REPORT

Results from the Swedish Screening programme 2012

Subreport 4: Pyrithiones

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Report Summary

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Summary

As an assignment from the Swedish Environmental Protection Agency, a screening study of pyrithiones and their major degradation products has been performed by IVL during 2012/2013. Zinc pyrithione (ZnPT) is a broad-spectrum biocide effective against bacteria, fungi and algae. ZnPT is commercially used as anti-dandruff agent in shampoos and as preservatives in cosmetics. Due to ZnPTs solubility in water it is suitable for use in outdoor and marine paints. Sodium pyrithione (HPT) is used as a preservative in cosmetics and as protective agent for fluids used in the metal industries.

The objective of the present screening was to determine concentrations and to illuminate the fate of pyrithiones in sewage treatment plants and in small boat marinas by broadening the chemical analysis also to 2-pyridinesulfonic acid (PSA) and other pyrithione residues.

ZnPT and/or cupper pyrithione (CuPT) were detected in 79% of influent and in 7% of effluent wastewaters from sewage treatment plants (STPs). A decrease in concentration from influent to effluent indicates that ZnPT and CuPT are degraded in the STPs. The absence of ZnPT and/or CuPT in sludge suggests that degradation will take place in contact with sludge. The degradation product PSA was the most abundant compound in the study. PSA was present in the majority of the wastewater samples and in all the sludge samples analysed. The concentration range of PSA in influents were 73 - 480 (median 240) ng/L (n=14), in effluents <2 - 330 (median 59) ng/L (n=28) and in sludge 25 - 280 (median 110) µg/kg dw (n=21). The decrease in concentration of PSA from influent to effluent wastewater shows that PSA may be further degraded or adsorbed to sludge in the STP.

In a small boat marina where ZnPT is probably constantly emitted from anti-fouling paints, (and could be trans-chelated to the even more toxic CuPT) none of these species could be detected in the water or in the sediment. PSA, however, was detected in the sediment. In the sediments from Gothenburg, where the ZnPT load is of a more diffuse character, accumulation of PSA was not detected. As the acute toxicity of pyrithiones decreases with increased degradation and PSA is the least toxic of the known pyrithione residues, the result indicates that a process leading to less toxic sediments is taking place. However, the absence of chronic tests of PSA in the scientific literature makes it impossible to estimate the long-term effect on the marine environment.

Keyword

Pyrithiones, Sewage treatment plants, Sludge, Seawater, Sediment.

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Sammanfattning

IVL Svenska Miljöinstitutet har på uppdrag av Naturvårdsverket genomfört en screening avseende pyritioner och deras viktigaste nedbrytningsprodukter under 2012/2013.

Zink pyrition (ZnPT) är en bredspektrumbiocid mot bakterier, svampar och alger. ZnPT används kommersiellt som antimjällmedel i schampon och som konserveringsmedel i kosmetika. ZnPTs löslighet i vatten gör ämnet lämpligt som tillsatts i båt- och utomhusfärger. Natrium pyrition (HPT) har samma användningsområden som ZnPT och förekommer som konserveringsmedel i kosmetika och i vätskor som används inom metallindustrin.

Syftet med studien var att bestämma halter och att bidra till förståelsen av pyritioners omvandling genom att även analysera 2-pyridinsulfonsyra (PSA) och andra nedbrytningsprodukter i prover från reningsverk och småbåtshamnar.

ZnPT och/eller kopparpyrition (CuPT) uppmättes i 79 % av inkommande och i 7 % av utgående avloppsvatten från kommunala reningsverk. Minskningen av halten ZnPT och/eller CuPT mellan inkommande och utgående avloppsvatten visar att nedbrytning kan ske i reningsverksprocessen. Att ZnPT och/eller CuPT inte kunde detekteras i slam tyder på att nedbrytning av dessa ämnen sker i kontakt med slam.

Nedbrytningsprodukten PSA var den mest frekvent förekommande föreningen och uppmättes i de flesta avloppsvatten samt i samtliga slamprover. Koncentrationen av PSA i inkommande vatten var 73 – 480 (median 240) ng/L (n=14), i utgående vatten <2 - 330 (median 59) ng/L (n=28) och i slam 25 - 280 (median 110) µg/kg TS (n=21). Minskningen i halten PSA mellan inkommande och utgående avloppsvatten tyder på att PSA kan brytas ner eller adsorberas till slam.

I en småbåtshamn där ZnPT troligen emitteras från båtbottenfärger, och också kan omvandlas till den ännu mer toxiska formen CuPT, kunde ingen av dessa former påvisas i vattenfasen eller sedimentet. Däremot kunde PSA detekteras i sedimentet. I sediment där belastningen av ZnPT är mer diffus (Göta älv, gradient från Göteborg), kunde ingen ackumulering av PSA påvisas.

Eftersom den akuta toxiciteten för pyritioner minskar med ökad nedbrytning och PSA är den minst giftiga av de kända nedbrytningsprodukterna indikerar resultatet att en process pågår i sedimentet som leder till minskande toxicitet. Avsaknaden av kroniska tester av PSA i den vetenskapliga litteraturen gör det inte möjligt att uppskatta långsiktig påverkan på den marina miljön.

Summary

As an assignment from the Swedish Environmental Protection Agency, a screening study of pyrithiones and their major degradation products has been performed by IVL during 2012/2013.

Zinc pyrithione (ZnPT) is a broad-spectrum biocide effective against bacteria, fungi and algae. ZnPT is commercially used as anti-dandruff agent in shampoos and as preservatives in cosmetics. Due to ZnPTs solubility in water it is suitable for use in outdoor and marine paints. Sodium pyrithione (HPT) is used as a preservative in cosmetics and as protective agent for fluids used in the metal industries.

The objective of the present screening was to determine concentrations and to illuminate the fate of pyrithiones in sewage treatment plants and in small boat marinas by broadening the chemical analysis also to 2-pyridinesulfonic acid (PSA) and other pyrithione residues.

ZnPT and/or cupper pyrithione (CuPT) were detected in 79% of influent and in 7% of effluent wastewaters from sewage treatment plants (STPs). A decrease in concentration from influent to effluent indicates that ZnPT and CuPT are degraded in the STPs. The absence of ZnPT and/or CuPT in sludge suggests that degradation will take place in contact with sludge.

The degradation product PSA was the most abundant compound in the study. PSA was present in the majority of the wastewater samples and in all the sludge samples analysed. The concentration range of PSA in influents were 73 - 480 (median 240) ng/L (n=14), in effluents <2 - 330 (median 59) ng/L (n=28) and in sludge 25 - 280 (median 110) µg/kg dw (n=21). The decrease in concentration of PSA from influent to effluent wastewater shows that PSA may be further degraded or adsorbed to sludge in the STP.

In a small boat marina where ZnPT is probably constantly emitted from anti-fouling paints, (and could be trans-chelated to the even more toxic CuPT) none of these species could be detected in the water or in the sediment. PSA, however, was detected in the sediment. In the sediments from Gothenburg, where the ZnPT load is of a more diffuse character, accumulation of PSA was not detected.

