Section A7.2.1-2 Anaerobic degradation in soil, initial study

Annex Point VII 4, XII.1.1

4.1.6	Intermediates/ degradation products	Dinotefuran mainly degraded to DN reaching a maximum of 33.1% AR at day 120; harsh extractions additionally extracted a further 8.7% of AR as DN. A further metabolite, UF, was detected at 7.7% AR by study completion. One more minor degradation product (coded as M12) was detected chromatographically at a maximum of 3.0% AR. Ultimate degradation to CO_2 was low, reaching a maximum amount of 4.2% AR after 120 d.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Guidelines: Degradation in water/sediment systems was performed according to the SETAC Europe: Procedures for assessing the environmental fate and ecotoxicity of pesticides, March 1995, Part 1 –2 Anaerobic degradation and EPA 540/9-82-021; Section 162-2 Anaerobic soil metabolism studies. No relevant deviations from test guidelines.	
5.2	**Results and discussion**	The degradation of dinotefuran in soil under anaerobic conditions was studied with ^{14}C labelled test substance in two different locations on its molecule (F-label and G-label). One soil system was set up at 20°C. In the experiment, total recoveries of radioactivity (mass balances) were 96.0±2.5% of the applied radioactivity (AR). The radioactivity in water decreased from 94.6% on day 0 to 58.8% on day 7 and 25.4% on day 120. There was a corresponding increase of the radioactivity in soil, reaching a maximum of 62.6% AR at the end of the test. The majority of this was extractable, amounting to 27.8% AR at day 3 and 53.5% at day 120. The maximum non-extractable radioactivity reached 10.7% on day 59. Organic matter fractionation indicated that the non-extractable radioactivity was mainly bound to the immobile humic acids and humic fraction. Volatile radioactivity was mostly associated with $^{14}CO_2$, representing a maximum of 4.2% AR. Only 0.1% AR were associated to organic volatiles. Dinotefuran mainly degraded to DN reaching a maximum of 33.1% AR; harsh extractions extracted additionally 8.7% of applied radioactivity as DN. Additionally, a further metabolite UF was detected at 7.7% AR on day 120. One more minor degradation product (M12) was detected chromatographically. Ultimate degradation to CO_2 was low, reaching a maximum of 4.2%.	
5.3	**Conclusion**	Dinotefuran declined in anaerobic soil systems with DT_{50} values of 22 and 77 days in the water phase and total system respectively. One major degradation product was formed – DN (i.e. l-methyl-3-(tetrahydro-3-furylmethyl)guanidine) that accounted for a maximum of 33.1% AR. Another metabolite, UF (i.e. l-methyl-3-(tetrahydro-3-furylmethyl)urea), reached a maximum of 7.7% AR by the end of the test.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No, however only single samples were taken on all sampling points except day 0 and day 120. Since these replicated well, this fact does not affect the quality of the study.	X

Table A7.2.1.2-1: Soil types and their characteristics

Parameters	System 1
Site location	Gartenacker, Switzerland
Batch No.	04/2002
Particle size analysis (USDA): < 0.002 (clay) % 0.002 – 0.05 (silt) % > 0.05 (sand) %	SILT LOAM 10.3 52.3 37.4
pH [0.01M $CaCl_2$]	7.22
Organic carbon [g/100 g dry soil]	1.78
Cation exchange capacity [meq/100 g dry soil]	13.11
Maximum water holding capacity at pF1 [g/100g wet soil]	67.5

Table A7.2.1.2-2: Test system

Criteria	Details		
Vessel type	Glass metabolism flasks connected to an adsorption trap with NaOH (CO_2) and then ethylene glycol (organic volatiles)		
Soil weight [g]	100		
Depth of soil layer [cm]	2		
Water volume [mL]	200		
Depth of water layer [cm]	2		
Vessel replicates			
Number of vessels	Test	Treatment rate [mg/100 g]	Radiolabel
13	Anaerobic 20°C	0.031	Mixture of F and G
	Control vessels for pH, oxygen and redox potential	-	-

Table A7.2.1.2-3: Test conditions

Parameters	
Test temperature [°C]	20 ± 2
Light conditions	dark
Aeration	nitrogen
Redox potential in soil [mV]	-16 ± 9
Redox potential in water [mV]	-129 ± 61
pH in water	8.56 ± 0.08
Oxygen concentration in water [mg/L]	0.2 ± 0.1
Microbial biomass at start of incubation [mg microbial C/100 g dry soil]	33.0
Microbial biomass at end of incubation [mg microbial C/100 g dry soil]	13.6

Table A7.2.1.2-4: Distribution and recovery of radioactivity [based on % AR]

Sample	Time after application [days]						
	0	3	7	14	28	59	120
Water phase	94.6	67.8	58.8	51.6	51.0	40.2	25.4
Total Extractables	2.3	27.8	33.5	40.6	39.0	42.0	44.7
Non-extractables	1.0	2.7	4.0	3.9	6.1	10.7	9.1
Organic volatiles	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
CO_2	-	0.1	0.4	0.7	0.8	1.9	4.2
Total recovery	97.9	98.4	96.7	96.8	96.9	94.7	92.1
Identified as parent	94.5	90.8	87.6	84.0	82.2	59.6	26.3
Identified as DN	0.5	0.7	0.8	2.1	2.3	14.6	33.1
Identified as UF	0.6	2.4	3.4	6.1	4.0	6.0	7.7

DN: 1-methyl-3-(tetrahydro-3-furylmethyl)guanidine
UF: 1-methyl-3-(tetrahydro-3-furylmethyl)urea
Minor degradation products are not listed in the table
Values on day 0 and day 120 are mean values (n=2)

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	25 October 2012
Materials and Methods	The Applicant's version is considered to be acceptable noting the following :- **3.4.8** It should be noted that the number of sampling points in the test system was 7 with 2 further duplicates (at day 0 and day 120) plus 2 sets of duplicate controls - this supports the number of vessels reported in **Table A7.2.1.2-2** as being 13. However, sampling was only performed at 0, 3, 7, 14, 28, 59 and 120 d so mention of a 10 d sampling point is erroneous.
Results and discussion	The Applicant's version is considered to be acceptable noting the following :- **4.1.1** The UK CA has repeated SFO kinetic modelling of results from total system for degradation of dinotefuran and can confirm the DT_{50} of 77 d, DT_{90} of 256 d and k1 of 0.009 d^{-1} (with r^2 of 0.965). Although SFO kinetics have been performed on results from dissipation of dinotefuran from the water phase giving rise to a DT_{50} of 44 d and k1 of 0.016 d^{-1}, an r^2 of only 0.825 suggests that this does not best model the degradation taking place. Biphasic modelling of dissipation performed by the Applicant would appear to be more appropriate. **4.1.2 & 5.3** It should be noted that the half-life (DT_{50}) plus DT_{90} for dinotefuran in the water phase actually represents a combination of degradation in this phase plus dissipation with relocation into the solid (soil) phase. However, the DT_{50} (77 d) and DT_{90} (256 d) derived for total system reflects actual anaerobic degradation.
Conclusion	The Applicant's version is considered to be acceptable noting the following :- **5.3.2** Test guidelines for OECD 307 (adopted April 2002) do indicate that well designed studies are considered to include sufficient flasks so that two may be sacrificed at each of at least 5 sampling events. Whilst duplicate samples were not taken at all 7 sampling points, there is good conformity between sampling results where they have been taken and recovery of AR (>92% for all samples). Therefore, the argument presented by the Applicant that these equate to minor deviations and minor methodological deficiencies that do not affect quality of the results can be supported. No reduction in reliability scoring would be necessary and results can be used where relevant for risk assessment purposes.
Reliability	Reliability to remain as 1.
Acceptability	The Applicant's version is considered to be acceptable.
Remarks	There was evidence of significant degradation of dinotefuran in soil under anaerobic conditions with levels dropping to 26.3% after 120 d, accompanied by formation of 33.1% of the metabolite DN (which increases to 41.8% at study completion following use of harsh extraction techniques). However, mineralisation was low, with only 4.2% of CO_2 being detected at study completion.

COMMENTS FROM...

Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Remarks

Section 7.2.2.1 Annex Point IIIA, VII.4 ; XII.1.1 ; XII.1.4	**Rate and route of degradation including identification of the processes involved and identification of any metabolites and degradation products in at least three soil types under appropriate conditions**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Indoor professional use is intended for the product. Therefore it is very unlikely that dinotefuran will enter the soil. This test is not required. See Section A7.2.1-1 and A7.2.1-2 for laboratory tested soil metabolism summaries in aerobic and anaerobic conditions respectively.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15 January 2013	
Evaluation of applicant's justification	It is clear from the use pattern of the representative product that direct exposure of the soil compartment is extremely unlikely and that any emissions from wet cleaning of indoor surfaces will be directed to STP and then surface waters. Due to dinotefuran demonstrating a low K_{oc} (< 50), binding to sewage sludge is unlikely and thus the compound is not expected to reach agricultural land in significant concentrations.	
Conclusion	The Applicant's justification is considered to be acceptable.	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section 7.2.2.2 Annex Point IIIA, XI.1.1	Field soil dissipation and accumulation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [X]	Technically not feasible [ ] Scientifically unjustified [X] Other justification [ ]	
Detailed justification:	Indoor professional use is intended for the product. Therefore it is very unlikely that dinotefuran will enter the soil. This test is not required. See Section A7.2.1-1 and A7.2.1-2 for laboratory tested soil metabolism summaries in aerobic and anaerobic conditions respectively.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15 January 2013	
Evaluation of applicant's justification	It is clear from the use pattern of the representative product that direct exposure of the soil compartment is extremely unlikely and that any emissions from wet cleaning of indoor surfaces will be directed to STP and then surface waters. Due to dinotefuran demonstrating a low K_{oc} (< 50), binding to sewage sludge is unlikely and thus the compound is not expected to reach agricultural land in significant concentrations.	
Conclusion	The Applicant's justification is considered to be acceptable.	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section 7.2.2.3 Annex Point IIIA, XI.1.1	**Extent and nature of bound residues**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Indoor professional use is intended for the product. Therefore it is very unlikely that dinotefuran will enter the soil. This test is not required. See Section A7.2.1-1 and A7.2.1-2 for laboratory tested soil metabolism summaries in aerobic and anaerobic conditions respectively.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15 January 2013	
Evaluation of applicant's justification	It is clear from the use pattern of the representative product that direct exposure of the soil compartment is extremely unlikely and that any emissions from wet cleaning of indoor surfaces will be directed to STP and then surface waters. Due to dinotefuran demonstrating a low K_{oc} (< 50), binding to sewage sludge is unlikely and thus the compound is not expected to reach agricultural land in significant concentrations.	
Conclusion	The Applicant's justification is considered to be acceptable.	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section 7.2.2.4 Annex Point IIIA, XI.1.1	**Other soil degradation studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Indoor professional use is intended for the product. Therefore it is very unlikely that dinotefuran will enter the soil. This test is not required. See Section A7.2.1-1 and A7.2.1-2 for laboratory tested soil metabolism summaries in aerobic and anaerobic conditions respectively.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15 January 2013	
Evaluation of applicant's justification	It is clear from the use pattern of the representative product that direct exposure of the soil compartment is extremely unlikely and that any emissions from wet cleaning of indoor surfaces will be directed to STP and then surface waters. Due to dinotefuran demonstrating a low K_{oc} (< 50), binding to sewage sludge is unlikely and thus the compound is not expected to reach agricultural land in significant concentrations.	
Conclusion	The Applicant's justification is considered to be acceptable.	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section 7.2.2 Annex Point IIIA, XI.2.1	**Aerobic degradation in soil, further studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Indoor professional use is intended for the product. Therefore it is very unlikely that dinotefuran will enter the soil. This test is not required. See Section A7.2.1-1 and A7.2.1-2 for laboratory tested soil metabolism summaries in aerobic and anaerobic conditions respectively.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15 January 2013	
Evaluation of applicant's justification	It is clear from the use pattern of the representative product that direct exposure of the soil compartment is extremely unlikely and that any emissions from wet cleaning of indoor surfaces will be directed to STP and then surface waters. Due to dinotefuran demonstrating a low K_{oc} (< 50), binding to sewage sludge is unlikely and thus the compound is not expected to reach agricultural land in significant concentrations.	
Conclusion	The Applicant's justification is considered to be acceptable.	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section 7.2.3.1 Annex Point IIIA XII.1.2	**Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant, adsorption and desorption of metabolites and degradation products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Indoor professional use is intended for the product. Therefore it is very unlikely that dinotefuran will enter the soil. Further testing is not required. See Section A7.1.3 for the adsorption/desorption of dinotefuran on soils.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15 January 2013	
Evaluation of applicant's justification	It is clear from the use pattern of the representative product that direct exposure of the soil compartment is extremely unlikely and that any emissions from wet cleaning of indoor surfaces will be directed to STP and then surface waters. Due to dinotefuran demonstrating a low K_{oc} (< 50), binding to sewage sludge is unlikely and thus the compound is not expected to reach agricultural land in significant concentrations.	
Conclusion	The Applicant's justification is considered to be acceptable.	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section 7.2.3.2 Annex Point IIIA XII.1.3	**Mobility in at least three soil types and where relevant mobility of metabolites and degradation products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Indoor professional use is intended for the product. Therefore it is very unlikely that dinotefuran will enter the soil. Further testing is not required. See Section A7.1.3 for the adsorption/desorption of dinotefuran on soils.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15 January 2013	
Evaluation of applicant's justification	It is clear from the use pattern of the representative product that direct exposure of the soil compartment is extremely unlikely and that any emissions from wet cleaning of indoor surfaces will be directed to STP and then surface waters. Due to dinotefuran demonstrating a low K_{oc} (< 50), binding to sewage sludge is unlikely and thus the compound is not expected to reach agricultural land in significant concentrations.	
Conclusion	The Applicant's justification is considered to be acceptable.	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section 7.2.3 Annex Point IIIA	Adsorption and mobility in soil, further studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [X]	Technically not feasible [ ] Scientifically unjustified [X] Other justification [ ]	
Detailed justification:	Indoor professional use is intended for the product. Therefore it is very unlikely that dinotefuran will enter the soil. Further testing is not required. See Section A7.1.3 for the adsorption/desorption of dinotefuran on soils.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15 January 2013	
Evaluation of applicant's justification	It is clear from the use pattern of the representative product that direct exposure of the soil compartment is extremely unlikely and that any emissions from wet cleaning of indoor surfaces will be directed to STP and then surface waters. Due to dinotefuran demonstrating a low K_{oc} (< 50), binding to sewage sludge is unlikely and thus the compound is not expected to reach agricultural land in significant concentrations.	
Conclusion	The Applicant's justification is considered to be acceptable.	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.3.1
Annex Point IIIA-VII.5

Phototransformation in air (estimation method) including identification of breakdown products

			Official use only
		1 REFERENCE	
1.1	**Reference**	Van der Gaauw, A., 2000, Estimation of the degradation of MTI-446 by photo-oxidation in air. RCC, Ltd., unpublished report no. 731160, (MRID 45640110), September 22, 2000.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Model calculation according to Atkinson.	
2.2	**GLP**	No, calculations are not subject to GLP requirements.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Dinotefuran	
3.1.1	Lot/Batch number	Not applicable as theoretical study	
3.1.2	Specification	Not applicable	
3.1.3	Purity	Assumes 100% pure for calculation	
3.2	**Reference substances**	Not applicable	
3.3	**Calculation method**	The degradation rate of dinotefuran in air was estimated using the computer program AOPWIN. The program is based on structure/activity relationships. The program estimates an overall OH-radical reaction rate constant by summing individual OH-radical reaction pathways.	X
		4 RESULTS	
4.1	**Hydroxyl radicals reaction**	In the troposphere, there are three important photochemical transformation processes that may contribute to the degradation on a chemical. These are direct photoreaction, indirect photoreaction with OH- radicals and oxidation with ozone. Of the direct and indirect phototransformation processes that are possible, the reaction with OH-radical is generally the most important. Reaction with ozone is generally of secondary importance. For dinotefuran, there is no reaction with ozone to consider as dinotefuran does not contain unsaturated carbon-carbon bonds. For dinotefuran, the overall OH- radical reaction rate constant was calculated to be 160.596 • 10^{-12} cm^3 • $molecule^{-1}$ • sec^{-1}. Based on this the half-life of dinotefuran was calculated as 0.067 days when a 12-hour day is considered and 0.033 days when a 24-hour day is considered.	X

Section A7.3.1
Annex Point IIIA-VII.5

Phototransformation in air (estimation method) including identification of breakdown products

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The degradation rate of dinotefuran in air was estimated using the computer program AOPWIN. The program estimates an overall OH-radical reaction rate constant by summing individual OH-radical reaction pathways.	**X**
5.2	**Results and discussion**	The total rate constant for the reaction of OH-radicals with the amine groups is K_{NSOH} = 126.000 x 10^{-12} cm^3 • $molecule^{-1}$ • sec^{-1}. For dinotefuran, the overall rate constant resulted in k_{OH} = 160.596 • 10^{-12} cm^3 • $molecule^{-1}$ • sec^{-1}.	
5.3	**Conclusion**	The half-life of MTI-446 was calculated as 0.067 days when a 12-hour daylight scenario is considered and 0.033 days when a 24-hour daylight scenario is considered.	**X**
5.3.1	Reliability	1	**X**
5.3.2	Deficiencies	None	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15 January 2013
Materials and Methods	The Applicant's version is considered to be acceptable, noting the following : **3.3** & **5.1**: The specific model used to address this endpoint is the Atmospheric Oxidation Program for Microsoft Windows 3.1 (Version 1.70; 1995).
Results and discussion	The Applicant's version is considered to be acceptable, noting the following : **4.1**: The UK CA notes that at the time that modelling was performed, AOPWIN was only available at Version 1.70. However, new modelling has been performed using Version 1.92 of AOPWIN and both SMILES notations provided for dinotefuran [C1(CCOC1)CNC(=NN(=O)(=O))NC] or [C(NC)(=NN(=O)(=O)) NCC1(CCOC1)] give rise to a k_{OH} value of 156.0666E-12 cm^3 mol^{-1} sec^{-1}. This rate is slightly lower than predicted by the applicant and will now be used in half-life determination. **4.1**: It is noted that guidance provided within the AOPWIN model recommends use of the 12-h default hydroxyl radical concentration (1.5 x 10^6 molecules cm^{-3}) during sunlight hours to determine half-life. However, it is EU policy to base half-life on the lower 24-h average rate of 5 x 10^5 molecules cm^{-3} as stated in section 2.3.6.3 of the Technical Guidance Document (TGD) on risk assessment. As a consequence, use of equation 28 from the TGD would give rise to a $kdeg_{air}$ pseudo first order rate constant of ~~160.596 x 10^{-12}~~ 156.0666 x 10^{-12} * 5.0 x 10^5 * 24 * 3600 = ~~6.938~~ 6.742 d^{-1}. This rate constant can then be converted into a half-life to predict photo-transformation in air [using ln2 / ~~6.938~~ 6.742] for dinotefuran of 0.10 d or 2.40 h.
Conclusion	The Applicant's version is considered to be acceptable, noting the following: **5.3**: It should be noted that when the appropriate value for OH^- radical concentration (5 x 10^5 radicals per cm^3 over a 24-h period) in EU air is used in the model, a half-life for dinotefuran of 0.1 d can be derived.
Reliability	Reduce reliability to 2
Acceptability	Reliability reduced as endpoint has been addressed by QSAR model rather than undertaking a specific study.
Remarks	This QSAR model is an acceptable method to determine half-life for photo-transformation in air and results may be used for risk assessment purposes.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.3.2 Annex Point IIIA, XII.3	Fate and behaviour in air, further studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Dinotefuran is not intended to be used in preparations for fumigants, or to be applied by a spray method, it is not volatile, and no other information indicates that further fate and behaviour in air studies are required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15 January 2013	
Evaluation of applicant's justification	It is clear from the use pattern of the representative product, its formulation type and low vapour pressure of dinotefuran (<1.7 x 10^{-6} Pa at 30 °C) that direct exposure of the air compartment is extremely unlikely and that any emissions from wet cleaning for indoor surfaces will be directed to STP and then surface waters. However, should dinotefuran become airborne, predictive AOPWIN modelling (presented in **A7.3.1**) suggests a half-life in air of approximately 0.1 d.	
Conclusion	The Applicant's justification is considered to be acceptable.	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.4.1.1-1 Acute toxicity to fish
Annex Point IIA7.1 **Rainbow Trout: *Oncorhynchus mykiss***
Static, 96-hour, limit test

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1999, Acute Toxicity of MTI-446 to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-Hour Static Test, [redacted] unpublished report no. 740924, December 20, 1999. [redacted] 2000a, First Amendment to Report: Acute Toxicity of MTI-446 to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-Hour Static Test, [redacted] unpublished report no. 740924, March 31, 2000.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes U.S. EPA OPPTS 850.1075 Commission Directive 92/69/EEC (C.1) OECD Guideline No. 203	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	5500310	
3.1.2	Specification		X
3.1.2.1	Purity	97.26%	
3.1.2.2	Stability	Expiration date: June 30, 2004	
3.1.2.3	Further relevant properties	Solubility in water: 54.3 g/L at 20 °C Stability in water: > 24 hours (Sponsor information)	
3.1.3	Method of analysis	Aqueous samples containing dinotefuran were analysed on a high performance liquid chromatographic (HPLC) system using ultraviolet (UV) detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	The test substance is not poorly soluble or volatile. See table A7.4.1.1.1-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	

Section A7.4.1.1-1 **Acute toxicity to fish**
Annex Point IIA7.1 **Rainbow Trout: *Oncorhynchus mykiss***
Static, 96-hour, limit test

3.4	**Testing procedure**	
3.4.1	Dilution water	See Table A7.4.1.1.1-2
3.4.2	Test organisms	See Table A7.4.1.1.1-3
3.4.3	Test system	See Table A7.4.1.1.1-4
3.4.4	Test conditions	See Table A7.4.1.1.1-5
3.4.5	Duration of the test	96-hours
3.4.6	Test parameter	Mortality and intoxication
3.4.7	Sampling	Test fish were observed after approximately 4, 24, 48, 72 and 96 hours
3.4.8	Monitoring of TS concentration	Yes Sampling at 0-hour (test initiation) and 96-hour (test termination)
3.4.9	Statistics	The NOEC and the LC_0 were determined directly from the raw data.

