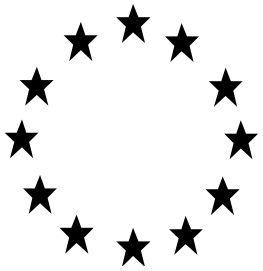
Competent Authority Report

Work Programme for Review of Active Substances in Biocidal Products Pursuant to Council Directive 98/8/EC



Copper pyrithione (PT 21)

Applicant: TNO Quality of Life

DOCUMENT III A4

Analytical methods

Rapporteur Member State: Sweden

Draft December 2010

KEMI
Kemikalieinspektionen
Swedish Chemicals Agency

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Section A4 Analytical Methods for Detection and Identification

General RMS comments on the Analytical Methods section

In the evaluation of the analytical methods the RMS has, where applicable, assessed the applicant's documentation in accordance with the Guidance document SANCO/825/00 rev. 7 used under Council Directive 91/414/EEC which is essentially equivalent to the new proposed TNsG on Analytical methods to be used under 98/8/EC (endorsed during the 29th CA meeting for release for a 6-month consultation period of stakeholders).

Section A4.1/01 Determination of the pure active substance and impurities in the technical material

Annex Point IIA, IV.4.1	Active substance and impurities

The contents of copper pyrithione and impurities in the technical material are determined simultaneously and the methodology employed is considered confidential and is therefore presented in the Confidential Annex. However, it should be noted that the methodology for determining the purity of the technical material cannot be confidential

Section A4.2(a)/01 Determination of residues in soil

Annex Point IIA, IV.4.2(a) **copper pyrithione**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [...]**	
Detailed justification:	An analytical method for the determination of copper pyrithione in soil is not required since due to the intended use no exposure of soil will occur. The formulated copper pyrithione is intended only for professional use as an antifouling for sea ship hulls. Inherent to this use, no contact with soil occurs, neither during nor after application of the formulation. This is confirmed by the environmental risk assessment (see document IIB and IIC).	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
Date	[redacted]
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	[redacted]

Section A4.2(b)/01 Determination of residues in air

Annex Point IIA, IV.4.2(b) **copper pyrithione**

Official use only

1. REFERENCE

1.1	**Reference**	Mol JGJ, Ravensberg JC (2005) Validation of an analytical method for the determination of copper pyrithione in air. TNO Quality of Life, report number V6110/01 [1] 01-04-2005 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	API Corporation, Japan
1.2.2	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**	SANCO/825/00 and SANCO/3029/99
2.2	**GLP**	Yes
2.3	**Deviations**	No

3. MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Glass fiber filters were fortified with copper pyrithione at two concentration levels in five-fold. Known amounts of copper pyrithione fortification solutions were added to the filters to achieve the following fortification levels: 0 µg/filter (control, 2x), 0.408 µg/filter (LOQ, 5x), 4.08µg/filter (10 times LOQ, 5x). Air was blown through the filters during 6 hours at approximately 2 L/min took place at 35.4°C and 84.9% relative humidity in a closed chamber
3.1.2	Cleanup	After suction with air as described above, the filters were extracted. The dust sampler consisted of two sections: a head section (spiked filter) and a breakthrough section (blank filter). These sections were extracted separately in a polypropylene tube, using 5 mL of acetonitrile. Three mL of each extract was concentrated by evaporation under N_2 till dryness and was redissolved in 200 µL of acetonitrile. To test the extraction efficiency, filters were extracted after fortification without suction of air.
3.2	**Detection**	Non-entry field
3.2.1	Separation method	HPLC, column properties: Symmetry® C18-column, 3.5 µm, 75/4.6 mm
3.2.2	Detector	UV (Applied Biosystems, type 759A) at 320 nm

Section A4.2(b)/01 Determination of residues in air

Annex Point IIA, IV.4.2(b) copper pyrithione

3.2.3	Standard(s)	External standards, calibration curve: calibration solutions (after evaporation of dichloromethane) were prepared in acetonitrile.	
3.2.4	Interfering substance(s)	No interference of the detection of copper pyrithone in air samples by other substances is expected.	
3.3	**Linearity**		
3.3.1	Calibration range	0 - 15 mg/L copper pyrithione.	X1
3.3.2	Number of measurements	Three concentration levels (0.31, 3.07 and 15 mg/L) were prepared and analysed in triplicate.	X1
3.3.3	Linearity	correlation coefficient $r^2 = 0.9998$	X2
3.4	**Specificity: interfering substances**	No interfering substances. No peaks higher than 30% of the LOQ were observed in the unfortified samples at the retention time of copper pyrithione.	X3
3.5	**Recovery rates at different levels**	Fortification level: 0.408 µg/filter; 4.08 µg/filter Recovery range (%): 71.1 – 86.8; 99.1 – 102.3 Average (%): 81.1; 100.5 Number: 5; 4[1)] [1)] *due to pump failure during 1 sampling*	X4
3.5.1	Relative standard deviation	Fortification level: 0.408 µg/filter; 4.08 µg/filter RSD (%): 7.4; 1.4 Number: 5; 4[1)] [1)] *due to pump failure during 1 sampling*	
3.6	**Limit of determination**	The limit of quantification could be calculated based on the added amount to the filters (0.908 µg) divided by the total flow through the filters (700 L). The LOQ accounts for 0.6 $\mu g/m^3$ for copper pyrithione.	X5
3.7	**Precision**		
3.7.1	Repeatability	See 3.5.1	
3.7.2	Independent laboratory validation	Not required.	