As the acute toxicity of pyrithiones decreases with increased degradation and PSA is the least toxic of the known pyrithione residues, the result indicates that a process leading to less toxic sediments is taking place. However, the absence of chronic tests of PSA in the scientific literature makes it impossible to estimate the long-term effect on the marine environment.

Contents

1. Introduction

As an assignment from the Swedish Environmental Protection Agency, a screening study of pyrithiones and their major degradation products has been performed by IVL during 2012/2013.

Zinc pyrithione (ZnPT) is a broad-spectrum biocide effective against bacteria, fungi and algae (Turley et al 2005). ZnPT is commercially used as an anti-dandruff agent in shampoos and as a preservative in cosmetics. Due to ZnPTs solubility in water it is suitable for use in outdoor and marine paints. Sodium pyrithione (HPT) is used as a preservative in cosmetics and as a protective agent for fluids used in the metal industries.

In a previous screening in 2006, ZnPT was found only in very few samples (Woldegiorgis et al 2007). The result indicated that ZnPT was not widely distributed in the Swedish environment.

The objective of the present screening was to determine concentrations and to further illuminate the fate of pyrithiones in sewage treatment plants and in small boat marinas by broadening the chemical analysis also to 2-pyridinesulfonic acid (PSA) and other pyrithione residues.

2.Chemical properties, fate and toxicity

2.1 Chemical properties

Table 1 and Table 2 present the pyrithiones that are included in this study. 2-Pyridine-sulfonic acid (PSA) and 2,2'-dithiodipyridine (PS_2) are common photolytic degradation products of ZnPT and other metal pyrithiones (MePT) (Sakkas et al 2007). Sodium pyrithione (HPT) can be used as a preservative in cosmetics and as a protective agent for fluids used in the metal industries, however, HPT can also be a degradation product of MePTs. Table 3 present the most reduced form of pyrithiones. This molecule is known as 2-mercaptopyridine (HPS) and was used in the study to determine a summed concentration of different pyrithions in a sample after reduction.

With respect to the relatively low octanol/water distribution coefficient it seems like the majority of the pyrithiones and pyrithione residues have high water solubilities.

Table 1. Substances commissioned by the Swedish Environmental Protection Agency in the original request.

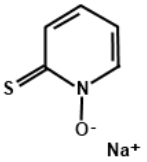
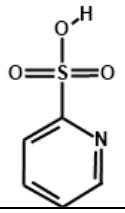
Substance	CAS-number	Mw (g/mol)	Log Kow	Structure
Sodium pyrithione HPT	3811-73-2	149.15	0.5	
2-Pyridinesulfonic acid PSA	15103-48-7	159.16	-0.1	

Table 2. Substances added to the Swedish Environmental Protection Agency original request.

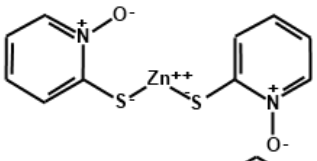
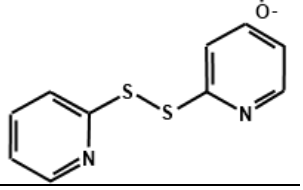
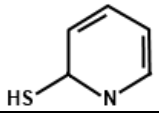
Substance	CAS-number	Mw (g/mol)	Log Kow	Structure
Zinc pyrithione ZnPT	13463-41-7	317.69	0.97	
2,2'-Dithiopyridine PS2	2127-03-9	220.31	2.6	

Table 3. Conversion product of pyrithiones after reduction.

Substance	CAS-number	Mw (g/mol)	Log Kow	Structure
2-Mercaptopyridine HPS	2637-34-5	111.16	-0.13	

2.2 Environmental fate

ZnPT undergoes rapid degradation in the environment. The half-live of ZnPT varies from 15 minutes to 30 days depending on the environmental composition (Turley et al 2000). For example, in the presence of cupper ions (Cu^{2+}) or in excess of sodium ions (Na^{+}) from e.g. seawater ZnPT will be transformed into CuPT and HPT (Figure 1) (Grunnet et al 2005). The resulting distribution of complexes is driven by the constant for each complex, as well as the concentration of the pyrithione and the metals (Sun et al 1964). The order of the complex strength is thought to be: Na<Fe<Mn<Zn<Cu. However, the most rapid degradation occurs when ZnPT is exposed to light (Turley et al 2000, Maraldo et al 2004).

In the presence of sulphur from e.g. sediment CuPT transforms into HPT (Bones et al 2006). Studies have shown that after 30 days the only detectable degradation product is PSA (Neihof et al 1979, Turley et al 2000 and 2005).

Considering that ZnPT is a relatively hydrophilic compound with a log Kow of 0.97 and a log Dow of -2.74 (at pH 7.4) it is expected that ZnPT and its metabolites mainly occurs in the water phase under normal environmental conditions.

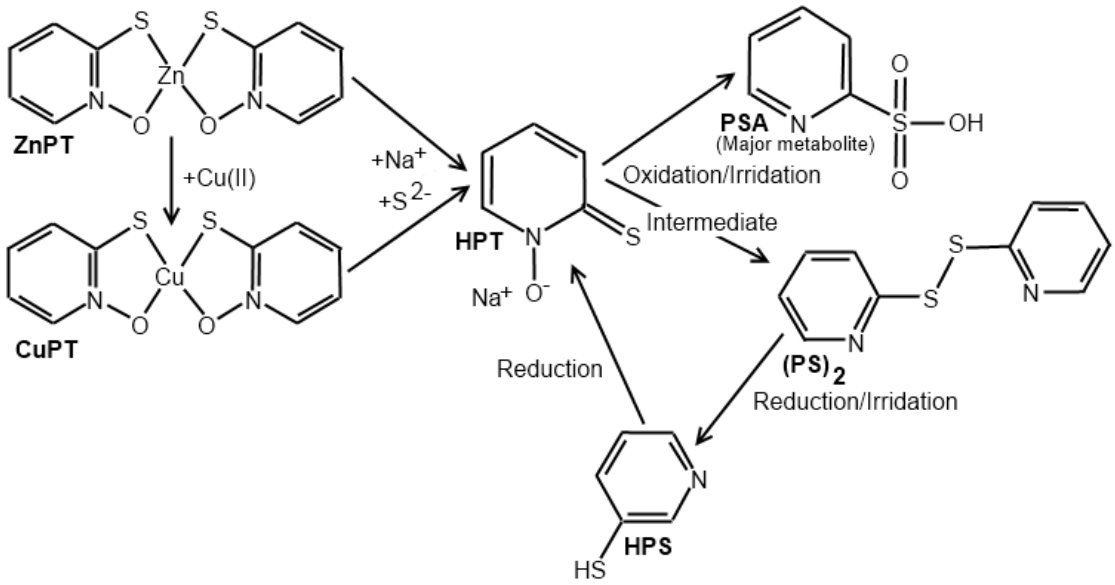


Figure 1. Degradation scheme of ZnPT.

