Section A5 **Effectiveness against target organisms and intended uses**

Subsection (Annex Point)			Official use only
5.1	**Function (IIA5.1)**	Biocide (Pesticide, non-agricultural): MG02Preservatives *Microbial control: Fungicide, mouldicide, algaecide.*	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)**	*Algae, yeasts, blue stain fungi and moulds.*	
5.2.1	**Organism(s) to be controlled (IIA5.2)**	<u>Typical microbes to be controlled</u> Algae, fungi and yeast including but not exclusively **Algae** Scenedesmus vacuolatus Chlorella pyrenoidosa Scenedesmus obliquus Stichococcus bacillaris Nostoc sp. **Yeasts** Candida albicans Rhodotorula ruba Saccharomyces cerevisiae Sporobolomyces roseus **Blue stain fungi** Aureobasidium pullulans Sydowia polyspora **Moulds** Alternaria alternate Aspergillus niger Aspergillus oryzae Aureobasidium pullulans Chaetomium globosum Cladosporium cladosporoides Cladosporium resinae Fusarium sp. Gliocladium virens Lentinus tigrinus Penicillium funiculosum Penicillium glaucum Penicillinium ochrochloron Phoma sp. Rhizopus stolonifer Sclerophoma pithyophila Trichoderma viride Ulocladium atrum Target organisms exist in all parts of the EU. Therefore, A.S./B.P. will be used in all parts of the Community.	x

Section A5 **Effectiveness against target organisms and intended uses**

5.2.2 Products, organisms or objects to be protected (IIA5.2)

Protection of damp surfaces and wood products from fungal growth in the whole EU. OIT is particularly suitable in formulations for the prevention of surface mould growth on timber, applied by dipping/immersion or as wood preservative by vacuum impregnation and for brush application in stains, varnishes and lasures.

The use concentration in PT8 should not be different in different parts of EU.

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)

Minimum inhibitory concentrations (MIC) of ACTICIDE OTW 8 (Product) and A.S. (OIT)

Names and Strains of Organisms	**Minimum Inhibitory Concentration [ppm]**	
Moulds	**B.P.**	**A.S.**
Alternaria alternata (solid)	19	1.5
Alternaria alternata (liquid)	31	2.5
Aspergillus niger (solid)	8	0.6
Aspergillus niger (liquid)	63	4.0
Fusarium spec.	31	2.5
Penicillium funiculosum (solid)	5	0.4
Penicillium funiculosum (liquid)	63	5.0
Penicillium ochrochloron	4	0.3
Yeasts		
Saccharomyces cerevisiae	19	1.5
Saccharomyces cerevisiae	63	5.0
Rhodotorula rubra	31	2.5

Grabbe R, 2008, report 26990, Grabbe R, 2008, report 26990a

Names and Strains of Organisms	**Minimum Inhibitory Concentration [ppm]**	
Blue stain fungi	**B.P.**	**A.S.**
Aureobasidium pullulans	10	0.8
Sydowia polyspora	31	2.5

Goldbach M 2010, report 28760.

x

Names and Strains of Organisms	Minimum Inhibitory Concentration [ppm]	
Algae	**B.P.**	**A.S.**
Scenedesmus vacuolatus	3.8	0.3
Stichococcus bacillaris	12.5	1.0
Nostoc sp.	6.3	0.5

Grabbe R, 2008, report 26991

5.3.2 Likely concentrations at which the A.S. will be used (IIA5.3)

The MIC values do not reflect the final use concentration due to fact that the MIC value is only the minimum inhibition concentration in sterile growth solutions infected with different strains of organisms. As soon as this concentration falls below this value, infections are most likely. In general MIC values are mainly to be used to compare single actives rather than to be served as use-concentration.
The use concentration in the final product is more dependent on the storage time, storage conditions and losses of the preservative due to infections in raw materials and incompatibility. The precise level required by a specific formulation is determined by the local Thor Microbiological Technical Centre.

Normal use concentrations of A.S. are in the range 40 ppm – 250 ppm A.S. depending on the product to be protected and the environmental conditions to which it will be exposed.

Typical use concentrations in the industrial process:

Dipping/immersion: 250 ppm (PT8)

Vacuum/pressure impregnation: 150 ppm (PT8)

5.4 Mode of action (including time delay) (IIA5.4)

1) Inhibition of growth and metabolism

2) Irreversible cell damage resulting in loss of viability

5.4.1 Mode of action

Electrophilically active microbiocides.

Nucleophilic attack at the activated N-S bound of Isothiazolinones by amino, amido, thiol groups of large molecular systems such as proteins or nucleic acids (PAULUS, 2005).
- Addition to membrane components or intracellular components
- Addition to nucleophiles by cleavage of the N-S-bound resulting in ring-opening/inactivation.

5.4.2 Time delay

Isothiazolones utilizes a two step mechanism involving rapid inhibition (minutes) of growth and metabolism, followed by irreversible cell damage resulting in loss of viability (hours). Cells are inhibited by disruption of the metabolic pathways involving dehydrogenase enzymes. Critical physiological functions are rapidly inhibited in microbes, including growth, respiration (oxygen consumption), and energy generation (ATP synthesis). Cell death results from the destruction of protein thiols and production of free radicals. The rate and extent of killing may be

Section A5		Effectiveness against target organisms and intended uses	
		enhanced by various adjuvants including surfactants. This unique mechanism results in a broad spectrum of activity, low use levels, and difficulty in attaining resistance. (Williams, 2006)	
5.5	**Field of use envisaged (IIA5.5)**		
	MG02: Preservatives	PT06.02 Other in-can preservatives PT07 Film preservatives **PT08 Wood preservative** PT09 Preservatives for fibres, leather, rubber and polymerised material PT10 Masonry preservatives PT11 Preservatives for liquid-cooling and processing systems PT13 Metalworking-fluid preservatives	x
	Further specification		
5.6	**User (IIA5.6)**	*See also Documents II-B and II-C of dossier.*	
	Industrial		
		Technical grade A.S. as described in section III.A.2 is used in the formulation process of B.P. of lower concentration.	
	Professional		
		PT6 PT07 **PT08** PT09 PT10 PT11 PT13	x
		Addition of B.P. to materials to be preserved or to cooling or processing liquids is by professionals only.	
	General public		
		OIT and its B.P.s in PTs 8 are not intended for use by the general public. The general public can only be exposed to preserved materials.	
	Industrial		
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)**	For industrial preservation using OIT resistance is not an issue. For all kinds of preservation with OIT-containing products, cases of resistance are not reported or known up to the time being. The mode of action of A.S. is quite non-specific (see above) both with respect to microbes as well as regarding the target molecules on cell surface or within a cell. This multiple attack mode precludes the possibility for organisms to develop mechanisms that can be passed on to future generations in the form of "resistance". Reference: Roden K, 1999; Rees R, 2006. Isothiazolinones and antibiotics do not share common behaviour and properties in their respective activity and in the resistance mechanisms developed by target organisms.	
5.7.1	**Development of resistance**	Not applicable. If the correct (effective/recommended) amount of biocide is used from the beginning, and the manufacturing	x