Section A4.2(b)/01 Determination of residues in air

Annex Point IIA, IV.4.2(b) **copper pyrithione**

4. APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	A validation of an analytical method for the determination of copper pyrithione in air was carried out using glass fiber filters fortified with copper pyrithione at levels of 0.408 µg/filter (LOQ) and 4.08 µg/filter (10xLOQ) in five-fold. A second filter was placed after the spiked filter in order to check for breakthrough. Air was lead through the filters at 35°C and >80% humidity; the total flow through the filters was 662-724 L. After suction with air, under the prescribed conditions, the filters were extracted in a polypropylene tube, using 5 ml of acetonitrile. Three mL of each extract was concentrated by evaporation under N_2 till dryness and was redissolved in 200 µl of acetonitrile (to test the extraction efficiency, filters were extracted after fortification without suction of air). The extracts were analyzed by RP-HPLC-UV. From the data obtained, the validation parameters were determined and tested against the criteria as stated in the guidelines.	
4.2	**Conclusion**	The method is suitable for the determination of copper pyrithione in air samples. All validation parameters fulfilled the stated criteria. The LOQ for copper pyrithione is 0.6 µg/m^3.	X6
4.2.1	Reliability	1	X7
4.2.2	Deficiencies	No	X6

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date [redacted]

Materials and methods [redacted]

Section A4.2(b)/01 Determination of residues in air

Annex Point IIA, IV.4.2(b) **copper pyrithione**

Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A4.2(c)/01 Determination of residues in water (drinking, sea and ground water)

Annex Point IIA, IV.4.2(c) **copper pyrithione**

			Official use only
		1. REFERENCE	
1.1	**Reference**	Mol JGJ, Engel R (2005) Validation of an analytical method for the determination of copper pyrithione in drinking-water, sea water and ground water. TNO Quality of Life, report number V6110/02, 12-07-2005 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	API Corporation, Japan	
1.2.2	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**	SANCO/825/00 and SANCO/3029/99	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Samples were fortified according to section 4.1 in a separation funnel, mixed and incubated for at least 30 seconds.	
3.1.2	Cleanup	The water sample (500 mL) was extracted twice, for at least 5 minutes, with 25 mL dichloromethane in a mechanical shaker at 300 strokes/min. The dichloromethane fractions were collected in a 100 mL Erlenmeyer flask and dried with sodium sulfate (about 3 gram). The dichloromethane was transferred in a 100 mL round bottom flask, the sodium sulfate was rinsed with another 25 mL dichloromethane, this fraction was also transferred in the 100 mL round bottom flask and both dichloromethane fractions were mixed and evaporated using a rotary evaporator at about 35°C at reduced pressure. The dry residue was dissolved in 1.5 mL HPLC eluent.	X1
3.2	**Detection**		
3.2.1	Separation method	HPLC, column properties Brownlee Spheri-5 Silica 5 µm, 220/4.6 mm	X1
3.2.2	Detector	UV (Applied Biosystems, type 759A) at 320 nm	
3.2.3	Standard(s)	External standards, calibration curve: for preparing the calibration standard, the stock standard solution in acetonitrile 5.1 mg/100 mL) was	X2

Section A4.2(c)/01 Determination of residues in water (drinking, sea and ground water)

Annex Point IIA, IV.4.2(c) **copper pyrithione**

		10x diluted in acetonitrile (concentration 5.1 mg/L). Calibration solutions were prepared by evaporating 0, 5, 10, 25, 50, 100 and 200 µL of the calibration standard solution under nitrogen. Before analysis, the calibration samples were dissolved in 1.5 mL HPLC eluent	
3.2.4	Interfering substance(s)	No interference of the detection of copper pyrithione in the various water samples by other substances occurred.	
3.3	**Linearity**		
3.3.1	Calibration range	0 - 2.04 µg/L copper pyrithione	X2
3.3.2	Number of measurements	Single measurement of 7 different concentrations: 0, 0.051, 0.102, 0.255, 0.51, 1.02 and 2.04 µg/L.	X2
3.3.3	Linearity	correlation coefficient $r^2 = 0.9995$	X3
3.4	**Specificity: interfering substances**	No interfering substances. No peaks higher than 30% of the LOQ were observed in the unfortified samples at the retention time of copper pyrithione.	X4
3.5	**Recovery rates at different levels**	drinking water:	X5