2.3 Toxicity

MePT have shown to be toxic, especially against algae (Onduka et al 2010, Table 4). A study by the Swedish Society for Nature Conservation, SNF, showed that "half a tea spoon" of commercial anti-dandruff shampoo (~2.6 mg of ZnPT) in 1 m^3 of water killed half the fish population within 4 days. In the experiment the fish were inserted into the aquarium after 2 days, when approximately 90% of the dosed ZnPT was assumed to be degraded (SNF 2004). The toxic effect of MePT is linked to its ability to cause membrane polarisation by inhibiting the activity of the proton pump. The energy produced by the proton pump is crucial for maintaining the transport mechanism over the cell membrane (Chandler et al 1978, Ermolayeva et al 1995).

Studies have shown that the acute toxicity of pyrithiones decreases with increased degradation (Neihof et al 1979, Turley et al 2000, Doose et al 2004, Omakura et al 2006, Onduka et al 2010). However, the degradation product sodium pyrithione (HPT) show similar toxicity as the MePT for algae (Table 4), probably because HPT is converted to CuPT in the presence of cupper (Cu^{2+}) (Onduka et al 2010).

Table 4. Toxicity of metal pyrithiones and their degradation products on three trophic levels from Oduka et al 2010.

	Skeletonema costatum (Algae)	**Tigriopus japonicus** (Crustacean)	**Pagrus major** (Fish)
Substance	72-h EC_{50} (µg/l)	24-h EC_{50} (µg/l)	96-h EC_{50} (µg/l)
CuPT	1.5	23	9.3[A]
ZnPT	1.6	280	98.2[A]
HPT	1.1	>12 500	4 500
PS_2	65	550	510[A]
HPS	730	76 000	45 000
PSA	>100 000	>100 000	>100 000

[A] Estimated values using measured concentrations.

3. Production and use

ZnPT is a broad-spectrum biocide effective against bacteria, fungi and algae (Turley et al 2005). It was first synthesised in the 1930s and has been used since the 1960s in various non-agricultural applications requiring microbial control. ZnPT is commercially used as an anti-dandruff agent in shampoos and as a preservative in cosmetics. Due to ZnPTs solubility in water (see above) it is suitable for use in outdoor paints, where it provides protection against mildew and algae. The ban of organotin biocides in anti-fouling paints for recreation boats in the Nordic countries by the International Marine Organisation (IMO), has resulted in the development of new organic booster biocides such as ZnPT. ZnPT is considered to be a biocide and therefore regulated within the European Union biocide directive (98/8/EC).

HPT can be used as a preservative in cosmetics and as a protective agent for fluids used in the metal industries. However HPT can also be a degradation product of MePT.

A previous study in Sweden estimated that the major sources for the discharge of ZnPT to the aquatic environment were personal care products, such as anti-dandruff shampoos (10 tonnes annually), and marine anti-fouling paints (2.4 tonnes annually) (SNF 2004).

4. Previous measurements in the environment

MePT have only been found in environmental samples on a few occasions, probably due to the rapid photolytic degradation (see above) (Neihof et al 1979, Galvin et al 1995). A study in Vietnam on sediments and clams reported pyrithiones in concentrations between <2 and 420 µg/kg dw (Harino et al 2006).

In the previous screening in 2006, ZnPT was measured in water (influent and effluent water from sewage treatment plants, industrial effluents, drinking water, surface water, landfill leachate), sediment, sludge, biota (fish) and human urine (Woldegiorgis et al 2007).

Out of 112 investigated samples only three (two influent waters to STPs and one industrial effluent) showed detectable levels of ZnPT (1.9, 17 and 32 µg/l).

5. Sampling programme

5.1 National sampling programme

A sampling strategy was developed in order to assess the contribution of point sources of ZnPT and its metabolites to the Swedish environment. A previous study in Sweden estimated that the major sources for the discharge of ZnPT to the aquatic environment was personal care products, such as anti-dandruff shampoos, and marine anti-fouling paints (SNF 2004). Samples from four sewage treatment plants and two small boat marinas were included in the sampling programme, which is summarised in Table 5.

Detailed information regarding the samples from the national screening programme is presented in Appendix 1.

Table 5. Number of samples for the screening program of pyrithiones.

Sampling site	Influent	Effluent	Sludge	Seawater	Sediment	Sum
Sewage treatment plant 1	2	2	2		3	9
Sewage treatment plant 2	2	2	2			6
Sewage treatment plant 3	2	2	2			6
Sewage treatment plant 4	2	2	2			6
Marina				2	2	4
					Total	31

5.2 Regional sampling programme

Swedish county administrative boards had the opportunity to contribute to a regional sampling program. Five county administrative boards participated. The samples originated from 21 different STPs, all contributed effluent waste water, 13 also sludge and six of those also influent waste water. There were also two influents to STPs representing wastewaters from industries which manufacture products containing ZnPT.

Detailed information regarding the samples from the regional screening programme is presented in Appendix 2.

6. Methods

6.1 Sampling

The staff at the different STPs collected influent and effluent waters in 1 litre PE bottles and sludge from the anaerobic chambers into PE-jars. All bottles were wrapped in aluminium foil to protect from light and were stored frozen.

Surface waters were sampled directly into 2 litre glass bottles wrapped in aluminium foil to protect from light and were stored refrigerated. Sediment was sampled in PE-jars wrapped in aluminium foil and was stored frozen.

6.2 Sample preparation

Extraction of zinc pyrithione and 2,2'-dithiodipyridine from water
Influent water (100 ml), effluent water (250 ml) or seawater (500 ml) was spiked with internal standard (Carbamazepine-$^{13}C^{15}N$). The extraction method was adopted and modified from Woldegiorgis et al 2007 and Bones et al 2006. An Oasis HLB solid-phase extraction (SPE) cartridge was pre-conditioned with 6 ml acetonitrile (ACN) followed by 6 ml MQ-water prior to loading of the water sample. Elution was performed with 6 ml dichloromethane (DCM). The extract was evaporated to a volume of 1 ml under a gentle stream of nitrogen at 40ºC. After evaporation 2 ml methanol (MeOH) was added to the extract and further evaporated to 0.5 ml. Thereafter 0.5 ml of 20 mM ammonium acetate (NH_4OAc) in MQ-water was added to the extract. The final extract was centrifuged at 10 000 rpm for 10 minutes and the supernatant was transferred to a vial for analysis.

Extraction of zinc pyrithione from sludge and sediment
Freeze-dried sludge or sediment (0.5 g) was spiked with internal standard (Carbamazepine-$^{13}C^{15}N$). The extraction method was adopted and modified from Woldegiorgis et al 2007 and Bones et al 2006. The sample was extracted with 10 ml of DCM on an ultrasonic bath for 5 minutes followed by shaking at 1400 rpm for 30 minutes. The supernatant was evaporated to a volume of 1 ml under a gentle stream of nitrogen at 40ºC. After evaporation 2 ml MeOH was added to the extract and further evaporated to 0.5 ml. Thereafter 0.5 ml of 20 mM NH_4OAc in MQ-water was added to the extract. The final extract was centrifuged at 10 000 rpm for 10 minutes and the supernatant was transferred to a vial for analysis.

