Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

5.3	**Conclusion**	All validity criteria according to OECD guideline 209 are fulfilled. The test item dinotefuran technical had no toxic effects on the oxygen uptake at the highest concentration tested (1000 mg/L). Hence, the EC_{50} of dinotefuran technical is > 1000 mg/L.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A7.4.1.4-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7.4.1.4-2: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	not applicable
Strain	not applicable
Source	Municipal wastewater treatment plant, collected from the aeration tank
Sampling site	Pforzheim, Germany
Laboratory culture	No
Method of cultivation	
Preparation of inoculum for exposure	The sludge was used one day after collection. It was settled for about 10 minutes and the upper layer with finer solids was decanted. Before starting the test, it was washed three times with chlorine free tap water by centrifugation (10 minutes at 3000 rpm). After centrifuging, the supernatant was decanted and discarded and the sludge was re-suspended in chlorine free tap water. This procedure was repeated twice. The mixed liquor suspended solids (MLSS) were adjusted to a concentration of 3.0 g/L (± 10 %). The activated sludge was continuously aerated at the test temperature, the solids did not settle down.
Pretreatment	None
Initial cell concentration	Not reported

Table A7.4.1.4-3: Test system

Criteria	Details
Culturing apparatus	BOD flasks
Number of culture flasks/concentration	3 replicates for the negative, the abiotic and the heterotrophic control (ATU) 3 replicates each at 1000mg/L for total oxygen uptake and heterotrophic oxygen uptake (ATU) 1 replicate each for the positive control (DCP), the 10 and 100 mg/L total oxygen uptake and heterotrophic uptake samples
Aeration device	air pump
Measuring equipment	O_2-electrode: Dissolved Oxygen Hand-Held Meter OXI 340, WTW
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.4-4: Test conditions

Criteria	Details
Duration	3 hours, during which the test assays were aerated
Test vessels	1 litre glass beakers
Test volume	500 mL
Nutrient solution	16 mL synthetic wastewater
Temperature	17.6 – 20.0 °C (measured during O_2-determination at 30 min and 3 h after start of respiration)
pH of the test solutions	7.78 – 8.22

Table A7.4.1.4-5: Deviations from the study plan

Section of study plan	Deviation	Reason	Impact on the study
2.1 Test item	The date of the certificate of analysis changed from 05 September 2011 to 24 November 2010.	Mistake during study plan preparation.	None.
2.2 Reference item	The stock solution of the reference item was not prepared with 0.5 g with a final volume of 500 mL but with 0.25 g filled up to a final volume of 250 mL.	Mistake during study plan preparation	None, since the concentration was the same
2.5 Microbial Inoculum	The sludge was not used on the same day as collec-tion but on the next day.	Mistake in study performance	None, since the guideline allows storage up to two days
2.5 Microbial Inoculum	The sludge was not centrifuged for 20 min at 4500 rpm but for 10 min at 3000 rpm.	Mistake in study performance.	None, since the sludge was centrifuged to produce a clear supernatant and pellet of sewage solids, therefore fulfilling guideline criteria as the guide-line does not stipulate a speed of centrifugation.
2.8 Test conditions	The temperature of the test decreased to 17.6 °C.	Mistake in study performance	None, since the deviation did not have an adverse effect on the validity and outcome of the study. The guideline recommends a temperature range of 20 ± 2 °C. The lowest recorded temperature was 17.6°C. However, the test item showed no adverse effect and the EC50 value of the reference item was within the recommended range. Therefore, the temperature difference of 0.4°C is considered not to have had an adverse effect on the validity and the outcome of the study.
2.7.2 Procedure of the Test	The pH measured ranged from 7.92 – 8.22. This is above the recommended range of 7.5 ± 0.5.	Mistake in study performance	None, since the deviation did not have an adverse effect on the validity and outcome of the study. The test item showed no adverse effect and the EC50 of the reference item was within the recommended range. Therefore, the pH difference of 0.2 is considered not to have had an adverse effect on the validity and the outcome of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	8 October 2012
Materials and Methods	Applicant's version considered acceptable, noting the following: 3.1.2 Section 2 of the report refers to the time schedule. Should read 'Section 4 of the report'. Include expiry date: 31 October 2012 and appearance of test item: white crystalline solid. 3.1.5 Solubility in water is reported as 40 g/l (difference may be due to rounding?)
Results and discussion	Applicant's version is considered acceptable, noting the following: 5.2 The first paragraph regarding the validity criteria is believed to refer to the reference substance but this is not stated explicitly.
Conclusion	Applicant's version is considered acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.2 Annex Point IIA7.5	**Bioconcentration in aquatic organisms** **Estimation of the intrinsic potential**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Bioconcentration by aquatic organisms is an important factor in the environmental risk assessment process. The bioconcentration factor is defined as the ratio between the concentration of the chemical in biota and the concentration in water at equilibrium. An estimation of the intrinsic potential for bioconcentration in aquatic organisms on the basis of physical and chemical properties (e.g. partition coefficient n-octanol/ water) or a relevant study available should be submitted. Dinotefuran is highly soluble in water with a solubility of 39.83 g/L (Malinski, 2000a; Section A3.5) and does not dissociate over the environmentally significant pH range. Dinotefuran has a partition coefficient n-octanol / water log K_{OW} = - 0.549 at 25°C (K_{OW} = 0.283). The requirement for measured bioconcentration studies in aquatic organisms is triggered when the log K_{OW} is > 3. Since the partition coefficient n-octanol / water is - 0.549, there is no risk of bioconcentration in fish and no requirement to measure experimentally directly. An estimation of the dinotefuran bioconcentration factor in fish has been determined. The QSAR models used are according to the Technical Guidance Document on Risk Assessment and published in literature, Veith *et al.*, 1979* and MacKay, 1982**: • Estimate of BCF_{fish} (Veith *et al.*, 1979) → log BCF_{fish} = 0.85 x log K_{OW} – 0.7 → log BCF_{fish} = -1.167 → BCF_{fish} = 0.068 • Estimate of BCF_{fish} (McKay, 1982) → log BCF_{fish} = -1.32 + log K_{OW} (equal: 0.048 x K_{OW}) → log BCF_{fish} = -1.869 → BCF_{fish} = 0.014 * Veith, G.D., *et al.*, 1979, Measuring and estimating the bioconcentration factor of chemicals on fish. J.Fish.Res. Board Can. 36: 1040-1048. ** Mackay, D., 1982. Correlation of bioconcentration factors. Env. Sci.Technol. 16: 274 – 278.	
Undertaking of intended data submission [ ]	Not applicable	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 November 2012
Evaluation of applicant's justification	Rapporteur supports the decision that a bioconcentration study was not required due to the low log K_{ow}. It is noted that the calculation for determining this is not necessary either.
Conclusion	
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section 7.4.3.1 Annex Point IIIA, XIII.2.1	**Prolonged toxicity to an appropriate species of fish**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Usually this test is not required, as it does not add information as needed in the risk assessment. Dinotefuran has been shown as non-toxic to fish in an acute study, therefore, no study has been submitted to address this data point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	22 November 2012	
Evaluation of applicant's justification	Applicant's justification considered acceptable. Additionally a fish early life stage test has been submitted.	
Conclusion		
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section 7.4.3.2 **Effects on reproduction and growth rate of fish**

Annex Point IIIA XIII 2.2 **Rainbow Trout: *Oncorhynchus mykiss***

Early Life Stage, 94 day, limit test

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 2001, Toxic effects of MTI-446 to Rainbow trout (*Oncorhynchus mykiss*) in an early-life stage toxicity test, [redacted] unpublished report no. 794338, December 6, 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. 210 US EPA OPPTS 850.1400	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	5400810	
3.1.2	Specification		
3.1.2.1	Purity	98.9	
3.1.2.2	Composition of Product	n.a.	
3.1.3	Further relevant properties	Solubility in water: 54.3 g/L at 20 °C Stability in water: > 24 hours (Sponsor information)	X
3.1.4	Method of analysis	Samples containing MTI-446 were analysed on a high performance liquid chromatographic (HPLC) system using UV/VIS 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 A7.4.3.2-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	see Table A7.4.3.2-2	
3.4.2	Test organisms	see Table A7.4.3.2-3	

Section 7.4.3.2 **Effects on reproduction and growth rate of fish**

Annex Point IIIA XIII 2.2 **Rainbow Trout: *Oncorhynchus mykiss***

Early Life Stage, 94 day, limit test

3.4.3	Handling of embryos and larvae (OECD 210/212)	The newly fertilised eggs were transferred in a cooled container. Before test start, the eggs were acclimated to test water for about 30 minutes. The eggs were transferred into stainless steel eggcups (diameter 8.5 cm, height 8 cm). The cups were moved up and down by means of a rocker arm (3 rounds per minute). After hatching was complete (d34 post fertilisation) the eggcups were removed and the larvae released into the corresponding 16 L test aquaria.
3.4.4	Test system	see Table A7.4.3.2-4
3.4.5	Test conditions	see Table A7.4.3.2-5
3.4.6	Duration of the test	94 days
3.4.7	Test parameter(s)	• egg development and hatching rate • time to hatch / development rate • survival of larvae and time to swim up • survival of juvenile fish and visible abnormalities • fish length and weight
3.4.8	Examination / Sampling	assessments on egg development, hatching, larvae survival, time to swim up, survival and development of juvenile fish were made at least three times a week, fish length and weight were determined after completion of the exposure phase
3.4.9	Monitoring of TS concentration	Yes sampling days: 0, 6, 13, 20, 27, 31, 35, 41, 48, 52, 55, 62, 69, 76, 84, and 94
3.4.10	Statistics	The results obtained for the 10 mg/L test concentration were nearly identical for all responses assessed. Therefore no statistical treatment of the data for evaluation of significant adverse effects was necessary.
		4 RESULTS
4.1	**Range finding test**	performed
4.1.1	Concentrations	not reported
4.1.2	Number/ percentage of animals showing adverse effects	not reported
4.1.3	Nature of adverse effects	not reported
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	10 mg/L

Section 7.4.3.2 **Effects on reproduction and growth rate of fish**

Annex Point IIIA XIII 2.2 **Rainbow Trout: *Oncorhynchus mykiss***

Early Life Stage, 94 day, limit test

4.2.2 Actual concentrations of test substance

sampling day [d]	measured [mg / L]	[% of nominal]
0	12.9	129
6	10.9	109
13	11.2	112
20	11.4	114
27	11.1	111
31	11.5	115
35	11.2	112
41	11.0	110
48	7.50	75
52	11.1	111
55	11.8	118
62	12.2	122
69	6.88	68.8
76	6.36	63.6
84	6.47	64.7
94	12.7	127
mean:	10.4	104

4.2.3 Effect data

Effect	Control	10mg/L (Test concentration)	NOEC
Fertilisation rate [%]	93 – 100 %	n.d.	n.a.
Start hatch [d]	31	31	n.a.
End hatch [d]	34	34	n.a.
Hatching rate [%]	92	97	≥ 10 mg/L
Development rate [1/d]	0.031	0.031	≥ 10 mg/L
Swim up [%]	98	97	≥ 10 mg/L
Mortality [n]	1	2	≥ 10 mg/L
Visible abnormalities [n]	0	0	≥ 10 mg/L
Length [mm]	41.0 ± 5.3	41.9 ± 4.5	≥ 10 mg/L
Dry weight [g]	0.15 ± 0.05	0.16 ± 0.06	≥ 10 mg/L

