** The results for barnacles showed that 10.0 ug dichlofluanid ml^{-1} produced a settlement rate of 69.1 $\pm$ 14.8 % against *Balanus amphitrite*, and that 100.0 µg dichlofluanid ml^{-1} produced a settlement rate of 0 %. From the statistical analysis of the results, the EC_{50} (i.e. the effective concentration at which settlement is reduced by 50.0 %) was estimated as 12.4 µg dichlofluanid ml^{-1}. The results for macro-algae spores showed that 1.0 ug dichlofluanid ml^{-1} produced a survival rate of 83.1 $\pm$ 9.8 % against *Enteromorpha* spp., and that 10.0 µg dichlofluanid ml^{-1} produced a survival rate of 0 %. From the statistical analysis of the results, the EC_{50} (i.e. the effective concentration at which survival is reduced by 50.0 %) was estimated as 1.72 µg dichlofluanid ml^{-1}. The results for marine diatoms showed that 1.0 µg dichlofluanid ml^{-1} produced 100.0 % pigmented cells of *Skeletonema costatum*, and that 10.0 µg dichlofluanid ml^{-1} produced 0 % pigmented cells. From the statistical analysis of the results, the EC_{50} (i.e. the effective concentration at which the percentage of pigmented cells is 50.0 %) was estimated to be 6.0 µg dichlofluanid ml^{-1}. The UK CA considers the results for *B. amphitrite*, *Enteromorpha* spp. and *S. costatum* as demonstrating the innate efficacy of dichlofluanid as an active substance against barnacles, algal spores and marine diatoms, respectively. The UK CA therefore considers the results to be acceptable in support of the approval of the active substance.
Conclusion	**5.4** The UK CA agrees with the Applicant's conclusion.
Reliability	2
Acceptability	The UK CA considers the data to be acceptable in support of active substance approval.
Remarks	All data and endpoints presented in the study summary have been checked against the original study and are correct.
	COMMENTS FROM ... (specify)

Evaluation by Competent Authorities
Date
Materials and methods
Results and discussion
Conclusion
Reliability
Acceptability
Remarks

Table A5_3_1-1a: Test organisms: barnacle

Criteria	Details
Species	*Balanus amphitrite*
Strain	-
Source	-
Laboratory culture	Yes
Stage of life cycle and stage of stadia	Larvae in cyprid stage
Method of cultivation	Adults maintained in containers with vigorous aeration and controlled temperature (27 ± 2 °C) and light conditions (15 hours light and 9 hours dark), were fed on a diet of the diatom *Skeletonema costatum* and larvae of the brine shrimp *Artemia salina.* Mass-spawned nauplii were collected, transferred to 8 litres carboys and fed on *Skeletonema costatum*. The vessels were kept at a constant temperature of 27 ± 2 °C and a 15:9 h light:dark photoperiod. Larvae reached the cyprid stage, which is the settling phase, after four days.
Pre-treatment	Cyprids were aged (at 4 – 6 °C in the dark) for 4 days prior to use in the experiments.
Initial density/number of test organisms in the test system	Between 20 and 66 cyprid larvae were injected (using a Finn pipette) in each well

Table A5_3_1-1b: Test organism: macro-algae

Criteria	Details
Species	*Enteromorpha* sp.
Strain	-
Source	Zoospores were obtained from mature algal tips collected from a local North Sea population. Spore release was induced by moist storage of tips for 24 hours and then flooding the individual tips with seawater. Zoospore suspensions were used immediately after collection.
Laboratory culture	No
Stage of life cycle and stage of stadia	Zoospores used
Method of cultivation	Not applicable
Pre-treatment	Heat sterilised glass cover slips were used as the substrate for adhesion. A 60 µL sample of zoospore suspension was placed centrally on top of each cover slip. The cover slips were then left in the dark at 18 °C for 2 hours to allow the zoospores to settle.
Initial density/number of test organisms in the test system	60 µL of zoospore suspension per well adhered at the surface of cover slips