Section A5		Effectiveness against target organisms and intended uses
		conditions and equipment are not highly contaminated, then acquired resistance or tolerance cannot occur, since the organisms will be killed before they can adapt or acquire resistance. For industrial/professional preservation using OIT cases of resistance are not reported or known up to the time being.
5.7.2	**Management strategies**	Under instances where the biocide is physically or chemically unstable in a system (or partitioned into any non-aqueous phase), then bacteria may grow and reproduce in the system and mimic "resistance" or tolerance. Therefore, it is essential that biocide suitability in the system should be assessed from the beginning. The Biocide manufacturer's Technical Department should be able to assist with this aspect. If tolerance is detected it is important to couple a clean-out (i.e. biofilm replacement) together with a biocide level elevation - using same biocide as before! The source of contamination must also be identified and eliminated.
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	For information on annual tonnage of A.S. placed on the market see "Confidential DocIII-A 2.10 (PT08)" in the confidential attachment. Cross reference to Doc II-B.

Section A5 **Effectiveness against target organisms and intended uses**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	19/07/11
Materials and methods	N/A
Conclusion	N/A
Reliability	N/A
Acceptability	The Applicant's version is considered acceptable in support of active substance approval.
Remarks	**5.2.1** This list of target organisms includes those for use in PT 6 and PT13 as well as those for PT 8. Only blue stain fungi and moulds are relevant to use in PT 8. **5.3.1** The MIC values for OIT reported from the studies carried out by Grabbe were generated from tests using a formulated product rather than the active substance itself (data have been provided to demonstrate that the other components of the formulation do not affect the efficacy), with the OIT values being calculated from the concentration in the formulation. **5.5** Only use in PT 8 is considered in this document. **5.6** OIT is to be used as an active substance in the pre-treatment of timber (i.e. industrial use). No professional use of OIT in PT 8 is intended. **5.7.1** Correct application of the product and maintenance of the equipment does not prevent resistance mutations occurring, but will decrease the chances of resistance developing. The non-specific mode of action also means that resistance is unlikely to develop.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 5.3: Summary table of experimental data on the effectiveness of the active substance against target organisms at different fields of use envisaged, where applicable

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*
ACTICIDE®OTW 8 [redacted]	**Moulds** *Alternaria alternata (solid)* *Alternaria alternata (liquid)* *Aspergillus niger (solid)* *Aspergillus niger (liquid)* *Fusarium spec.* *Penicillium funiculosum (solid)* *Penicillium funiculosum (liquid)* *Penicillium ochrochloron* **Yeasts** *Saccharomyces cerevisiae* *Saccharomyces cerevisiae* *Rhodotorula rubra*	Thor Microbiological Test Method 711 Minimum Inhibitory Concentrations against Moulds, Yeasts and Bacteria	Standard growth medium for MIC testing: Serial dilution of ACTICIDE®OTW 8 were incubated with Inoculum ([redacted]) in liquid culture medium at 25°C +/- 2°C for 3 (yeasts) and 7 (moulds) days.	*Moulds:* *The MIC values of the tested moulds against ACTICIDE®OTW 8 were in a concentration range from 4 to 63 ppm.* *Yeasts:* The MIC values of the tested yeasts against ACTICIDE®OTW 8 were in a concentration range from 19 to 63 ppm.	Grabbe R, 2008 report 26990
ACTICIDE®OTW 8-vehicle	Alternaria alternate (solid)	Thor Microbiological Test Method 711 Minimum Inhibitory Concentrations against Moulds, Yeasts and Bacteria	Standard growth medium for MIC testing: Serial dilution of ACTICIDE®OTW 8-vehicle were incubated with Inoculum ([redacted]) in liquid culture medium at 25°C +/- 2°C for 3 (yeasts) and 7 (moulds) days.	The vehicle control produced no inhibitory effect against alternaria alternate at concentrations up to 50 ppm. The test data demonstrates that MIC values stated for ACTICIDE® OTW 8	Grabbe R, 2008 report 26990a

				were due to the action of the active substance itself and not to the other components of the biocidal product.	
ACTICIDE®OTW 8 [redacted]	**Algae** *Scenedesmus vacuolatus,* *Stichococcus bacillaris,* *Nostoc sp.*	Test Method 711 (Algae)	Standard growth medium for MIC testing: Serial dilution of ACTICIDE®OTW 8 were incubated with Inoculum ([redacted]) in liquid culture medium [redacted] at 19°C +/- 1°C for 14-28 (algae) days.	The MICs of the tested algae against ACTICIDE®OTW 8 were in a concentration range from 4 to 13 ppm.	Grabbe R, 2008 report 26991
ACTICIDE®OTW 8 [redacted]	**Blue Stain Fungi** *Aureobasidium pullulans,* *Sydowia polyspora*	Thor Microbiological Test Method 711 Minimum Inhibitory Concentrations against Moulds, Yeasts and Bacteria	Standard growth medium for MIC testing: Serial dilution of ACTICIDE®OTW 8 or vehicle were incubated with Inoculum ([redacted]) in liquid culture medium at 25°C +/- 2°C for 96 h.	The vehicle control produced no inhibitory effect against alternaria alternate at concentrations up to 50 ppm. The test data demonstrates that MIC values stated for ACTICIDE® OTW 8 were due to the action of the active substance itself and not to the other components of the biocidal product. MICs were found in the range of 10 – 31 ppm ACTICIDE® OTW 8 corresponding to 0.8 – 2.5 ppm OIT.	Goldbach M 2010, report 28760

* References: Refer to main reference list for full details.