Fortification level	0.1 µg/L	1.0 µg/L
Recovery (%)	85.9	92.2
Number	5	5
ground water:		
Fortification level	0.1 µg/L	1.0 µg/L
Recovery (%)	74.3	97.7
Number	5	5
sea water:		
Fortification level	0.1 µg/L	1.0 µg/L
Recovery (%)	84.3	98.4
Number	5	5

Section A4.2(c)/01 Determination of residues in water (drinking, sea and ground water)

Annex Point IIA, IV.4.2(c) **copper pyrithione**

3.5.1	Relative standard deviation	drinking water:			
		Fortification level	0.1 µg/L	1.0 µg/L	
		RSD (%)	6.06	2.76	
		Number	5	5	
		ground water:			
		Fortification level	0.1 µg/L	1.0 µg/L	
		RSD (%)	18	7.60	
		Number	5	5	
		sea water:			
		Fortification level	0.1 µg/L	1.0 µg/L	
		RSD (%)	8.39	6.16	
		Number	*5*	*5*	

3.6 Limit of determination

The limit of quantification for copper pyrithione analysis in drinking-water, sea water and ground water is 0.1 µg/L.. X6

3.7 Precision

3.7.1 Repeatability

See 3.5.1

3.7.2 Independent laboratory validation

Not required.

4. APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Water samples

Drinking water: Spa Reine, natural mineral water, blue label, ref. 28-08-06 N17.

Sea water: Oosterschelde (Jacobahaven), The Netherlands

Ground water: Well water from Keizersberg, Blitterswijk, The Netherlands

Known amounts of copper pyrithione fortification solution were added to each water sample to achieve the following fortification levels:

0 µg/L (control, 2x), 0.1 µg/L (LOQ, 5x) and 1.0 µg/L (10 times LOQ, 5x).

After a short incubation period, the fortified water samples and two blank control samples of each matrix were extracted with dichloromethane. The dichloromethane extracts were dried with sodium sulfate and evaporated. The dry residue was dissolved in HPLC eluent and analysed using SP-HPLC-UV.

4.2 Conclusion

The method is suitable for the determination of copper pyrithione in drinking-water, sea water and ground water samples. X7

All validation parameters fulfilled the stated criteria. The limit of quantification for copper pyrithione analysis in drinking-water, sea water and ground water is 0.1 µg/L.

Section A4.2(c)/01 Determination of residues in water (drinking, sea and ground water)

Annex Point IIA, IV.4.2(c) **copper pyrithione**

4.2.1	Reliability	1	X8
4.2.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date [redacted]

Materials and methods [redacted]

Section A4.2(c)/01 Determination of residues in water (drinking, sea and ground water)

Annex Point IIA, IV.4.2(c) **copper pyrithione**

Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A4.2(c)/02 Determination of residues in water (sea water)

Annex Point IIA, IV.4.2(c) Pyritione

			Official use only
		1. REFERENCE	
1.1	**Reference**	Thomas KV (1999) Determination of the antifouling agent zinc pyrithione in water samples by copper chelate formation and high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, J Chromatogr A, 833, 105-109.	
1.2	**Data protection**	No	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No data	
2.3	**Deviations**	Not applicable	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	No data	
3.1.2	Cleanup	Zinc pyrithione in the water sample (2 l) was extracted by the addition of $Cu(NO_3)_2$ solution (1 M, 10 ml) and dichloromethane (25 ml) for 15 minutes in a mechanical shaker. The phases were allowed to separate for 10 minutes before the dichloromethane fractions were transferred to a Teflon Soveril tube. This was repeated twice. The tubes were sealed and centrifuged at 3000 rpm after which residual water was drawn off and the organic layer decanted into a round bottom flask. After the dichloromethane was reduced in volume to 2 ml using a rotary evaporator at 25°C, a further reduction to 200 µl was achieved using a TurboVap at 37°C.	X1
3.2	**Detection**		
3.2.1	Separation method	HPLC, PEEK analytical column (15 cm x 4.6 mm) and guard column (4 cm x 4.6 mm) packed with Prodigy ODS33 (particle size 5 µm). The mobile phase was methanol-water (50:50%). Column temperature was 30°C. Injections volume was 25 µl.	
3.2.2	Detector	MS, positive ion mode	X2
3.2.3	Standard(s)	Copper pyrithione standards were made in dichloromethane saturated with water. Zinc pyrithione calibration standards were made in 0.43-µm filtered natural sea water. Typically, zinc pyrithione (25 mg) was suspended in reagent-grade water (100 ml) and stirred in the dark for 24	