Table A5_3_1-1c: Test organisms: marine diatom

Criteria	Details
Species	*Skeletonema costatum*
Strain	-
Source	This species is found in coastal fouling communities and is also commonly used in biocide testing (recommended by CCAP, UK).
Laboratory culture	Yes
Stage of life cycle and stage of stadia	Actively growing 5 day old cultures of *Skeletonema costatum* were used for this test.
Method of cultivation	Diatom cultures were maintained in the growth room (18 °C) at TNO/MML in enriched filtered sterilised seawater (EFSW) with silicate enriched F2 growth media.
Pre-treatment	No
Initial density/number of test organisms in the test system	1mL of cell suspension per well

Table A5_3_1-2: Test system

Criteria	Details
Culturing apparatus / test chamber	Polystyrene multiwell plates from Greiner for barnacles. Macro-algae and diatoms were placed on cover slips and inserted in multiwell plates.
Number of vessels / concentration	4 replicates for barnacles and 3 for both macro-algae and diatoms
Test culture media and/or carrier material	Test suspension consisted of seawater and for the diatoms a silicate enriched F2 growth media (EFSW) was additionally used
Nutrient supply	Sterile filtered natural seawater (pH 7.4) from the stock solution
Measuring equipment	Stereo microscope to observe barnacle settlement and bright field microscope to observe settlement of macro-algae and diatoms

Table A5_3_1-3: Application of test substance

Criteria	Details
Application procedure	An aliquot of a solution containing the test substance in aqueous solution (seawater) and DMSO was introduced into the wells
Delivery method	Single application
Dosage rate	0.01, 0.1, 1.0, 10, 100 ppm
Carrier	DMSO was used
Concentration of liquid carrier	0.3 % v/v
Liquid carrier control	Yes
Other procedures	To ensure that the concentrations of the active ingredient were maximised at the start of each assay, fresh suspensions were prepared immediately prior to use.

Table A5_3_1-4: Test conditions

Criteria	Details
Substrate	Barnacles were injected into multiwell plates. Macro-algae and diatoms were placed on sterilised glass cover slips and introduced into the well.
Incubation temperature	27 ± 2 °C for barnacles 18°C for macro-algae and diatoms
Moisture	Not applicable
Aeration	Not stated
Method of exposure	Individual subsamples
Aging of samples	No extra procedure for aging was used. Biological tests were run for 48 h (barnacles), 5d (macro-algae) and 24h (diatoms)
Other conditions	Light conditions were 15:9 light/dark cycle for barnacles and constant illumination for macro-algae and diatoms

Table A5_3_1-5: Relative settlement of barnacle larvae to various concentrations of dichlofluanid

Concentration (µg/mL)	Relative settlement (%) ± STDEV
0.01	96.9 ± 19.5
0.1	98.5± 22.0
1	130 ± 14.5
10	69.1 ± 14.8
100	0

Table A5_3_1-6: Appearance of zoospores after a 5-day germination period and % relative survivorship

Concentration	White cells	Green cells	Relative survivorship (%) ± STDEV
SW+DMSO	-	264	-
0.01 ppm	10	189	99.0 ± 0.6
0.1 ppm	24	179	90.4 ± 0.7
1 ppm	18	168	83.1 ± 9.8
10 ppm	129	-	0
100 ppm	145	-	0

Table A5_3_1-7: Diatom population after 24 hors settlement and growth and % pigmented cells

Concentration (µg/mL)	White cells	Green cells	Percentage pigmented cells (%)
SW+DMSO	-	272	-
0.01 ppm	-	301	100
0.1 ppm	-	216	100
1 ppm	-	93	100
10 ppm	16	-	0
100 ppm	10	-	0

