Section 7.4.3.2 Effects on reproduction and growth rate of fish
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			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]; FISH EARLY LIFE STAGE TEST (PIMEPHALES PROMELAS) WITH CYPERMETHRIN; [redacted] (826-001), 15th March 2012 *The definitive test was initiated on 12 December 2011 and concluded on 13 January 2012.*	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Arysta LifeScience Benelux (formerly Agriphar S.A.)	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline 210	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	25163S63	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	95.83% w/w	
3.1.4	Composition of Product	Not applicable, test carried out on active substance	
3.1.5	Further relevant properties	Low water solubility, therefore test was a continuous flow design.	
3.1.6	Method of analysis	Concentrations of Cypermethrin in treated mains water were extracted by partitioning with hexane, followed by quantification by gas chromatography (GC) using µECD detection	

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3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A concentrated solvent stock solution was prepared by weighing ca. 6.52 mg of the test substance and diluting to 100 mL with acetone to produce a 61 µg/mL solution of Cypermethrin. Serial dilutions of this stock solution were prepared in acetone to produce additional solvent stock solutions at 20.25, 6.74, 2.25, 0.75 and 0.25 µg/mL. A 0.3 mL aliquot of the appropriate solvent stock solution was then injected into 15 L of treated mains water in glass aspirators (ca. 22 L in volume) to produce nominal test media concentrations of 0.005, 0.015, 0.045, 0.135, 0.405 and 1.22 µg/L.
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	The dilution water used for conducting the definitive test was dechlorinated mains water that had been passed through particulate and activated carbon filters and treated with ozone (O_3).
3.4.2	Test organisms	See table A7_4_3_2_2-2
3.4.3	Test system	See table A7_4_3_2_2-3
3.4.4	Test conditions	See table A7_4_3_2_2-4
3.4.5	Duration of the test	28 days post hatch
3.4.6	Test parameter(s)	The post-hatch phase started once all of the viable eggs were considered to have hatched in the control groups. Egg hatch at each treatment was then assessed relative to control group performance. Once all viable eggs had hatched across all treatments and fish were actively feeding and mobile, the chambers were removed. On Day 28 of the post-hatch phase, hatching success was confirmed by definitive counts of all surviving fish. These counts, for each vessel, were then corrected for all larvae lost during the post-hatch phase. Furthermore, the total numbers of surviving larvae were counted and individual total fish lengths (mm) and wet weights (mg) of all remaining fish were determined. The percentage post-hatch survival was determined by expressing the number of surviving larvae on Day 28 post-hatch as a percentage of the hatched larvae at Day 0 post-hatch. The nominal test concentrations were 0.005, 0.015, 0.045, 0.135, 0.405 and 1.22 µg a.s./L.
3.4.7	Examination / Sampling	Chemical analysis of samples of test media taken from each test vessel on Day 0 pre-hatch and Days 0, 6, 13, 20 and 27 post-hatch, gave geometric mean measured concentrations of <LOQ, 0.00777, 0.0211, 0.0582, 0.198 and 0.463 µg/L, equivalent to <LOQ, 51.8, 46.9, 43.1, 48.9 and 38.0% of nominal, respectively.