4 RESULTS

4.1	**Limit Test**	Performed
4.1.1	Concentration	Nominal: 100 mg/L
4.1.2	Number/ percentage of animals showing adverse effects	0
4.1.3	Nature of adverse effects	No adverse effects
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	100 mg/L (nominal concentration)

4.2.2 Actual concentrations of test substance

Nominal test Concen-tration (mg/L)	Sample date (day)	Sample Age (hours)	Measured Concent-ration (mg/L)[1]	% of Nominal[2]	Mean Measured Concentrat-ion (mg/L)	Mean Measured % of Nominal
100	0	0	98.94	99	99.4	99
	0	0	99.88	100		
	4	96	99.26	99	99.5	100
	4	96	99.80	100		

4.2.3	Effect data (Mortality)	Mortality data as absolute numbers of immobile fish and as percent of exposed animals in tabular form (see Table A7.4.1.1.1-6).
4.2.4	Concentration / response curve	No concentration/response curves were reported
4.2.5	Other effects	None
4.3	**Results of controls**	

Section A7.4.1.1-1 **Acute toxicity to fish**
Annex Point IIA7.1 **Rainbow Trout: *Oncorhynchus mykiss***
Static, 96-hour, limit test

4.3.1	Number/ percentage of animals showing adverse effects	0
4.3.2	Nature of adverse effects	No adverse effects
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	
4.4.2	Results	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Guidelines: U.S. EPA OPPTS 850.1075, Commission Directive 92/69/EEC (C.1), OECD Guideline No. 203. No relevant deviations from test guidelines. Method: In a 96-hour acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to dinotefuran at 0 mg/L (control) and a single nominal concentration of 100 mg/L under static conditions.
5.2	**Results and discussion**	No remarkable observations were made concerning the appearance of the test medium. The pH values in the test medium and the control ranged from 7.7 to 8.0. The oxygen concentration was always 8.9 mg/L or higher, and thus higher than 60% oxygen saturation. The water temperature ranged from 12 to 13 °C. In the control and at the test concentration of 100 mg/L no mortality or other signs of intoxication were observed during the test period of 96 hours. Therefore, the 96-hour NOEC was 100 mg/L. The 96-hour LC_0 was determined to be ≥ 100 mg/L and the 96-hour LOEC, the 96-hour LC_{50}, and the 96-hour LC_{100} of dinotefuran to rainbow trout were all determined to be > 100 mg/L.
5.2.1	LC_0	≥ 100 mg/L
5.2.2	LC_{50}	> 100 mg/L
5.2.3	LC_{100}	> 100 mg/L
5.3	**Conclusion**	Validity criteria can be considered fulfilled (see validity criteria summarised in Table A7.4.1.1.1-8). After 96 hours of exposure, there were no mortalities or signs of intoxication. Based on the results of this study, dinotefuran would be classified as practically nontoxic to juvenile rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis.
5.3.1	Other Conclusions	
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Table A7.4.1.1.1-1: Preparation of TS solution for test substance

Criteria	Details
Dispersion	Yes
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	No
Other procedures	Not applicable

Table A7.4.1.1.1-2: Dilution water

Criteria	Details
Source	Reconstituted test water according to the Commission Directive 92/69/EEC and the OECD Guideline, however for the EPA requirements the hardness was lowered to one-half of the normal hardness. Analytical grade salts were added to purified water to obtain the following nominal concentrations, and the test water was aerated until oxygen saturation was reached.
Alkalinity	0.4mmol/L
Hardness	1.25 mmol/L (= 125 mg/L) as $CaCO_3$
pH	Not reported
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	No

Table A7.4.1.1.1-3: Test organisms

Criteria	Details
Species/strain	*Oncorhynchus mykiss*
Source	[redacted]
Wild caught	No
Age/size	Mean body length at test initiation: 5.5 ± 0.32 cm (Mean ± SD; range: 5.1 - 5.9 cm) Mean body wet weight at test initiation 1.9 ± 0.31 g (Mean ± SD; range: 1.4 - 2.4 g).
Kind of food	Commercial fish diet (HOKOVIT 502, 1.2 mm, supplied by H.U. Hoffmann AG, CH-4922 Bützberg, Switzerland)
Amount of food	Not reported
Feeding frequency	Not reported
Pretreatment	The test fish were treated for diseases immediately after arriving at the test facility, then held for more than two weeks without any further medication. The fish were then acclimated for one additional week prior to the test start to the test water and temperature (12 – 13°C).
Feeding of animals during test	No

Table A7.4.1.1.1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	Not applicable
Volume of test vessels	Not reported
Volume/animal	2.75 L
Number of animals/vessel	20
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1.1-5: Test conditions

Criteria	Details
Test temperature	12 – 13°C
Dissolved oxygen	8.9 – 9.6 mg/L
pH	7.7 – 8.0
Adjustment of pH	No
Aeration of dilution water	Yes Test water aerated slightly until oxygen saturation was reached
Intensity of irradiation	Not reported
Photoperiod	16 hours light/8 hours dark daily

Table A7.4.1.1.1-6: Effect of dinotefuran on mortality of Rainbow Trout (*Oncorhynchus mykiss*)

Test-Substance Concentration measured and (nominal) [mg/l]	**Mortality**							
	Number				**Percentage**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
Negative control	0	0	0	0	0	0	0	0
99.5 (100)	0	0	0	0	0	0	0	0
NOEC (mortality)	100 mg/L							
LC_{50} (95% C.I.)	>100 mg/L							
Positive control, if used mortality: LC_{50}	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Temperature [°C]	13	13	12	12				
pH	7.8 – 7.9	7.9 – 8.0	7.7	7.7				
Oxygen [mg/l]	9.5 – 9.6	9.4	9.2 – 9.3	8.9 – 9.0				

Table A7.4.1.1.1-7: Sub-lethal effect of dinotefuran on Rainbow Trout (*Oncorhynchus mykiss*)

Treatment, mg/L, Measured and (Nominal conc.)	Observation period (endpoint at) (Hours)			
	24	48	72	96
	% affected	% affected	% affected	% affected
Negative control	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
99.5 (100)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
NOEC (sub-lethal)	100 mg/L			
LOEC (sub-lethal)	>100 mg/L			
EC_{50}	Not determined			
Positive control, if used % sub-lethal effect: EC_{50}:	N/A	N/A	N/A	N/A

Table A7.4.1.1.1-8: Validity criteria for acute fish test according to OECD Guideline 203

Criteria	Fulfilled	Not fulfilled
Mortality of control animals <10%	X	
Concentration of dissolved oxygen in all test vessels > 60% saturation	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	03October 2012
Materials and Methods	Applicant's version considered acceptable, noting the following: 3.1 Test material is MTI-446 (dinotefuran) as given in Section 2 of the report. 3.1.2 Test material is a white solid
Results and discussion	Applicants version considered acceptable
Conclusion	Applicants version considered acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.1.1-2 **Acute toxicity to fish**

Annex Point IIA7.1 **Rainbow Trout: *Oncorhynchus mykiss***

Semi - static, 96-hour, extended limit test

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 2002a, DN Phosphate Determination of Acute Toxicity to Rainbow Trout (96 h, Semi-static), [redacted] unpublished report no. 19814, January 18, 2002.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD No. 203 OPPTS 850.1075	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	DN Phosphate	
3.1.1	Lot/Batch number	MU-9428M	
3.1.2	Specification	DN Phosphate is a degradation product of the parent molecule MTI-446	
3.1.3	Purity	not reported	X
3.1.4	Composition of Product	n.a.	
3.1.5	Further relevant properties	n.a.	
3.1.6	Method of analysis	Test item concentrations in the test solutions were determined by HPLC with UV detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	n.a., see Table A7.4.1.1.2-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	n.a.	
3.4	**Testing procedure**		
3.4.1	Dilution water	see Table A7.4.1.1.2-2	
3.4.2	Test organisms	see Table A7.4.1.1.2-3	
3.4.3	Test system	see Table A7.4.1.1.2-4	
3.4.4	Test conditions	see Table A7.4.1.1.2-5	X

Section A7.4.1.1-2 **Acute toxicity to fish**

Annex Point IIA7.1 **Rainbow Trout: *Oncorhynchus mykiss***

Semi - static, 96-hour, extended limit test

3.4.5	Duration of the test	96-hours
3.4.6	Test parameter	Mortality and other signs of toxicity
3.4.7	Sampling	Duplicate samples were taken at test initiation, after 24 hours (before medium change, 72 hours (after medium change) and at the end of exposure (96 hours).
3.4.8	Monitoring of TS concentration	Yes The test item concentration was analysed in the control and at 100 mg/L at 0, 24, 72 and 96 hours.
3.4.9	Statistics	No statistics was carried out since the study was conducted as an (extended) limit test.

4 RESULTS

4.1	**Limit Test**	Performed
4.1.1	Concentration	100 mg/L
4.1.2	Extended concentrations	Additionally 3 fish each were tested at 10 and 1.0 mg/L. No test item analysis was carried out.
4.1.3	Number/ percentage of animals showing adverse effects	Cumulative number of mortalities

Time [h]	Nominal concentration of DN Phosphate [mg/L]			
	Control	1	10	100
0	0	0	0	0
1	0	0	0	0
3	0	0	0	0
6	0	0	0	0
24	0	0	0	0
48	0	0	0	0
72	0	0	0	0
96	0	0	0	0

4.1.4	Nature of adverse effects	No adverse effects were reported
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 1, 10 and 100 mg/L

Section A7.4.1.1-2 **Acute toxicity to fish**

Annex Point IIA7.1 **Rainbow Trout: *Oncorhynchus mykiss***

Semi - static, 96-hour, extended limit test

4.2.2 Actual concentrations of test substance

Measured concentrations of DN Phosphate

Time [h]	Nominal concentration [mg/L]	Measured concentration [mg/L]	
0	0	ND	ND
24*		ND	ND
72		ND	ND
96*		ND	ND
0	100	102.58	102.62
24*		108.75	108.58
72		107.06	108.11
96*		106.04	108.40

ND: not detected

*: sample taken before medium change

4.2.3 Effect data (Mortality): Mortality data as absolute numbers of immobile fish and as percent of exposed animals in tabular form (see Table A7.4.1.1.2-6)

4.2.4 Concentration / response curve: n.a.

4.2.5 Other effects: None

4.3 Results of controls

4.3.1 Number/ percentage of animals showing adverse effects: None

4.3.2 Nature of adverse effects: n.a.

4.4 Test with reference substance: Not performed

4.4.1 Concentrations: n.a.

4.4.2 Results: n.a.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

OECD No. 203, OPPTS 850.1075

No relevant deviations from test guidelines.