4.2.4 Concentration / response curve — No concentration/response curves were reported

4.2.5 Other effects — No other effects were reported.

4.3 Results of controls

4.3.1 Number/ percentage of animals showing adverse effects — None

4.3.2 Nature of adverse effects — None

4.4 Test with reference substance — Not performed

4.4.1 Concentrations — n.a.

Section 7.4.3.2 **Effects on reproduction and growth rate of fish**

Annex Point IIIA XIII 2.2 **Rainbow Trout: *Oncorhynchus mykiss***

Early Life Stage, 94 day, limit test

4.4.2	Results	n.a.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Guidelines: OECD Guideline No. 210, US EPA OPPTS 850.1400 No relevant deviations from test guidelines Method: In a 94 day early life stage test, freshly fertilised eggs of the rainbow trout were tested at a limit test concentration of 10 mg/L and a blank control. The test was started with sixty eggs divided into 4 replicates (15 eggs each) in the control and test item group. The eggs, developing larvae and juvenile fish were assessed for adverse signs of toxicity on their development, growth and survival.	
5.2	**Results and discussion**	The analytical determined test item concentration in the test solutions ranged from 64 – 129 % of the nominal test item concentration of 10 mg/L. The mean temperature ranged from 10.3 – 10.5 °C (day 0 – 66) and the range was from 11.0 – 12.9 °C (day 69 – 94). The dissolved oxygen concentration was ≥ 71 %. The pH ranged from 7.0 – 7.8. Hatching started on day 31 and was completed on day 34 post fertilisation in the control and in the treatment group respectively. The hatching rates in the control were 87 – 93 % and 93 – 100 % in the treatment group. The development rate of the larvae was 0.031 day $^{-1}$ in the control and in the treatment group. Swim up of juvenile fish stated at day 16 and completed by day 23 in the control and in the treatment group. The mean percent swimming up was calculated to be 98 and 97 % for the control and the treatment group, respectively. No mortalities or visible abnormalities were observed for the juvenile fish (day 23 – 60 post hatch). The total mean fish length in the control and in the treatment group was 41.0 ± 5.3 and 41.9 ± 4.5 mm, respectively. The total mean body dry weights in the control and in the treatment group were 0.15 ±0.05 and 0.16 ± 0.06 g, respectively.	
5.2.1	NOEC	≥ 10 mg/L (based on nominal concentrations)	X
5.2.2	LOEC	> 10 mg/L (based on nominal concentrations)	X
5.3	**Conclusion**	The following validity criteria according to OECD guideline No. 210 are fulfilled: • the dissolved oxygen concentration was at least 71 % of the air saturation value • the water temperature did not differ by more than 1.5 °C between test chambers or between successive days and was within the recommended range for the rainbow trout • overall survival of fertilised eggs in the controls was greater than the limit for the rainbow trout The following validity criteria was not fulfilled: • the measured concentration of the test item was not	

Section 7.4.3.2 **Effects on reproduction and growth rate of fish**

Annex Point IIIA XIII 2.2 **Rainbow Trout: *Oncorhynchus mykiss***

Early Life Stage, 94 day, limit test

		satisfactorily maintained within ± 20 % of the mean measured concentration Dinotefuran tested a 10 mg/L (nominal concentration) had no adverse effects on the survival, development and growth of rainbow trout in a 94 day early life stage test.	
5.3.1	Other Conclusions	None	
5.3.2	Reliability	1	
5.3.3	Deficiencies	Yes The test item concentration on the sampling days 48, 69, 64 and 65 ranged from 64-75 % and ranged from 109 to 129 % of the nominal concentration at any other sampling day, resulting in an overall mean measured test concentration of 104 %. There was no indication for malfunction of the dosing system, the water flow or the consumption of the application solution. Since no adverse effects were observed throughout the course of the study, using the lowest measured test item concentration would be a worst-case scenario still leading to a robust endpoint. Therefore the author of this summary recommends to use an overall NOEC of 6.4 mg/L rather than the reported overall NOEC of 10 mg/L.	X

Table A7.4.3.2-1: 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	n.a.
Other procedures	n.a.

Table A7.4.3.2-2: Dilution water

Criteria	Details
Source	Local non-chlorinated tap water (well water of drinking water quality mixed with deionised water for reduction of total hardness.
Salinity	n.a.
Hardness	198 – 342 mg/L as $CaCO_3$
pH	Not reported
Oxygen content	Not reported
Conductance	480 – 647 µS/cm
Holding water different from dilution water	n.a.

Table A7.4.3.2-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Oncorhynchus mykiss*)
Source	[redacted]
Wild caught	No
Age/size	Newly fertilised eggs
Kind of food	Hokovit 500 and Hokovit 502 (H.U. Hofmann AG, CH-4922 Bützberg, Switzerland)
Amount of food	Fish were fed *ad libitum*
Feeding frequency	Twice a day
Post-hatch transfer time	Not applicable
Time to first feeding	Before total consumption of the yolk sack
Feeding of animals during test	Yes
Treatment for disease within 2 weeks preceding test	n.a.

Table A7.4.3.2-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	3.2 L/h per replicate (6 fold theoretical volume exchange per day)
Volume of test vessels	13 L
Volume/animal	13/15 L
Number of animals/vessel	15
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.2-5: Test conditions

Criteria	Details
Test temperature	

Treatment / replicate		Day 0- 66, post fertilization				Day 69 – 94, post fertilization			
		mean	n	min	max	mean	n	min	max
Control	A	10.5	21	9.9	11.7	12.4	9	11.6	12.9
	B	10.5	21	9.9	11.7	12.3	9	11.5	12.8
	C	10.3	21	9.9	10.9	12.1	9	11.3	12.8
	D	10.4	21	9.9	10.8	12.1	9	11.2	12.7
10 mg/L	A	10.4	21	9.9	11.0	12.0	9	11.0	12.8
	B	10.4	21	9.9	11.0	12.2	9	11.2	12.9
	C	10.3	21	10.0	11.0	12.0	9	11.3	12.8
	D	10.4	21	10.0	11.0	11.9	9	11.1	12.6

Dissolved oxygen

Treatment / replicate		min	max
Control	A	8.1	9.6
	B	8.2	10.0
	C	8.1	9.9
	D	8.2	9.7
10 mg/L	A	8.2	9.9
	B	8.2	9.9
	C	8.3	10.0
	D	8.4	10.0

Dissolved oxygen concentration express in [mg/L]

pH

Treatment / replicate		min	max
Control	A	7.4	7.7
	B	7.1	7.7
	C	7.0	7.8
	D	7.2	7.7
10 mg/L	A	7.4	7.7
	B	7.1	7.7
	C	7.1	7.6
	D	7.2	7.7

Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	• study start – one week after hatching: in the dark • one week after hatch – end of exposure: 340 – 470 Lux
Photoperiod	16 hour light / 8 hour dark with a 30 min transition period each

Table A7_4_3_2-6: Validity criteria for fish tests according to OECD Guidelines 210/212

	Fulfilled	Not fulfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	X	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	X	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	X	
Test substance concentrations maintained within ± 20% of mean measured values		X
No effect on survival nor any other adverse effect found in solvent control	X	
Further criteria for poorly soluble test substances	n.a.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10 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. 3.1.3 Further relevant properties, Solubility in water is 40 g/l in report
Results and discussion	Applicant's summary considered acceptable
Conclusion	Applicant's version considered acceptable, noting the following: The applicant's conclusion that 6.4 mg/l should be taken as the NOEC is not given in the body of the report. However, given that for over half of the test the measured concentration of test item was greater than the nominal 10 mg/l and taking the biological results into account this approach is considered conservative. Normally, the geometric mean of measured concentrations would be considered acceptable thus making the NOEC 10.1 mg/l
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.3.3.1 Annex Point IIIA, XIII.2.3	**Bioaccumulation in an appropriate species of fish**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	The test is required when there is the risk for secondary poisoning. There may be also other grounds for testing, for example, when the substance has surface activity. There is no risk of secondary poisoning from dinotefuran. Dinotefuran is not surface active, therefore a test for bioaccumulation in an appropriate species of fish is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	22 November 2012	
Evaluation of applicant's justification	The rapporteur agrees with the justification reasons given by the applicant.	
Conclusion		
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section 7.4.3.3.2 Annex Point IIIA, XIII.2.3	**Bioaccumulation in an appropriate invertebrate species**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	This test may be required for some product types, especially if a direct release to marine/brackish water occurs. Dinotefuran is not intended for use where direct release to marine/brackish waters occurs. Therefore a test for bioaccumulation in an appropriate invertebrate species is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	22 November 2012	
Evaluation of applicant's justification	Applicant's justification for non-submission of data considered acceptable	
Conclusion		
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section 7.4.3.4
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Daphnia magna

21 day reproduction, dose response test

			Official use only
		1 REFERENCE	
1.1	**Reference**	Peither, A., 2000d, Influence of MTI-446 on survival and reproduction of *Daphnia magna* in a semistatic test over three weeks, RCC Ltd., unpublished report no. 752106, August 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 No. 211 OPPTS 850.1300	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
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	Composition of Product	n.a.	
3.1.3	Further relevant properties	Solubility in water: 54.3 g/L at 20 °C Stability in water: > 24 hours (Sponsor information)	
3.1.4	Method of analysis	Samples containing MTI-446 were analysed on a high performance liquid chromatographic (HPLC) system using UV/VIS 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 A7.4.3.4-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	Reconstituted water M7 (see Table A7.4.3.4-2)	
3.4.2	Test organisms	*Daphnia magna* (see Table A7.4.3.4-3)	
3.4.3	Handling of	Mortality of adult daphnids and number of offspring were recorded on	

Section 7.4.3.4
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Daphnia magna

21 day reproduction, dose response test

	offspring	days 0, 1, 2, 5. 7, 9, 12, 14, 16, 19 and 21 (before renewal of the test media)	
3.4.4	Test system	see Table A7.4.3.4-4	
3.4.5	Test conditions	see Table A7.4.3.4-5	X
3.4.6	Duration of the test	21 days	
3.4.7	Test parameter	Survival, reproduction and growth	
3.4.8	Examination / Sampling	Duplicate samples were taken from the freshly prepared test media of all concentrations and the control at days 0, 12 and 16. Additional samples from the old test solution were taken on days 14 and 19. All samples were stored frozen at approximately -20°C until analysis.	
3.4.9	Monitoring of TS concentration	Yes Since the NOEC was determined to be 100 mg/L, both replicates from the test item concentration 100 mg/L were analysed. Only one replicate of the corresponding control was analysed.	
3.4.10	Statistics	Significant differences in the reproduction rate and the body length of the adult daphnids were evaluated by testing the mean reproduction rate and the mean body length for statistically significant differences to the corresponding control values by the multiple Williams-test after a one-way analysis of variance (ANOVA).	
		4 RESULTS If appropriate, include tables.	
4.1	**Range finding test**	Not performed	
4.1.1	Concentrations	n.a.	
4.1.2	Number/ percentage of animals showing adverse effects	n.a.	
4.1.3	Nature of adverse effects	n.a.	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0, 6.25, 12.5, 25, 50 and 100 mg/L	

Section 7.4.3.4
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Daphnia magna

21 day reproduction, dose response test

4.2.2 Actual concentrations of test substance

Concentration nominal [mg/L]	Sampling day [d]	Concentration measured [mg/L]	[% nominal]
100	0	96.0	96
	12	94.4	94
	14*	95.7	96
	16	94.6	95
	19*	96.0	96
Control	0	n.d.	n.a.
	12	n.d.	n.a.
	14*	n.d.	n.a.
	16	n.d.	n.a.
	19*	n.d.	n.a.