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3.4.8	Monitoring of TS concentration	The test was conducted under continuous flow conditions, using embryos produced by brood fish which, on exposure, were at the tail bud to late embryo stage of development. Chemical analysis of samples of test media taken from each test vessel on Day 0 pre-hatch and Days 0, 6, 13, 20 and 27 post-hatch, gave geometric mean measured concentrations of <LOQ, 0.00777, 0.0211, 0.0582, 0.198 and 0.463 µg/L, equivalent to <LOQ, 51.8, 46.9, 43.1, 48.9 and 38.0% of nominal, respectively.
3.4.9	Statistics	Day 28 total lengths and wet weights were analysed using analysis of variance, fitting fixed effects for group and vessel within group. The test concentrations were compared with the control using Dunnett's test. All tests were interpreted with two-sided risk. The residuals from the model were examined and, in all cases, this showed no evidence of non-normality. The control and solvent control groups were compared and the results were interpreted with two-sided risk. Where there was a statistically significant difference between the control and solvent control, the test concentrations were compared with the solvent control. As there were significant differences between the control and solvent control length and hatching success, all analyses were compared to the solvent control only.
		4 RESULTS
4.1	**Range finding test**	Not performed.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Geometric mean measured concentrations within the test vessels were <LOQ, 0.00777, 0.0211, 0.0582, 0.198 and 0.463 µg a.s./L, equivalent to <LOQ, 51.8, 46.9, 43.1, 48.9 and 38.0 % of nominal, respectively. The nominal test concentrations were 0.005, 0.015, 0.045, 0.135, 0.405 and 1.22 µg a.s./L, respectively. As the measured concentrations were not within the range of 80 - 120 % of the nominal concentration, the results have been reported in terms of geometric mean measured concentrations.
4.2.2	Actual concentrations of test substance	See Table A7_4_3_2_2-5

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4.2.3	Effect data	Hatching success in the control vessels ranged from 70 to 90 % and provided a mean value of 80 %. Hatching success in the solvent control group ranged between 97 and 100 % with a mean value of 98 %. The percentage hatching success for the control and treatment groups based on pooled replicate vessels at each treatment are presented in Table A7_4_3_2_2-6 The mean post-hatch survival in the control and solvent control groups was 96 and 95 %, respectively and was between 96 and 98 % in the Cypermethrin treatment groups. The mean length of fish in the control group was statistically significantly lower compared to the solvent control and the wet weight of fish at the lowest treatment concentration (< LOQ) was significantly higher compared to the solvent control. Neither of these differences are considered to be biologically significant as the total length and wet weights were well within the usual ranges for this species and the higher treatment rates did not show any statistically significant differences. See Table A7_4_3_2_2-6 A review of the data indicated that the validation criteria of the revised draft guideline (OECD Testing Guideline No 210 (December 2012) and the final version from 2013 were achieved: The dissolved oxygen concentration was maintained above 60 % of the air saturation value. The water temperature did not differ by more than 1.5°C between test chambers or successive days during the test. The hatching success was > 70 % and post-hatch survival of fish larvae was > 75 % in the control treatments. The NOEC, EC_{10} and EC_{20} values for hatching success, post-hatch survival, total length and wet weight were all determined to be ≥ 0.463 µg a.s./L, the maximum concentration tested
4.2.4	Other effects	-
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	Hatching success in the solvent control group was 77% and fry survival 73%, showing the test was valid.
4.4	**Test with reference substance**	Not performed

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The effects of Cypermethrin (cis:trans, 40:60) on the early life stages of Fathead minnow (Pimephales promelas) were determined under flow-through conditions according to OECD guideline 210 (July 1992).

Fertilized eggs of Fathead minnow (Pimephales promelas) were exposed to six initial test item concentrations for 28 days post-hatch. The nominal test concentrations were 0.005, 0.015, 0.045, 0.135, 0.405 and 1.22 µg a.s./L. Chemical analysis of samples of test media taken from each test vessel on Day 0 pre-hatch and Days 0, 6, 13, 20 and 27 post-hatch, resulted in geometric mean measured concentrations of <LOQ, 0.00777, 0.0211, 0.0582, 0.198 and 0.463 µg/L, respectively, and equivalent to <LOQ, 51.8, 46.9, 43.1, 48.9 and 38.0 % of the nominal Cypermethrin concentrations, respectively. The biological results have been reported in terms of geometric mean measured concentrations.

5.2 Results and discussion

5.2.1 NOEC

Cypermethrin was not considered to have any effect on the hatching success or post-hatch survival of Pimephales promelas up to the maximum concentration tested. It was also considered that there were no treatment related effects on the growth (total length and wet weight) of the fish.