Method:

An extended limit test was carried out to determine the toxicity of DN phosphate to rainbow trout (*Oncorhynchus mykiss*), over a 96 h period. The test was carried out under semi-static conditions, with the test solution being renewed at 24-hour intervals throughout the test period.

The nominal concentrations of DN phosphate tested were 100, 10, 1 and

Section A7.4.1.1-2 **Acute toxicity to fish**

Annex Point IIA7.1 **Rainbow Trout: *Oncorhynchus mykiss***

Semi - static, 96-hour, extended limit test

		0 mg/L. Analysis of test solutions was conducted for the highest concentration and the control at 0, 24, 72 and 96 h post initial exposure. Water quality parameters (pH, temperature, conductivity and dissolved oxygen) were measured at the beginning and at 24 h intervals in all vessels.
5.2	**Results and discussion**	The mean measured concentration for the 100 mg/L test item treatment group was 106.5 mg/L. No test item was detected in the control. There was no evidence for any instability over two 24-hour medium change intervals. There were no mortalities or other adverse effects in any of the test item treatment groups or the control.
5.2.1	LC_0	> 100 mg/L
5.2.2	LC_{50}	> 100 mg/L
5.2.3	LC_{100}	> 100 mg/L
5.3	**Conclusion**	The validity criteria for the study are fulfilled. The LC_{50} for DN phosphate was determined to be > 100 mg/L and the NOEC is concluded to be 100 mg/L.
5.3.1	Other Conclusions	None
5.3.2	Reliability	1
5.3.3	Deficiencies	Yes The test temperature was above the range recommended in the OPPTS 850.1075 guideline. However, it was within the recommended temperature range for the rainbow trout according to the OECD No. 203 guideline. The deviation from the OPPTS 850.1075 guideline is not regarded as significant, particularly as the temperature range was less than 1°C over duration of the test.

Table A7.4.1.1.2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	n.a.
Vehicle control performed	No
Other procedures	None

Table A7.4.1.1.2-2: Dilution water

Criteria	Details
Source	Not reported
Alkalinity	Not reported

Hardness	Not reported
pH	Not reported
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	Not reported

Table A7.4.1.1.2-3: Test organisms

Criteria	**Details**
Species/strain	Rainbow trout *(Oncorhynchus mykiss)*
Source	[redacted]
Wild caught	No
Age/size	length 4 – 5 cm
Kind of food	not reported
Amount of food	not reported
Feeding frequency	not reported
Pretreatment	not reported
Feeding of animals during test	No

Table A7.4.1.1.2-4: Test system

Criteria	**Details**
Test type	Semistatic
Renewal of test solution	Test solution renewal in 24 h intervals
Volume of test vessels	25 L
Volume/animal	1.25 L at 100 mg/L and in the control 4.17 at 1 and 10 mg/L
Number of animals/vessel	10 at 100 mg/L and in the control 3 at 1 and 10 mg/L
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1.2-5: Test conditions

Criteria	Details
Test temperature	14.2 – 14.7
Dissolved oxygen	89.0 – 93.7
pH	6.2 – 8.0
Adjustment of pH	No
Aeration of dilution water	Yes Test vessels were continuously aerated throughout the exposure phase
Intensity of irradiation	artificial daylight fluorescent tubes provided illumination
Photoperiod	16 h light and 8 h dark

Table A7.4.1.1.2-6: Mortality data

Test-Substance Concentration (nominal/measured)[1] [mg/l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0
Temperature [°C]	14.2 – 14.7	14.5 - 14.7	14.4 – 14.7	14.4 – 14.7				
pH	6.2 – 7.6	6.5 – 8.0	6.3 – 7.1	6.5 – 7.7				
Oxygen [mg/l]	90.4 – 93.7	89.0 – 92.5	90.1 – 92.6	89.9 – 92.4				

[1] TS concentrations nominal

Table A7.4.1.1.2-7: Effect data

	48 h [mg/l][1]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0	> 100 mg/L	n.a.	> 100 mg/L	n.a.
LC_{50}	> 100 mg/L	n.a.	> 100 mg/L	n.a.
LC_{100}	> 100 mg/L	n.a.	> 100 mg/L	n.a.

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7.4.1.1.2-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	X	
Concentration of dissolved oxygen in all test vessels > 60% saturation	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	02 October 2012
Materials and Methods	Applicant's version considered acceptable noting the following: 3.1.3 The purity of the test item has not been presented in the report and purity may have an impact on the toxicity. However, the RMS has obtained a copy of the appropriate certificate of analysis and can confirm that the purity of the test item is 99.5%. 3.4.4 The pH range in the study report is contradictory. Section 4 (Results and Discussion) of the study report states that the pH ranged from 6.5 to 7.7 however Section 7 (Tables) states that the pH range is 6.2 to 8.0. Since both these ranges are within the pH range recommended in OCED Guideline 203 (1992) acceptability is not affected.
Results and discussion	Applicant's version considered acceptable
Conclusion	Applicant's version considered acceptable.
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.1.2-1 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

Static, 48-hour, limit test

			Official use only
		1 REFERENCE	
1.1	**Reference**	Peither, A., 2000b, Acute toxicity of MTI-446 to *Daphnia magna* in a 48-hour immobilization test, RCC Ltd., unpublished report no. 740968, February 10, 2000. Peither, A., 2000c, First Amendment tot report: Acute toxicity of MTI-446 to *Daphnia magna* in a 48-hour immobilization test, RCC Ltd., unpublished report no. 740968, March 31, 2000.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline No. 202 (Part 1) Directive 92/69/EEC (Part C.2) U.S. EPA OPPTS 850.1010	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	5500310	
3.1.2	Specification		X
3.1.2.1	Purity	97.26%	
3.1.2.2	Stability	Expiration date: June 30, 2004	
3.1.2.3	Further relevant properties	Solubility in water: 39.83 g/L at 20 °C Stability in water: > 24 hours (Sponsor information)	X
3.1.3	Method of analysis	Aqueous samples containing dinotefuran were analysed on a high performance liquid chromatographic (HPLC) system using ultraviolet (UV) detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	- The test substance is not poorly soluble or volatile. See Table A7.4.1.2.1-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	

Section A7.4.1.2-1 Acute toxicity to invertebrates
Annex Point IIA7.2 *Daphnia magna*
Static, 48-hour, limit test

3.4	**Testing procedure**		
3.4.1	Dilution water	See Table A7.4.1.2.1-2	
3.4.2	Test organisms	See Table A7.4.1.2.1-3	
3.4.3	Test system	See Table A7.4.1.2.1-4	
3.4.4	Test conditions	See Table A7.4.1.2.1-5	
3.4.5	Duration of the test	48-hours	
3.4.6	Test parameter	Immobility and mortality	
3.4.7	Sampling	At 0-hour and 48 hour	
3.4.8	Monitoring of TS concentration	Yes Sampling at 0-hour (without daphnids) and after 48-hour (test termination, without daphnids).	
3.4.9	Statistics	The NOEC and EC_0 were determined directly from the raw data.	
		4 RESULTS	
4.1	**Limit Test**	Performed	
4.1.1	Concentration	Nominal: 1000 mg/L	X
4.1.2	Number/ percentage of animals showing adverse effects	0	
4.1.3	Nature of adverse effects	No adverse effects	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	1000 mg/L (nominal concentration)	
4.2.2	Actual concentrations of test substance	See table below	
4.2.3	Effect data (Immobilisation)	Immobilisation data as absolute numbers of immobile daphnia and as percent of exposed animals in tabular form (see Table A7.4.1.2.1-6).	
4.2.4	Concentration / response curve	No concentration/response curves were reported	
4.2.5	Other effects	None	
4.3	**Results of controls**		

Nominal test Concentration (mg/L)	Sample (h)	Measured Concentration (mg/L)
1000	0	965.8
	0	974.8
	48	964.7
	48	967.8

Section A7.4.1.2-1 Acute toxicity to invertebrates
Annex Point IIA7.2 *Daphnia magna*
Static, 48-hour, limit test

4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations		
4.4.2	Results		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Guidelines: OECD Guideline No. 202 (Part 1), Directive 92/69/EEC (Part C.2), U.S. EPA OPPTS 850.1010 No relevant deviations from test guidelines. Method: The 48-hour acute toxicity of dinotefuran to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at a single nominal test concentration of 1000 mg/L as compared to the control (0 mg/L).	
5.2	**Results and discussion**	The analytically determined mean test item concentrations at the start and the end of the test were 97% of the nominal value. The test item MTI-446 was stable under the test conditions during the test period of 48 hours. Therefore all results are reported as nominal test concentrations. In the control (0 mg/L) and the test item concentration of 1000 mg/L, no immobilized or dead test organisms or other signs of intoxication were determined during the entire test period (48 hours). The 48-hour NOEC and the 48-hour EC_0 of dinotefuran to *Daphnia magna* were determined to be ≥ 1000 mg/L. The 48-hour EC_{50} and the 48-hour EC_{100} were >1000 mg/L. No remarkable observations were made concerning the appearance of the test medium. It was a clear solution throughout the whole test duration. At the beginning and the end of the test period, the oxygen concentrations in the test medium and the control was 8.7 mg/L and the pH-values ranged from pH 7.7 - 7.8. The EC_{50} and EC_{100} values (with 95% C.I.) could not be calculated due to insufficient immobility. The NOEC and LOEC were determined visually.	X
5.2.1	EC_0	≥1000 mg/L (based on nominal concentration)	
5.2.2	EC_{50}	>1000 mg/L	
5.2.3	EC_{100}	>1000 mg/L	
5.3	**Conclusion**	The 48-hour EC_{50} was > 1000 mg/L, which categorizes dinotefuran as nontoxic to the water flea (*Daphnia magna*) on an acute toxicity basis. The 48-hour NOEC and LOEC levels were ≥ 1000 and > 1000 mg/L, respectively.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7.4.1.2.1-1: Preparation of TS solution for test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	No
Other procedures	Not applicable

Table A7.4.1.2.1-2: Dilution water

Criteria	Details
Source	Reconstituted test water according to the Commission Directive 92/69/EEC and the OECD Guideline, however for the EPA requirements the hardness was lowered to one-half of the normal hardness. Analytical grade salts were added to purified water to obtain the following nominal concentrations, and the test water was aerated until oxygen saturation was reached.
Alkalinity	0.4mmol/L
Hardness	1.25 mmol/L (= 125 mg/L) as $CaCO_3$
pH	Not reported
Ca / Mg ratio	1.00/0.25 mmol/L (= 147/61.5 mg/L)
Na / K ratio	0.38/0.038 mmol/L (= 32.5/2.9 mg/L)
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	No

Table A7.4.1.2.1-3: Test organisms

Criteria	Details
Strain	*Daphnia magna* Straus
Source	The test subjects were clones of the species Daphnia magna, originally supplied by the University of Sheffield/UK in 1992, defined from the supplier as clone 5.
Age	6-24 hours old
Breeding method	The Daphnids from the clone line were bred in the laboratories of RCC in reconstituted water under identical conditions as in the tests.
Kind of food	Not reported
Amount of food	Not reported
Feeding frequency	Not reported
Pretreatment	No
Feeding of animals during test	No

Table A7.4.1.2.1-4: Test system

Criteria	Details
Renewal of test solution	Static test, no renewal
Volume of test vessels	250 mL glass beaker containing 150 mL test medium
Volume/animal	30 mL
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No, although beakers were covered with glass plates.