		Efficacy Data ***Minimum Inhibitory Concentrations*** against Moulds and Yeasts	
Doc III-A: 1		**1 REFERENCE**	**Official use only**
1.1	**Reference**	Grabbe R, 2008, Evaluation of Minimum Inhibitory Concentrations (MIC) for ACTICIDE®OTW 8 against Moulds and Yeasts, [redacted], unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	THOR GmbH, Germany	
1.2.2		n.a.	
1.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.	
1.3	**Guideline study**	THOR Microbiological Test Method 711	
1.4	**Deviations**	No	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**		
2.1.1	Trade name/ proposed trade name	ACTICIDE OTW 8	
2.1.2	Composition of Product tested	[redacted]	X
2.1.3	Physical state and nature	Aqueous dispersion	
2.1.4	Monitoring of active substance concentration	No – nominal concentration, only.	X
2.1.5	Method of analysis	-	
2.2	**Reference substance**	No	X
2.2.1	Method of analysis for reference substance	-	
2.3	**Testing procedure**	Non-entry field	
2.3.1	Test population / inoculum / test organism	see Table 1.1 or see Table 1.2	
2.3.2	Test system	see Table 1.3	
2.3.3	Application of TS	Give relevant details in tabular form (see Table 1.4)	
2.3.4	Test conditions	Give relevant test conditions in tabular form (see Table 1.5)	

		Efficacy Data ***Minimum Inhibitory Concentrations*** against Moulds and Yeasts	
2.3.5	Duration of the test / Exposure time	yeasts assessment after 3 days moulds assessment after 7 days	
2.3.6	Number of replicates performed	1 replicate was carried out. As the MIC value is taken from a dilution series this is considered to be sufficient.	X
2.3.7	Controls	none	X
2.4	**Examination**	Non-entry field	
2.4.1	Effect investigated	growth	
2.4.2	Method for recording / scoring of the effect	In the case of yeasts any growth giving a visible turbidity or cloudiness, however slight, is recorded as +. Some fungal spores may initially germinate but then be inhibited. In such instances slight cloudiness of the growth medium may be observed but an assessment of 0 should be made nonetheless.	
2.4.3	Intervals of examination		X
2.4.4	Statistics	none	X
2.4.5	Post monitoring of the test organism	no	X
		3 RESULTS	
3.1	**Efficacy**		
3.1.1	Dose/Efficacy curve	The MIC value is the lowest concentration of biocide inhibiting the test organism in both tubes. Streaking out onto agar plates can be used for determining the MMC (Minimum Microbicidal Concentration).	
3.1.2	Begin and duration of effects	Not evaluated	
3.1.3	Observed effects in the post monitoring phase	Not applicable	
3.2	**Effects against organisms or objects to be protected**	Not applicable	
3.3	**Other effects**	-	
3.4	**Efficacy of the reference substance**	Blank sample (not preserved) showed growth.	X

3.5	**Tabular and/or graphical presentation of the summarised results**	**Names and Strains of Organisms** — **Minimum Inhibitory Concentration [ppm]** **Moulds** *Alternaria alternate* [redacted] (solid) — 19 *Alternaria alternata* [redacted] (liquid) — 31 *Aspergillus niger* [redacted] (solid) — 8 *Aspergillus niger* [redacted] (liquid) — 63 *Fusarium spec.* — 31 *Penicillium funiculosum* [redacted] (solid) — 5 *Penicillium funiculosum* [redacted] (liquid) — 63 *Penicillium ochrochloron* [redacted] (solid) — 4 **Yeasts** *Saccharomyces cerevisiae* [redacted] (solid) — 19 *Saccharomyces* [redacted] (liquid) — 63 *Rhodotorula rubra* [redacted] (Solid) — 31	X
3.6	**Efficacy limiting factors**	*Non-entry field*	
3.6.1	Occurrences of resistances	*Include observations of the test; refer to data on active substance*	X
3.6.2	Other limiting factors	*e.g. from observations on physico-chemical properties*	X
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	In general MIC values are mainly to be used to compare single actives rather than to be served as use-concentration. The use concentration in the final product is more dependent on the storage time, storage conditions and losses of the preservative due to infections in raw materials and incompatibility. The precise level required by a specific formulation is determined by the local Thor Microbiological Technical Centre. Normal use concentrations of A.S. are in the range 40 ppm – 250 ppm A.S. depending on the product to be protected and the environmental conditions to which it will be exposed. Wet state preservation: 40 – 100 ppm Film preservation: 100 – 250 ppm	
4.2	**Intended actual scale of biocide application**	ditto	
4.3	**Relevance compared to field conditions**	*non-entry field*	

4.3.1	Application method	The application method is relevant with regard to field conditions. But stability of the biocide under field conditions is very different.	
4.3.2	Test organism	Relevant.	
4.3.3	Observed effect	Growth is the relevant effect. However, in vitro test conditions are very different to field conditions regarding to stability so that under field conditions higher use concentrations are necessary.	
4.4	**Relevance for read-across**	See 4.1	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Determination of Minimum Inhibitory Concentrations (MIC) according to THOR Microbiological Test Method 711 using relevant yeast and moulds strains.	
5.2	**Reliability**	2	X
5.3	**Assessment of efficacy, data analysis and interpretation**	The MIC values do not reflect the normal use concentration due to fact that the MIC value is only the minimum inhibition concentration in sterile growth solutions infected with different strains of organisms. As soon as this concentration falls below this value, infections are most likely. In general MIC values are mainly to be used to compare single actives rather than to be served as use-concentration. The use concentration in the final product is more dependent on the storage time, storage conditions and losses of the preservative due to infections in raw materials and incompatibility. The precise level required by a specific formulation is determined by the local Thor Microbiological Technical Centre.	
5.4	**Conclusion**	Moulds: The MIC values of the tested moulds against ACTICIDE®OTW 8 were in a concentration range from 4 to 63 ppm. Yeasts: The MIC values of the tested yeasts against ACTICIDE®OTW 8 were in a concentration range from 19 to 63 ppm.	X
5.5	**Proposed efficacy specification**	Under the applied conditions **ACTICIDE®OTW 8** is an effective biocide against the tested micro-organisms in concentrations of 4 to 63 ppm.	X