STUDY SUMMARY 2	1 REFERENCE	Official use only
1.1 Reference	Callow, M.E. (2005). Toxicity of Dichlofluanid (Preventol A4S) to algae University of Birmingham., Report No. not specified, 02 November 2005 (unpublished).	
1.2 Data protection	Yes.	
1.2.1 Data owner	International Paint Ltd.	
1.2.2 Companies with letter of access	None.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
	2 METHOD	
2.1 Test Substance (Biocidal Product)	--	
2.1.1 Trade name/ proposed trade name	[redacted]	X
2.1.2 Composition of Product tested	[redacted]	X
2.1.3 Physical state and nature	[redacted]	
2.1.4 Monitoring of active substance concentration	No.	X
2.1.5 Method of analysis	Not applicable.	
2.2 Reference substance	No.	X
2.2.1 Method of analysis for reference substance	Not applicable.	
2.3 Testing procedure	--	
2.3.1 Test population / inoculum / test organism	Unicellular alga *Amphora coffeaeformis* var. *perpusilla*. *Amphora* is a member of the class Bacillariophyceae (diatoms). This is the test organism of choice for measuring the efficacy of biocides used in antifouling paints since it is ubiquitously found on surfaces in the marine environment.	
2.3.2 Test system	A suspension of *Amphora* was adjusted to 0.125 µg chlorophyll/mL. 10 mL of culture was pipetted into glass tubes, plugged with cotton wool and incubated on an illuminated orbital shaker (150 rpm) at 20°C for 24 h.	
2.3.3 Application of TS	The study was initiated by adding 10 µl of Dichlofluanid in DMF to each of the prepared tubes, the tubes shaken and returned to the illuminated orbital shaker for 96 h. Five replicates were set up for each concentration of biocide; controls contained 10µl DMF. The following final concentrations of Dichlofluanid were tested: 0.25, 0.5, 0.75, 1.0, 2.5, 5.0 and 7.5 mg/L. Chlorophyll concentrations were recorded for 5 replicate tubes of culture at time zero i.e. at the time the biocide was added.	
2.3.4 Test conditions	20°C.	
2.3.5 Duration of the test	96 hours.	

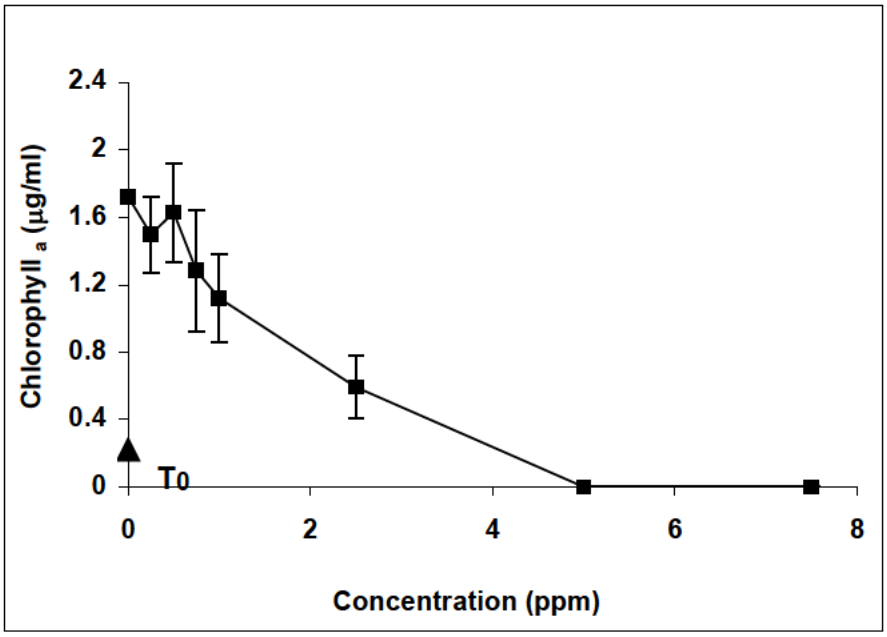
	/ Exposure time		
2.3.6	Number of replicates performed	Five replicates for each test concentration.	
2.3.7	Controls	Yes, 10µL DMF.	
2.4	**Examination**	--	
2.4.1	Effect investigated	Increase in growth (biomass) of cells measured as extracted chlorophyll.	
2.4.2	Method for recording / scoring of the effect	The contents of each tube were filtered through a 3µm pore cellulose nitrate filter with suction. The filter with trapped cells was returned to the assay tube and 5 mL of dimethylsulfoxide (DMSO) added. Growth was measured as chlorophyll *a* after extraction in DMSO (1 h in darkness) using the method of Shoaf & Lium (1976). The absorbance of the DMSO extracts was measured in a spectrophotometer at 630 and 664 nm. Chlorophyll concentration was calculated using the equations of Jeffrey & Humphrey (1975)	
2.4.3	Intervals of examination	One examination at 96 hours.	
2.4.4	Statistics	Mean chlorophyll *a* concentration ± 95% confidence limits was calculated for each concentration of biocide (n=5). Mean percentage inhibition was also calculated; error bars show 95% confidence limits. The data were also analysed by probit analysis using the programme in Minitab version 13.31.	
2.4.5	Post monitoring of the test organism	No.	**X**
		3 RESULTS	
3.1	**Efficacy**	--	
3.1.1	Dose/Efficacy curve	See Figures 1 and 2.	**X**
3.1.2	Begin and duration of effects	See Figures 1 and 2.	**X**
3.1.3	Observed effects in the post monitoring phase	Not recorded.	
3.2	**Effects against organisms or objects to be protected**	Not recorded.	
3.3	**Other effects**	Not applicable.	
3.4	**Efficacy of the reference substance**	Not applicable.	
3.5	**Tabular and/or graphical presentation of the summarised results**	See Figures 1 and 2.	**X**
3.6	**Efficacy limiting factors**	--	
3.6.1	Occurrences of resistances	None stated.	