* samples with food
n.d.: not detectable
n.a.: not applicable

4.2.3 Effect data

Total number of alive, young daphnids per test concentration (cumulative numbers)

Exposure day	Nominal concentration [mg/L]					
	Control	6.25	12.5	25	50	100
0	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
5	0	0	0	0	0	0
7	0	0	0	0	0	0
9	87	92	85	83	84	66
12	306	252	290	264	265	239
14	335	252	314	264	265	244
16	583	467	526	477	516	473
19	722	598	679	602	600	634
21	722	598	679	602	621	653
% of control*	100	82.8	94.0	83.4	86.0	90.4

* based on results of d 21

Section 7.4.3.4
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Daphnia magna

21 day reproduction, dose response test

Number of alive offspring reproduced per surviving adult within 21 days of exposure

Replicate number	Nominal concentration [mg/L]					
	Control	6.25	12.5	25	50	100
1	80	85	74	81	54	73
2	58	68	59	77	59	74
3	76	66	71	30	64	63
4	80	59	71	71	62	60
5	85	66	67	35	75	59
6	63	72	79	71	66	75
7	81	*	69	73	73	79
8	63	77	69	79	56	72
9	74	45	51	55	44	55
10	62	58	69	30	68	43
mean	**72.2**	**66.2**	**67.9**	**60.2**	**62.1**	**65.3**
±SD	9.8	11.6	7.8	21.0	9.3	11.2
n	10	9	10	10	10	10
CV %	13.5	17.5	11.5	34.8	15.0	17.2
Mean in %	100	91.7	94.0	83.4	86.0	90.4
STAT	-	n.s.	n.s.	n.s.	n.s.	n.s.

CV %: coefficient of variation in %: $(SD_x/mean_x) \times 100\%$

STAT: result of a Williams-test with the mean values of alive offspring (one-sided smaller, $\alpha = 0.05$)

n.s.: mean value not significantly lower than in the control

*: test animal died throughout the exposure phase

$NOEC_{survival, reproduction}$: 100 mg/L

$LOEC_{survival, reproduction}$: ≥ 100 mg/L

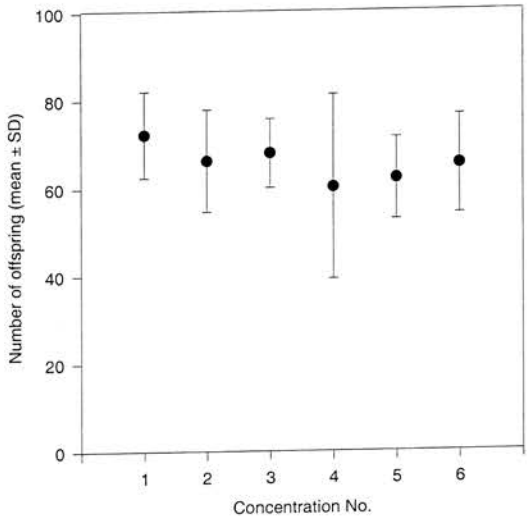
Section 7.4.3.4
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Daphnia magna

21 day reproduction, dose response test

4.2.4	Concentration / response curve	Number of alive offspring (mean ± SD) per parent animal surviving at the end of the test



Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6
Control	6.25 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L

4.2.5	Other effects	The body length of each surviving adult at the end of exposure was determined by the use of a binocular. The mean body length of the daphnids in the control was 3.77 ± 0.08 mm. The mean body length in the 6.25, 12.5, 25, 50 and 100 mg/L test item groups were 3.77 ± 0.04, 3.75 ± 0.08, 3.77 ± 0.07, 3.75 ± 0.08 and 3.78 ± 0.05 mm , respectively. There was not statistically significant effect on the mean body weight length of adult daphnia using a Williams-test (one-sided smaller, α = 0.05)
4.3	**Results of controls**	see 4.2.3 and 4.2.4
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. 211 and OPPTS 8500.1300 No relevant deviations from test guidelines.
5.2	**Results and discussion**	The effect of MTI-446 on the survival, reproduction and growth was determined in a 21 day semistatic dose response test. The nominal test concentrations were 6.25, 12.5, 25, 50 and 100 mg/L. The measured concentration in the 100 mg/L treatment group ranged from 94 – 96% of

Section 7.4.3.4
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Daphnia magna

21 day reproduction, dose response test

		nominal. Therefore, all biological results are reported based on nominal concentrations. The NOEC for survival, reproduction and growth was 100 mg/L
5.2.1	NOEC	100 mg/L
5.2.2	LOEC	> 100 mg/L
5.2.3	EC_{50} (EC_x)	n.a.
5.3	**Conclusion**	All validity criteria for the study were fulfilled. MTI-446 had no toxic effects on survival, reproduction and growth of adult *Daphnia magna* in a 21 day test.
5.3.1	Reliability	1
5.3.2	Deficiencies	The temperature records were not reported in detail (only target temperature).

Table A7.4.3.4-1: 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	n.a.
Other procedures	none

Table A7.4.3.4-2: Dilution water

Criteria	Details
Source	reconstituted water "M7" according to OECD No. 211
Salinity	n.a.
Hardness	250 mg/L (as $CaCO_3$)
pH	not reported
Ca / Mg ratio	not reported
Na / K ratio	not reported
Oxygen content	The water was aerated until oxygen saturation before use
Conductance	not reported
TOC	not reported
Holding water different from dilution water	No

Table A7.4.3.4-3: Test organisms

Criteria	Details
Strain / Clone	Daphnia magna Straus, Clone 5
Source	University of Sheffield, UK
Age	< 24 hours
Breeding method	The daphnia were cultured und the same environmental conditions as used in the test
Kind of food	1:1mixture of green algae (Scenedesmus subspicatus) and fish food suspension (Tetra Min Hauptfutter)
Amount of food	0.10 – 0.25 mg TOC/daphnia/day
Feeding frequency	each working day
Pretreatment	not reported
Feeding of animals during test	Yes see above

Table A7.4.3.4-4: Test system

Criteria	Details
Test type	Semistatic
Renewal of test solution	Test media was renewed on days: 2, 5, 7, 9, 12, 14, 16 and 19. Surviving adults were carefully transferred from the old into the corresponding freshly prepared test item solution using glass tubes.
Volume of test vessels	100 mL
Volume/animal	80 mL
Number of animals/vessel	1
Number of vessels/ concentration	10
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.4-5: Test conditions

Criteria	Details
Test temperature	20°C
Dissolved oxygen	
pH	
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	300-800 Lux
Photoperiod	16 hours light 8 hours dark with a 30 min transition period

Dissolved oxygen

Exposure period [d]	Start			End		
	Nominal concentration [mg/L]					
	control	6.25	100	control	6.25	100
0-2	7.9	7.9	7.9	7.7	7.7	7.8
2-5	7.8	7.8	7.8	7.9	7.9	7.9
5-7	8.0	8.0	8.0	7.8	7.8	7.8
7-9	8.0	8.0	8.0	7.7	7.7	7.8
9-12	8.0	8.0	8.0	7.7	7.7	7.8
12-14	8.0	8.0	8.0	7.7	7.7	7.7
14-16	7.9	7.8	7.8	7.7	7.7	7.6
16-19	7.9	7.9	7.9	7.8	7.8	7.8
19-21	7.9	7.9	7.9	7.7	7.7	7.7

pH

Exposure period [d]	Start			End		
	Nominal concentration [mg/L]					
	control	6.25	100	control	6.25	100
0-2	8.4	8.4	8.5	8.2	8.2	8.2
2-5	8.4	8.4	8.4	8.3	8.2	8.3
5-7	8.3	8.3	7.9	8.0	7.9	7.9
7-9	8.3	8.3	8.3	8.1	8.0	8.0
9-12	8.3	8.4	8.3	7.9	8.0	8.0
12-14	8.3	8.2	8.2	8.0	8.0	8.1
14-16	8.5	8.5	8.5	8.3	8.3	8.1
16-19	8.6	8.6	8.4	8.1	8.0	8.1
19-21	8.4	8.4	8.3	8.0	8.0	8.0

Table A7.4.3.4-6: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

	Fulfilled	Not fulfilled
Mortality of parent animals < 20% at test termination	X	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	X	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10 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. 3.4.5 Test Conditions: In Table A7.4.3.4-5 the headings have been transposed such that the values given for pH actually refer to dissolved oxygen content and *vice verca*
Results and discussion	Applicant's version considered acceptable
Conclusion	Applicant's version considered acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.3.5.1-1 Effects on sediment dwelling organisms

Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

			Official use only
		1 REFERENCE	
1.1	**Reference**	Memmert, U., 2000, Acute toxicity of MTI-446 to first-instar larvae of *Chironomus riparius*, RCC Ltd., unpublished report no. 752128, July 4, 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**	No guideline available, therefore study followed guidelines OECD 202 and directive 92/69/EEC, C2 for test item administration. Test species and age followed proposal for a BBA guideline, 1995 "Effect of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water sediment system" and the OECD guidelines for testing of chemicals, proposal for a new guideline 219, draft document February 2000: "Sediment-water chironomid toxicity test using spiked water".	
2.2	**GLP**	Yes	
2.3	**Deviations**	Not a guideline study	
		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	Description	White solid	X
3.1.2.2	Purity	97.26%	
3.1.2.3	Stability	Expiration date: June 30, 2004	
3.1.3	Further relevant properties	Solubility in water: 54.3 g/L at 20 °C Stability in water: > 24 hours (Sponsor information) Stability in test water: ≥ 96 h; examined within this study	
3.1.4	Method of analysis	Diluted stock solution samples and aliquots of the test samples were analysed by HPLC and UV/VIS 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 A7.4.3.5.1.1-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		

Section A7.4.3.5.1-1 Effects on sediment dwelling organisms

Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

3.4.1	Dilution water	Table A7.4.3.5.1.1-2
3.4.2	Sediment	None
3.4.3	Test organisms	Table A7.4.3.5.1.1-4
3.4.4	Test system	Table A7.4.3.5.1.1-5
3.4.5	Test conditions	Table A7.4.3.5.1.1-6
3.4.6	Duration of the test	48 hours
3.4.7	Test parameter	Survival and symptoms of intoxication
3.4.8	Sampling	0 and 48 hours, stored at ~-20°C
3.4.9	Monitoring of TS concentration	Yes, 0 and 48 hours
3.4.10	Statistics	The 48 hours LC_{50} and the 95 % confidence limits were calculated by Moving Average Interpolation. The NOEC, LOEC, LC_0 and LC_{100} were determined directly from the raw data without any data transformation.