Extraction of pyridinsulfonic acid from water
Influent water (100 ml), effluent water (250 ml) or seawater (500 ml) was spiked with internal standard (Ibuprofen-D_3). An Oasis WCX solid-phase SPE cartridge was pre-conditioned with 6 ml ACN followed by 6 ml MQ-water prior to loading of the water sample. Elution was performed with 6 ml ACN followed by 6 ml 2% trifluoroacetic acid (TFA) in ACN . The extract was evaporated to dryness under a gentle stream of nitrogen at 40ºC. The sample was reconstituted in 1 ml MeOH: water (1:1) on an ultrasonic bath for 5

minutes. The final extract was centrifuged at 10 000 rpm for 10 minutes and the supernatant was transferred to a vial for analysis.

Extraction of pyridinsulfonic acid from sludge
Freeze-dried sludge or sediment (0.5 g) was spiked with internal standard (Carbamazepine-$^{13}C^{15}N$). The sample was extracted with 1 ml 1M acetic acid (HAc) in MQ-water and 10 ml ACN on an ultrasonic bath for 5 minutes followed by shaking at 1400 rpm for 30 minutes. The supernatant was evaporated to dryness under a gentle stream of nitrogen at 40ºC. The sample was reconstituted in 1 ml MeOH: water (1:1) on an ultrasonic bath for 5 minutes. The final extract was centrifuged at 10 000 rpm for 10 minutes and the supernatant was transferred to a vial for analysis.

Extraction of total pyrithiones from water in the form of 2-mercaptopyridine
Influent water (100 ml), effluent water (250 ml) or seawater (500 ml) was spiked with internal standard (Carbamazepine-$^{13}C^{15}N$). An Oasis HLB SPE cartridge was pre-conditioned with 6 ml ACN followed by 6 ml MQ-water prior to loading of the water sample. Elution was performed with 5 ml MeOH followed by 5 ml DCM. After elution 0.5 ml of a solution containing 0.1% ethylenediaminetetraacetic acid (EDTA) and 0.1% ascorbic acid in MQ-water (w/w %) was added to the extract and shaken at 1400 rpm for 1 hour. Thereafter the extract was evaporated to a volume of 1 ml under a gentle stream of nitrogen at 40ºC. The final extract was centrifuged at 10 000 rpm for 10 minutes and the supernatant was transferred to a vial for analysis.

6.3 Instrumental analysis

The samples were analysed applying a high performance liquid chromatography system consisting of a Prominence UFLC system (Shimadzu) with two pumps LC 20AD, degasser DGU-20A5, auto sampler SIL-20ACHT and column oven CTO-20AC. The analytical column was an X Bridge™ BEH Amide 50 mm x 3 mm, particle size 2.5 µm (Waters, Ireland). The mobile phase A was a solution of 10 mM acetic acid in water and solvent B was methanol. The flow rate of the mobile phase was 0.3 ml/min. A gradient elution for the detection of 2-pyridinesulfonic acid (PSA) and 2-mercaptopyridine (HPS) were performed: 0-1 minutes 0% B, 1-13 minutes linear increase to 90% B, 13-15 minutes isocratic 90% B. Equilibration time 4 min when B reached 0% again. A gradient elution for the detection of zinc pyrithione (ZnPT) as cupper pyrithione (CuPT) and 2,2'-dithiodipyridine (PS_2) were performed: 0-1 minutes 0% B, 1-8 minutes linear increase to 90% B, 8-12 minutes isocratic 90% B. Equilibration time 4 min when B reached 0% again.

The effluent was directed to an API 4000 triple quadruple mass spectrometer (Applied Biosystems). For analysis, 10 µl sample extract was injected. ESI in positive ion mode and multiple reaction monitoring (MRM) were used to detect ZnPT, PSA and PS_2. ESI in negative ion mode and MRM were used to detect HPS. The masses used for quantification are shown in Table 6. Identification was done by retention time and quantification was done using authentic reference compounds.

Table 6. Precursor ions and fragment ions for detection and quantification of pyrithiones.

	Precursor ion $[M+H]^{1+}$/ $[M-H]^{1-}$	**Product ion used for quantification**	**Product ion used as qualifier**
Substance	m/z	m/z	m/z
2-Pyridinesulfonic acid	158.1	79.5	94.0
Zinc pyrithione*	316.0	189.0	142.0
2,2'-Dithiopyridine	221.1	110.9	187.0
2-Mercaptopyridine	112.0	78.0	96.1

*Detected as Cupper pyrithione (CuPT)

6.4 Quality control

To ensure the quality of the identification of the target compounds, two MRM transitions were used for each compound (Table 6). Also, the retention time should match those of the authentic standard compounds within ± 0.2 min.

For each series of ten samples, two solvent method blanks were prepared in parallel with the samples to assess possible interferences and contamination from the background.

Carbamazepine-$^{13}C^{15}N$ and/or Ibuprofen-D_3 were used as internal standard in all samples.

The background contamination in the blank samples was subtracted from the measured sample values and the limit of detection (LOD) was defined as three times the standard deviation of the blank samples noise (S/N=3).

A minimum of 5 samples per analytical method were spiked with known amounts of the four authentic standards and pre-concentrated on different days in order to establish the method recovery and relative standard deviation (RSD) (Table 7).

Table 7. Detection limits (LOD), recovery and relative standard deviation (RSD) for the investigated pyrithiones in water, sludge and sediment based on 5 replicates.

	LOD (S/N=3)		**Recovery**		**RSD**	
	Water	Sludge/Sediment	Water	Sludge/Sediment	Water	Sludge/Sediment
Substance	ng/L	µg/kg dw*	%	%	%	%
2-Pyridinesulfonic acid	<2	<24	97	51	11	22
Zinc pyrithione	<6	<30	88	17	23	26
2,2'-Dithiopyridine	<14	-	90	-	16	-
2-Mercaptopyridine	<310	-	28	-	29	-

*dw = Dry weight

7. Results and discussion

The concentrations of pyrithiones and pyrithione residues found in samples from the national and regional screening programmes are presented in tabular format in Appendix 1 and Appendix 2.

The quantification of HPT proved to be impossible, despite extensive method development. The problem with HPT is that it is highly hydrophilic, which makes it impossible to retain on the chromatographic column or on the solid-phase extraction cartridges investigated. The problem with lack of retention for HPT has previously been described in the scientific literature (Sakkas et al 2007). One way of overcoming this problem is to reduce HPT to HPS by adding EDTA and ascorbic acid to the final extract. However, pyrithiones other than HPT present in the sample will also be reduced to HPS. For example, during an experiment where a solution containing 1.57 mmol/ml of ZnPT was treated with an increased amount of EDTA and ascorbic acid an increase in HPS was observed (
Figure 2). The increase of HPS corresponded to a 50% conversion of the initial concentration of ZnPT in the solution. In addition, the experiment revealed that PS_2, formed during ionization of ZnPT in the analytical instrument, also was reduced (Figure 2). The only pyrithione residue that was not affected by the reduction agent was PSA (data not shown). The implication of this result is that the concentration of HPT in the present study is quantified as the total sum of pyrithione and pyrithione residues in the samples, except for the presence of PSA.