3.6.2 Other limiting factors	None.	
	4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1 Reasons for laboratory testing	This laboratory study provides important information on the efficacy of the product in terms of growth reduction, and provides valuable data in support of field studies.	
4.1 Intended actual scale of biocide application	This test is representative of some uses of the product.	
4.2 Relevance compared to field conditions		
4.2.1 Application method	Yes, the algae were exposed to concentrations of Dichlofluanid.	
4.2.2 Test organism	The test was carried out using laboratory algae. Algae are the target organism.	
4.2.3 Observed effect	Yes.	
4.3 Relevance for read-across	Yes. The test demonstrates efficacy in terms of growth inhibition which is applicable to both laboratory and field situations.	**X**
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The growth inhibition of the unicellular alga *Amphora coffeaeformis* var. *perpusilla* by Dichlofluanid was investigated. *A. coffeaeformis* var. *perpusilla* was exposed for 96 h to concentrations of 0.25, 0.5, 0.75, 1.0, 2.5, 5.0 and 7.5 mg/L Dichlofluanid.	
5.2 Results and discussion	Following an incubation period of 96 h, Dichlofluanid achieved 50% efficacy at a concentration of 1.97 mg/L and 100% efficacy at 6.35 mg/L (calculated).	**X**
5.3 Conclusion	The 96 hour EC_{50} (based on algal growth inhibition) for *Amphora coffeaeformis* var. *perpusilla*, was 1.97 mg/L.	**X**
5.3.1 Reliability	1.	**X**
5.3.2 Deficiencies	No.	