Table A7.4.1.2.1-5: Test conditions

Criteria	Details
Test temperature	20 – 21 °C
Dissolved oxygen	8.7 mg/L
pH	7.7 – 7.8
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Between 200 and 1200 lux
Photoperiod	16 hours light/8 hours dark daily

Table A7.4.1.2.1-6: Immobility of dinotefuran on *Daphnia magna*

Treatment mg/L (Nominal conc.)	Observation period			
	24 hours		48 hours	
	endpoint	% affected	endpoint	% affected
Dilution water Control	Immobile	0	Immobile	0
Solvent Control	N/A	N/A	N/A	N/A
Positive Control, if used	N/A	N/A	N/A	N/A
968.3 (1000)	Immobile	0	Immobile	0

Table A7.4.1.2.1-7: Effect data of dinotefuran on *Daphnia magna*

Treatment mg/L (Nominal conc.)	Observation period	
	24 hours	48 hours
NOEC, mg/L	≥1000	≥1000
LOEC, mg/L	>1000	>1000
EC_{50} (with 95% C.I.), mg/L	>1000	>1000

Table A7.4.1.2.1-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

Criteria	Fulfilled	Not fulfilled
Immobilisation of control animals <10%	X	
Control animals not staying at the surface	X	
Concentration of dissolved oxygen in all test vessels >3 mg/l	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04 October 2012
Materials and Methods	Applicant's version considered acceptable, noting the following: 3.1 Test material is MTI-446 (dinotefuran) as given in section 2 of the report 3.1.2 Test material is a white solid 3.1.2.3 Further relevant properties Solubility in water: 54.3 g/l at 20°C
Results and discussion	Applicant's version considered acceptable, noting the following: 4.1.1 Limit test exceeds maximum current guideline recommended dose level of 100 mg/l 5.2 The absence of 'other signs of intoxication' is not mentioned in the report
Conclusion	Applicant's version considered acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.1.2-2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 ***Daphnia magna***

Static, 48-hour, extended limit test

			Official use only
		1 REFERENCE	
1.1	**Reference**	Kelly, C.R., Murphy, C.M., Allan, J., 2001, DN phosphate determination of acute toxicity to *Daphnia* (48 h, Static), Inveresk Research, unpublished report no. 20122, September 24, 2001.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline No. 202 (Part 1) Directive 92/69/EEC (Part C.2) U.S. EPA OPPTS 850.1010	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	DN Phosphate	
3.1.1	Lot/Batch number	MU-9428M	
3.1.2	Specification	DN Phosphate is a degradation product of the parent molecule MTI-446	
3.1.3	Purity	not reported	X
3.1.4	Composition of Product	n.a.	
3.1.5	Further relevant properties	n.a.	
3.1.6	Method of analysis	Test item concentrations in the test solutions were determined by HPLC with UV detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	n.a., see Table A7.4.1.2.2-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	n.a.	
3.4	**Testing procedure**		
3.4.1	Dilution water	see Table A7.4.1.2.2-2	
3.4.2	Test organisms	see Table A7.4.1.2.2-3	
3.4.3	Test system	see Table A7.4.12.2-4	X
3.4.4	Test conditions	see Table A7.4.1.2.2-5	

Section A7.4.1.2-2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 ***Daphnia magna***

Static, 48-hour, extended limit test

3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Immobility
3.4.7	Sampling	Duplicate samples for analysis were taken from the highest concentration (100 mg/L) and the control at the start and at the end of exposure.
3.4.8	Monitoring of TS concentration	Yes 0 and 48 hours
3.4.9	Statistics	The NOEC was calculated using a Fisher's Exact Test.

4 RESULTS

4.1	**Limit Test**	Performed as extended limit test
4.1.1	Concentration	100 mg/L
4.1.2	Extended concentrations	Two replicates were tested at the additional test concentrations of 1 and 10 mg/L. No analytical test item determination was carried out.
4.1.3	Number/ percentage of animals showing adverse effects	Cumulative number of immobile daphnia

Time [h]	Nominal concentration [mg/L]			
	100	10	1	0
0	0 (0)	0 (0)	0 (0)	0 (0)
24	0 (0)	0 (0)	0 (0)	0 (0)
48	3 (10)	0 (0)	0 (0)	0 (0)

( ) % immobile daphnia

4.1.4	Nature of adverse effects	Immobility
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	100, 10, 1 and 0
4.2.2	Actual concentrations of test substance	

Time [h]	Nominal concentration [mg/L]	Mean measured concentration [mg/L]
0	0	0
48		0
0	100	114.31
48		106.79

4.2.3	Effect data (Immobilisation)	Immobilisation data as absolute numbers of immobile daphnia and as percent of exposed animals in tabular form (see Table A7.4.1.2.2-6)
4.2.4	Concentration / response curve	No dose response curve is available since a limit test was carried out
4.2.5	Other effects	None
4.3	**Results of controls**	

Section A7.4.1.2-2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 ***Daphnia magna***

Static, 48-hour, extended limit test

4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	n.a.	
4.4.2	Results	n.a.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Guidelines: OECD Guideline No. 202 (Part 1), Directive 92/69/EEC (Part C.2), U.S. EPA OPPTS 850.1010 No relevant deviations from test guidelines. Method: The 48-hour acute toxicity of DN Phosphate to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test item in an extended limit test. 30 daphnids in 6 replicates (five daphnia each) were exposed to the test item at 100 mg/L and in a blank control. 10 daphnids in 2 replicates (5 daphnia each) were exposed to the test item at 1 and 10 mg/L. No analytical determination of the test item was carried out at 1 and 10 mg/L.	
5.2	**Results and discussion**	The analytically determined mean test item concentrations at the start and the end of the test were 114 and 107 % of the nominal value. The test item DN Phosphate was stable under the test conditions during the test period of 48 hours. Therefore biological results are reported as nominal test concentrations. In the control (0 mg/L), at 1 and 10 mg/L no immobilized test organisms or other signs of toxicity were determined during the entire test period (48 hours). In the 100 mg/L treatment group, 3 out of 30 daphnids (10 %) were immobile. The 48-hour EC_{50} of DN Phosphate to Daphnia magna was determined to be > 100 mg/L. The 48-hour NOEC was determiend to be 100 mg/L. The pH ranged from 6.96 – 8.23, conductivity from 0.57 – 0.63 mS, and dissolved oxygen from 80.5 – 91.8 %. The temperature ranged from 21.0 – 22.1 °C. This is slightly outside the recommended range according to OECD guideline No. 202. Since the tepmperature deviation is only very little (0.1 °C) this is not considered as a relevant deviation and had no impact on the validity and outcome of the study.	
5.2.1	EC_0	n.d.	
5.2.2	EC_{50}	> 100 mg/L	
5.2.3	EC_{100}	> 100 mg/L	
5.3	**Conclusion**	The 48 h EC50 of DN Phosphate was > 100 mg/L under the conditions of the test. The NOEC was 100 mg/L, the highest concentration tested. All validity criteria for the study are fulfilled.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7.4.1.2.2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	n.a.
Vehicle control performed	No
Other procedures	None

Table A7.4.1.2.2-2: Dilution water

Criteria	Details
Source	Elendt M7 reconstituted water according to OECD 202
Alkalinity	not reported
Hardness	not reported
pH	7.75
Ca / Mg ratio	not reported
Na / K ratio	not reported
Oxygen content	not reported
Conductance	0.57 mS
Holding water different from dilution water	not reported

Table A7.4.1.2.2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	not reported
Age	6 – 24 hours
Breeding method	not reported
Kind of food	*Selenastrum capricornutum*
Amount of food	not reported
Feeding frequency	not reported
Pretreatment	not reported
Feeding of animals during test	No

Table A7.4.1.2.2-4: Test system

Criteria	Details
Renewal of test solution	None
Volume of test vessels	not reported
Volume/animal	20 mL
Number of animals/vessel	5
Number of vessels/ concentration	6 at 100 mg/L and in the control 2 at 1 and 10 mg/L
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2.2-5: Test conditions

Criteria	Details
Test temperature	21.0 – 22.1 °C
Dissolved oxygen	80.5 – 91.8 %
pH	6.96 – 8.23
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	not reported
Photoperiod	16 h light and 8 h dark

Table A7.4.1.2.2-6: Immobilisation data

Test-Substance Concentration (nominal/effective) [1] [mg/l]	Immobile *Daphnia*						
	Number		Percentage		Oxygen [mg/l]	pH	Tempera-ture [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
0	0	0	0	0	91.8	7.79	21.0
1	0	0	0	0	91.8	7.84	21.2
10	0	0	0	0	90.0	7.81	21.2
100	0	3	0	10	91.4	7.61	21.2

[1] specify, if TS concentrations were nominal or measured

Table A7.4.1.2.2-7: Effect data

	EC_{50} [1]	95 % c.l.	EC_0 [1]	EC_{100} [1]
24 h [mg/l]	> 100mg/L	n.a.	n.d.	> 100mg/L
48 h [mg/l]	> 100mg/L	n.a.	n.d.	> 100mg/L

[1] effect data are based on nominal concentrations

Table A7.4.1.2.2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	Fulfilled	Not fullfilled
Immobilisation of control animals <10%	X	
Control animals not staying at the surface	X	
Concentration of dissolved oxygen in all test vessels >3 mg/l	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	03 October 2012
Materials and Methods	Applicant's version considered acceptable, noting the following: 3.1.3 The purity of the test item has not been presented in the report and purity may have an impact on the toxicity. However, the RMS has obtained a copy of the appropriate certificate of analysis and can confirm that the purity of the test item is 99.5%. 3.4.3 Test System: Table A7 4.1.2.2-4 Volume of test vessels is reported as 200 ml.
Results and discussion	Applicant's version considered acceptable.
Conclusion	Applicant's version considered acceptable noting the following: 5.3 Appendix 3 of the report states that it was not possible to determine the NOEC concentration of DN Phosphate but in the same paragraph states there were no statistically significant differences between the 0 mg/l and 100 mg/l dose groups (p=0.12). This is contradictory and the RMS considers the NOEC to be 100 mg/L as stated in the main study report and RSS.
Reliability	1
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.1.3-1 **Growth inhibition test on algae**
Annex Point IIA7.3 ***Pseudokirchneriella Subcapitata***
(formerly *Selenastrum Capricornutum*)
Static, 96-hour