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	
Materials and methods	The UK CA accepts the Applicant's version, with the following comments. **2.1.2** This study was carried out using a formulated product, Acticide OTW 8, rather than a simple dilution of the active substance. Apart from OIT and water, the formulation contained 4.1 % of other ingredients, which is too much to be considered insignificant. Therefore the Applicant provided an addendum to this test report (Doc III-A: 2), in which the formulation without the active substance was tested, to demonstrate that the results obtained were not due to these other substances. **2.1.4** As monitoring of the active substance concentration is not required, the UK CA does not consider this to be a significant omission. **2.2** A reference substance is not required, so the UK CA does not consider this to be a significant omission. **2.3.6** Only 1 replicate was carried out. Although this reduces the usefulness of the study, it still provides evidence of the innate activity of the active substance. **2.3.7** Although the RSS states that there were no controls, a control was in fact carried out, as a test was conducted using no OIT (0 ppm). **2.4.3** Table 1.5 indicates that the yeasts were examined at 48 and 72 h, and the moulds at 72 and 96 h. **2.4.4** As statistics are not required, the UK CA does not consider this to be a significant omission. **2.4.5** As post monitoring of the test organism is not required, the UK CA does not consider this to be a significant omission. **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 methodology used to be acceptable. The UK CA therefore considers the reliability indicator to be 2 (see below).
Results and discussion	**3.4** The blank samples mentioned in this section were the control plates (0 ppm). **3.5** The MIC results presented in this section refer to ppm of Acticide OTW 8, and not ppm OIT. The results expressed as ppm OIT (calculated from the concentration in the formulation) are:

Names and Strains of Organisms	**MIC [ppm OIT]**	**MIC [mg l^{-1} OIT]**
Moulds		
Alternaria alternata [redacted] (solid)	1.5	1.5
Alternaria alternata [redacted] (liquid)	2.5	2.5
Aspergillus niger [redacted] (solid)	0.6	0.6
Aspergillus niger [redacted] (liquid)	5.0	5.0
Fusarium spec.	2.5	2.5
Penicillium funiculosum [redacted] (solid)	0.4	0.4
Penicillium funiculosum [redacted] (liquid)	5.0	5.0

Evaluation by Competent Authorities			
	Penicillium ochrochloron [redacted] (solid)	0.3	0.3
	Yeasts		
	Saccharomyces cerevisiae [redacted] (solid)	1.5	1.5
	Saccharomyces cerevisiae [redacted] (liquid)	5	5
	Rhodotorula rubra [redacted] (Solid)	2.5	2.5
	3.6.1 The Applicant has left this section blank. As this test is using standard strains of fungi, these should be susceptible strains.		
	3.6.2 No other limiting factors were reported.		
Conclusion	**5.4** The results presented here are expressed in terms of ppm Acticide OTW 8 rather than ppm OIT. The values correspond to 0.3 - 5.0 ppm OIT for moulds and 1.5 - 5 ppm OIT for yeasts.		
	5.5 The values presented here are expressed in terms of ppm Acticide OTW 8 rather than ppm OIT. They correspond to 0.3 - 5.0 ppm OIT.		
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)
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Tables for Method

1.1 (single) Population / Inoculum

Criteria	**Details**
Nature	
Origin	*details, e.g. on sampling site are given in table 1.2*
Initial biomass	*No data*
Reference of methods	Determine the total count of suspensions thus prepared using a haemocytometer or other type of counting chamber.
Collection / storage of samples	*Not applicable.*
Preparation of inoculum for exposure	*micro-organism cultures are pure*
Pretreatment	*e.g. pre-incubation, adaptation procedure*
Initial density of test population in the test system	*The concentration of the suspension was for each micro-organism* [redacted]

1.2 Test organism (if applicable)

Names and Strains of Organisms	**National Collection* Number**
Moulds	
Alternaria alternata	[redacted]
Aspergillus niger	[redacted]
Fusarium spec.	[redacted]
Penicillium funiculosum	[redacted]
Penicillium funiculosum	[redacted]
Penicillium ochrochloron	[redacted]
Yeast	
Saccharomyces cerevisiae	[redacted]
Rhodotorula rubra	[redacted]

* [redacted]

1.3 Test system

Criteria	**Details**
Culturing apparatus / test chamber	[redacted]
Number of vessels / concentration	*2*
Test culture media and/or carrier material	[redacted]
Nutrient supply	*Complete medium*
Measuring equipment	*Haemocytometer or other counting chamber*

1.4 Application of test substance

Criteria	**Details**
Application procedure	aqueous solutions of the biocide/s under test at three times the required final concentration
Delivery method	
Dosage rate	
Carrier	*water*
Concentration of liquid carrier	*Not applicable*
Liquid carrier control	*Not applicable*
Other procedures	-

1.5 Test conditions

Criteria	**Details**
Substrate	[redacted]
Incubation temperature	Incubate at 25 °C +/- 2 °C or other appropriate temperature:-
Moisture	-
Aeration	-
Method of exposure	*Addition in culture medium*
Aging of samples	-
Other conditions	

		Efficacy Data Minimum Inhibitory Concentrations – vehicle control	
Doc III-A: 2		**1 REFERENCE**	**Official use only**
1.1	**Reference**	Grabbe R, 2008, Addendum (vehicle control) to Evaluation of Minimum Inhibitory Concentrations (MIC) for ACTICIDE®OTW 8 against Moulds and Yeasts, [redacted], unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	THOR GmbH, Germany	
1.2.2		n.a.	
1.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.	
1.3	**Guideline study**	THOR Microbiological Test Method 711 (Bacteria, Moulds and Yeasts)	
1.4	**Deviations**	None	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**		
2.1.1	Trade name/ proposed trade name	ACTICIDE OTW 8-vehicle in which the content of OIT is replaced with water.	X
2.1.2	Composition of Product tested	[redacted]	
2.1.3	Physical state and nature	Aqueous dispersion	
2.1.4	Monitoring of active substance concentration	No.	X
2.1.5	Method of analysis	-	
2.2	**Reference substance**	*No*	X
2.2.1	Method of analysis for reference substance	-	
2.3	**Testing procedure**	Non-entry field	
2.3.1	Test population / inoculum / test organism	see Table 1.1 or see Table 1.2	
2.3.2	Test system	see Table 1.3	
2.3.3	Application of TS	0, 4, 8, 16, 25, 50 ppm ACTICIDE OTW 8-vehicle (see Table 1.4)	X
2.3.4	Test conditions	Give relevant test conditions in tabular form (see Table 1.5)	
2.3.5	Duration of the test /	moulds assessment after 3 days	