	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	7/10/2014	
Materials and Methods	The UK CA accepts the Applicant's version, with the following comments. **2.1.1 & 2.1.2** The applicant originally cited this study in support of the product Interspeed Ultra. However, the test substance is not the product but the active substance dichlofluanid. **2.1.4** Although no monitoring of the active substance concentration was conducted, this, in the UK CA's view, is not required in efficacy testing. The UK CA does not, therefore, consider the absence of such data to be an issue. **2.2** Although a reference substance was not used, this, in the UK CA's view, is not required in efficacy testing. The UK CA does not, therefore, consider the absence of a reference substance to be an issue. **2.4.5** Although no post monitoring of the test organisms was conducted, this, in the UK CA's view, is not required in efficacy testing. The UK CA does not, therefore, consider the absence of such monitoring to be an issue. **4.3** As the study investigated the innate efficacy of dichlofluanid in inhibiting algal growth under laboratory conditions, it cannot be read-across to demonstrate the efficacy of the active substance in a formulated product under field conditions. **5.3.1.** The efficacy template does not require the Applicant to state a number for the reliability indicator. Although the study was not conducted to an internationally recognised test standard, the UK CA considers the methodologies used to be acceptable. The UK CA therefore considers the reliability indicator to be 2 (see below).	
Results and discussion	The UK CA accepts the Applicant's version, with the following comments. **3.1.1, 3.1.2., 3.5 & 5.2** The results showed that the 96 hour EC_{50} (based on algal growth inhibition) for dichlofluanid against *A. coffeaeformis* var. *perpusilla* was 1.97 mg dichlofluanid l^{-1}, and that the 96 hour EC_{100} was 6.35 mg dichlofluanid l^{-1}. The UK CA considers the results as demonstrating the innate efficacy of dichlofluanid as an active substance against algae. The UK CA therefore considers the results to be acceptable in support of the approval of the active substance.	
Conclusion	**5.3** The UK CA agrees with the Applicant's conclusion.	
Reliability	2	
Acceptability	The UK CA considers the data to be acceptable in support of active substance approval.	
Remarks	All data and endpoints presented in the study summary have been checked against the original study and are correct.	
	COMMENTS FROM…	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		

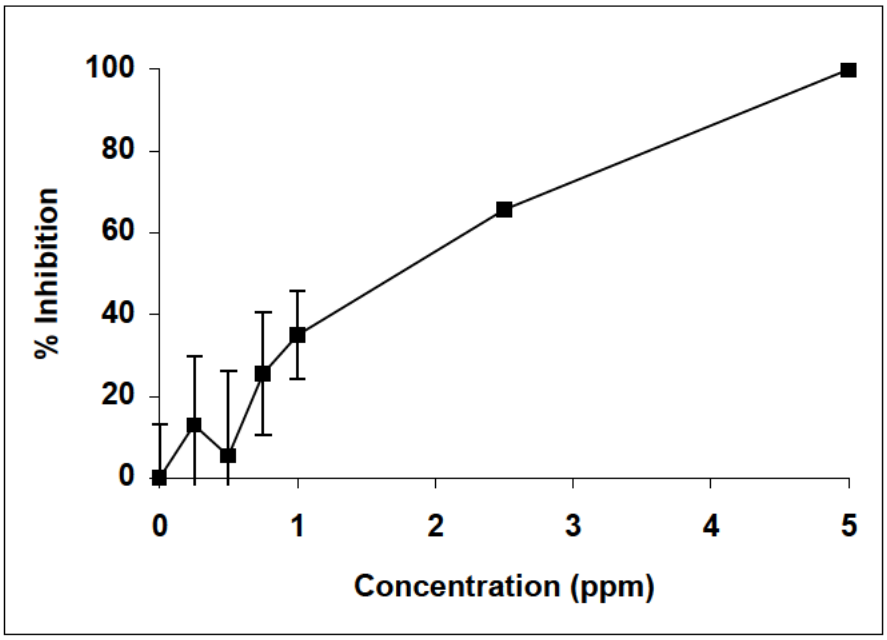
Remarks	

Figure 1: Chlorophyll concentration (µg/ml) of *Amphora* culture after 96 h growth in the presence of various concentrations of Dichlofluanid



The chlorophyll concentration at the time of biocide addition (To) is indicated on the y axis. Points are the mean of 5 replicates; bars show 95% confidence limits.