Section A4.2(c)/02 Determination of residues in water (sea water)

Annex Point IIA, IV.4.2(c) Pyritione

		h. The mixture was then passed through a pre-weighed GFC filter (1 µm) and the weight in solution calculated. This stock was then used to prepare sample spikes.	
3.2.4	Interfering substance(s)	No interference in the detection by other substances occurred.	
3.3	**Linearity**		
3.3.1	Calibration range	0.25 – 125 ng/µl copper pyrithione 1 – 500 ng/l zinc pyrithione	X3
3.3.2	Number of measurements	>100	
3.3.3	Linearity	Copper pyritione: linear in range 2.5 – 125 ng/µl; correlation coefficient $r^2 = 0.999$	X3
3.4	**Specificity: interfering substances**	No interfering substances.	X4
3.5	**Recovery rates at different levels**	Sea water: Concentration zink pyrithione 100 ng/l Recovery (%) 77 Number 6	X5
3.5.1	Relative standard deviation	Sea water: Concentration zink pyrithione 100 ng/l RSD (%) 17 Number 6	
3.6	**Limit of determination**	The limit of detection in sea water is 20 ng/l.	X7
3.7	**Precision**		
3.7.1	Repeatability	See 3.5.1.	
3.7.2	Independent laboratory validation	Not required.	

Section A4.2(c)/02 Determination of residues in water (sea water)

Annex Point IIA, IV.4.2(c) **Pyritione**

4. APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Methanol, dichloromethane and water were obtained from Rathburn Chemicals (Walkerburn, UK). Copper(II) nitrate hemipentahydrate, polyethylene glycol (mixture of PEG 300, 600 and 1000) and zinc pyrithione, purity >95%, were obtained from Aldrich (Gillingham, Dorset, UK). Copper pyrithione was prepared from zinc pyrithione and recrystallised to >95% purity. The samples were extracted with dichloromethane. The dichloromethane extracts were dried by evaporation.	
4.2	**Conclusion**	A method has been developed for the analysis of zinc pyrithione using copper transchelation and HPLC coupled to mass spectrometric detection using APCI. The method has been shown to have both good precision and accuracy, and also yielding a limit of detection that is adequate for the detection of environmental concentrations. Therefore, this method can be used to analyse both zinc pyrithione and copper pyrithione.	X8
4.2.1	Reliability	2	X9
4.2.2	Deficiencies	No GLP, not completely according to SANCO.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date [redacted]

Materials and methods [redacted]

Section A4.2(c)/02 Determination of residues in water (sea water)

Annex Point IIA, IV.4.2(c) **Pyritione**

	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A4.2(c)/03 Determination of residues in water (river water)

Annex Point IIA, IV.4.2(c) **Pyrithione**

			Official use only
		1. REFERENCE	
1.1	**Reference**	Bones J, Thomas KV, Paull B (2006) Improved method for the determination of zinc pyrithione in environment water samples incorporating on-line extraction and preconcentration coupled with liquid chromatography atmospheric pressure chemical ionization mass spectrometry, J Chromatogr A, 1132, 157-164.	
1.2	**Data protection**	No	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No data	
2.3	**Deviations**	Not applicable	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	No data	
3.1.2	Cleanup	On-line SPE was performed using a six-port column-switching valve. The extraction column used was a 10.0mm×4.6mm I.D. monolithic silica guard cartridge. An isocratic pump was used for sample delivery at flow rates of 10ml/min. The monolithic column was conditioned with 20ml acetonitrile and 20mL of water, respectively prior to use. Environmental samples were filtered through 0.45 µm glass fibre filters to remove particulate matter and adjusted to pH7.0 prior to extraction. A 200ml portion of sample was extracted using the monolithic column and elution was performed using mobile phase back flushing onto the analytical monolithic column. Removal of matrix interference was achieved by the incorporation of sacrificial strong anion exchange (SAX) column prior to the monolithic concentrator column along with a solventwash step. The sorbent used was anion exchange silica (Si-SAX) packed into a 33.0mm×4.6mm stainless steel column housing. The Si-SAX column was conditioned with 0.2M ammonium acetate and water before use. The clean-up column was switched off-line prior to elution of ZnPT from the concentrator column onto the analytical column.	X1
3.2	**Detection**		
3.2.1	Separation method	LC, gradient separations performed on a Merck Chromolith Performance RP-18e, 100.0 mm x 4.6 mm I.D. monolithic silicacolumn with a mobile phase of methanol and 10 mM ammonium acetate	