		Efficacy Data Minimum Inhibitory Concentrations – vehicle control	
	Exposure time		
2.3.6	Number of replicates performed	1 replicate was carries out. As the MIC value is taken from a dilution series this is considered to be sufficient.	X
2.3.7	Controls	none	X
2.4	**Examination**	Non-entry field	
2.4.1	Effect investigated	growth	
2.4.2	Method for recording / scoring of the effect	In the case of yeasts any growth giving a visible turbidity or cloudiness, however slight, is recorded as +. Some fungal spores may initially germinate but then be inhibited. In such instances slight cloudiness of the growth medium may be observed but an assessment of 0 should be made nonetheless.	
2.4.3	Intervals of examination		X
2.4.4	Statistics	none	X
2.4.5	Post monitoring of the test organism	no	X
		3 RESULTS	
3.1	**Efficacy**		
3.1.1	Dose/Efficacy curve	The MIC value is the lowest concentration of biocide inhibiting the test organism in both tubes. Streaking out onto agar plates can be used for determining the MMC (Minimum Microbicidal Concentration). No inhibitory effect towards the microorganism is observed up to an addition level of 50 ppm (ACTICIDE OTW 8-vehicle).	
3.1.2	Begin and duration of effects	Not evaluated	
3.1.3	Observed effects in the post monitoring phase	Not applicable	
3.2	**Effects against organisms or objects to be protected**	Not applicable	
3.3	**Other effects**	-	
3.4	**Efficacy of the reference substance**	Not applicable	
3.5	**Tabular and/or graphical presentation of the summarised results**	**Names and Strains of Organisms** / **Minimum Inhibitory Concentration [ppm]** **Moulds** Alternaria alternata — Not found up to 50 ppm ACTICIDE OTW 8-vehicle	
3.6	**Efficacy limiting factors**	Non-entry field	
3.6.1	Occurrences of resistances	none	X

		Efficacy Data Minimum Inhibitory Concentrations – vehicle control	
3.6.2	Other limiting factors	none	X
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	The MIC data having its source from testing a B.P. do support the innate efficacy of the A.S. itself because ACTICIDE OTW 8 does contain OIT as single biocide active substance. This test proofs for the efficacy of the vehicle in the range of the B.P. level applied.	
4.2	**Intended actual scale of biocide application**	ditto	
4.3	**Relevance compared to field conditions**	non-entry field	
4.3.1	Application method	The application method is relevant with regard to field conditions. But stability of the biocide under field conditions is very different.	
4.3.2	Test organism	Relevant.	
4.3.3	Observed effect	Growth is the relevant effect. However, in vitro test conditions are very different to field conditions regarding to stability so that under field conditions higher use concentrations are necessary.	
4.4	**Relevance for read-across**	See 4.1	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Determination of Minimum Inhibitory Concentrations (MIC) according to THOR Microbiological Test Method 711 (Bacteria, Moulds and Yeasts) using a relevant Mould species.	X
5.2	**Reliability**	1	X
5.3	**Assessment of efficacy, data analysis and interpretation**	Not applicable	
5.4	**Conclusion**	The vehicle control produced no inhibitory effect towards *Alternaria alternatria* at concentrations up to 50 ppm (ACTICIDE OTW 8-vehicle).	X
5.5	**Proposed efficacy specification**	Under the applied conditions **ACTICIDE®OTW 8-vehicle** is non-effective against microorganisms.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	
Materials and methods	The UK CA accepts the Applicant's version, with the following comments. **2.1.1** This test was carried out as an addition to the previous study (Grabbe, R. 2008, test report number 26990), in order to demonstrate that the other components of the formulation tested were not responsible for the effects noted. This is why the test material in this study does not contain any active substance. **2.1.4** As monitoring of the active substance concentration is not required, the UK CA does not consider this to be a significant omission. **2.2** A reference substance is not required, so the UK CA does not consider this to be a significant omission. **2.3.3** While this study was conducted using concentrations up to 50 ppm Acticide OTW 8-vehicle, the previous study also tested at one higher concentration (63 ppm). **2.3.6** Only 1 replicate was carried out. Although this reduces the usefulness of the study, it still provides evidence of the innate activity of the active substance. **2.3.7** Although the RSS states that there were no controls, a control was in fact carried out, as a test was conducted using 0 ppm of the test solution. **2.4.3** Table 1.5 indicates that the samples will have been examined at 72 and 96 h. **2.4.4** As statistics are not required, the UK CA does not consider this to be a significant omission. **2.4.5** As post monitoring of the test organism is not required, the UK CA does not consider this to be a significant omission. **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	**3.6.1** The Applicant has left this section blank. As this test used a standard strain of fungus, this would have been a susceptible strain. **3.6.2** No other limiting factors were reported.
Conclusion	**5.1** The Applicant has stated that both trials were carried out according to THOR Microbiological Test Method 711, however there are differences in how the trial has been conducted in each case. In test report 26990, it is stated that the incubation time for moulds was 7 days, but in this test report (26990a), the incubation time is given as 3 days. The UK CA does not consider the lack of reporting of the water control data to be an issue. **5.4** This section should say "*Alternaria alternata*" instead of "*Alternaria alternatria*". The conclusion that "no inhibitory effect" was produced cannot be drawn from the results, as the data recorded were whether or not the sample was cloudy due to microbial growth, and not the degree of cloudiness. Therefore there is the potential for the test material to cause a reduction in growth without causing complete inhibition. However, what the data do show is that the highest concentration of test material tested (50 ppm) did not completely inhibit the growth of *A. alternata*, while the full Acticide OTW 8 caused complete inhibition at 19 ppm of the test solution (equivalent to 1.5 ppm OIT). Therefore the innate activity of the OIT in the

	formulation has been demonstrated.
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)
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Tables for Method

1.1 (single) Population / Inoculum

Criteria	Details
Nature	
Origin	*details, e.g. on sampling site are given in table 1.2*
Initial biomass	*No data*
Reference of methods	Determine the total count of suspensions thus prepared using a haemocytometer or other type of counting chamber.
Collection / storage of samples	*Not applicable.*
Preparation of inoculum for exposure	*micro-organism cultures are pure*
Pretreatment	*e.g. pre-incubation, adaptation procedure*
Initial density of test population in the test system	*The concentration of the suspension was for each micro-organism:* [redacted]

1.2 Test organism (if applicable)

Names and Strains of Organisms	National Collection* Number
Moulds	
Alternaria alternata	[redacted]

*
[redacted]

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	[redacted]
Number of vessels / concentration	2
Test culture media and/or carrier material	[redacted]
Nutrient supply	*Complete medium*
Measuring equipment	*Haemocytometer or other counting chamber*

1.4 Application of test substance

Criteria	Details
Application procedure	aqueous solutions of the biocide-vehicle under test at three times the required final concentration
Delivery method	
Dosage rate	
Carrier	*water*
Concentration of liquid carrier	*0, 4, 8, 16, 25, 50 ppm*
Liquid carrier control	*Not applicable*
Other procedures	-