Figure 2: shows data from Figure 1 plotted as percentage inhibition



Bars are calculated from arcsine transformed data

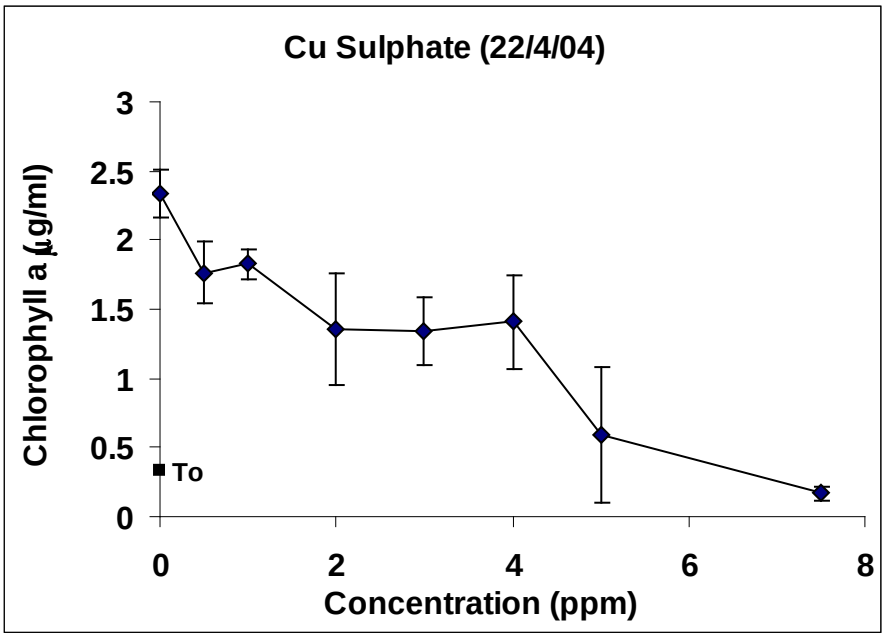
STUDY SUMMARY 3		1 REFERENCE	Official use only
1.1	**Reference**	Callow, M.E. (2005). Toxicity of copper to algae. University of Birmingham, Report No. not specified, 31 October 2005 (unpublished).	
1.2	**Data protection**	Yes.	
1.2.1	Data owner	International Paint Ltd.	
1.2.2	Companies with letter of access	None.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**	--	
2.1.1	Trade name/ proposed trade name	[redacted].	**X**
2.1.2	Composition of Product tested	[redacted].	**X**
2.1.3	Physical state and nature	Not stated.	
2.1.4	Monitoring of active substance concentration	No.	**X**
2.1.5	Method of analysis	Not applicable.	
2.2	**Reference substance**	No.	**X**
2.2.1	Method of analysis for reference substance	Not applicable.	
2.3	**Testing procedure**	--	
2.3.1	Test population / inoculum / test organism	Unicellular alga *Amphora coffeaeformis* var. *perpusilla*. *Amphora* is a member of the class Bacillariophyceae (diatoms). This is the test organism of choice for measuring the efficacy of biocides used in antifouling paints since it is ubiquitously found on surfaces in the marine environment.	
2.3.2	Test system	A suspension of *Amphora* was adjusted to 0.125 µg chlorophyll/mL. 10 mL of culture were pipetted into glass tubes, plugged with cotton wool and incubated on an illuminated orbital shaker (150 rpm) at 20°C for 24 h.	
2.3.3	Application of TS	The study was initiated by the addition of 10 µL of copper sulphate in deionised water to each of the prepared tubes, the tubes shaken and returned to the illuminated orbital shaker for 96 h. Five replicates were set up for each concentration of biocide; controls contained 10µL deionised water. The following final concentrations of copper sulphate were tested: 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, and 10.0 mg/L. Chlorophyll concentrations were recorded for 5 replicate tubes of culture at time zero i.e. at the time the biocide was added.	
2.3.4	Test conditions	20°C.	
2.3.5	Duration of the test	96 hours.	