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	
4.1.2	Number/ percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	2.2, 4.6, 10, 22, 46 and 100 µg/L
4.2.2	Actual concentrations of test substance	

Nominal test concentration [µg/L]	Sample [h]	Measured Concentration [µg/L]
100	0	94
	48	92
46	0	43
	48	43
22	0	22
	48	22
Control	0	< LOD
	48	< LOD

Only samples ≥ 48 hour NOEC were analysed

4.2.3	Effect data	Mortality data and sublethal effects: Table A7.4.3.5.1.1-8
4.2.4	Concentration / response curve	Not available
4.2.5	Other effects	Larvae showed convulsions and unusual body movements
4.3	**Results of controls**	No adverse effects observed in the control

Section A7.4.3.5.1-1 Effects on sediment dwelling organisms

Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

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: No guideline available, therefore study followed guidelines OECD 202 and directive 92/69/EEC, C2 for test item administration. Test species and age followed proposal for a BBA guideline, 1995 "Effect of plant protection products on the development of sediment-dwelling larvae of chironomus riparius in a water sediment system" and the OECD guidelines for testing of chemicals, proposal for a new guideline 219, draft document February 2000: "Sediment-water chironomid toxicity test using spiked water". Method: The 48-hour acute toxicity of dinotefuran to the first instar larvae of the midge *Chironomus riparius* was studied under static conditions for 48 hours. Larvae were exposed to the test item at nominal concentrations of 100, 46, 22, 10, 4.6, 2.2 µg/L and a blank control (0 µg/L).	
5.2	**Results and discussion**	The analytical determined concentrations of dinotefuran in the analysed test media from the start and the end of the exposure period ranged from 92 – 98 % of the nominal concentrations. Therefore biological results are based on nominal concentrations. After 24 hours no mortality was determined in the control or at any concentration tested. However at 100 µg/L, all larvae showed convulsions and unusual body movements. After 48 hours 17 of 20 larvae exposed to 100 µg/L test item concentration were dead and surviving larvae showed sublethal effects like convulsions and unusual body movements. Larvae in the 46 µg/L test item concentration showed the same sublethal effect as in the 100 µg/L dose level. The 48 hour LC50 was calculated to be 72.1 µg/L with 95 % confidence limits of 64.5 and 80.6 µg/L. The 48 hour NOEC was determined to be 22 µg/L and the 48 hour LOEC was 46 µg/L. All test media appeared as clear solution throughout the exposure period. Oxygen concentrations ranged from 8.8 – 8.8 mg/L and pH ranged from 7.9 – 8.0 at the start and the end of exposure.	
5.2.1	LC_0 (48 hours)	46 µg/L	
5.2.2	LC_{50} (48 hours)	72.1 µg/L	
5.2.3	LC_{100} (48 hours)	100 µg/L	X
5.2.4	NOEC (48 hours)	22 µg/L	
5.2.5	LOEC (48 hours)	46 µg/L	
5.3	**Conclusion**	Validity criteria of the combined guidelines are fulfilled. A clear dose response of the test item for survival could be determined.	
5.3.1	Reliability	1	

Section A7.4.3.5.1-1 Effects on sediment dwelling organisms
Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

5.3.2 Deficiencies Not a guideline study

Table A7.4.3.5.1.1-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7.4.3.5.1.1-2: Dilution water

Criteria	Details
Source	Reconstituted water, "M7" according to OECD 219
Alkalinity	0.9 mmol/L
Hardness	2.5 mmol/L
pH	7.9 ± 0.3
Ca / Mg ratio	Not determined
Na / K ratio	Not determined
Oxygen content	Not determined
Conductance	Not determined
Holding water different from dilution water	Not described

Table A7.4.3.5.1.1-3: Sediment

Criteria	Details
Type/source	Not applicable
Composition (% w/w)	Not applicable
Total organic carbon (TOC)	Not applicable
pH adjustment	Not applicable
pH of final sediment mixture	Not applicable

Table A7.4.3.5.1.1-4: Test organisms

Criteria	Details
Species/strain	*Chironomus riparius*
Source	In-house culture. The culture originated from Novartis Crop Protection AG, Basel.
Age	First instar larvae, 2-3 days old
Breeding method	Not described
Kind of food	Green alga/TetraMin® fish food : 1/1 contributing 5 mg carbon /L test solution
Amount of food	Contributing 5 mg carbon /L test solution (as TOC)
Feeding frequency	Before start of exposure
Pretreatment	Not applicable
Feeding of animals during test	None

Table A7.4.3.5.1.1-5: Test system

Criteria	Details
Type of test	Acute 48 hour toxicity
Renewal of test solution	No
Volume of test vessels	100 mL
Amount/volume of sediment per vessel	None
Volume of test medium per vessel	50 mL
Volume/animal	10 mL
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.5.1.1-6: Test conditions

Criteria	Details
Test temperature	20°C
Dissolved oxygen	8.8 mg/L
pH	7.9- 8.0
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Light intensity 300 – 700 lux
Photoperiod	16 hours light / 8 hours dark

Table A7.4.3.5.1.1-8: Mortality of larvae of *Chironomus riparius* after 48 hours of exposure

Nominal test item concentration [µg/L]	No. of larvae tested	No. dead larvae after 24 hours	No. dead larvae after 48 hours	Mortality after 24 hours [%]	Mortality after 48 hours [%]
0	20	0	0	0	0
2.2	20	0	0	0	0
4.6	20	0	0	0	0
10	20	0	0	0	0
22	20	0	0	0	0
46	20	0	0*	0	0
100	20	0*	17**	0	85

* all larvae showing sublethal effects, e.g. convulsion, unusual body movements

** surviving larvae showing sublethal effects, e.g. convulsion, unusual body movements

Table A7._3.5.1.1-10: Validity criteria for the *Chironomus riparius* toxicity test according to OECD Guideline 202 and OECD draft Guideline 219

Criteria	Fulfilled	Not fulfilled
In the control, not more than 10 per cent of the test organisms should have been immobilised or trapped at the surface of the water	X	
The dissolved oxygen concentration at the end of the test should be ≥ 60 % of the air saturation value at the temperature used.	X*	

* Oxygen concentrations were reported as mg/L, temperature was not reported, and hence the conversion into % water saturation value is not possible. At 20°C (the target temperature of the study) and normal air pressure the 100 % oxygen saturation value for fresh water is 9.1 mg/L. Therefore, the measured oxygen concentration of 8.8 mg/L is highly likely to be > 60%.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	11 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. 3.1.2.1 Test material is described as light blue in the report (although other reports with this test item have been amended to describe the test material as white)
Conclusion	Applicant's version considered acceptable, noting the following: 5.2.3 the LC100(48 hours) of 100µg/l is an approximate value as 85 % of larvae were dead after 48 hours and the remainder showed sublethal effects (which may have resulted in death beyond 48 hours).
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.3.5.1-2 Effects on sediment dwelling organisms

Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

			Official use only
		1 REFERENCE	
1.1	**Reference**	Memmert, U., 2003, Effects of MTI-446 on the development of sediment-dwelling larvae of *Chironomus riparius* in a water sediment system, RCC Ltd, Environmental Chemistry & Pharmanalytics Division, Itingen, Switzerland; unpublished report no. 844569, December 10, 2003.	
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 draft 219 (February 2001)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		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	Description	White solid	
3.1.2.2	Purity	97.26%	
3.1.2.3	Stability	Expiration date: June 30, 2004	
3.1.3	Further relevant properties	Solubility in water: 54.3 g/L at 20 °C Stability in water: > 24 hours (Sponsor information)	X
3.1.4	Method of analysis	The concentrations of dinotefuran in the overlaying water columns, in the pore water and in the sediment were monitored throughout the exposure period using HPLC with MS 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 A7.4.3.5.1.2-1	
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	Table A7.4.3.5.1.2-2	
3.4.2	Sediment	Table A7.4.3.5.1.2-3	

Section A7.4.3.5.1-2 Effects on sediment dwelling organisms

Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

3.4.3	Test organisms	Table A7.4.3.5.1.2-4	
3.4.4	Test system	Table A7.4.3.5.1.2-5	
3.4.5	Test conditions	Table A7.4.3.5.1.2-6	
3.4.6	Duration of the test	27 days	
3.4.7	Test parameter	The number of emerged adults and their sex was recorded daily from day 10 after application until day 27 (9 days after last emergence in the controls). After sex identification midges were removed from the test vessels and discarded. Only the number of fully emerged male and female midges was counted. The number of visible pupae that have failed to emerge was counted separately for each vessel. Other signs of toxicity were recorded. The emergence ratio and the development time and rate were calculated according to guideline OECD 219.	
3.4.8	Sampling	• 2 samples each of every application solution immediately after application • Sediment, pore water and overlaying water samples from the 8 and 32 µg/L treatment were taken on day 0, 7 and 27 • Sediment, pore water and overlaying water samples from the control (0 µg/L) were taken on days 0 and 27 • all samples were stored frozen at ~-20°C immediately after sampling until analysis	
3.4.9	Monitoring of TS concentration	Yes, day 0, 7* and 27 * 8 and 32 µg/L treatment only	X
3.4.10	Statistics	For emergence rate and development rate the arithmetic mean values (mean), standard deviation (SD), minimum and maximum (min/max) were calculated from the four replicates per treatment. The values of the emergence rates and the development rates were normally distributed and were statistically evaluated on significant differences to the control by the multivariate Williams-test after a one-way analysis of variance (ANOVA). Statistical evaluations were done separately for emerged males and females (development rate) and with pooled sexes (emergence rate). The 27-day EC_{10} and EC_{50} and the 95% confidence limits of the emergence rate (pooled sexes) were calculated by Probit analyses. The 27-day EC_x values and the 95% confidence limits of the development rate of the midges could not be calculated due to the low inhibitory effect up to the highest test concentration that could be evaluated.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration		
4.1.2	Number/ percentage of animals showing adverse effects		
4.1.3	Nature of adverse effects		

Section A7.4.3.5.1-2 Effects on sediment dwelling organisms

Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

4.2 Results test substance

4.2.1 Initial concentrations of test substance: Control (0 µg/L), 2, 4, 8, 16, 32 µg/L (nominal concentration in water)

4.2.2 Actual concentrations of test substance

Application solutions		
Nominal concentration dinotefuran [mg/L]	Measured concentration dinotefuran [mg/L]	Measured concentration [%] of nominal
0.32	0.296	93
0.64	0.596	93
1.28	1.19	93
2.56	2.34	92
5.12	4.86	95

Water samples			
Nominal concentration [µg/L]	Sampling day [d]	measured concentration[1] [µg/L]	[% nominal]
0 (control)	0	n.d.	n.a.
	27	n.d.	n.a.
8	0	5.73	72
	7	4.66	58
	27	4.50	56
32	0	24.3	76
	7	21.7	68
	27	19.4	61
Pore water samples			
0 (control)	0	0.286*	n.a.
	27	n.d.	n.a.
8	0	0.247	n.a.
	7	2.53	n.a.
	27	3.50	n.a.
32	0	1.35	n.a.
	7	10.1	n.a.
	27	17.1	n.a.
Sediment samples			
0 (control)	0	n.d.	n.a.
	27	n.d.	n.a.
8	0	0.275	n.a.
	7	0.654	n.a.
	27	1.13	n.a.
32	0	0.263	n.a.
	7	2.64	n.a.
	27	5.67	n.a.

1: corrected for recovery rate of spiked samples
*: excluded as outlier because of absence of test item in the corresponding water and sediment samples
n.d.: no test item detected
n.a.: not applicable

4.2.3 Effect data: Emergence rate: Table A7.4.3.5.1.2-7
Development rate: Table A7.4.3.5.1.2-8

Section A7.4.3.5.1-2 **Effects on sediment dwelling organisms**
Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

4.2.4	Concentration / response curve	Not available
4.2.5	Other effects	No symptoms of toxicity were observed for larvae emerged in the control or at 2, 4 and 8 µg/L. Some dead larvae or not completely emerged midges were observed at 16 µg/L. No emergence at 32 µg/L.
4.3	**Results of controls**	Development ratio females:0.065 / day Development ratio males:0.074 / day Validity criteria: 0.05 – 0. 1
4.4	**Test with reference substance**	None
4.4.1	Concentrations	
4.4.2	Results	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

Toxic effects of the test item on the development of sediment dwelling larvae of the midge *Chironomus riparius* in water-sediment systems were investigated following the proposal for a BBA-Guideline "Effects on plant protection products on the development of the sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system" (1995) and the OECD guideline "Proposal for a new guideline 219, draft document: Chironomid Toxicity Test Using Spiked Water" (2001).