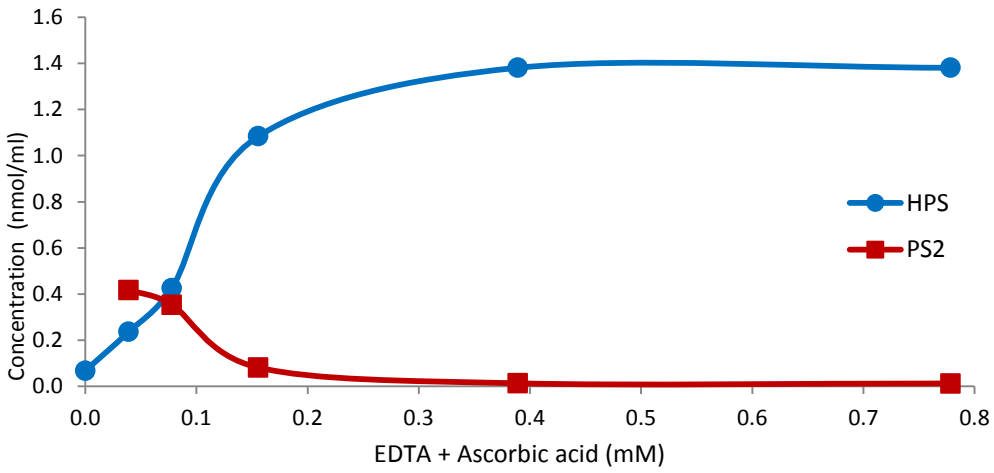


Figure 2. Reduction of 1.57 mmol/ml ZnPT to HPS by addition of EDTA and ascorbic acid.

It is impossible with the current analytical method to distinguish between ZnPT and CuPT, because ZnPT is converted to >95% to CuPT in the analytical instrument. That ZnPT trans-chelate to CuPT in the analytical instrument have previously been reported in the scientific literature (Bones et al 2006). The trans-chelation results in that ZnPT and CuPT will be quantified as a single substance.

7.1 National sampling programme

7.1.1 Sewage treatment plants

All four sewage treatment plants (STPs) showed detectable concentrations of ZnPT and/or CuPT in their influent (range 10 - 260 ng/L, Figure 3). In effluent the concentration of ZnPT and/or CuPT were below the detection limit (LOD=6.0 ng/L) in all four STPs. The result indicates that ZnPT and CuPT are degraded in the STPs.

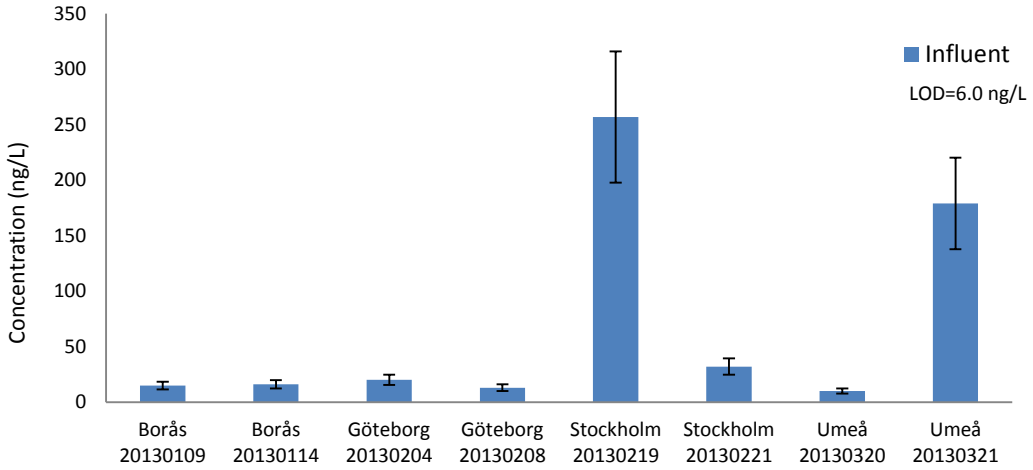


Figure 3. Concentration of the sum of ZnPT and CuPT in influent waste water from the four sewage treatment plants in the national programme, each sampled at two occasions. The concentration in the corresponding effluent waters were <6 ng/L in all cases. Error bars represent the relative standard deviation (RSD) established for the method (Table 7).

The degradation product PSA was detected in all influent and in all effluent wastewaters with the exception of one. A decrease in concentration from influent (range 73 - 340 ng/L) to effluent wastewater (range <2 - 86 ng/L) was observed for in all four STPs (Figure 4).

The degradation product PS_2 was not detected in any of the wastewater samples even though the detection limit was low (LOD=14 ng/L) and the recovery was sufficient (90%). This shows that PS_2 is not a major degradation product of ZnPT in STPs, even though earlier laboratory experiments have proposed the opposite (Sakkas et al 2007).

The determination of summed pyrithione residues as HPS showed no detectable concentrations in wastewater from the four STPs. The detection limit was relatively high (LOD=310 ng/L).

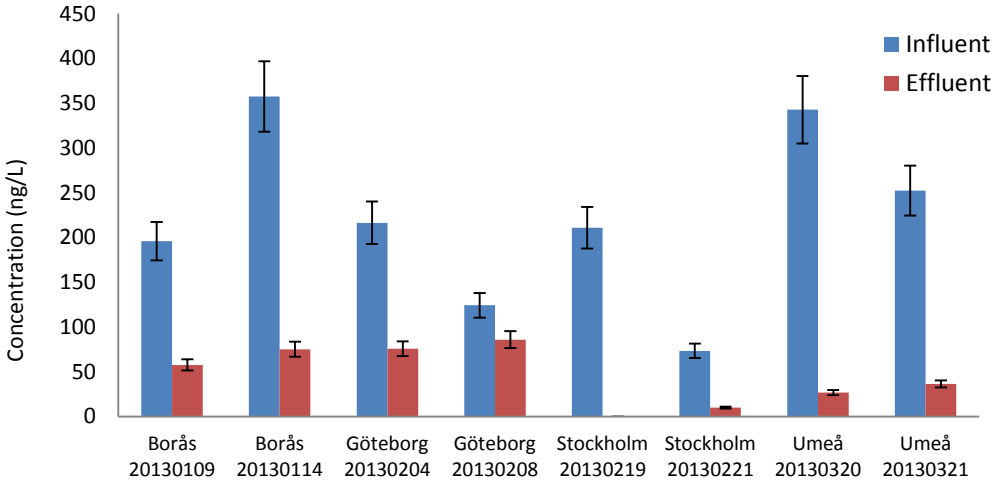


Figure 4. Concentration of PSA in influent and effluent wastewater from four sewage treatment plants each sampled at two occasions.

PSA was detected in all sludges from the four STPs (Figure 5) while ZnPT, CuPT or any of the other investigated degradation products could not be detected. An explanation to the absence of detectable concentrations of ZnPT in sludge could be that ZnPT will trans-chelate to CuPT and thereafter sulphur, present in the sludge, will react with cupper and break the complex to form HPT (Bones et al 2006).

PS_2 was not measured in sludge due to low or zero recovery. The reason for the poor recovery is that PS_2 probably binds irreversibly to sludge.