	/ Exposure time		
2.3.6	Number of replicates performed	Five replicates for each test concentration.	
2.3.7	Controls	Yes, 10µL deionised water only.	
2.4	**Examination**	--	
2.4.1	Effect investigated	Increase in growth (biomass) of cells measured as extracted chlorophyll.	
2.4.2	Method for recording / scoring of the effect	The contents of each tube were filtered through a 3µm pore cellulose nitrate filter with suction. The filter with trapped cells was returned to the assay tube and 5 ml of dimethylsulfoxide (DMSO) added. Growth was measured as chlorophyll *a* after extraction in DMSO (1 h in darkness) using the method of Shoaf & Lium (1976). The absorbance of the DMSO extracts was measured in a spectrophotometer at 630 and 664 nm. Chlorophyll concentration was calculated using the equations of Jeffrey & Humphrey (1975)	
2.4.3	Intervals of examination	One examination at 96 hours.	
2.4.4	Statistics	Mean chlorophyll *a* concentration ± 95% confidence limits was calculated for each concentration of biocide (n=5). Mean percentage inhibition was also calculated; error bars show 95% confidence limits. The data were also analysed by probit analysis using the programme in Minitab version 13.31.	
2.4.5	Post monitoring of the test organism	No.	**X**
		3 RESULTS	
3.1	**Efficacy**	--	
3.1.1	Dose/Efficacy curve	See Figures 1 and 2.	**X**
3.1.2	Begin and duration of effects	See Figures 1 and 2.	**X**
3.1.3	Observed effects in the post monitoring phase	Not recorded.	
3.2	**Effects against organisms or objects to be protected**	Not recorded.	
3.3	**Other effects**	Not applicable.	
3.4	**Efficacy of the reference substance**	Not applicable.	
3.5	**Tabular and/or graphical presentation of the summarised results**	See Figures 1 and 2.	**X**
3.6	**Efficacy limiting factors**		
3.6.1	Occurrences of	None stated.	

	resistances		
3.6.2	Other limiting factors	None.	
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	This laboratory study provides important information on the efficacy of the product in terms of growth reduction, and provides valuable data in support of field studies.	
4.2	**Intended actual scale of biocide application**	This test is representative of some uses of the product.	
4.3	**Relevance compared to field conditions**	--	
4.3.1	Application method	Yes, the algae were exposed to concentrations of copper sulphate.	
4.3.2	Test organism	The test was carried out using laboratory algae. Algae are the target organism.	
4.3.3	Observed effect	Yes.	
4.4	**Relevance for read-across**	Yes. The test demonstrates efficacy in terms of growth inhibition which is applicable to both laboratory and field situations.	**X**
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The growth inhibition of the unicellular alga *Amphora coffeaeformis* var. *perpusilla* by copper sulphate was investigated. *A. coffeaeformis* var. *perpusilla* was exposed for 96 h to concentrations of 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, and 10.0 mg/L copper sulphate.	
5.2	**Results and discussion**	Following an incubation period of 96 h, copper sulphate achieved 50% efficacy at a concentration of 3.46 mg/L and 100% efficacy at 10.33 mg/L (calculated).	**X**
5.3	**Conclusion**	The 96 hour EC_{50} (based on algal growth inhibition) for *Amphora coffeaeformis* var. *perpusilla*, was 3.46 mg/L.	**X**
5.3.1	Reliability	1.	**X**
5.3.2	Deficiencies	No.	