No relevant deviations from test guidelines.

Method:

First instar larvae of *Chironomus riparius* were exposed for a period of 27 days until full maturation of the larvae to adult midges. The test parameters of the study were the development rate of the midges and the emergence rate as the number of fully emerged males and females.

The test item was applied to the water column in static water-sediment systems. The nominal test item concentrations in the overlaying water columns were 2, 4, 8, 16 and 32 µg/L. A negative control was tested in parallel.

5.2 Results and discussion

The mean measured concentrations of the test item in the application solutions ranged from 92 – 95 % of the nominal concentrations.

One hour after application, the mean measured concentration of the test item in the water column corresponded to 72 and 76 % of the nominal concentrations of 8 and 32 µg/L.

Seven days after application, the mean measured concentration of the test item in the water column corresponded to 58 and 68 % of the nominal concentrations of 8 and 32 µg/L.

At the end of exposure, the mean measured concentration of the test item in the water column corresponded to 56 and 61 % of the nominal concentrations of 8 and 32 µg/L.

Test item concentrations in the pore water and sediment increased continuously throughout the exposure phase. In the 8 µg/L treatment group, measured concentrations reached a maximum of 3.5 µg/L in pore water and 1.13 µg/kg in sediment. In the 32 µg/L treatment group, measured concentrations reached a maximum of 17.1 µg/L in pore water and 5.7 µg/kg in sediment.

Section A7.4.3.5.1-2 Effects on sediment dwelling organisms

Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

		The correct preparation of the application solutions was confirmed. The low recoveries in the water samples 1 hour after application were presumably caused by transfer of the test item into the sediment and the pore water phase. The biological results are presented based on nominal concentrations. Based on nominal concentrations, the 27 day EC_{10} for the arcsin-transformed emergence rate of pooled sexes was calculated by Probit analyses to be 4.6 µg/L (95% confidence limits 3.7 – 5.8 µg/L), the EC_{50} was calculated to be 14.5 µg/L (95 % confidence limits 11.8 – 17.8). At nominal concentrations of 2, 4 and 8 µg/L there was no statistically significant difference in the development rate of male midges. At nominal concentrations of 2, 4, 8 and 16 µg/L there was no statistically significant difference in the development rate of female midges. At a nominal concentration of 32 µg/L no development rate could be calculated due to 0 % emergence. The EC_{10} and EC_{50} for development rate could not be calculated due to the low inhibition rates. The 27 day NOEC and the LOEC for development and emergence for *Chironomus riparius* were 4 and 8 µg/L, respectively.	
5.2.1	EC_{10} emergence (27-day)	4.6 µg/L (95% confidence limits 3.7 – 5.8 µg/L)	
5.2.2	EC_{50} emergence (27-day)	14.5 µg/L (95 % confidence limits 11.8 – 17.8)	X
5.2.3	NOEC (27-day)	4 µg/L	X
5.2.4	LOEC (27-day)	8 µg/L	
5.3	**Conclusion**	All validity criteria of guideline OECD 219 are fulfilled: • the emergence rate in the controls is ≥ 70 % • emergence to adults from the control occurred between 12 and 23 days after insertion of egg masses into the vessels • pH and dissolved oxygen were measured in each vessel at the end of exposure and was 7.6 – 8.7 and ≥ 69 % respectively • the water temperature did not differ by not more than 1 °C A clear dose response of the test item for development and emergence rate could be determined.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A7.4.3.5.1.2-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7.4.3.5.1.2-2: Dilution water

Criteria	Details
Source	Reconstituted water, "M7" according to OECD 219
Alkalinity	Not determined
Hardness	Ca^{2+}: approx. 2.0 mmol/L (as $CaCO_3$) Ca^{2+} and Mg^{2+}: approx 2.5 mmol/L
pH	7.9 ± 0.3
Ca / Mg ratio	Not determined
Na / K ratio	Not determined
Oxygen content	Not determined
Conductance	Not determined
Holding water different from dilution water	Not described

Table A7.4.3.5.1.2-3: Sediment

Criteria	Details
Type/source	Artificial sediment according to OECD 219
Composition (% w/w)	Sphagnum peat (air dried, ground to ≤ 1mm): 4 % Kaolin clay (content of Al_2O_3: 36.4 5): 20 % Sand (Sihelco 36): 76 % Calcium carbonate ($CaCO_3$): 0.4 %
Total organic carbon (TOC)	2.0 %
pH adjustment	Yes
pH of final sediment mixture	7.3

Table A7.4.3.5.1.2-4: Test organisms

Criteria	Details
Species/strain	*Chironomus riparius*
Source	Novartis Crop Protection AG
Age	2 – 3 days (first instar larvae)
Breeding method	Similar temperature and light conditions and in the same kind of water as used in the test
Kind of food	TetraMin Hauptfutter (TETRA-Werke, D-49304 Melle, Germany)
Amount of food	Day -1 – 5: 23 mg / vessel Day 7 – 24: 47 mg/vessel
Feeding frequency	3 times a week
Pretreatment	Finely ground and suspended in test water
Feeding of animals during test	Yes

Table A7.4.3.5.1.2-5: Test system

Criteria	Details
Type of test	Chronic 27 days
Renewal of test solution	No
Volume of test vessels	3 L
Amount/volume of sediment per vessel	462 g (dry weight)
Volume of test medium per vessel	1600 mL
Volume/animal	80
Number of animals/vessel	20
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No, vessels covered with a watch-glass

Table A7.4.3.5.1.2-6: Test conditions

Criteria	Details
Test temperature	19.4 – 19.8
Dissolved oxygen	≤ 69 %
pH	7.6 – 8.7
Adjustment of pH	No
Aeration of dilution water	Yes
Quality/Intensity of irradiation	640 – 860 lux
Photoperiod	16/8 hours light/dark with a 30 min transition period

Table A7.4.3.5.1.2-7: Emergence rate of *Chironomus riparius* (males and females pooled) after 27 days of exposure

	Nominal test item concentration [µg/L]					
	Control	2	4	8	16	32
sum inserted larvae / treatment	80	80	80	80	80	80
sum emerged midges / treatment	76	77	74	69	30	0
% emerged midges / treatment	95	96	93	86	38	0
emergence rate ER_{arc}	1.4099	1.4016	1.2971	1.1920	0.6545	0
% of control	100	99.4	92.0	84.5	46.4	0
STAT	-	n.s.	n.s.	s	s	s

ERarc: arcsin-transformed emergence rate
STAT: results of a Williams t-test ($\alpha = 0.05$, one-sided smaller)
n.s.: mean ERarc not significantly lower than in the control
s: mean ERarc significantly lower than in the control

Table A7.4.3.5.1.2-8: Development rate for males and females

Females						
Development rate / treatment [day^{-1}]	Nominal initial test item concentration [µg/L]					
	control	2	4	8	16	32
Mean	0.06465	0.06283	0.06377	0.06495	0.06325	n.a.
SD	0.00336	0.00189	0.00178	0.00060	0.00209	n.a.
min	0.06120	0.06070	0.06190	0.06440	0.06100	n.a.
max	0.06810	0.06470	0.06600	0.06580	0.06600	n.a.
n	4	4	4	4	4	4
% of control	100	97.2	98.6	100.5	97.8	n.a.
STAT	-	n.s.	n.s.	n.s.	n.s.	n.a.

STAT: results of a Williams t-test ($\alpha = 0.05$, one-sided smaller)
n.s. mean ERarc not significantly lower than in the control:
s: mean ERarc significantly lower than in the control
n.a.: not applicable (no midge emerged)

Males						
Development rate / treatment [day^{-1}]	Nominal initial test item concentration [µg/L]					
	control	2	4	8	16	32
Mean	0.07412	0.07360	0.07295	0.07240	0.07085	n.a.
SD	0.00126	0.00226	0.00234	0.00062	0.00413	n.a.
min	0.07230	0.07080	0.07120	0.07150	0.06480	n.a.
max	0.07510	0.07580	0.07640	0.07290	0.07410	n.a.
n	4	4	4	4	4	4
% of control	100	99.3	98.4	97.7	95.6	n.a.
STAT	-	n.s.	n.s.	n.s.	s	n.a.

STAT: results of a Williams t-test ($\alpha = 0.05$, one-sided smaller)
n.s.: mean ERarc not significantly lower than in the control
s: mean ERarc significantly lower than in the control
n.a.: not applicable (no midge emerged)

Table A7.4.3.5.1.2-10: Validity criteria for the sediment/water Chironomus toxicity test according to OECD Guideline 219.

Criteria	Fulfilled	Not fulfilled
Mortality in the controls <30% at the end of the test	X	
Emergence of adults (days after insertion in test vessels): - *C. riparius*, *C. yoshimatsui*: 12 – 23 days - *C. tentans*: 20 – 65 days	X	
Emergence in controls: 50 – 70%	X	
Concentration of dissolved oxygen: > 60% of the air saturation value of the temperature used	X	
pH of overlying water: 6 – 9	X	
Water temperature does not differ by more than ± 1.0°C between test vessels, and is be maintained within the temperature ranges specified for the test species	X	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15 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 3.1.3 Further relevant properties: Solubility in water given in report as 39.83 g/l 3.4.9 Believe that '*' refers to all time points, not just Day 7. 5.2.2 and 5.2.3 Results are based on nominal concentrations as opposed to measured concentrations in the water column after one hour. The report gives a value of 3 µg/l for the NOEC 'based on the analytically measured initial concentrations in the water columns.' However, only the 8 and 32 µg/l levels were analysed and there is no explanation as to how the value of 3 µg/l was reached. Nor is this value presented in the summary. The RMS considers it appropriate to calculate the NOEC based on analysed concentrations: lowest percentage of test item present in analyzed concentrations at the start and end of the test (72 and 56 % respectively) multiplied by the nominal NOEC and the geomean taken of the two resulting values. This results in a value of 2.54 µg/L. Currently, the EC_{50} value is based on nominal concentrations and would change if based on the lowest initial analysed concentrations.
Conclusion	Applicant's version considered acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.3.5.1-3 Effects on sediment dwelling organisms

Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

Official use only

		1 REFERENCE
1.1	**Reference**	Memmert, U., 2007, Effects of DN phosphate on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system with spiked sediment, RCC Ltd, unpublished report no. 844571, February 1, 2007.
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 draft 218 (February 2001)
2.2	**GLP**	Yes
2.3	**Deviations**	None
		3 MATERIALS AND METHODS
3.1	**Test material**	DN Phosphate
3.1.1	Lot/Batch number	OFU-1290
3.1.2	Specification	DN Phosphate is a degradation product of the parent molecule MTI-446
3.1.2.1	Description	Solid white
3.1.2.2	Purity	97.27%
3.1.2.3	Stability	Hydrolytically stable at pH 4, 7 and 9
3.1.3	Further relevant properties	water solubility: 601.5 g/L at 20 °C
3.1.4	Method of analysis	Test item concentrations in the test solutions were determined by HPLC with MS-detection
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	n.a, see Table A7.4.3.5.1.3-1
3.3	**Reference substance**	n.a
3.3.1	Method of analysis for reference substance	n.a
3.4	**Testing procedure**	
3.4.1	Dilution water	Table A7.4.3.5.1.3-2
3.4.2	Sediment	Table A7.4.3.5.1.3-3
3.4.3	Test organisms	Table A7.4.3.5.1.3-4
3.4.4	Test system	Table A7.4.3.5.1.3-5