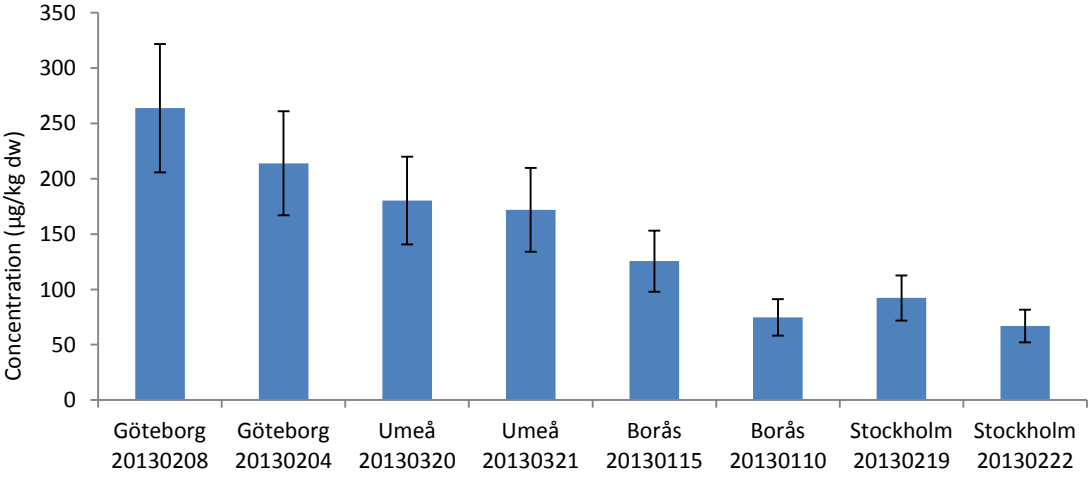


Figure 5. Concentration of PSA in sludge from four sewage treatment plants each sampled at two occasions.

The determination of total pyrithione residues in the form of HPS was not possible in sludge due to low or zero recovery. The poor recovery of HPS can be a result of irreversible binding of the amine in the pyridine ring to sludge, which is positively charged

under environmental pH (Güven et al 2005). The positively charged amine in the pyridine ring is probably also the reason why PSA partitions to sludge, even though the substance is highly hydrophilic (log Kow= -0.13).

7.1.2 Marinas

In the two sediments from small boats marinas PSA was detected in concentrations of 43 - 66 µg/kg (dry weight) (Figure 6). However PSA was not detected in the water phase of the marinas or in the sediments along a gradient from Gothenburg harbour to the archipelago. None of the other investigated compounds was detected in the marine water or sediment.

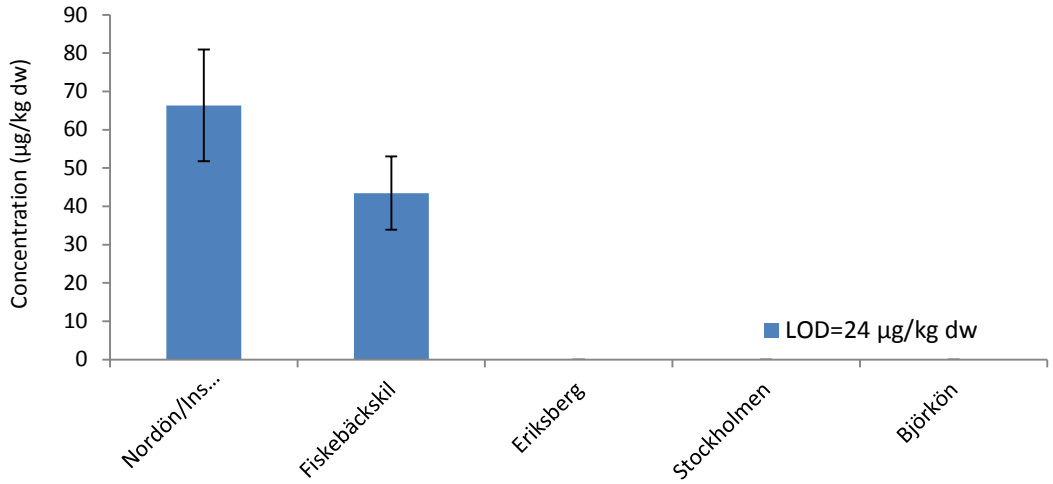


Figure 6. Concentration of PSA in sediment from two small boat marinas (Nordön and Fiskebäkskil), and in sediment from a three point gradient out from Gothenburg (Eriksberg, Stockholmen and Björkön).

7.2 Regional sampling programme

Three of the six influents showed detectable concentrations of ZnPT and/or CuPT (range 6 - 48 ng/L, Figure 7) while only two of the 21 effluents showed detectable concentrations (range 23 - 40 ng/L, Figure 7).The detection limit was 6 ng/L.

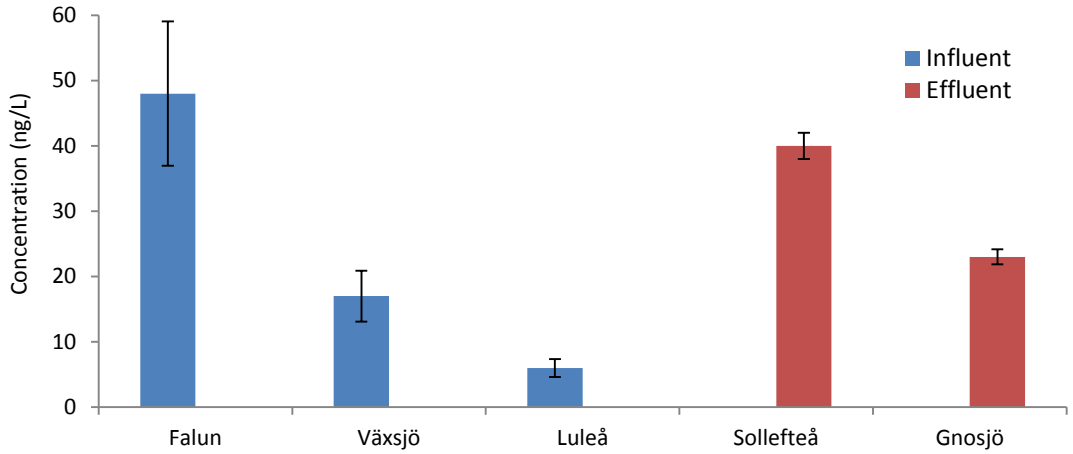


Figure 7. Concentration of the sum of ZnPT and CuPT in influent and effluent wastewater from five regional sewage treatment plants from the regional programme. In remaining influent and effluent samples (n=26) the concentration was <6 ng/L.

All influents showed detectable concentrations of PSA (range 78 - 480 ng/L, Figure 8). In the corresponding effluents the PSA concentrations were lower, or in one case similar, to the influent concentrations (range 4.1 - 130 ng/L, Figure 8).