Section A4.2(c)/03 Determination of residues in water (river water)

Annex Point IIA, IV.4.2(c) Pyrithione

3.2.2	Detector	APCI-MS, positive ion mode, solutions of the analyte in dichloromethane were infused using a syringe pump at a rate 600 µl/h into a flow of methanol at a rate of 190 µl/min from the LC pump through a mixing tee and then into the APCI source. The optimisation was performed using a nebuliser pressure of 50. psi, a dry gas flow of 10.0 l/min, a drying temperature of 325°C, a corona voltage of +3200 V and an APCI temperature of 500°C.	X1
3.2.3	Standard(s)	In order to examine the effect of contact with common inorganic anions solutions of 5mg L−1 ZnPT were prepared in water containing varying concentrations of chloride, sulphate, nitrate and phosphate in the range of 5–20 mg L−1 and5mgL−1 Cu(II). The solutions were then analysed using LC–UV and then reanalysed after both 24 and 48 h, respectively in order to determine the effect of contact time.	
3.2.4	Interfering substance(s)	In this study a method is developed to overcome the difficulties that have been reported concerning the chromatographic analysis of pyrithione complexes due to problematic unwanted interactions with the silica stationary phase. The results of this study are positive, therefore no interfering substances are present.	
3.3	**Linearity**		
3.3.1	Calibration range	Not applicable.	
3.3.2	Number of measurements	Not applicable.	
3.3.3	Linearity	Linear in range 0.25 – 10 µg/l; correlation coefficient $r^2 = 0.9802$.	X3
3.4	**Specificity: interfering substances**	No interfering substances.	X4
3.5	**Recovery rates at different levels**	River water: Concentration zinc pyrithione 1 µg/l Recovery (%) 72±9 Number 3	X5
3.5.1	Relative standard deviation	River water: Concentration zinc pyrithione 1 µg/l RSD (%) 27 Number 10	X6
3.6	**Limit of determination**	The limit of detection in river water is 18 ng/l. The limit of quantitation in river water is 62 ng/l.	X7
3.7	**Precision**		
3.7.1	Repeatability	See 3.5.1.	

Section A4.2(c)/03 Determination of residues in water (river water)

Annex Point IIA, IV.4.2(c) **Pyrithione**

3.7.2	Independent laboratory validation	Not required.	

4. APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	This method is a further refinement of the method by Thomas (1999) (see A4.2/02_02) Reagent water was obtained from a Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA). Methanol, acetonitrile and dichloromethane were received from Labscan (Dublin, Ireland). Zinc pyrithione, sulphate pentahydrate, copper(II) acetate, 2,6-pyridine dicarboxylic acid, sodium chloride, formic acid, ammonium formate, ammonium acetate, disodium hydrogen phosphate, pyridine and phenol were obtained from Sigma-Aldrich (Steinheim, Germany). Ferric nitrate nonahydrate, hydrochloric acid, nitric acid, sulphuric acid, glacial acetic acid and ammonia solution (33%) were received from BDH Chemicals Ltd. (Poole, UK). Copper(II) nitrate trihydrate and anhydrous sodium sulphate were received from Riedel de Haen (Seelze, Germany). Potassium nitrate was purchased from Merck KGaA (Darmstadt, Germany). Stock 1000 mg/l solutions of zinc pyrithione were prepared in dichloromethane and stored in the refrigerator in darkness and replaced monthly.	
4.2	**Conclusion**	An on-line sample clean-up and preconcentration procedure coupled with LC-APCI-MS detection has been developed for the analysis of zinc pyrithione (as copper pyrithione) in aquatic environmental samples. The method was validated in a real sample matrix and showed high sensitivity with acceptable analyte recovery and reproducibility. Therefore, this method can be used to analyse both zinc pyrithione and copper pyrithione.	X8
4.2.1	Reliability	2	X9
4.2.2	Deficiencies	No GLP, not completely according to SANCO.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date [redacted]

Materials and methods [redacted]

Section A4.2(c)/03 Determination of residues in water (river water)

Annex Point IIA, IV.4.2(c) **Pyrithione**

ratio of m/z 316 to m/z 318.0 (~100:45) was used for qualitative confirmation.