Section A7.4.3.5.1-3 **Effects on sediment dwelling organisms**

Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

3.4.5	Test conditions	Table A7.4.3.5.1.3-6
3.4.6	Duration of the test	27 days
3.4.7	Test parameter	The number of emerged adults and their sex was recorded daily from day 10 after application until day 27 (7 days after last emergence in the control). After sex identification midges were removed from the test vessels and discarded. Only the number of fully emerged male and female midges was counted. The number of visible pupae that have failed to emerge was counted separately for each vessel. Any other signs of toxicity were recorded. The test vessels were searched for deposited egg masses to prevent re-introduction of new larvae into the sediment.
3.4.8	Sampling	• duplicate samples from each application solution were taken immediately after spiking of the sediment on Day -7 • water, pore water and sediment samples from the test systems were taken at study start on Day 0, Day 7 and at study termination on Day 27 from of a middle and the highest concentration (nominal 1.25 and 5.0 mg/kg). The control was sampled on Day 0 and Day 27. • all samples were stored frozen at approx. -20°C immediately after sampling until analysis
3.4.9	Monitoring of TS concentration	Yes, day 0, 7* and 27 * 1.25 and 5.0 mg/kg treatment only
3.4.10	Statistics	The emergence ratio (ER) and the development time and rate were calculated for each vessel as proposed by the testing guideline. The emergence ratio is defined as the sum of fully emerged midges by the number of inserted larvae. To obtain an approximate normal distribution and to equalize variances, ER_{arc} was calculated by transforming ER to arcsin-values. The mean development time represents the mean time span between the insertion of the larvae (Day 0) and the emergence of the experimental cohort of midges. The development rate is the reciprocal of the development time and represents the portion of larval development that takes place per day. For both parameters, emergence ratio and development rate, the arithmetic mean values, standard deviation and min/max were calculated from the four replicates per treatment. The mean emergence ratio and development rate of all test concentrations were statistically evaluated on significant differences to the control by the multivariate Dunnett's test after a one-way analysis of variance (ANOVA). Statistical evaluations were done separately for emerged males and females (development rate and with pooled sexes (emergence ratio). The 27-day EC_{50} and the 95 % confidence limits could not be calculated because no signs of toxicity were observed.
		4 RESULTS
4.1	**Limit Test**	not performed
4.1.1	Concentration	n.a.
4.1.2	Number/ percentage of animals showing adverse effects	n.a.

Section A7.4.3.5.1-3 Effects on sediment dwelling organisms

Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

4.1.3 Nature of adverse effects: n.a.

4.2 Results test substance

4.2.1 Initial concentrations of test substance: Nominal concentrations: control, 0.32, 0.63, 1.25, 2.5, 5.0 mg/kg dry sediment

4.2.2 Actual concentrations of test substance

Application solutions		
Nominal concentration DN Phosphate [mg/L]	Measured concentration DN Phosphate [mg/L]	Measured concentration [%] of nominal
1.43	1.62	113
2.81	2.97	106
5.58	5.63	101
11.15	11.9	106
22.3	23.2	104

Water samples			
Nominal concentration [µg/L]	Sampling day [d]	measured concentration [mg/kg resp. L]	[% nominal][1]
control	0	n.d.	n.a.
	27	n.d.	n.a.
1.25	0	0.0290	-
	7	0.0452	-
	27	0.0257	-
5.0	0	0.255	-
	7	0.250	-
	27	0.171	-
Pore water samples			
control	0	n.d.	n.a.
	27	n.d.	n.a.
1.25	0	0.0194	-
	7	0.0455	-
	27	0.0190	-
5.0	0	0.239	-
	7	0.158	-
	27	0.212	-
Sediment samples			
control	0	n.d.	n.a.
	27	n.d.	n.a.
1.25	0	0.860	130
	7	0.997	151
	27	0.769	117
5.0	0	2.67	101
	7	1.70	64
	27	2.45	93

1: corrected for recovery rate of spiked samples
n.d.: no test item detected
n.a.: not applicable

Section A7.4.3.5.1-3 **Effects on sediment dwelling organisms**
Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

4.2.3	Effect data	Emergence rate: Table A7.4.3.5.1.3-7 Development rate: Table A7.4.3.5.1.3-8
4.2.4	Concentration / response curve	n.a.
4.2.5	Other effects	At all test concentrations some dead midges were observed at the water surface of the test vessels. They were not taken into account for the calculation of emergence and development rates.
4.3	**Results of controls**	Emergence ratio: 90 – 95 % Development rate: 0.064 day^{-1} for the females and 0.071 day^{-1} for the males
4.4	**Test with reference substance**	None
4.4.1	Concentrations	n.a.
4.4.2	Results	n.a.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Guidelines: Toxic effects of the test item on the development of sediment dwelling larvae of the midge *Chironomus riparius* in water-sediment systems were investigated following OECD guideline "Proposal for a new guideline 218, draft document February 2001: Chironomid Toxicity Test Using Spiked Sediment" (2001). No relevant deviations from test guidelines. Method: First instar larvae of *Chironomus riparius* were exposed for a period of 27 days until full maturation of the larvae to adult midges. The test parameters of the study were the development rate of the midges and the emergence rate as the number of fully emerged males and females. The test item was applied mixed into the sediment 7 days prior to insertion of the larvae. The nominal test item concentrations in the overlaying sediment were 0.32, 0.63, 1.25, 2.5 and 5.0 mg/kg. A negative control was tested in parallel.
5.2	**Results and discussion**	The mean measured concentrations of the test item in the application solutions ranged from 101 – 113 % of the nominal concentrations. At the highest test concentration of nominal 5.0 mg/kg dry sediment 101% of the theoretical value was found in the sediment sample at Day 0. On Day 7 and 27, the measured concentrations corresponded to 64% and 93% of the nominal concentration, respectively. At the test concentration of nominal 1.25 mg/kg the actual concentration at Day 0 corresponded to 130% of the nominal concentration in the sediment. On Day 7 and 27, the measured concentrations corresponded to 151% and 117% of the nominal concentration, respectively. The concentrations in sediment were corrected by the mean recovery of 53% obtained for spiked samples. All reported biological results are related to the nominal concentrations of the test item since the analytically measured concentrations in the application solutions corresponded well with the theoretical values. Under consideration of the recovery rates of the analytical method, e.g. correction of measured values, the same was true for the concentrations

Section A7.4.3.5.1-3 Effects on sediment dwelling organisms
Annex Point IIIA-XIII.3.4 ***Chironomus riparius***

		of DN Phosphate in the sediment samples on Day 0. The emergence ratios per vessel in the controls ranged from 90 to 95% (and thus fulfilled the validity criterion of the test guideline). The mean development rate of fully emerged midges in the control amounted to 0.064 day^{-1} for the females, and 0.071 day^{-1} for the males, respectively (and thus fulfilled the validity criterion of the test guideline requesting a development rate between 0.05 and 0.1 day^{-1}). The EC_{10} and EC_{50} for emergence ratio and development rate was > 5.0 mg/kg dry sediment. The 27 day NOEC and the LOEC for development and emergence for *Chironomus riparius* were 5.0 and > 5mg/kg dry sediment, respectively.
5.2.1	EC_{10} emergence (27-day)	> 5mg/kg
5.2.2	EC_{50} emergence (27-day)	> 5mg/kg
5.2.3	EC_{10} development (27-day)	> 5mg/kg
5.2.4	EC_{50} development (27-day)	> 5mg/kg
5.2.5	NOEC (27-day)	5 mg/kg
5.2.6	LOEC (27-day)	> 5mg/kg
5.3	**Conclusion**	All validity criteria of guideline OECD 218 are fulfilled: • the emergence rate in the controls is ≥ 70 % • emergence to adults from the control occurred between 12 and 23 days after insertion of egg masses into the vessels • pH and dissolved oxygen were measured in each vessel at the end of exposure and was 7.1 – 8.5 and ≥ 60 % respectively • the water temperature did not differ by not more than 1 °C The test item DN Phosphate had no adverse effect on the emergence and development of *Chironomus riparius* in a 27-day sediment spiked test.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Table A7.4.3.5.1.3-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7.4.3.5.1.3-2: Dilution water

Criteria	Details
Source	Reconstituted water, "M7" according to OECD 218
Alkalinity	Not determined
Hardness	Ca^{2+}: approx. 2.0 mmol/L (as $CaCO_3$) Ca^{2+} and Mg^{2+}: approx 2.5 mmol/L
pH	7.9 ± 0.3
Ca / Mg ratio	Not determined
Na / K ratio	Not determined
Oxygen content	Not determined
Conductance	Not determined
Holding water different from dilution water	No

Table A7.4.3.5.1.3-3: Sediment

Criteria	Details
Type/source	Artificial sediment according to OECD 218
Composition (% w/w)	Sphagnum peat (air dried, ground to ≤ 1mm): 4 % Kaolin clay (content of Al_2O_3: 36.4 5): 20 % Sand (Sihelco 36): 76 % Calcium carbonate ($CaCO_3$): 0.25 %
Total organic carbon (TOC)	1.7 %
pH adjustment	Yes
pH of final sediment mixture	6.4

Table A7.4.3.5.1.3-4: Test organisms

Criteria	Details
Species/strain	*Chironomus riparius*
Source	Novartis Crop Protection AG
Age	2 – 3 days (first instar larvae)
Breeding method	Similar temperature and light conditions and in the same kind of water as used in the test
Kind of food	TetraMin Hauptfutter (TETRA-Werke, D-49304 Melle, Germany)
Amount of food	Day 0 – 6: 23 mg / vessel Day 8 – 25: 47 mg/vessel
Feeding frequency	3 times a week
Pretreatment	None
Feeding of animals during test	Yes

Table A7.4.3.5.1.3-5: Test system

Criteria	Details
Type of test	Chronic 27 days
Renewal of test solution	No
Volume of test vessels	600 mL
Amount/volume of sediment per vessel	about 130 g wet weight corresponding to 1.8 cm
Volume of test medium per vessel	250 mL
Volume/animal	12.5 mL
Number of animals/vessel	20
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No, vessels covered with a watch-glass

Table A7.4.3.5.1.3-6: Test conditions

Criteria	Details
Test temperature	18.8 – 19.8
Dissolved oxygen	4.5 – 8.9 mg/L, ≥ 7.3 at the end of the test
pH	7.1 – 8.5
Adjustment of pH	Yes
Aeration of dilution water	Yes
Quality/Intensity of irradiation	approx. 780 Lux
Photoperiod	16 h light / 8 h dark with a 30 min transition period each

Table A7.4.3.5.1.3-7: Emergence rate of *Chironomus riparius* (males and females pooled) after 27 days of exposure

	Nominal initial test item concentration [mg/kg]					
	control	0.32	0.63	1.25	2.5	5.0
Sum of inserted larvae per treatment	80	80	80	80	80	80
Sum of emerged midges per treatment	75	71	67	72	69	71
% of emerged midges per treatment (mean)	94	89	84	90	86	89
Emergence ratio ER_{arc}:						
mean	1.321	1.243	1.168	1.262	1.263	1.230
SD	0.0482	0.1215	0.1288	0.1126	0.2851	0.0380
min	1.249	1.107	1.047	1.107	0.886	1.173
max	1.345	1.345	1.345	1.345	1.571	1.249
n	4	4	4	4	4	4
% of control	100.0	94.1	88.4	95.5	95.6	93.1
STAT	---	n.s.	n.s.	n.s.	n.s.	n.s.