			Official use only
		1 REFERENCE	
1.1	**Reference**	Seyfried, B., 2000a, Toxicity of MTI-446 to *Pseudokirchneriella subcapitata* (Formerly *Selenastrum capricornutum*) in a 96-hour algal growth inhibition test, RCC Ltd., unpublished report no. 740981, February 10, 2000. Seyfried, B., 2000b, First amendment to report: Toxicity of MTI-446 to *Pseudokirchneriella subcapitata* (Formerly *Selenastrum capricornutum*) in a 96-hour algal growth inhibition test, RCC Ltd., unpublished report no. 740981, March 28, 2000. Seyfried, B., 2000c, Second amendment to report: Toxicity of MTI-446 to *Pseudokirchneriella subcapitata* (Formerly *Selenastrum capricornutum*) in a 96-hour algal growth inhibition test, RCC Ltd., unpublished report no. 740981, April 11, 2000.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes U.S. EPA OPPTS 850.5400 OECD Guideline No. 201 1984 Directive 92/69/EEC (C.3)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	5500310	
3.1.2	Specification		
3.1.2.1	Purity	97.26%	
3.1.2.2	Stability	Expiration date: June 30, 2004	
3.1.2.3	Further relevant properties	Solubility in water: 39.83 g/L at 20 °C Stability in water: > 24 hours (Sponsor information) Stability in test water ≥ 96 h; examined within this study	X
3.1.3	Method of analysis	Aqueous samples containing dinotefuran were analysed on a high performance liquid chromatographic (HPLC) system using UV detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test**	- The test substance is not poorly soluble or volatile. See Table A7.4.1.3.1-1	

Section A7.4.1.3-1 **Growth inhibition test on algae**
Annex Point IIA7.3 ***Pseudokirchneriella Subcapitata***
(formerly *Selenastrum Capricornutum*)
Static, 96-hour

	substances	
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Culture medium	Macro-nutrients: $NaHCO_3$ 50.0 mg/L $CaCl_2$ x 2 H_2O 18.0 mg/L NH_4Cl 15.0 mg/L $MgSO_4$ x 7 H_2O 15.0 mg/L $MgCl_2$ x 6 H_2O 12.0 mg/L KH_2PO_4 1.6 mg/L Trace elements: Na_2EDTA x 2 H_2O 100.0 µg/L $FeCl_3$ x 6 H_2O 80.0 µg/L $MnCl_2$ x 4 H_2O 415.0 µg/L H_3BO_3 185.0 µg/L Na_2MoO_4 x 2 H_2O 7.0 µg/L $ZnCl_2$ 3.0 µg/L $CoCl_2$ x 6 H_2O 1.5 µg/L $CuCl_2$ x 2 H_2O 0.01 µg/L Hardness: 0.24 mmol/L (= 24 mg/L) as $CaCO_3$ pH: 8.1
3.4.2	Test organisms	See Table A7.4.1.3.1-2
3.4.3	Test system	See Table A7.4.1.3.1-3
3.4.4	Test conditions	See Table A7.4.1.3.1-4
3.4.5	Duration of the test	96-hours
3.4.6	Test parameter	Biomass and growth rate
3.4.7	Sampling	After 24, 48, 72, and 96 hours of exposure, small volumes of the test media and the control were taken out to determine cell densities.
3.4.8	Monitoring of TS concentration	Yes Sampling prior to test initiation and after 96-hours (both with and without algae) for the highest test concentration.
3.4.9	Statistics	- The E_bC_{50} and $E_\mu C_{50}$ and the corresponding EC_{10} and EC_{90} and their 95%-confidence limits could not be calculated due to the absence of a toxic effect of the test item. - The LOEC and the NOEC were determined directly from the mean biomass and the mean growth rates

Section A7.4.1.3-1 **Growth inhibition test on algae**
Annex Point IIA7.3 ***Pseudokirchneriella Subcapitata***
(formerly *Selenastrum Capricornutum*)
Static, 96-hour

4 RESULTS

4.1 Limit Test Not performed

4.1.1 Concentration Not applicable

4.1.2 Number/ percentage of animals showing adverse effects Not applicable

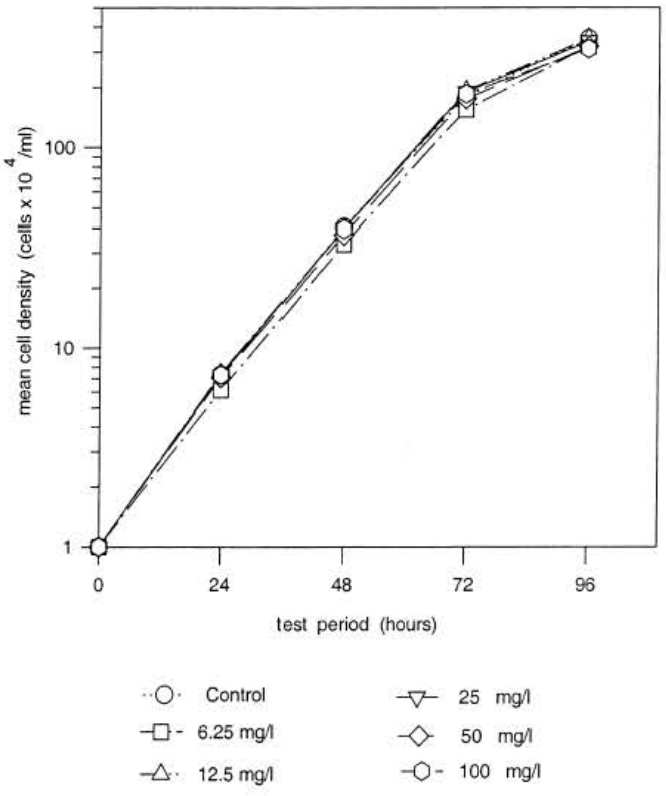
4.2 Results test substance

4.2.1 Initial concentrations of test substance Nominal: 6.25, 12.5, 25, 50, and 100 mg/L

4.2.2 Actual concentrations of test substance

Nominal test Concen-tration (mg/L)	Sample date (day)	Sample Age (hours)	Measured Concent-ration (mg/L)[1]	% of Nominal	Mean Measured Concentration (mg/L)	Mean Measured % of Nominal
100	0	0	99.5	100	99.6	100
	0	0	99.7	100		
	4	96	95.2	95	95.6	96
	4	96	95.9	96		

4.2.3 Growth curves



Section A7.4.1.3-1 **Growth inhibition test on algae**
Annex Point IIA7.3 ***Pseudokirchneriella Subcapitata***
(formerly *Selenastrum Capricornutum*)
Static, 96-hour

4.2.4	Concentration / response curve	No concentration/response curves were reported
4.2.5	Cell concentration data	See Table A7.4.1.3.1-5
4.2.6	Effect data (cell multiplication inhibition)	LOEC: > 100 mg/L EC_{50}: > 100 mg/L NOEC (growth rate): 100 mg/L
4.2.7	Other observed effects	None
4.3	**Results of controls**	The mean algal cell densities in the treated test media were nearly identical with those in the parallel control culture throughout the entire test duration. At microscopic examination of the shape of the algal cells after 96 hours, no difference was observed between the algae growing in the test concentration of 100 mg/L and those in the control. The control cell density increased from nominal N = 1 x 10^4 cells/mL at the start of the test (0 hours) to N = 179 x 10^4 cells/mL (mean value) after 72 hours. Algal cell densities during the test period of 96 hours (control):

Nominal (mg/L)	Flask No.	Density of algal cells (cell number x 10,000/mL) after							
		24 h		48 h		72 h		96 h	
Control	1	6.9	7.0	37.8	38.0	179.3	178.7	356.3	352.8
	2	7.3	7.3	39.2	40.5	190.9	187.4	341.0	345.2
	3	8.2	8.4	39.8	39.9	182.2	181.0	345.8	347.1
	4	6.9	6.6	38.4	38.6	166.0	175.1	350.8	354.3
	5	7.0	7.3	39.3	39.3	192.4	191.3	356.2	355.1
	6	7.8	7.2	44.5	45.5	165.9	163.0	335.3	348.1
	m	7.33		40.07		179.43		349.00	
	s	0.54		2.54		10.55		6.04	
	n	6		6		6		6	
m: mean value, s: standard deviation, n: number of flasks									
At the start. 10,000 algal cells/mL were incubated									

4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not applicable
4.4.2	Results	Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Guidelines: U.S. EPA OPPTS 850.5400, OECD Guideline No. 201, Directive 92/69/EEC (C.3) No relevant deviations from test guidelines. Method: A study was conducted to investigate the effects of MTI-446 on the

Section A7.4.1.3-1 **Growth inhibition test on algae**
Annex Point IIA7.3 ***Pseudokirchneriella Subcapitata***
(formerly *Selenastrum Capricornutum*)
Static, 96-hour

		growth of the green algal species *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a 96-hour static test. The test concentrations were 6.25, 12.5, 25, 50, and 100 mg/L, and one control.	
5.2	**Results and discussion**	No difference was observed between the algae growing in the test concentration of 100 mg/L and the algal cells in the control at the microscopic examination of the algal cell shape after 96 hours test period. The shape of the algal cells growing up to this concentration was obviously not affected. The control cell density increased from nominal N = 1 x 10^4 cells/mL at the start of the test (0 hours) to N = 179 x 10^4 cells/mL (mean value) after 72 hours. As a result, the algal growth in the control was sufficiently high under the test conditions, and the validity criterion of increase by at least a factor of 16 was fulfilled. The cell density further increased to N = 350 x 10^4 cells/mL (mean value) at the end of the test (96 h). There were no remarkable test media appearance observations. Throughout the test period, all test media were clear solutions. The test media and the control pH values were between 8.1 and 8.2 at the start and the end of the test.	
5.2.1	NOEC	100 mg/L	X
5.2.2	$E_\mu C_{50}$	> 100 mg/L	X
5.2.3	$E_b C_{50}$	> 100 mg/L	X
5.3	**Conclusion**	Validity criteria can be considered fulfilled (see validity criteria summarized in Table A7.4.1.3.1-6 Dinotefuran did not significantly inhibit the growth of *Pseudokirchneriella subcapitata* during the exposure period of 96 hours up to and including the nominal test concentration of 100 mg/L. This concentration was determined as the 96-hour NOEC (highest concentration tested without toxic effects after a test period of 96 hours). The 96-hour LOEC (lowest concentration tested with toxic effects), and the 96-hour EC_{50} for the algal biomass b and growth rate μ could not be quantified due to the absence of any dinotefuran toxic effect at the tested concentrations. However, these parameters were clearly higher than 100 mg/L.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7.4.1.3.-1: Preparation of TS solution for test substance

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	No
Other procedures	Not applicable

Table A7.4.1.3.1-2: Test organisms

Criteria	Details
Species	*Pseudokirchneriella subcapitata (*formerly *Selenastrum capricornutum)*
Strain	SAG-61.81
Source	Sammlung von Algenkultuen, Pflanzenphysiologisches Institut der Universität Göttingen, D-37073
Laboratory culture	Yes
Method of cultivation	Cultured under standardised conditions according to the test guidelines.
Pretreatment	Not reported
Initial cell concentration	Approximately 10^4 cells/ml.