[redacted]

Conclusion

[redacted]

Reliability

[redacted]

Acceptability

[redacted]

Section A4.2(c)/03 Determination of residues in water (river water)

Annex Point IIA, IV.4.2(c) **Pyrithione**

	[redacted]
Remarks	[redacted]

Section A4.2(c)/04 Determination of residues in sediment (and water)

Annex Point IIA, IV.4.2(c) Monitoring

			Official use only
		1. REFERENCE	
1.1	**Reference**	Woldegiorgis A, Remberger M, Kaj L, Green J, Ekheden Y, Palm Cousins A, Brorström-Lundén E, Dye C, Aspmo K, Vadset M, Schlabach M, Langdford K (2007) Results from the Swedish National Screening Programme 2006, Subreport 3: Zinc pyrithione and Irgarol 1051, IVL Swedish Environmental Research Institute Ltd., IVL Report B1764, September 2007.	
1.2	**Data protection**	No	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No data	
2.3	**Deviations**	Not applicable	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	No data	
3.1.2	Cleanup	Water samples were acidified (pH 2) and extracted onto Oasis HLB SPE cartridges. Elution was performed by 4 ml dichloromethane. 1 ml of extract was removed for another analysis and 2 ml methanol was added to the remaining extract. Sludge and sediment samples to be analysed were air dried before extraction. 3 g aliquots were subjected to ultrasonic extraction with 30 ml dichloromethane:methanol (2:1) for 45 minutes. After centrifugation (10 minutes, 3500 rpm) the extracts were filtered (0.45 µm) after which 2 ml methanol was added.	X1
3.2	**Detection**		
3.2.1	Separation method	HPLC, to reduce matrix interference an anion exchange silica column was positioned before the extraction guard column. The guard column was then flushed onto a monolithic column by gradient elution.	X2
3.2.2	Detector	MS, dual electro spray chemical ionisation in positive mode.	
3.2.3	Standard(s)	None.	
3.2.4	Interfering substance(s)	None reported.	
3.3	**Linearity**		

Section A4.2(c)/04 Determination of residues in sediment (and water)

Annex Point IIA, IV.4.2(c) Monitoring

3.3.1	Calibration range	Not applicable.		
3.3.2	Number of measurements	Not reported.		
3.3.3	Linearity	Not applicable.		
3.4	**Specificity: interfering substances**	None reported.		
3.5	**Recovery rates at different levels**	Not reported.		
3.5.1	Relative standard deviation	Not reported.		
3.6	**Limit of determination**	Limit of detection in		X3
		Water	0.015 µg/l	
		Sludge	20 µg/kg dw	
		Sediment	20 µg/kg dw	
		Fish	-	
		Urine	0.05 µg/l	
3.7	**Precision**			
3.7.1	Repeatability	Not reported.		
3.7.2	Independent laboratory validation	Not required.		

4. APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	The method used is based on the method developed by Bones, *et al* (2006) (see Section A4.2/02_03). See 3.1.2.	
4.2	**Conclusion**	Zinc pyrithione was only detected in three out of 124 environmental samples. This limited detection frequency despite its extensive use indicates a high degradation rate of zinc pyrithione. In addition, as a monitoring method exists based on a valid analytical method, the need for developing a new analytical method for sediment does not seem to be necessary.	X4
4.2.1	Reliability	2	X5
4.2.2	Deficiencies	No GLP, not completely according to SANCO.	

Section A4.2(c)/04 Determination of residues in sediment (and water)

Annex Point IIA, IV.4.2(c) Monitoring

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A 4.2(c)/05 Determination of residues in sediment (and water)

Annex Point IIA, IV.4.2(c)

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	A search was performed in Toxline for publications from the year 2000 up to the most recent on copper pyrithione. This resulted in the hits shown below, Table 1. Most of the publications concern toxicity and/or degradation of copper pyrithione. When analytical methods to determine the concentration copper pyrithione in (sea) water or sediment are provided, these methods are either only briefly described or follow the same approach as reported in the publications by Thomas (1999) and Bones *et al* (2006). Therefore, it is not considered necessary to perform any more studies for analytical methods in water and sediment.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
Date	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	[redacted]	

Table 1
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Harino H, Midorikawa S, Arai T, Madoka O, Nguyen DC and Miyazaki N.
J Mar Biol Ass UK 86, 2006: 1163-1170

Analysis, fate and toxicity of zinc-and copper pyrithione in the marine environment.
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TemaNord 550, 2005.

Pyrithiones as antifoulants: environmental fate and loss of toxicity.
Turley PA; Fenn RJ; Ritter JC; Callow ME

Biofouling. 2005; 21(1):31-40.

Toxicity reduction of metal pyrithiones by near ultraviolet irradiation.
Okamura H; Kobayashi N; Miyanaga M; Nogami Y
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Seasonal variations in the effect of zinc pyrithione and copper pyrithione on pelagic phytoplankton communities.
Maraldo K; Dahllöf I
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Effects of new antifouling compounds on the development of sea urchin.
Kobayashi N; Okamura H
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Effects of zinc pyrithione and copper pyrithione on microbial community function and structure in sediments.
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Environ Toxicol Chem. 2004, Apr; 23(4):921-8.