ER_{arc}:arcsin-transformed emergence ratio
STAT:results of a Dunnett-test (α= 0.05, one-sided smaller)
n.s.:mean ER_{arc} not significantly lower than in the control
s.:mean ER_{arc} significantly lower than in the control

Table A7.4.3.5.1.3-8: Development rate for males and females

Males						
Development rate per treatment	Nominal initial test item concentration (mg/kg)					
(day^{-1})	control	0.32	0.63	1.25	2.5	5.0
Mean	0.0707	0.0688	0.0675	0.0672	0.0679	0.0680
SD	0.00093	0.00017	0.00207	0.00127	0.00061	0.00112
min	0.0697	0.0686	0.0645	0.0663	0.0672	0.0671
max	0.0717	0.0689	0.0691	0.0691	0.0686	0.0696
n	4	4	4	4	4	4
% of control	100.0	97.2	95.5	95.0	96.0	96.2
STAT	---	n.s.	n.s.	n.s.	n.s.	n.s.

Females						
Development rate per treatment	Nominal initial test item concentration (mg/kg)					
(day^{-1})	control	0.32	0.63	1.25	2.5	5.0
Mean	0.0641	0.0629	0.0626	0.0636	0.0633	0.0631
SD	0.00120	0.00088	0.00119	0.00081	0.00283	0.00074
min	0.0624	0.0618	0.0614	0.0631	0.0599	0.0621
max	0.0652	0.0638	0.0642	0.0648	0.0667	0.0637
n	4	4	4	4	4	4
% of control	100.0	98.1	97.7	99.2	98.8	98.4
STAT	---	n.s.	n.s.	n.s.	n.s.	n.s.

STAT: results of a Dunnett-test ($\alpha = 0.05$, one-sided smaller)
n.s.: mean development rate not significantly lower than in the control
s.: mean development rate significantly lower than in the control

Table A7.4.3.5.1.3-10: Validity criteria for the sediment/water Chironomus toxicity test according to OECD Guideline 219.

Criteria	Fulfilled	Not fulfilled
Mortality in the controls <30% at the end of the test	X	
Emergence of adults (days after insertion in test vessels): - *C. riparius*, *C. yoshimatsui*: 12 – 23 days - *C. tentans*: 20 – 65 days	X	
Emergence in controls: 50 – 70%	X	
Concentration of dissolved oxygen: > 60% of the air saturation value of the temperature used	X	
pH of overlying water: 6 – 9	X	
Water temperature does not differ by more than ± 1.0°C between test vessels, and is be maintained within the temperature ranges specified for the test species	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: 3.4.9 RMS believes that the '*' refers to all sampling days and not just Day 7.
Conclusion	Applicant's version considered acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.3.5.2 Aquatic plant toxicity
Annex Point IIIA-XIII.3.4 *Lemna gibba*
Semi-static, 7-day

			Official use only
		1 REFERENCE	
1.1	**Reference**	Bätscher, R., 2002, Toxicity of MTI-446 to the aquatic higher plant *Lemna gibba* in a 7-day semistatic growth inhibition test, RCC Ltd., unpublished report no. 827752, January 16, 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 OPPTS 850.4400 under consideration of OECD 221 (draft October 2000)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	2100910	
3.1.2	Specification		
3.1.2.1	Description	white solid	
3.1.2.2	Purity	99.2 %	
3.1.2.3	Stability	Expiration date: July 22, 2004	
3.1.3	Further relevant properties	Solubility in water: 40 g/L Stability in water: > 24 hours (Sponsor information)	
3.1.4	Method of analysis	The concentrations of dinotefuran in the test medium were monitored throughout the exposure period using HPLC with UV/VIS 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 A7.4.3.5.2-1	
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	Table A7.4.3.5.2-2	
3.4.2	Sediment	Table A7.4.3.5.2-3	
3.4.3	Test organisms	Table A7.4.3.5.2-4	
3.4.4	Test system	Table A7.4.3.5.2-5	

Section A7.4.3.5.2 **Aquatic plant toxicity**

Annex Point IIIA-XIII.3.4 ***Lemna gibba***

Semi-static, 7-day

3.4.5	Test conditions	Table A7.4.3.5.2-6
3.4.6	Duration of the test	7 days
3.4.7	Test parameter	The *Lemna* colonies in each test vessel were inspected for changes in frond and colony number and appearance (discoloration, sinking root length or other abnormalities) on days 2, 4 and 7. The number of living and dead fronds and the number of colonies were counted. Fronds visibly projecting over the edge of the mother frond were counted as separate fronds. Additionally, the dry weight of a sample of fronds identical to that used to inoculate the test vessels was determined at the start of the test. At test termination, the dry weight of all colonies per vessel was determined. The colonies were dried at approx. 60°C to constant weight. Inhibition of Lemna growth was determined from: • mean frond numbers • the average specific growth rates • the area under the growth curves (AUC) • the final biomass determined on the basis of dry weight (DW)
3.4.8	Sampling	Duplicate samples of the fresh prepared test solutions were taken on days 0 and 4. Duplicate samples of the old test solutions were taken on days 2 and 7. All samples were stored at about -20°C.
3.4.9	Monitoring of TS concentration	The test item concentration was determined from the highest test solution only since all other test concentrations were < the 7-day NOEC. One sample of the corresponding control solutions was analysed.
3.4.10	Statistics	Frond number, growth rate (r), AUC, and dry weight were determined or calculated for each test vessel, followed by calculation of the arithmetic mean frond number, mean growth rate, mean area, and mean dry weight, respectively, per test concentration and control. For the growth of Lemna after the 7-day exposure period, the 7-day NOEC (highest concentration without toxic effects) and the LOEC (lowest concentration with toxic effects) were determined by testing the growth parameters (mean frond numbers, average specific growth rate (r), the AUC, and the mean dry weight of the plants) at the test concentrations on statistically significant differences to the control values by multiple Dunnett-tests. The EC values (EC_5, EC_{10}, EC_{50}, and EC_{90}) for the growth parameters could not be calculated since no toxic effect of the test item on the growth of the plants was determined up to and including the highest test concentration.
		4 RESULTS
4.1	**Limit Test**	None
4.1.1	Concentration	
4.1.2	Number/ percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	

Section A7.4.3.5.2 **Aquatic plant toxicity**
Annex Point IIIA-XIII.3.4 ***Lemna gibba***
Semi-static, 7-day

4.2 Results test substance

4.2.1 Initial concentrations of test substance: 11, 20, 35, 62 and 110 mg/L

4.2.2 Actual concentrations of test substance:

Analytical results, Treatment 110 g/L nominal			
Sampling day	Age of sample	Measured concentration	
[d]	[h]	[mg/L]	[% of nominal]
0	0	117	106
2	48	102	92
4	0	117	106
7	72	109	99
	mean:	111	101
Analytical results, Control 0 mg/L nominal			
0	0	n.d.	n.a.
2	48	n.d.	n.a.
4	0	n.d.	n.a.
7	72	n.d.	n.a.

n.d.: no test item detected
n.a.: not applicable

X

4.2.3 Effect data: Growth rate (r) and inhibition of r: Table A7.4.3.5.2-7
Areas under growth curve (AUC) and inhibition of AUC: Table A7.4.3.5.2-8
Final biomass (dry weight) after 7 days: Table A7.4.3.5.2-9
Total number of fronds and colonies per test vessel at the counting dates: Table A7.4.3.5.2-10

4.2.4 Concentration / response curve: None

4.2.5 Other effects: None

4.3 Results of controls: $T_{d\ control}$: 1.9 days

4.4 Test with reference substance: None

4.4.1 Concentrations

4.4.2 Results

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:
OPPTS 850.4400 under consideration of OECD 221 (draft October 2000)
No relevant deviations from test guidelines
Method:
The duckweed *Lemna gibba* G3 (family Lemnaceae, Macrophyta) was the test organism used for this study. The plants were cultivated for more than two weeks prior to the test under standardized conditions in the same nutrient medium as used in the test. Plants from an exponentially growing

culture were used in the test, and only young, rapidly growing colonies without visible lesions were used.

A semi-static test with test medium renewal after 2 and 4 days was utilized to keep the concentrations of the test item and nutrients in the test media as constant as possible during the 7-day test period. Under aseptic conditions, the test plants were transferred in a clean test vessel with freshly prepared test medium of the corresponding concentration, and the plants were cultivated and tested in sterile synthetic test water. The pH of the test water was adjusted to pH 7.5 with 1 M HCl solution.

The test was designed with three replicates per test concentration and control. Each replicate consisted of a glass dish partly filled with test medium, resulting in a water depth of approximately 25 mm. All test vessels were covered with glass lids, arranged randomly, and were incubated in a temperature-controlled water bath at about 24 °C. The temperature ranged from 23.4 to 24.6 °C, and they were continuously illuminated at a light intensity of about 6500 Lux (mean value), range: 6100 to 6700 Lux.

Lemna colonies were transferred at test initiation from the pre-culture into the test vessels in a randomized order. With three randomly selected colonies per vessel, the test was started. Each colony had 4 fronds resulting in 12 fronds per vessel.

Dinotefuran concentrations of 11, 20, 35, 62, and 110 mg/L were tested. A negative control (test water without test item) was tested in parallel. The test media were freshly prepared at the start of the test and prior to each test. The highest test item concentration medium of 110 mg/L was prepared by dissolving 154 mg of the test item (range: 154-155 mg) completely in 1400 mL water by ultrasonic treatment (15 minutes) and intense stirring (5 minutes at room temperature). Portions of this medium were diluted with water to prepare the test media with the lower test item concentrations. The actual test item concentration in the test medium of the highest test concentration was analytically determined.

At the beginning and the end of each test medium renewal period, the pH values and the appearance of the test media were recorded. At the start and end of the test medium renewal, the water temperature was measured in a vessel filled with water and incubated under the same conditions as the test vessels.

On Days 2 and 4, and at the end of the test (Day 7), the Lemna colonies in each test vessel were inspected for changes in frond and colony number and appearance (discoloration, sinking root length, or other abnormalities). Living and dead fronds and the number of colonies were counted on these dates. The dry weight of a sample of fronds identical to that used to inoculate the test vessels was also determined at test initiation. The colonies were dried at about 60 °C to a constant weight (weighed by an analytical balance). The dry weight of all colonies per test vessel was determined at the test termination.