Table A7.4.1.3.1-3: Test system

Criteria	Details
Volume of culture flasks	50 mL Erlenmeyer flasks; 15 mL medium/flask
Culturing apparatus	Water bath for temperature regulation
Light quality	Continuous illumination at mean 8400 Lux (range: 7900 to 9000 Lux), fluorescent tubes.
Procedure for suspending algae	Continuous stirring by magnetic stirrers
Number of vessels/ concentration	Test concentrations: 3 vessels/concentration Control: 6 vessels/concentration
Test performed in closed vessels due to significant volatility of TS	No, although flasks were covered with glass dishes

Table A7.4.1.3.1-4: Test conditions

Criteria	Details
Test temperature	22 – 23℃
pH	8.1 –8.2
Aeration of dilution water	No
Light intensity	Mean 8400 Lux (range: 7900 to 9000 Lux)
Photoperiod	Continuous illumination

Table A7.4.1.3.1-5: Cell concentration data

Test-Substance Nominal Concentration [mg/l]	Mean density of algal cells (cell number x 10,000/mL) after:			
	Number			
	24 h	48 h	72 h	96 h
Control	7.33	40.07	179.43	349.00
6.25	6.13	33.08	154.45	320.30
12.5	7.45	39.38	192.28	350.47
25	7.28	39.42	189.72	339.68
50	7.10	36.87	175.02	318.80
100	7.30	39.27	185.22	311.40
Temperature [°C]	22	23	23	23
pH	8.1 – 8.2	8.1 – 8.2	8.1 – 8.2	8.1 – 8.2

Table A7.4.1.3.1-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201

Criteria	Fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	8 October 2012
Materials and Methods	Applicant's version considered acceptable, noting the following: 3.1 Test material is MTI-446 (dinotefuran) as given in section 2 of the report 3.1.2 Test material is a white solid 3.1.2.3 Further relevant properties Solubility in water: 54.3 g/l at 20°C
Results and discussion	Applicant's version considered acceptable, noting the following: 5.2.1-3 Concentrations used are nominal.
Conclusion	Applicant's version considered acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.1.3-2 Growth inhibition test on algae

Annex Point IIA7.3

			Official use only
		1 REFERENCE	
1.1	**PReference**	Kelly, C.R., Ferguson, K., 2002b, DN phosphate alga, growth inhibition test (96 h), Inveresk Research, unpublished report no. 19849, January 18, 2002.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes U.S. EPA OPPTS 850.5400 and general agreement with OECD Guideline No. 201 1984	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	DN Phosphate	
3.1.1	Lot/Batch number	MU-9428M	
3.1.2	Specification	DN Phosphate is a degradation product of the parent molecule MTI-446	
3.1.3	Purity	not reported	X
3.1.4	Composition of Product	n.a.	
3.1.5	Further relevant properties	n.a.	
3.1.6	Method of analysis	Test item concentrations in the test solutions were determined by HPLC with UV detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	n.a., see Table A7.4.1.3.2-2	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	n.a.	
3.4	**Testing procedure**		

Section A7.4.1.3-2 Growth inhibition test on algae

Annex Point IIA7.3

3.4.1	Culture medium	Macro-nutrients: $NaHCO_3$ 50.0 mg/L $CaCl_2 \times 2 H_2O$ 18.0 mg/L NH_4Cl 15.0 mg/L $MgSO_4 \times 7 H_2O$ 15.0 mg/L $MgCl_2 \times 6 H_2O$ 12.0 mg/L KH_2PO_4 1.6 mg/L Trace elements: $Na_2EDTA \times 2 H_2O$ 100.0 µg/L $FeCl_3 \times 6 H_2O$ 80.0 µg/L $MnCl_2 \times 4 H_2O$ 415.0 µg/L H_3BO_3 185.0 µg/L $Na_2MoO_4 \times 2 H_2O$ 7.0 µg/L $ZnCl_2$ 3.0 µg/L $CoCl_2 \times 6 H_2O$ 1.5 µg/L $CuCl_2 \times 2 H_2O$ 0.01 µg/L	
3.4.2	Test organisms	see Table A7.4.1.3.2-2	
3.4.3	Test system	see Table A7.4.1.3.2-3	X
3.4.4	Test conditions	see Table A7.4.1.3.2-4	
3.4.5	Duration of the test	96-hours	
3.4.6	Test parameter	Cell concentrations and growth rate	
3.4.7	Sampling	After 24, 48, 72, and 96 hours of exposure, small volumes of the test media and the control were taken out to determine cell densities.	
3.4.8	Monitoring of TS concentration	Yes 0 and 96-hours	
3.4.9	Statistics	The analysis for each of the NOEC was based on a parametric ANOVA followed by a Dunnett's test, given that Levene's test was not significant at the 1 % significance level. As a one sided Dunnett's test has been used it should be noted that in several instances the AUC and growth rate values were higher for the active dose levels than for the control.	
		4 RESULTS	
4.1	**Limit Test**	Performed as extended limit test	
4.1.1	Concentration	100	
4.1.2	Extended concentrations	Six test flasks (100 mL) were prepared at 100 and 0 mg/L and three flasks were prepared at 1 and 10 mg/L. For the purpose of chemical analysis an extra flask was prepared at 100 and 0 mg/L. The extra flasks were not inoculated with algae. No analytical determination of the test item was carried out at the 1 and 10 mg/L does levels.	
4.1.3	Number/ percentage of animals showing adverse effects	n.a.	
4.2	**Results test substance**		
4.2.1	Initial concentrations of	100, 10, 1 and 0 mg/L	

Section A7.4.1.3-2 Growth inhibition test on algae

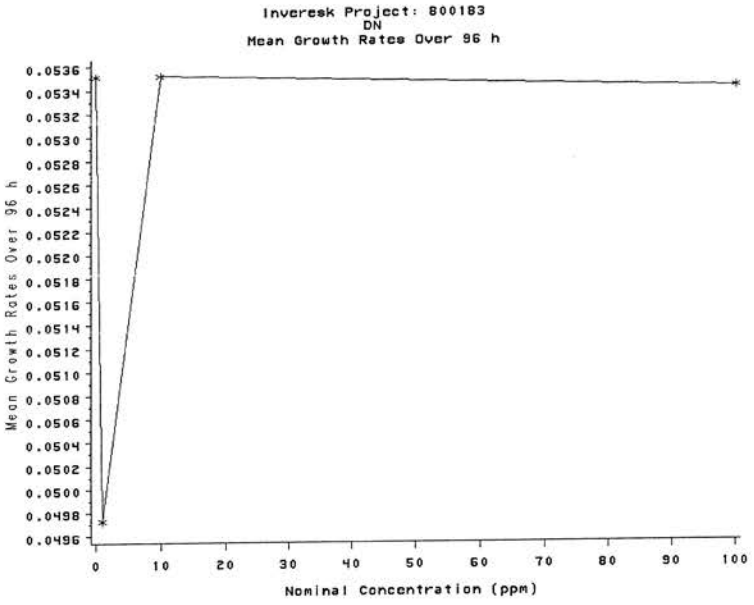
Annex Point IIA7.3

test substance

4.2.2 Actual concentrations of test substance

Time [h]	Nominal concentration [mg/L]	Mean measured concentration [mg/L]
0	0	ND
96		ND
0	100	95.8
96		105.1

4.2.3 Growth curves



X

4.2.4 Concentration / response curve

n.a.

4.2.5 Cell concentration data

see Table A7.4.1.3.2-5

4.2.6 Effect data (cell multiplication inhibition)

Growth function	Tine interval [h]	EC_{50}	NOEC [mg/L]
Average specific growth rate (μ_{ave} x day $^{-1}$)	0 - 24	> 100	100
	0 -48	> 100	100
	0 – 72	> 100	100
	0 - 96	> 100	100
Area under growth curve (AUC)	0 - 24	> 100	100
	0 -48	> 100	100
	0 – 72	> 100	100
	0 - 96	> 100	100

Section A7.4.1.3-2 Growth inhibition test on algae

Annex Point IIA7.3

4.2.7 Other observed effects

The pH range of the test solutions deviated from that specified in the OECD guideline No. 201. This is not a consideration for the OPPTS 850.5400 guideline for a 96-hours test. The high level of growth of the algae cultures is likely to have caused this pH change. This is not considered to have affected the validity of the test result.

4.3 Results of controls

Time [h]	Mean cell concentration [x 10^5 cells mL^{-1}]
0	0.10
24	0.52
48	2.77
72	8.29
96	17.53

	Average specific growth rate [μ_{ave} x day^{-1}]	Area under growth curve [x 10^5 cells.h.mL^{-1}]
0 – 24	0.071	0.225
	0.058	0.150
	0.058	0.150
	0.065	0.190
	0.086	0.340
	0.065	0.190
0 – 48	0.071	0.958
	0.066	0.720
	0.062	0.625
	0.065	0.728
	0.073	1.165
	0.075	1.060
0 – 72	0.060	2.367
	0.056	1.810
	0.061	2.022
	0.061	2.215
	0.065	3.060
	0.064	2.937
0 – 96	0.055	4.983
	0.058	5.220
	0.050	4.058
	0.051	4.328
	0.055	6.145
	0.052	5.290

4.4 Test with reference substance

Not performed

4.4.1 Concentrations

n.a.

4.4.2 Results

n.a.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

U.S. EPA OPPTS 850.5400 and general agreement with OECD Guideline No. 201 1984

No relevant deviations from test guidelines.

Method:

The effect of DN Phosphate upon the growth of *Selenastrum capricornutum* was determined in an extended limit test over a 96 h period.

The nominal concentrations tested were 100, 10, 1 and 0 mg/L.

Six test flasks (100 mL) were prepared at 100 and 0 mg/L and three flasks were prepared at 1 and 10 mg/L. For the purpose of chemical analysis, an extra flask was prepared at 100 and 0 mg/L. The extra flasks were not inoculated with algae. No analytical determination of the test item was carried out at the 1 and 10 mg/L does levels.

5.2 Results and discussion

The initial measured concentrations were 95.8 and 0 mg/L for the 100 mg/L treatment and the control, respectively. After 96 hours the measured concentrations were 105.1 and 0 mg/L for the 100 mg/L and the control respectively. Therefore, biological results are based on nominal concentrations.

The results are reported as daily cell concentrations (cells. mL^{-1}), average specific growth rates (day^{-1}) and areas under the growth curves (cells.h.mL^{-1}). In addition the NOEC was calculated.

Growth function	Tine interval [h]	EC_{50}	NOEC [mg/L]
Average specific growth rate (μ_{ave} x day^{-1})	0 - 24	> 100	100
	0 -4 8	> 100	100
	0 – 72	> 100	100
	0 - 96	> 100	100
Area under growth curve (AUC)	0 - 24	> 100	100
	0 -4 8	> 100	100
	0 – 72	> 100	100
	0 - 96	> 100	100

The pH range of the test solutions deviated from that specified in the OECD guideline No. 201. This is not a consideration for the OPPTS 850.5400 guideline for a 96-hours test. The high level of growth of the algae cultures is likely to have caused this pH change. This is not considered to have affected the validity of the test result.