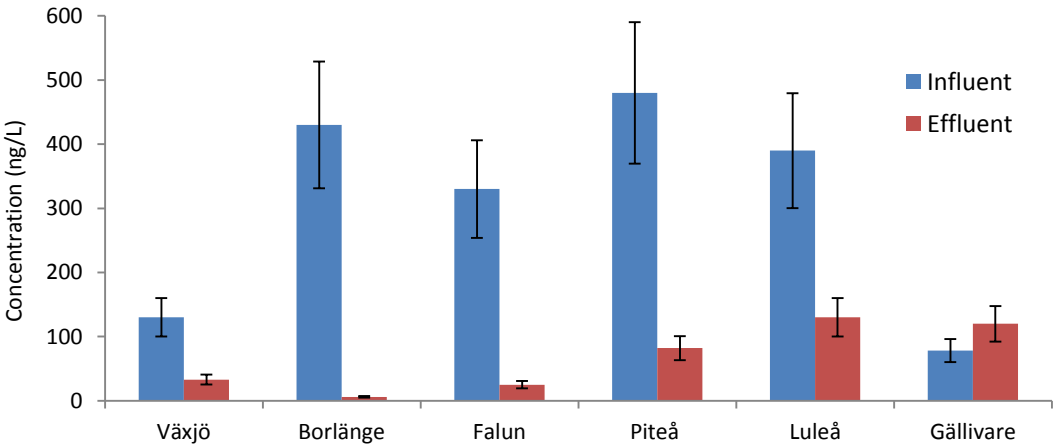


Figure 8. Concentration of PSA in influent and effluent wastewater from six sewage treatment plants from the regional programme.

The PSA-concentration in all effluents is illustrated in Figure 9.

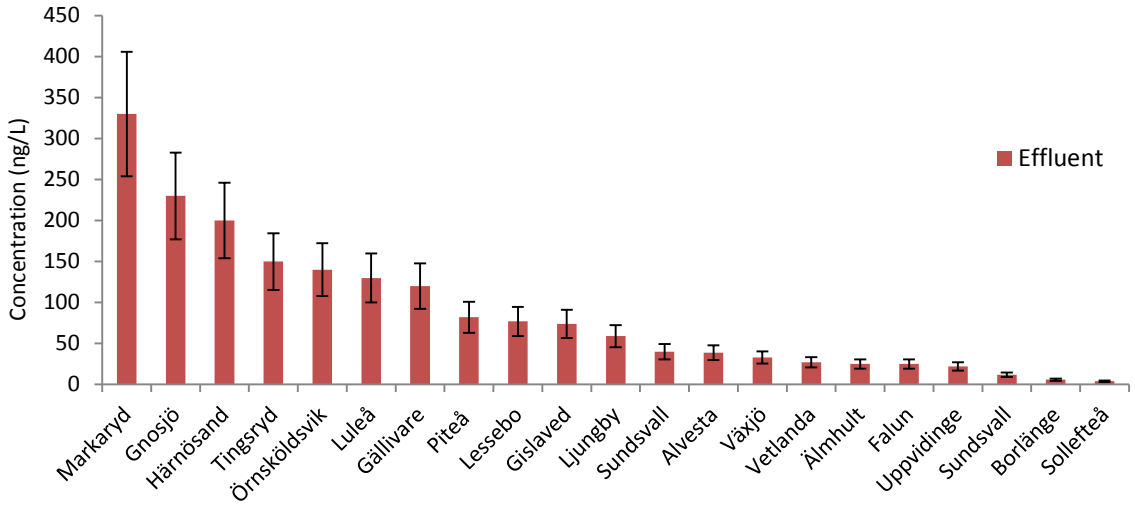


Figure 9. Concentration of PSA in effluent wastewater from 21 sewage treatment plants from the regional programme.

Two separately sampled influents to STPs originating from industries using ZnPT were also analysed. Due to the complex matrix those samples had to be diluted (100 times) before analysis leading to a correspondingly higher detection limit. None of the analytes could be detected.

Thirteen STPs participated with sludge. All sludges showed detectable concentrations of PSA (range 25 - 278 µg/kg dw, Figure 10).

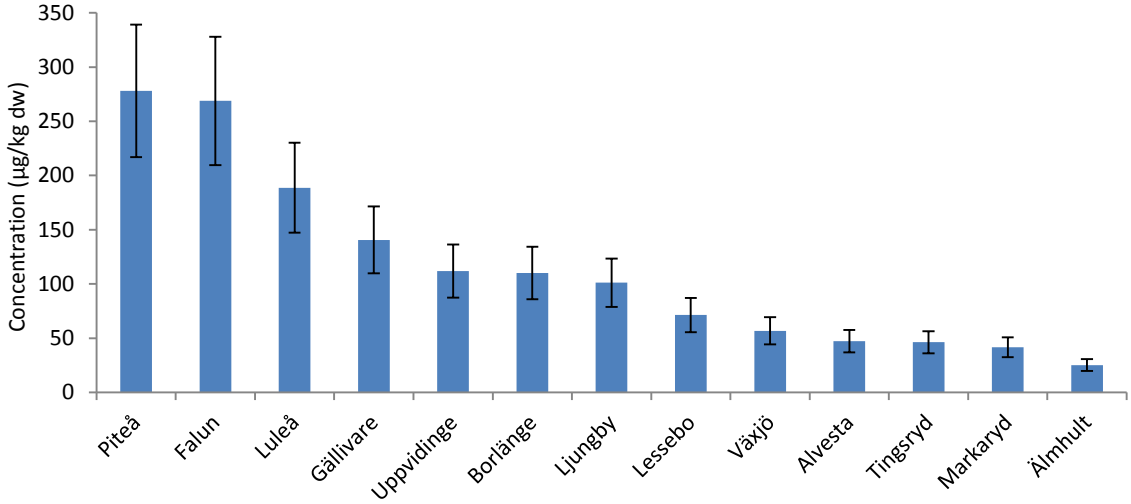


Figure 10. Concentration of PSA in sludge from 13 regional sewage treatment plants (STPs).

None of the other investigated compounds were detected in sludge. The previous explanations for the absence of the other investigated pyrithiones in wastewater and sludge are consistent with these findings.

8. Conclusions

When the national and regional sampling programs are taken together a total of 14 STP influent and 28 STP effluent samples were analysed. Detection frequencies and concentration ranges for Zn/Cu PT and PSA are summarised in Table 8.

Table 8 Summery of detection frequencies and concentration ranges for Zn/Cu PT and PSA in all STP influents and effluents.

		Zn/Cu PT		PSA	
	number of samples	Det. freq.	min - max (median), ng/L	Det. freq.	min - max (median), ng/L
Influent	14	79%	<6 - 260 (17)	100%	73 - 480 (240)
Effluent	28	7%	<6 - 40 (32)	96%	<2 - 330 (59)

The decrease in detection frequency and in concentration range for Zn/Cu PT from influent to effluent shows that degradation may take place in the STP.

The degradation product PSA was the most abundant compound and it was present in the majority of the wastewater samples. The decrease in concentration from influent to effluent shows that PSA may be urther degraded or absorbed to sludge in the STP.

PSA was found in all sludge samples. The concentration range was 25 - 280 (median 110) µg/kg dw (n=21). The concentration in sludge on a wet weight basis was usually higher than in the corresponding influent or effluent waters which indicates enrichment to sludge.