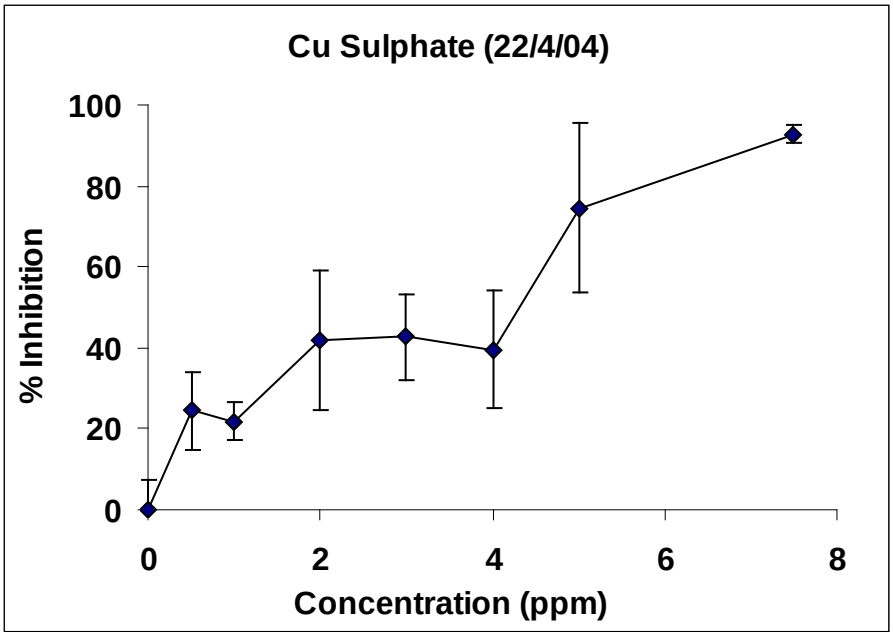
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	7/10/2014	
Materials and Methods	The UK CA accepts the Applicant's version, with the following comments. **2.1.1 & 2.1.2** The applicant originally cited this study in support of the product Interspeed Ultra. However, the test substance is not the product but copper sulphate. **2.1.4** Although no monitoring of the active substance concentration was conducted, this, in the UK CA's view, is not required in efficacy testing. The UK CA does not, therefore, consider the absence of such data to be an issue. **2.2** Although a reference substance was not used, this, in the UK CA's view, is not required in efficacy testing. The UK CA does not, therefore, consider the absence of a reference substance to be an issue. **2.4.5** Although no post monitoring of the test organisms was conducted, this, in the UK CA's view, is not required in efficacy testing. The UK CA does not, therefore, consider the absence of such monitoring to be an issue. **4.4** As the study investigated the innate efficacy of copper in inhibiting algal growth under laboratory conditions, it cannot be read-across to demonstrate the efficacy of copper in a formulated product under field conditions. **5.3.1.** The efficacy template does not require the Applicant to state a number for the reliability indicator. Although the study was not conducted to an internationally recognised test standard, the UK CA considers the methodologies used to be acceptable. The UK CA therefore considers the reliability indicator to be 2 (see below).	
Results and discussion	The UK CA accepts the Applicant's version, with the following comments. **3.1.1, 3.1.2., 3.5 & 5.2** The results showed that the 96 hour EC_{50} (based on algal growth inhibition) for copper against *A. coffeaeformis* var. *perpusilla* was 3.46 mg copper l^{-1}, and that the 96 hour EC_{100} was 10.33 mg copper l^{-1}. The UK CA considers the results as demonstrating the innate efficacy of copper as an active substance against algae. However, as the active substance for which approval is being sought is dichlofluanid, the UK CA does not consider the results to be acceptable in support of the approval of dichlofluanid.	
Conclusion	**5.3** The UK CA agrees with the Applicant's conclusion.	
Reliability	2	
Acceptability	The UK CA considers the data to be unacceptable in support of active substance approval.	
Remarks	All data and endpoints presented in the study summary have been checked against the original study and are correct.	
	COMMENTS FROM…	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Figure 1: Chlorophyll concentration (µg/ml) of *Amphora* culture after 96 h growth in the presence of various concentrations of Cu sulphate



The chlorophyll concentration at the time of biocide addition (To) is indicated on the y axis. Points are the mean of 5 replicates; bars show 95% confidence limits.

Figure 2: shows data from Figure 1 plotted as percentage inhibition



Bars are calculated from arcsine transformed data