5.2.1 NOE_rC 100 mg/L

5.2.2 EC_{50} > 100 mg/L

5.3 Conclusion

Validity criteria can be considered fulfilled (see validity criteria summarized in Table A7.4.1.3.2-6.

DN Phosphate did not significantly inhibit the growth of *Selenastrum capricornutum* during the exposure period of 96 hours up to and including the nominal test concentration of 100 mg/L. This concentration was determined as the 96-hour NOEC (highest concentration tested without toxic effects after a test period of 96 hours). The 96-hour EC_{50} was > 100 mg/L.

5.3.1 Reliability 1

5.3.2 Deficiencies No

Table A7.4.1.3.2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	n.a.
Vehicle control performed	No
Other procedures	None

Table A7.4.1.3.2-2: Test organisms

Criteria	Details
Species	*Selenastrum capricornutum*
Strain	278/4
Source	CCAP, Ambleside, UK
Laboratory culture	Yes
Method of cultivation	axenic subcultures
Pretreatment	not reported
Initial cell concentration	10^4 cells/mL

Table A7.4.1.3.2-3: Test system

Criteria	Details
Volume of culture flasks	250 mL
Culturing apparatus	Improved Neubauer Counting Chamber
Light quality	uniform illumination in the spectral range 400 -700 nm by artificial daylight fluorescent tubes
Procedure for suspending algae	Shaking on vertical shaker
Number of vessels/ concentration	
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.3.2-4: Test conditions

Criteria	Details
Test temperature	22 – 26 °C
pH	7.6 – 10.1
Aeration of dilution water	No
Light intensity	light intensity 4190 Lux
Photoperiod	continuous

Table A7.4.1.3.2-5: Cell concentration data

Test Substance Concentration (nominal/effective)[1] [mg/l]	Cell concentrations (mean values) [cells x 10^5/ml]								
	measured					Percent of control			
	0 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
100	0.1	0.65	2.66	7.63	17.34	125%	96%	92%	99%
10	0.1	0.52	3.09	7.13	18.61	100%	112%	86%	106%
1	0.1	0.64	2.93	7.75	12.03	123%	106%	93%	69%
0	0.1	0.52	2.77	8.29	17.53	100%	100%	100%	100%
Temperature [°C]		22 - 25	24 - 26	23 - 25	23 - 24				
pH	7.4- 8.2	n.d.	n.d.	n.d.	7.6 – 10.1				

[1]: TS concentrations were nominal

Table A7.4.1.3.2-6 Validity criteria for algal growth inhibition test according to OECD Guideline 201

	Fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 17 October 2012

Materials and Methods Applicant's version considered acceptable, noting the following:

3.1.3 The purity of the test item has not been presented in the report and purity may have an impact on the toxicity. However, the RMS has obtained a copy of the appropriate certificate of analysis and can confirm that the purity of the test item is 99.5%.
3.4.3 Table A7 4.1.3.2-3 Number of vessels at 100 mg/l and 0 mg/l was 6 with 3 vessels at other dose levels.

Results and discussion Applicant's version considered acceptable, noting the following:

4.2.3 Growth curves: The graph presented would suggest that there is a marked drop in the growth rate. However, this is due to the small scale used which has enhanced the appearance of a very slight decrease.

Conclusion Applicant's version considered acceptable

Reliability 2

Acceptability Acceptable

Remarks

COMMENTS FROM ...

Date

Materials and Methods

Results and discussion

Conclusion
Reliability
Acceptability
Remarks

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Falk, S., 2012, Toxicity testing of dinotefuran technical - on microorganisms with the activated sludge respiration inhibition test, Eurofins Agroscience Services, unpublished report no. S11-03209, March 19, 2012	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline 209 (2010)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, see Table A7.4.1.4-5	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Dinotefuran technical	
3.1.1	Lot/Batch number	K09A3559	
3.1.2	Specification	As given in section 2	X
3.1.3	Purity	99.4 %	
3.1.4	Composition of Product	Test item is not a product. See 3.1.3 for purity.	
3.1.5	Further relevant properties	Solubility in water: 39.83 g/L Stability in water: > 24 hours (Sponsor information)	X
3.1.6	Method of analysis	Not applicable	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	Yes 3,5-dichlorophenol (DCP) as positive control	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**	Non-entry field	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

3.4.1	Culture medium	The test was performed in a synthetic wastewater that was prepared with the following amounts of substances in 1 litre of deionised water: • 16 g peptone • 11 g meat extract • 3 g urea • 0.7 g NaCl • 0.4 g $CaCl_2 \times 2\ H_2O$ • 0.2 g $MgSO_4 \times 7\ H_2O$ • 2.8 g K_2HPO_4 The solution was sterilized prior to storage
3.4.2	Inoculum / test organism	See Table A7.4.1.4-2
3.4.3	Test system	See Table A7.4.1.4-3
3.4.4	Test conditions	See Table A7.4.1.4-4
3.4.5	Duration of the test	3 hours
3.4.6	Test parameter	Total inhibition, inhibition of the heterotrophic organisms and inhibition of nitrifying bacteria
3.4.7	Analytical parameter	Oxygen uptake
3.4.8	Sampling	30 min and 3 hours
3.4.9	Monitoring of TS concentration	None
3.4.10	Controls	• negative control without test substance • positive control (with DCP: reference substance) • nitrification control (with N-Allylthiourea (ATU)) • abiotic control (without inoculum)
3.4.11	Statistics	The statistical evaluation was performed for specific respiration rate of the control and highest concentration using SAS® (2002–2008). The calculation was performed using SAS Software service pack 9.2. The NOEC was determined using the t-Test pooled. P-values below 0.05 showed statistically significant differences to the control. A test for normality of the data was performed by calculating the Shapiro-Wilk's statistic. The EC_{50}-values for the reference test item DCP were evaluated using probit analysis following normal and logistic distribution. In the case of the nitrifiers the EC_{50} value for DCP after 3 h was extrapolated with a linear regression curve. The EC_{50} calculations were based on the percentage of inhibition.
		4 RESULTS
4.1	**Preliminary test**	Not performed
4.2	**Results test substance**	Non-entry field
4.2.1	Initial concentrations of test substance	10, 100 and 1000 mg/L

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

4.2.2	Actual concentrations of test substance	Not applicable
4.2.3	Growth curves	Not applicable
4.2.4	Cell concentration data	Not applicable
4.2.5	Concentration/ response curve	Not applicable
4.2.6	Effect data	NOEC = 1000 mg/L; EC_{50} > 1000 mg/L
4.2.7	Other observed effects	None
4.3	**Results of controls**	Negative control: The total oxygen uptake rates of the negative controls were determined to be 27.85 and 30.83 mg O_2/(g × h) dry matter after 30 min and 3 h, respectively. Positive control: See subsection 4.4 Nitrification control: For nitrification the oxygen uptake rates were 11.74 and 13.02 mg O_2/(g × h) dry matter after 30 min and 3 h, respectively. These values are the difference between the control and the average of the heterotrophic rates. Abiotic control: The total oxygen uptake rates of the abiotic controls ranged from – 0.83 to 0.09 mg O_2/(g x h) after 30 mins and ranged from – 0.40 to 1.38 mg O_2/(g x h) after 3 h.
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	2, 5, 10, 25 mg/L DCP. Additionally, for each concentration and replicate of the test item assay and of the reference concentrations a nitrification control with ATU was prepared.
4.4.2	Results	For the total oxygen uptake, the EC_{50} was determined to be 11.29 mg/L after 30 min and 6.17 mg/L after 3 h. These values were within the recommended range of 2 to 25mg/L. For the heterotrophic oxygen uptake, the EC_{50} was determined to be 23.03 after 30 min and 15.60 mg/L after 3h. These values were within the recommended range of 5 to 40 mg/L. For the oxygen uptake due to nitrification the EC_{50} was determined to be 2 mg/L after 30 min and 1.67 mg/L after 3h. Both values were within the recommended range of 0.1 to 10 mg/L

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Guidelines: OECD Guideline 209 (2010) No relevant deviations from test guidelines.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

		Method: Test item: dinotefuran technical, batch No. K09A3559. Activated sludge from the municipal wastewater treatment plant of Pforzheim, Germany, was used as microbial inoculum for the test. This plant predominantly is treating domestic sewage. The sludge was adjusted to a content of 1.5 g/L dry matter and was exposed to the test item under continuous aeration. After stopping the aeration, the O_2 consumption was measured for approx. 5 minutes. The slope of the O_2 consumption straight line is an indication for toxic effects on respiration activity of microorganisms. A range-finding test was performed with concentrations from 10, 100 and 1000 mg/L in a limit test design. The highest concentration of 1000 mg/L was tested in three replicates whereas 10 and 100 mg/L were tested in one replicate. A water control consisting of three replicates was also included. Four concentrations of DCP as toxic reference item were also tested to demonstrate the sensitivity of the test system. To test the differences between total oxygen uptake, heterotrophic oxygen uptake and oxygen uptake due to nitrification, two test assays were performed, one with and one without ATU respectively.	
5.2	**Results and discussion**	The total oxygen uptake was measured at the limit test concentration of 1000 mg/L. After 30 min, an inhibitory effect of -1.8 % (average) was determined. After 3 hours, an inhibitory effect of 1.7 % (average) was determined. This effect was statistically not significant (p-value was 0.3293, determined to be above 0.05). For the heterotrophic oxygen uptake, after 30 min an average inhibitory effect of 10.8 % was determined. After 3 hours, an average inhibitory effect of 2.8 % was determined. This effect was statistically not significant (p-value was 0.4193 determined to be above 0.05). Due to the fact that the observed effects were not statistically significant the NOEC was determined to be 1000 mg/L. The test fulfils the criteria of validity, since: · For the total oxygen uptake, the EC_{50} was determined to be 11.29 mg/L after 30 min and 6.17 mg/L after 3 h. These values were within the recommended range of 2 to 25mg/L. For the heterotrophic oxygen uptake, the EC_{50} was determined to be 23.03 after 30 min and 15.60 mg/L after 3h. These values were within the recommended range of 5 to 40 mg/L. For the oxygen uptake due to nitrification the EC_{50} was determined to be 2 mg/L after 30 min and 1.67 mg/L after 3h. Both values were within the recommended range of 0.1 to 10 mg/L. · The coefficient of variation of the control was 6 % and 3 % after 30 min and 3 h for total oxygen uptake, respectively and did not exceed 30 %. · The oxygen uptake rate of the blank controls were 27.85 (mean) and 30.83 (mean) mg O_2/(g x h) dry matter after 30 min and 3 h, which is within the recommended range. There was not less than 20 mg oxygen uptake per g of activated sludge (dry weight of suspended solids) in 1 hour.	X
5.2.1	EC_{20}	Not reported	
5.2.2	EC_{50}	> 1000 mg/L	
5.2.3	EC_{80}	Not reported	