1.5 Test conditions

Criteria	Details
Substrate	[redacted]
Incubation temperature	Incubate at 25 °C +/- 2 °C or other appropriate temperature:-
Moisture	-
Aeration	-
Method of exposure	*Addition in culture medium*
Aging of samples	-
Other conditions	

		Efficacy Data *Minimum Inhibitory Concentrations against Algae*	
Doc III-A: 3		**1 REFERENCE**	**Official use only**
1.1	**Reference**	Grabbe R, 2008, Evaluation of Minimum Inhibitory Concentrations (MIC) for ACTICIDE®OTW 8 against Algae, [redacted], unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	THOR GmbH, Germany	
1.2.2		n.a.	
1.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.	
1.3	**Guideline study**	THOR Microbiological Test Method 711 (Algae)	
1.4	**Deviations**	No	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**		
2.1.1	Trade name/ proposed trade name	ACTICIDE OTW 8	
2.1.2	Composition of Product tested	[redacted]	X
2.1.3	Physical state and nature	Aqueous dispersion	
2.1.4	Monitoring of active substance concentration	No – nominal concentration, only.	X
2.1.5	Method of analysis	-	
2.2	**Reference substance**	No	X
2.2.1	Method of analysis for reference substance	-	
2.3	**Testing procedure**	Non-entry field	
2.3.1	Test population / inoculum / test organism	see Table 1.1 or see Table 1.2	
2.3.2	Test system	see Table 1.3	
2.3.3	Application of TS	Give relevant details in tabular form (see Table 1.4)	
2.3.4	Test conditions	Give relevant test conditions in tabular form (see Table 1.5)	

		Efficacy Data *Minimum Inhibitory Concentrations against Algae*	
2.3.5	Duration of the test / Exposure time	algae assessment after 14 and 28 days	
2.3.6	Number of replicates performed	1 replicate was carries out. As the MIC value is taken from a dilution series this is considered to be sufficient.	X
2.3.7	Controls	none	X
2.4	**Examination**	Non-entry field	
2.4.1	Effect investigated	growth	
2.4.2	Method for recording / scoring of the effect	Any growth giving visible or green colour, however slight, is recorded as "+".	
2.4.3	Intervals of examination		X
2.4.4	Statistics	none	X
2.4.5	Post monitoring of the test organism	no	X
		3 RESULTS	
3.1	**Efficacy**		
3.1.1	Dose/Efficacy curve	The MIC value is the lowest concentration of biocide inhibiting the test organism in both tubes. Streaking out onto agar plates can be used for determining the MMC (Minimum Microbicidal Concentration).	
3.1.2	Begin and duration of effects	Not evaluated	
3.1.3	Observed effects in the post monitoring phase	Not applicable	
3.2	**Effects against organisms or objects to be protected**	Not applicable	
3.3	**Other effects**	-	
3.4	**Efficacy of the reference substance**	Not applicable	
3.5	**Tabular and/or graphical presentation of the summarised results**	**Names and Strains of Organisms** / **Minimum Inhibitory Concentration [ppm]** **Algae** *Scenedesmus vacuolatus* [redacted] — 3.8 *Stichococcus bacillaris* [redacted] — 12.5 *Nostoc sp.* [redacted] — 6.3	X
3.6	**Efficacy limiting factors**	*Non-entry field*	

3.6.1	Occurrences of resistances	*Include observations of the test; refer to data on active substance*	X
3.6.2	Other limiting factors	*e.g. from observations on physico-chemical properties*	X
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	In general MIC values are mainly to be used to compare single actives rather than to be served as use-concentration. The use concentration in the final product is more dependent on the storage time, storage conditions and losses of the preservative due to infections in raw materials and incompatibility. The precise level required by a specific formulation is determined by the local Thor Microbiological Technical Centre. Normal use concentrations of A.S. are in the range 40 ppm – 250 ppm A.S. depending on the product to be protected and the environmental conditions to which it will be exposed. Wet state preservation: 40 – 100 ppm Film preservation: 100 – 250 ppm	
4.2	**Intended actual scale of biocide application**	ditto	
4.3	**Relevance compared to field conditions**	*Non-entry field*	
4.3.1	Application method	The application method is relevant with regard to field conditions. But stability of the biocide under field conditions is very different.	
4.3.2	Test organism	Relevant.	
4.3.3	Observed effect	Growth is the relevant effect. However, in vitro test conditions are very different to field conditions regarding to stability so that under field conditions higher use concentrations are necessary.	
4.4	**Relevance for read-across**	See 4.1	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Determination of Minimum Inhibitory Concentrations (MIC) according to THOR Microbiological Test Method 711 (Algae) using relevant Algae species.	
5.2	**Reliability**	2	X
5.3	**Assessment of efficacy, data analysis and interpretation**	The MIC values do not reflect the normal use concentration due to fact that the MIC value is only the minimum inhibition concentration in sterile growth solutions infected with different strains of organisms. As soon as this concentration falls below this value, infections are most likely. In general MIC values are mainly to be used to compare single actives rather than to be served as use-concentration. The use concentration in the final product is more dependent on the storage time, storage conditions and losses of the preservative due to infections in raw materials and incompatibility. The precise level required by a specific formulation is determined by the local Thor Microbiological Technical Centre.	

5.4	**Conclusion**	The MICs of the tested algae against ACTICIDE®OTW 8 were in a concentration range from 4 to 13 ppm.	X
5.5	**Proposed efficacy specification**	Under the applied conditions **ACTICIDE®OTW 8** is an effective biocide against the tested micro-organisms in concentrations of 4 to 13 ppm.	X