Acute toxicity of pyrithione antifouling biocides and joint toxicity with copper to red sea bream (Pagrus major) and toy shrimp (Heptacarpus futilirostris).
Mochida K; Ito K; Harino H; Kakuno A; Fujii K
Environ Toxicol Chem. 2006, Nov; 25(11):3058-64.

Indirect estimation of degradation time for zinc pyrithione and copper pyrithione in seawater.
Maraldo K; Dahllöf I
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Environmental fate of the antifouling compound zinc pyrithione in seawater.
Grunnet KS; Dahllöf I
Environ Toxicol Chem. 2005, Dec; 24(12):3001-6.

Toxicity evaluation of new antifouling compounds using suspension-cultured fish cells.
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Toxicity of four antifouling biocides and their mixtures on the brine shrimp Artemia salina.
Koutsaftis A; Aoyama I
Sci Total Environ. 2007, Nov 15; 387(1-3):166-74.

Synergistic toxic effects of zinc pyrithione and copper to three marine species: Implications on setting appropriate water quality criteria.
Bao VW; Leung KM; Kwok KW; Zhang AQ; Lui GC
Mar Pollut Bull. 2008; 57(6-12):616-23.

The interactive effects of binary mixtures of three antifouling biocides and three heavy metals against the marine algae Chaetoceros gracilis.
Koutsaftis A; Aoyama I
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Early life-stage toxicity test for copper pyrithione and induction of skeletal anomaly in a teleost, the mummichog (Fundulus heteroclitus).
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Environ Toxicol Chem. 2008, Feb; 27(2):367-74.

Concentrations of antifouling biocides in sediment and mussel samples collected from Otsuchi bay, Japan.
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Prog Mol Subcell Biol. 2006; 42:105-24.

Chemical genetics suggests a critical role for lysyl oxidase in zebrafish notochord morphogenesis.
Anderson C; Bartlett SJ; Gansner JM; Wilson D; He L; Gitlin JD; Kelsh RN; Dowden J
Mol Biosyst. 2007, Jan; 3(1):51-9.

New biocide-free anti-fouling paints are toxic.
Karlsson J; Eklund B
Mar Pollut Bull. 2004, Sep; 49(5-6):456-64.

Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review.
Konstantinou IK; Albanis TA
Environ Int. 2004, Apr; 30(2):235-48.

Section A4.2(d)/01 Determination of residues in animal and human body fluids and tissues (urine)

Annex Point IIA, IV.4.2(d) **2-pyridinethiol-1-oxid glucoronide**

			Official use only
		Since copper pyrithione is metabolised in the rat and mainly excreted as 2-pyridinethiol-1-oxid glucoronide in urine (see section A6.2), an analytical method for the determination of that metabolite in urine is developed.	X1
		1. REFERENCE	
1.1	**Reference**	Wyma-Teitsma, G.R (2005): Validation of an analytical method for the determination of 2-pyridinethiol-1-oxid glucuronide in urine. TNO Quality of Life, report number V6110/03, 10-10-2005 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	API Corporation, Japan	
1.2.2	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**	SANCO/825/00 and SANCO/3029/99	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Not applied – direct determination of the analyte in the fortified urine samples.	
3.1.2	Cleanup	Not applied – direct determination of the analyte in the fortified urine samples.	
3.2	**Detection**		
3.2.1	Separation method	HPLC, column properties: Insertil 5 ODS 3, 5 µm, length 100 mm, i.d. 3 mm	
3.2.2	Detector	MS/MS (API-4000, PE Sciex) Ionisation mode: positive (+) Fragments: m/z 304 → 128 (quantification) *m/z 304 → 110*	X2

Section A4.2(d)/01 Determination of residues in animal and human body fluids and tissues (urine)

Annex Point IIA, IV.4.2(d) **2-pyridinethiol-1-oxid glucoronide**

3.2.3	Standard(s)	External standards, calibration curve: calibration solutions of 2-pyridinethiol-1-oxid glucuronide were prepared in 0.1% formic acid.	X3
3.2.4	Interfering substance(s)	No interference	
3.3	**Linearity**		
3.3.1	Calibration range	0.0037 – 0.19 mg/L in diluted urine (5 times diluted, corresponds to 0.019 – 0.93 mg/L urine)	
3.3.2	Number of measurements	6	
3.3.3	Linearity	$r^2 = 0.9975$	X4
3.4	**Specificity: interfering substances**	No interfering substances. No peaks higher than 30% of the LOQ were observed in the unfortified samples at the retention time of 2-pyridinethiol-1-oxid glucuronide	X5
3.5	**Recovery rates at different levels**	Fortification level: 0.0466 mg/L; 0.466 mg/L Recovery range (%): 72.1 – 76.6; 93.1 – 98.3 Average (%): 74.6; 96.4 Number: 5; 5	X6
3.5.1	Relative standard deviation	Fortification level: 0.0466 mg/L; 0.466 mg/L RSD (%): 2.8; 2.1 Number: 5; 5	
3.6	**Limit of determination**	0.05 mg/L	
3.7	**Precision**		
3.7.1	Repeatability	See 3.5.1	
3.7.2	Independent laboratory validation	Not required.	

4. APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Human urine samples were fortified with POG at two concentration levels in five fold. Two control samples were taken. The urine samples were diluted in 0.1% (V/V) formic acid in purified water and analysed by liquid chromatography with tandem mass spectrometry. An aliquot of the extracts was analysed by LC-MS/MS. The conditions are given below:

Section A4.2(d)/01 Determination of residues in animal and human body fluids and tissues (urine)

Annex Point IIA, IV.4.2(d) **2-pyridinethiol-1-oxid glucoronide**

Injection volume: 5 µl
Eluent A: 0.4% (v/v) formic acid in purified water
Eluent B: 0.1% (v/v) formic acid in acetonitrile
gradient:

Time (min)	% A	% B
0.00	100	0
0.50	100	0
10.00	70	30
10.01	0	100
13.00	0	100
13.01	100	0
16.50	100	0

Flow: 0.6 ml/min
Analytical column: i.d. Inertsil 5 ODS 3, 5µm, length 100 mm, 3 mm
Detector: API-4000 (PE Sciex)
Scan parameters:
-Polarity: Positive
-Scan mode: N/A
-Scan type: MRM
-Precursor/product ion:
304 → 128 (quantification)
304 → 110
-Collision gas: Nitrogen

4.2 Conclusion

The analytical method as described in this report is suitable for the determination of POG in human urine down to a level 0.05 mg/L according to SANCO/825/00. All validation parameters were within the stated criteria. The average recovery of POG at 0.0466 mg/L and 0.466 mg/l was 74.6% and 96.3% respectively, the RSD was 2.8% and 2.1% respectively. The specificity was met and the linearity of POG was demonstrated over the range of 0.019 – 0.93 mg/L

X7

4.2.1 Reliability: 1

X8

4.2.2 Deficiencies: None

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date [redacted]

Materials and methods [redacted]

Section A4.2(d)/01	Determination of residues in animal and human body fluids and tissues (urine)
Annex Point IIA, IV.4.2(d)	2-pyridinethiol-1-oxid glucoronide

[redacted]

Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A4.3/01 Determination of residues in in/on food or feedstuffs

Annex Point IIIA, IV.4.3 **copper pyrithione**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Due to the use of copper pyrithione, on marine vessels only, only contact to marine food might occur, such as (shell)fish. However, copper pyrithione is known to degrade quickly in marine environment. In addition, the bioconcentration study with fish shows that the use of (very) low concentrations of copper pyrithione results in full metabolisation. Furthermore, this study shows full integration of the radio label as either insoluble or polar degradation products in the natural constituents (non-soluble pellets) of the fish. Therefore, no analysis of copper pyrithione is possible and no analytical method for the active substance seems to be required.	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
Date	[redacted]
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	[redacted]

Reference list of studies submitted (by Section No.)

Section No. / Reference No	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes / No)	Owner*
A4.2/01	Mol JGJ, Ravensberg JC	2005	Validation of an analytical method for the determination of copper pyrithione in air. TNO Quality of Life, report number V6110/01 [1] GLP, unpublished	Y	API
A4.2/02	Mol JGJ, Engel R	2005	Validation of an analytical method for the determination of copper pyrithione in drinking-water, sea water and ground water. TNO Quality of Life, report number V6110/02 GLP, unpublished	Y	API
A4.2/03	Wyma-Teitsma, G.R.	2005	Validation of an analytical method for the determination of 2-pyridinethiol-1-oxid glucuronide in urine. TNO Quality of Life, report number V6110/03 GLP, unpublished	Y	API

* API=API Corporation, Japan

Reference list of studies submitted (by Author)

Author(s)	Section No. / Reference No	Year	Title Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes / No)	Owner*
Mol JGJ, Ravensberg JC	A4.2/01	2005	Validation of an analytical method for the determination of copper pyrithione in air. TNO Quality of Life, report number V6110/01 [1] GLP, unpublished	Y	API
Mol JGJ, Engel R	A4.2/02	2005	Validation of an analytical method for the determination of copper pyrithione in drinking-water, sea water and ground water. TNO Quality of Life, report number V6110/02 GLP, unpublished	Y	API
Wyma-Teitsma, G.R.	A4.2/03	2005	Validation of an analytical method for the determination of 2-pyridinethiol-1-oxid glucuronide in urine. TNO Quality of Life, report number V6110/03 GLP, unpublished	Y	API

* API=API Corporation, Japan