The NOEC, EC10 and EC20 values for hatching success, post-hatch survival, total length and wet weight were all determined to be ≥ 0.463 µg a.s./L, the maximum concentration tested.

5.2.2 LOEC

There was no treatment related effect on the post-hatch survival of the fish. As no significant effects were determined the LOEC could not be accurately defined. In terms of geometric mean measured concentrations, the NOEC and LOEC were therefore considered to be 0.463 µg/L and >0.463 µg/L, respectively.

5.3 Conclusion

Cypermethrin was not considered to have any effect on the hatching success or post hatch survival of Pimephales promelas up to the maximum concentration tested. It was also considered that there were no treatment related effects on the growth (total length and wet weight) of the fish.

In terms of the geometric mean measured concentrations, the NOEC for hatching success, post-hatch survival, total length and wet weight was 0.463 µg/L; the maximum concentration used during this test.

5.3.1 Other Conclusions

5.3.2 Reliability 1

5.3.3 Deficiencies No

Evaluation by Competent Authorities
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
EVALUATION BY RAPPORTEUR MEMBER STATE

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Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_3_2_2-1: Dilution water

Criteria	Details
Source	Reconstituted fresh water prepared by the test laboratory.
Chloride	47 mg Cl/L
Sodium	5 mg Na/L
Potassium	<1 mg K/L
Magnesium	4 mg Mg/L
Hardness	80 mg $CaCO_3$
pH	6.66 as $CaCO_3$
Alkalinity	7 mg/L
Conductance	190 µS/cm at 20 ºC
Holding water different from dilution water	No

Table A7_4_3_2_2-2: Test organisms

Criteria	Details
Species/strain	Fathead Minnow *(Pimephales promelas)*
Source	[redacted]
Wild caught	No
Age/size	25 hours
Pre-treatment	The eggs were deposited approximately 24 hours prior to testing and were collected in glass scintillation vials containing treated mains water. The embryos were acclimated to laboratory conditions for at least one hour prior to being added to the vessels. During this time the embryos were examined under a microscope so that the stage of development could be assessed
Kind of food	Brine Shrimp *(Artemia salina)*
Amount of food	*Ad libitum*
Feeding frequency	

Table A7_4_3_2_2-3: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	Approx. 10 ml/min
Volume of test vessels	nominal 3.5 L constructed silanised glass aquariums. Egg incubation chambers used in the test were 17 cm long glass cylinders (6.5 cm internal diameter) with a 0.5 - 1.0 mm non-reactive mesh attached to the bottom
Number of animals/vessel	*Nominally 30 eggs were added to each of 16 glass scintillation vials half filled with treated mains water and each vial was randomly assigned a test or control vessel.*
Number of vessels/ concentration	*Each replicate tank contained one incubation chamber.*
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2_2-4: Test conditions

Criteria	Details
Test temperature	24.9-26.8ºC
Aeration of dilution water	*To ensure adequate movement of water within the test vessels and maintain dissolved oxygen concentrations in the egg incubation chambers, two aeration tubes were added to each replicate vessel. The first was maintained in the test medium surrounding the incubation chamber and the second aerator was maintained within the incubation chamber, positioned with the tube end at approximately 1 cm from the mesh on the end of the cylinder. The agitation/turbulence caused by the aerators on the water column within the chambers ensured that a dynamic equilibrium of concentration was achieved between the environment within the chamber and surrounding test medium. The turbulence created slight movement of the embryos and maintained good levels of aeration within the chamber.*
Photoperiod	The test was conducted in a light-controlled facility, with a 16-hour photoperiod