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	
Materials and methods	The UK CA accepts the Applicant's version, with the following comments. **2.1.2** This study was carried out using a formulated product, Acticide OTW 8, rather than a simple dilution of the active substance. Apart from OIT and water, the formulation contained 4.1 % of other ingredients, which is too much to be considered insignificant. The Applicant addressed this by providing an addendum to the test report on moulds and fungi (Grabbe, R. 2008, test report number 26990a), in which the formulation without the active substance was tested, to demonstrate that the results obtained were not due to these other substances. However, this read across only compared the activity against fungi, and no data have been provided on the activity of the base formulation against algae. This reduces the usefulness of the study. **2.1.4** As monitoring of the active substance concentration is not required, the UK CA does not consider this to be a significant omission. **2.2** A reference substance is not required, so the UK CA does not consider this to be a significant omission. **2.3.6** Only 1 replicate was carried out. Although this reduces the usefulness of the study, it still provides evidence of the innate activity of the active substance. **2.3.7** Although the RSS states that there were no controls, a control was in fact carried out, as a test was conducted using no OIT (0 ppm). **2.4.3** Examinations were carried out at 14, 21 and 18 d. **2.4.4** As statistics are not required, the UK CA does not consider this to be a significant omission. **2.4.5** As post monitoring of the test organism is not required, the UK CA does not consider this to be a significant omission. **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 methodology used to be acceptable. The UK CA therefore considers the reliability indicator to be 2 (see below).
Results and discussion	**3.5** The MIC results presented in this section refer to ppm of Acticide OTW 8, and not ppm OIT. The results expressed as ppm OIT (calculated from the concentration in the formulation) are: **Names and Strains of Organisms** — **Minimum Inhibitory Concentration [ppm OIT]** *Scenedesmus vacuolatus* [redacted] — 0.3 *Stichococcus bacillaris* [redacted] — 1.0 *Nostoc sp.* [redacted] — 0.5 **3.6.1** The Applicant has left this section blank. As this test used standard strains of algae, these would have been susceptible strains. **3.6.2** No other limiting factors were reported.

Evaluation by Competent Authorities	
Conclusion	**5.4** The results presented here are expressed in terms of ppm Acticide OTW 8 rather than ppm OIT. The values correspond to 0.3 - 1.0 ppm OIT. **5.5** The values presented here are expressed in terms of ppm Acticide OTW 8 rather than ppm OIT. They correspond to 0.3 - 1.0 ppm OIT.
Reliability	**2**
Acceptability	The UK CA considers that these data are acceptable to support use against algae for 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)
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

5.6 Tables for Method

1.1 (single) Population / Inoculum

Criteria	Details
Nature	
Origin	*details, e.g. on sampling site are given in table 1.2*
Initial biomass	*No data*
Reference of methods	*Determine the total count of suspensions thus prepared using a haemocytometer or other type of counting chamber*.
Collection / storage of samples	*Not applicable.*
Preparation of inoculum for exposure	*micro-organism cultures are pure*
Pretreatment	*e.g. pre-incubation, adaptation procedure*
Initial density of test population in the test system	*The concentration of the suspension was for each micro-organism:* [redacted]

1.2 Test organism (if applicable)

Names and Strains of Organisms	National Collection* Number
Algae	
Scenedesmus vacuolatus	[redacted]
Stichococcus bacillaris	[redacted]
Nostoc sp.	[redacted]

*
[redacted]

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	[redacted]
Number of vessels / concentration	*2*
Test culture media and/or carrier material	[redacted] *other appropriate media*
Nutrient supply	*Complete medium*
Measuring equipment	*Haemocytometer or other counting chamber*

1.4 Application of test substance

Criteria	Details
Application procedure	*aqueous solutions of the biocide/s under test at three times the required final concentration*
Delivery method	
Dosage rate	
Carrier	*water*
Concentration of liquid carrier	*Not applicable*
Liquid carrier control	*Not applicable*
Other procedures	-

1.5 Test conditions

Criteria	Details
Substrate	[redacted]
Incubation temperature	*Incubate at 19°C +/-1°C*
Moisture	[redacted]
Aeration	-
Method of exposure	*Addition in culture medium*
Aging of samples	-
Other conditions	

		Efficacy Data Minimum Inhibitory Concentrations **against Moulds and Yeasts**	
Doc III-A: 4		**1 REFERENCE**	**Official use only**
1.1	**Reference**	Goldbach M, 2010, Evaluation of Minimum Inhibitory Concentrations (MIC) for ACTICIDE®OTW 8 against Moulds, [redacted] unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	THOR GmbH, Germany	
1.2.2		n.a.	
1.2.3	Criteria for data protection	Data submitted on existing A.S. for the purpose of its entry into Annex I.	
1.3	**Guideline study**	THOR Microbiological Test Method 711	
1.4	**Deviations**	No	X
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**		
2.1.1	Trade name/ proposed trade name	ACTICIDE OTW 8	
2.1.2	Composition of Product tested	[redacted]	
2.1.3	Physical state and nature	Aqueous dispersion	
2.1.4	Monitoring of active substance concentration	No – nominal concentration.	X
2.1.5	Method of analysis	-	
2.2	**Reference substance**	No	X
2.2.1	Method of analysis for reference substance	-	
2.3	**Testing procedure**	Non-entry field	
2.3.1	Test population / inoculum / test organism	see Table 1.1 or see Table 1.2	
2.3.2	Test system	see Table 1.3	
2.3.3	Application of TS	Give relevant details in tabular form (see Table 1.4)	
2.3.4	Test conditions	Give relevant test conditions in tabular form (see Table 1.5)	
2.3.5	Duration of the test	In method 711 incubation time for mould is 7 days. Here, moulds	

		Efficacy Data Minimum Inhibitory Concentrations **against Moulds and Yeasts**	
	/ Exposure time	growth assessment after incubation time of 96 hours was considered sufficient because the blank and the vehicle control showed unambiguous results.	
2.3.6	Number of replicates performed	1 replicate was carried out. As the MIC value is taken from a dilution series this is considered to be sufficient.	X
2.3.7	Controls	None 1 replicate was carried out . As the MIC value is taken from a dilution series this is considered to be sufficient.	X
2.4	**Examination**	Non-entry field	
2.4.1	Effect investigated	growth	
2.4.2	Method for recording / scoring of the effect	Some fungal spores may initially germinate but then be inhibited. In such instances slight cloudiness of the growth medium may be observed but an assessment of 0 should be made nonetheless.	
2.4.3	Intervals of examination		X
2.4.4	Statistics	none	X
2.4.5	Post monitoring of the test organism	no	X
		3 RESULTS	
3.1	**Efficacy**		
3.1.1	Dose/Efficacy curve	The MIC value is the lowest concentration of biocide inhibiting the test organism in both tubes. Streaking out onto agar plates can be used for determining the MMC (Minimum Microbicidal Concentration).	
3.1.2	Begin and duration of effects	Not evaluated	
3.1.3	Observed effects in the post monitoring phase	Not applicable	
3.2	**Effects against organisms or objects to be protected**	Not applicable	
3.3	**Other effects**	-	
3.4	**Efficacy of the reference substance**	Blank sample (not preserved) showed growth. Vehicle control sample (Blank with vehicle of ACTICIDE OTW 8) showed growth.	