For test item concentration analysis, duplicate samples were taken from the freshly prepared test media at all concentrations and the control at the start of the test (Day 0) and at the start of the last test medium renewal (Day 4). For the test concentration maintenance determination during the test period, duplicate samples were taken from the test media of all test concentrations and the control at the end of the first test medium renewal (Day 2) and at the end of the test (Day 7). The aged test media samples from the three replicates per test concentration were pooled. All samples were

Section A7.4.3.5.2 **Aquatic plant toxicity**
Annex Point IIIA-XIII.3.4 ***Lemna gibba***
Semi-static, 7-day

		immediately deep-frozen after sampling and were stored at about -20 °C until the analyses were performed. Dinotefuran concentrations were measured in all test medium samples from the highest test concentration of nominal 110 mg/L from all sampling dates. Only one of the duplicate samples per corresponding sampling date was analyzed from the control. Since the samples from the lower test concentrations of nominal 11 to 62 mg/L were below the 7-day NOEC, they were not analyzed.	
5.2	**Results and discussion**	Dinotefuran concentration in the freshly prepared test medium of the highest test concentration of nominal 110 mg/L was 106% of the nominal value at the start of the first and last test medium renewal. At the end of the first and last test medium renewal, 92% and 99% of the nominal value were found after 48 and 72 hours, respectively. Test item mean concentration (calculated as the average over all measurements in the test medium) was 101% of the nominal value. The growth of *Lemna gibba* was not inhibited up to and including the highest test concentration of 110 mg/L. The mean frond and colony number, the average specific growth rates (r), and the AUC after the test period of 7 days were not statistically significantly reduced compared to the control values at any concentration (results of Dunnett-tests, one-sided, á = 0.05). The dry weight of the plants after the test period of 7 days was statistically not significantly lower compared to the control at any concentration. The plants showed no mortality or other abnormalities at any concentration or in the control. Dinotefuran did not inhibit the growth of *Lemna gibba* during the exposure period of 7 days up to and including 110 mg/L. As a result, this concentration was determined as the 7-day NOEC (highest concentration tested without toxic effects after a test period of 7 days). This value could be higher, but concentrations greater than 110 mg/L were not tested. After the 7 days test duration, the 7-day LOEC (lowest concentration tested with toxic effects) and the EC-values (EC_5, EC_{10}, EC_{50}, and EC_{90}) for the growth parameters could not be quantified due to the absence of a toxic effect of Dinotefuran up to and including the highest test concentration of 110 mg/L. The doubling time (T_d = In 2 / r) of *Lemna* growth in the control was determined to be 1.9 days. This shows that the growth of *Lemna gibba* was sufficiently high. The pH values in the freshly prepared test media ranged from 7.4 to 7.6 at the start of the test medium renewal periods. Measured pH values were between 8.6 to 9.2 at the end of the test medium renewal periods. This increase of the pH during the test was caused by the CO_2-consumption of the plants due to their rapid growth. All test media were clear solutions and remained clear throughout the test medium renewal periods.	
5.2.1	$EC_{5, 10, 50, 90}$ (7-day)	> 110 mg/L	X
5.2.2	NOEC (10-day)	110 mg/L	X
5.2.3	LOEC (7-day)	> 110 mg/L	X

Section A7.4.3.5.2 Aquatic plant toxicity
Annex Point IIIA-XIII.3.4 *Lemna gibba*
Semi-static, 7-day

5.3	**Conclusion**	Dinotefuran did not inhibit the growth of *Lemna gibba* during the exposure period of 7 days up to and including 110 mg/L. This concentration was determined as the 7-day NOEC (highest concentration tested without toxic effects after a test period of 7 days). Due to the absence of a toxic effect of dinotefuran up to and including the highest test concentration of 110 mg/L, the 7-day LOEC (lowest concentration tested with toxic effects) and the EC_{10} and EC_{50} values after the 7-day test period could not be quantified for the different growth parameters, nonetheless, these parameters were clearly higher than 110 mg/L.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Table A7.4.3.5.2-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7.4.3.5.2-2: Dilution water

Criteria	Details
Source	Sterile synthetic test water "20X AAP groth medium" according to guidelines
Alkalinity	not determined
Hardness	not determined
pH	7.5
Ca / Mg ratio	not determined
Na / K ratio	not determined
Oxygen content	not determined
Conductance	not determined
Holding water different from dilution water	No

Table A7.4.3.5.2-3: Sediment

Criteria	Details
Type/source	None
Composition (% w/w)	n.a.
Total organic carbon (TOC)	n.a.
pH adjustment	n.a.
pH of final sediment mixture	n.a.

Table A7.4.3.5.2-4: Test organisms.

Criteria	Details
Species/strain	*Lemna gibba G3*
Source	Bayer AG, Crop Protection Institute for Environmental Biology, D-40789 Monheim, Germany
Age	more than two weeks
Breeding method	n.a.
Kind of food	n.a.
Amount of food	n.a.
Feeding frequency	n.a.
Pretreatment	n.a.
Feeding of animals during test	n.a.

Table A7.4.3.5.2-5: Test system.

Criteria	Details
Type of test	Growth inhibition
Renewal of test solution	Yes
Volume of test vessels	250 mL
Amount/volume of sediment per vessel	n.a.
Volume of test medium per vessel	150 mL
Volume/colony	50 mL
Number of colonies/vessel	3
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No, vessels covered with a glass lid

Table A7.4.3.5.1.2-6: Test conditions.

Criteria	Details
Test temperature	24 °C
Dissolved oxygen	Not reported
pH	7.4 – 7.6 at start of test medium renewals 8.6 – 9.2 at the end of test medium renewals
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	6100 – 6700 lux
Photoperiod	Continuously illuminated

Table A7.4.3.5.2-7: Influence of the test item on *Lemna gibba* growth: growth rates (r) and percentage inhibition of r

Nominal test item concentration [mg/L]	Growth rate r (1/day) and % inhibition of r					
	0 – 48 h		0 – 96 h		0 – 168 h	
	r	%	r	%	r	%
Control	0.35	0	0.36	0	0.36	0
11	0.32	8.0	0.34	4.3	0.34	4.8
20	0.35	0.2	0.37	-2.0	0.35	2.3
35	0.31	12.2	0.33	9.4	0.34	6.9
62	0.31	12.9	0.34	6.5	0.34	4.7
110	0.32	8.0	0.35	3.9	0.35	3.2

Growth rates (r) were not significantly lower than the corresponding control value at any test item concentration (Dunett-test, one-sided smaller, $\alpha = 0.05$)

Table A7.4.3.5.2-8: Influence of the test item on *Lemna gibba* growth: areas under growth curves (AUC) and percentage inhibition of AUC

Nominal test item concentration [mg/L]	Areas under the growth curve (AUC) and percentage inhibition					
	0 – 48 h		0 – 96 h		0 – 168 h	
	AUC	%	AUC	%	AUC	%
Control	296	0	1520	0	7952	0
11	264	10.8	1384	8.9	7060	11.2
20	296	0	1560	-2.6	7716	3.0
35	248	16.2	1272	16.3	6564	17.5
62	248	16.2	1320	13.2	6960	12.5
110	264	10.8	1392	8.4	7284	8.4

AUC was not significantly lower than the corresponding control value at any test item concentration (Dunett-test, one-sided smaller, $\alpha = 0.05$)

Table A7.4.3.5.2-9: Final biomass on the basis of dry weight (mg per test vessel) of *Lemna* colonies after 7 days

Vessel No.	Nominal test item concentration [mg/L]					
	Control	11	20	35	62	110
1	20.1	14.9	19.9	14.1	17.1	16.2
2	14.0	16.2	14.3	18.3	17.6	17.6
3	21.3	12.9	19.7	16.3	19.6	19.4
Mean*	18.5	14.7	18.0	16.2	18.1	17.7
SD	3.9	1.7	3.2	2.1	1.3	1.6
% inhibition# compared to control	-	21.5	2.9	12.9	2.3	4.3

Mean: arithmetic mean
SD: standard deviation
* all mean values not significantly lower than in control (Dunett-test, one sided, smaller, $\alpha = 0.05$)
# % inhibition based on the increase in biomass (mean final dry weight minus starting dry weight).
Dry weight of three colonies with in total 12 fronds at test start: 0.86 mg

Table A7.4.3.5.2-10: Total number of fronds and colonies per test vessel at the counting dates

Nominal test item concentration (mg/L)	Vessel No.	Frond number (#F) and colony number (#C) per test vessel								Inhibition of mean frond number*
		0 h (Day 0)		48 h (Day 2)		96 h (Day 4)		168 h (Day 7)		
		#F	#C	#F	#C	#F	#C	#F	#C	
Control	1	12	3	24	6	52	12	167	34	
	2	12	3	25	6	51	12	126	32	
	3	12	3	24	6	49	12	163	33	
	Mean	12.0	3.0	24.3	6.0	50.7	12.0	152.0	33.0	-
	SD	0.0	0.0	0.6	0.0	1.5	0.0	22.6	1.0	
11	1	12	3	24	6	49	12	126	30	
	2	12	3	23	6	50	12	150	35	
	3	12	3	22	5	44	12	126	29	
	Mean	12.0	3.0	23.0	5.7	47.7	12.0	134.0	31.3	12.9%
	SD	0.0	0.0	1.0	0.6	3.2	0.0	13.9	3.2	
20	1	12	3	23	6	47	12	142	34	
	2	12	3	24	6	53	12	128	28	
	3	12	3	26	7	57	12	158	34	
	Mean	12.0	3.0	24.3	6.3	52.3	12.0	142.7	32.0	6.7%
	SD	0.0	0.0	1.5	0.6	5.0	0.0	15.0	3.5	
35	1	12	3	23	6	48	12	130	31	
	2	12	3	21	6	43	11	130	28	
	3	12	3	23	6	42	10	120	28	
	Mean	12.0	3.0	22.3	6.0	44.3	11.0	126.7	29.0	18.1%
	SD	0.0	0.0	1.2	0.0	3.2	1.0	5.8	1.7	
62	1	12	3	19	6	40	10	120	28	
	2	12	3	24	6	51	12	133	33	
	3	12	3	24	5	48	11	150	35	
	Mean	12.0	3.0	22.3	5.7	46.3	11.0	134.3	32.0	12.6%
	SD	0.0	0.0	2.9	0.6	5.7	1.0	15.0	3.6	
110	1	12	3	22	6	45	11	125	29	
	2	12	3	24	6	46	12	140	28	
	3	12	3	23	6	53	12	154	35	
	Mean	12.0	3.0	23.0	6.0	48.0	11.7	139.7	30.7	8.8%
	SD	0.0	0.0	1.0	0.0	4.4	0.6	14.5	3.8	

Mean: arithmetic mean
SD: standard deviation
* % inhibition of mean frond number compared to the control at the test end (after 168 hours)

Mean frond and colony numbers at the test end (after 168 hours) were not significantly lower than the control values at any test item concentration (according to Dunnett-tests, one-sided smaller, $\alpha = 0.05$).

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	16 October 2012
Materials and methods	Applicant's version considered acceptable, noting the following: 3.1 Test material is MTI-446 as given in section 2 of the report. 4.2.2 Heading is incorrect for analytical results table – should read 110 mg/l and not 110 g/l Results and discussion: for clarity, the biological results are presented in terms of the nominal concentrations.
Conclusion	Applicant's version considered acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.3 Annex Point IIIA, XIII.2	**Effects on aquatic organisms, further studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Dinotefuran has been shown as non-toxic to fish in an acute study, therefore, no further studies are required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	22 November 2012	
Evaluation of applicant's justification	The applicant has supplied acute and chronic data for three trophic levels in species of appropriate sensitivity, thus no further testing is considered necessary and the justification is accepted.	
Conclusion		
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Völkel, D., 2000, The effects of MTI-446 20% SG on soil respiration and nitrification, RCC Ltd, unpublished report no. 747281, July 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 SETAC-Europe: Procedures for assessing the environmental fate and ecotoxicity of pesticides, March 1995 Under consideration of: • Biologische Bundesanstalt für Land-und Forstwirtschaft (BBA), Deutschland. Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln Teil VI 1-1 (2.Auflage). Auswirkungen auf die Aktivität der Bodenmikroflora, März 1990 • Draft OPPTS 850.5100 Soil microbial community toxicity test; United States Environmental Protection Agency (EPA), April 1996	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	99I-5311	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.3	Identity	MTI-446 20 % SG	X
3.1.4	Aggregate state of the formulation	solid	
3.1.5	Content a.i.	21.09 %	
3.1.6	Composition of Product	n.a.	
3.1.7	Further relevant properties	Active ingredient: Solubility in water: 39.83 g/L at 20 °C Stability in water: > 24 hours (Sponsor information)	X
3.1.8	Method of analysis	No test item analysis was conducted	
3.2	**Reference substance**	Yes Dinoseb acetate	
3.2.1	Method of analysis for reference substance	none	