Table A7_4_3_2_2-5: Measured concentration of cypermethrin during the test

Nominal Cypermethrin concentration (µg/L)	Measured Cypermethrin concentration (µg/L)						Geometric mean measured concentration corrected for mean control value (µg/L)	Mean as % of nominal
	Day 0 pre-hatch	Day 0 post-hatch	Day 6 post-hatch	Day 13 post-hatch	Day 20 post-hatch	Day 27 post-hatch		
Control	0.00515	0.00537	0.00572	0.00588	0.00804	<LOQ	-	-
	<LOQ	<LOQ	<LOQ	0.0100	0.00733	0.00548		
Solvent Control	0.00679	0.00740	<LOQ	0.00682	0.00735	<LOQ		
	0.00696	0.00629	<LOQ	0.00657	0.00743	<LOQ		
0.005	0.00757 (151)	0.00684 (137)	0.00514 (103)	0.00723 (145)	0.00747 (149)	<LOQ	0.00116 (<LOQ)	<LOQ
	0.00626 (125)	0.0146 (292)	0.00540 (108)	[a]0.0654 (1308)	0.00784 (157)	<LOQ		
0.015	0.00826 (55.1)	0.0114 (76.0)	0.0108 (72.0)	0.0140 (93.3)	0.0135 (90.0)	0.0145 (96.7)	0.00777	51.8
	0.00955 (63.7)	0.0148 (98.7)	0.0118 (78.7)	0.0277 (185)	0.0130 (86.7)	0.00946 (63.1)		
0.045	0.0226 (50.2)	0.0249 (55.3)	0.0276 (61.3)	0.0234 (52.0)	0.0379 (84.2)	0.0199 (44.2)	0.0211	46.9
	0.0242 (53.8)	0.0306 (68.0)	0.0303 (67.3)	0.0268 (59.6)	0.0316 (70.2)	0.0177 (39.3)		
0.135	0.0598 (44.3)	0.0616 (45.6)	0.096 (71.1)	0.0511 (37.9)	0.0954 (70.7)	0.0618 (45.8)	0.0582	43.1
	0.0464 (34.4)	0.0675 (50.0)	0.088 (65.2)	0.0452 (33.5)	0.0822 (60.9)	0.0360 (26.7)		
0.405	0.195 (48.1)	0.234 (57.8)	0.300 (74.1)	0.130 (32.1)	0.275 (67.9)	0.159 (39.3)	0.198	48.9
	0.165 (40.8)	0.233 (57.5)	0.399 (98.5)	0.114 (28.1)	0.194 (47.9)	0.186 (45.9)		
1.22	0.385 (31.5)	0.328 (26.9)	0.539 (44.2)	0.512 (42.0)	0.475 (38.9)	0.389 (31.9)	0.463	38.0
	0.320 (26.3)	0.671 (55.0)	0.376 (30.8)	0.450 (36.9)	1.27 (104)	0.370 (30.3)		

-	Not applicable	(x)	Numbers in italics are % nominals
<LOQ	Not detected above the limit of quantitation (0.005 µg/L). Values of half the LOQ (0.0025 µg/L) have been used for calculation of the geometric mean values		
[a]	Measurement excluded from the calculation of the mean due to interference from co-extracted material in the water. Not considered to be contamination		

Table A7_4_3_2_2-6: Hatchability and survival data

Nominal Concentration of Cypermethrin [mg a.s./L]	**Control**	**Solvent Control**	**0.005**	**0.015**	**0.045**	**0.135**	**0.405**	**1.22**
Geometric Mean Measured Concentration [mg a.s./L]	nd	nd	< LOQ	0.00777	0.0211	0.0582	0.198	0.463
Egg no. at test start [n]	60	60	60	60	60	60	60	60
Egg Survival, Hatching, mean [%]	80*	98	92	93	93	97	85*	95
Post Hatch Survival day 28 [%]	96	95	98	98	96	98	98	98
Geometric Mean Wet Weight, day 28 of the single replicates [mg]*	76.3	71.4	83.7*	81.9	68.4	65.0	76.6	71.9
Geometric Mean Total Length, day 28 [mm]*	19.3*	20.0	20.1	20.4	19.5	20.0	20.8	19.9

* Statistically significant difference to the solvent control ($p < 0.05$)

nd = not determined

LOQ = 0.005 µg a.s./L