3.5	**Tabular and/or**	**Names and Strains of Organisms**	**Minimum Inhibitory**	X

	graphical presentation of the summarised results		**Concentration [ppm OIT]**	
		Moulds		
		Aureobasidium pullans [redacted]	0.8	
		Syndowia polyspora [redacted]	2.5	
3.6	**Efficacy limiting factors**	Non-entry field		
3.6.1	Occurrences of resistances	Not applicable to this test.		
3.6.2	Other limiting factors	-		
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS		
4.1	**Reasons for laboratory testing**	In general MIC values are mainly to be used to compare single actives rather than to obtain use-concentration. The use concentration is more dependent on the application conditions and losses of the preservative due to infections in raw materials and incompatibility. The precise level required by a specific formulation is determined by the local Thor Microbiological Technical Centre.		
4.2	**Intended actual scale of biocide application**	Normal use concentrations of A.S. in wood preservation applications are in the range of 250 ppm OIT in treatment solution for dipping/immersion; 150 ppm OIT in the treatment solution for vacuum/pressure impregnation		
4.3	**Relevance compared to field conditions**	non-entry field		
4.3.1	Application method	The application method is relevant with regard to field conditions. But stability of the biocide under field conditions is very different.		
4.3.2	Test organism	Relevant strains of Moulds (blue stain fungi).		
4.3.3	Observed effect	Growth is the relevant effect. However, in vitro test conditions are very different to field conditions regarding to stability so that under field conditions higher use concentrations are necessary.		
4.4	**Relevance for read-across**	See 4.1		
		5 APPLICANT'S SUMMARY AND CONCLUSION		
5.1	**Materials and methods**	Determination of Minimum Inhibitory Concentrations (MIC) according to THOR Microbiological Test Method 711 using relevant yeast and moulds strains.		
5.2	**Reliability**	1		X
5.3	**Assessment of efficacy, data analysis and interpretation**	The MIC values do not reflect the normal use concentration in wood preservation due to fact that the MIC value is only the minimum inhibition concentration in sterile growth medium infected with different strains of organisms. In general MIC values are mainly to be used to compare single actives rather than to be served as use-concentration. The use concentration in the final product is more dependent on the application conditions and losses of the preservative due to infections in raw materials and incompatibility. Growth is the relevant effect. However, in vitro test conditions are very different to field conditions regarding to stability so that under field conditions higher use		

		concentrations are necessary. The precise level required by a specific formulation is determined by the local Thor Microbiological Technical Centre.	
5.4	**Conclusion**	Moulds: The MIC values of the tested moulds against OIT were in a concentration range from 0.8 to 2.5 ppm.	X
5.5	**Proposed efficacy specification**	Under the applied conditions **ACTICIDE®OTW 8** is an effective biocide against the tested micro-organisms in concentrations of 10 to 31 ppm.	X

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20/7/2011
Materials and methods	The UK CA accepts the Applicant's version, with the following comments. **1.4** The growth assessment was carried out after 96 h (4 days) rather than 7 days as specified in the protocol. As the blank and control samples showed "unambiguous results" in terms of growth at this stage, the UK CA does not consider this to be a problem. **2.1.4** Although no monitoring of the active substance concentration was conducted, the UK CA does not consider the absence of such monitoring to be an issue. **2.2** A reference substance is not required for a minimum inhibitory concentration (MIC) study. Therefore the UK CA does not consider this to be a problem. **2.3.6** As only 1 replicate per dilution was carried out, this reduces the usefulness of the test. However it still provides evidence of the innate activity of the active substance. **2.3.7** The study was carried out using both blank control samples (sterile water) and vehicle controls (the other components of Acticide OTW 8 without OIT, diluted in sterile water). **2.4.3** The evaluation was carried out after 96 h. **2.4.4** Statistics are not appropriate for this type of dilution series test. **2.4.5** As post monitoring of the test organism is not required, the UK CA does not consider this to be a significant omission.
Results and discussion	The UK CA accepts the Applicant's version, with the following comments. **3.5** *Syndowia polyspora* should read *Sydowia polyspora*. 0.8 ppm OIT is equivalent to 0.000008 % OIT. 2.5 ppm OIT is equivalent to 0.000025 % OIT. **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). **5.5** The amount of product Acticide OTW 8 is not relevant to the active substance. These amounts are equivalent to 0.8 - 2.5 ppm OIT. The UK CA considers the results to be acceptable in support of active substance approval.
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

Tables for Method

1.1 (single) Population / Inoculum

Criteria	**Details**
Nature	Single strain inoculum
Origin	details, e.g. on sampling site are given in table 1.2
Initial biomass	No data
Reference of methods	Determine the total count of suspensions thus prepared using a haemocytometer or other type of counting chamber.
Collection / storage of samples	Not applicable.
Preparation of inoculum for exposure	micro-organism cultures are pure
Pretreatment	none
Initial density of test population in the test system	The concentration of the suspension was for each micro-organism: [redacted]

1.2 Test organism (if applicable)

Names and Strains of Organisms	**National Collection* Number**
Moulds	
Aureobasidium pullans	[redacted]
Sydowia polyspora	[redacted]

*

[redacted]

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	[redacted]
Number of vessels / concentration	1
Test culture media and/or carrier material	[redacted]
Nutrient supply	Complete medium
Measuring equipment	Haemocytometer or other counting chamber

1.4 Application of test substance

Criteria	Details
Application procedure	An aqueous solutions of the biocide under test at three times the required final concentration (as) is mixed gently with liquid culture medium (3x concentrated) and with the microorganism suspension.
Delivery method	Liquid to liquid (no immersion or vacuum-pressure)
Dosage rate	0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.3, 1.5, 1.8, 2.0, 2.5, 3.0, 4.0, 5.0 ppm OIT
Carrier	[redacted]
Concentration of liquid carrier	In the range of 0-63 ppm ACTICIDE OTW 8 tested, there is 0-5 ppm OIT and 0-2.5 ppm carrier-auxiliaries (without the water content).
Liquid carrier control	0-50 ppm vehicle formulation (=Acticide OTW 8 without OIT)
Other procedures	-

1.5 Test conditions

Criteria	Details
Substrate	[redacted]
Incubation temperature	Incubate at 25 °C +/- 2 °C or other appropriate temperature:-
Moisture	None
Aeration	None
Method of exposure	Addition in culture medium
Aging of samples	None
Other conditions	None