The absence of Zn/Cu PT in sludge shows that these substances undergo degradation in contact with sludge.

In a small boat marina where ZnPT is probably constantly emitted from anti-fouling paints, and could be trans-chelated to the even more toxic CuPT (2.3), none of these species could be detected in the water or in the sediment. PSA, however, which from earlier studies (Neihof et al 1979, Turley et al 2000 and 2005) is assumed to be the major pyrithione residue in the marine environment was detected in the sediment from the marina. In the sediments from Gothenburg, where the ZnPT load is of a more diffuse character, accumulation of PSA was not detected.

As the acute toxicity of pyrithiones decreases with increased degradation and PSA is the least toxic of the known pyrithione residues, with a LC_{50} value of >100 000 mg/l for algae, crustacean and fish (Onduka et al 2010) the result indicates that a process leading to less toxic sediments is taking place. However, the absence of chronic tests of PSA in the scientific literature makes it impossible to estimate the long-term effect on the marine environment.

9. Acknowledgements

The staffs at the municipal sewage treatment plants are acknowledged for their help during sampling. This study was funded by Environmental Monitoring at the Swedish Environmental Protection Agency.

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Appendix 1. National sampling programme.

MR nr	County	Municipality	Site	Matrix	Sampling date	Sample Information	dw (%)	Unit	PSA	ZnPT and/or CuPT	PS_2	Total Pyrithione
2114	Nationellt	Borås	Gässlösa STP	Influent	2013-01-09	Composite sample, 24h		ng/L	200	15	<14	<310
2115	Nationellt	Borås	Gässlösa STP	Effluent	2013-01-09	Composite sample, 24h		ng/L	58	<6	<14	<310
2116	Nationellt	Borås	Gässlösa STP	Sludge	2013-01-10	Grab sample	26	µg/kg dw	75	<30	*	*
2117	Nationellt	Borås	Gässlösa STP	Influent	2013-01-14	Composite sample, 24h		ng/L	360	16	<14	<310
2118	Nationellt	Borås	Gässlösa STP	Effluent	2013-01-14	Composite sample, 24h		ng/L	75	<6	<14	<310
2119	Nationellt	Borås	Gässlösa STP	Sludge	2013-01-15	Grab sample	22	µg/kg dw	130	<30	*	*
2130	Nationellt	Göteborg	Ryaverket STP	Influent	2013-02-04	Composite sample, 24h		ng/L	220	20	<14	<310
2131	Nationellt	Göteborg	Ryaverket STP	Effluent	2013-02-04	Composite sample, 24h		ng/L	76	<6	<14	<310
2132	Nationellt	Göteborg	Ryaverket STP	Sludge	2013-02-04	Grab sample	29	µg/kg dw	210	<30	*	*
2133	Nationellt	Göteborg	Ryaverket STP	Influent	2013-02-08	Composite sample, 24h		ng/L	120	13	<14	<310
2134	Nationellt	Göteborg	Ryaverket STP	Effluent	2013-02-08	Composite sample, 24h		ng/L	86	<6	<14	<310
2135	Nationellt	Göteborg	Ryaverket STP	Sludge	2013-02-08	Grab sample	28	µg/kg dw	260	<30	*	*
2153	Nationellt	Stockholm	Henriksdal STP	Influent	2113-02-19	Composite sample, 24h		ng/L	210	260	<14	<310
2154	Nationellt	Stockholm	Henriksdal STP	Effluent	2113-02-19	Composite sample, 24h		ng/L	<2	<6	<14	<310
2155	Nationellt	Stockholm	Henriksdal STP	Sludge	2113-02-19	Grab sample	26	µg/kg dw	92	<30	*	*
2156	Nationellt	Stockholm	Henriksdal STP	Influent	2013-02-21	Composite sample, 24h		ng/L	73	32	<14	<310
2157	Nationellt	Stockholm	Henriksdal STP	Effluent	2013-02-21	Composite sample, 24h		ng/L	10	<6	<14	<310
2158	Nationellt	Stockholm	Henriksdal STP	Sludge	2013-02-22	Dewatered sludge	25	µg/kg dw	67	<30	*	*
2205	Nationellt	Umeå	Ön STP	Influent	2013-03-20	Composite sample, 24h		ng/L	340	10	<14	<310
2206	Nationellt	Umeå	Ön STP	Effluent	2013-03-20	Composite sample, 24h		ng/L	27	<6	<14	<310
2207	Nationellt	Umeå	Ön STP	Sludge	2013-03-20	Dewatered sludge	33	µg/kg dw	180	<30	*	*
2209	Nationellt	Umeå	Ön STP	Influent	2013-03-21	Composite sample, 24h		ng/L	250	180	<14	<310
2210	Nationellt	Umeå	Ön STP	Effluent	2013-03-21	Composite sample, 24h		ng/L	36	<6	<14	<310
2208	Nationellt	Umeå	Ön STP	Sludge	2013-03-21	Dewatered sludge	33	µg/kg dw	170	<30	*	*
2183	Nationellt	Kungälv	Nordön / Instöränna	Sediment	2012-06-08	Grab sample	23	µg/kg dw	66	<30	*	*
2184	Nationellt	Lysekil	Fiskebäckskil	Sediment	2012-06-08	Grab sample	34	µg/kg dw	43	<30	*	*
2185	Nationellt	Kungälv	Nordön / Instöränna	Surface water	2012-06-08	Grab sample		ng/L	<2	<6	<14	<310
2186	Nationellt	Lysekil	Fiskebäckskil	Surface water	2012-06-08	Grab sample		ng/L	<2	<6	<14	<310
9602	Nationellt	Göteborg	Björkö	Sediment	2011-10-20	Grab sample, Gradient from Gothenburg	29	µg/kg dw	<24	<30	*	*
9601	Nationellt	Göteborg	Stockholmen	Sediment	2011-10-17	Grab sample, Gradient from Gothenburg	27	µg/kg dw	<24	<30	*	*
9615	Nationellt	Göteborg	Eriksberg	Sediment	2011-10-17	Grab sample, Gradient from Gothenburg	35	µg/kg dw	<24	<30	*	*

Appendix 2. Regional sampling programme.

MR nr	County	Municipality	Site	Matrix	Sampling date	Sample Information	dw (%)	Unit	PSA	ZnPT and/or CuPT	PS_2	Total Pyrithione
1994	Dalarna	Borlänge	Fagersta STP	Influent	2012-11-26	Composite sample, 24h		ng/L	430	<6	<14	<310
1995	Dalarna	Borlänge	Fagersta STP	Effluent	2012-11-26	Composite sample, 24h		ng/L	6	<6	<14	<310
1996	Dalarna	Borlänge	Fagersta STP	Sludge	2012-11-27	Digested sludge, Grab sample	35	µg/kg dw	110	<30	*	*
1997	Dalarna	Borlänge	Fagersta STP	Influent from industry	2012-11-28	Grab sample, Industrial		ng/L	<200	<600	<1400	<31000
2109	Dalarna	Falun	Främby STP	Influent	2013-01-15	Composite sample, 24h		ng/L	330	48	<14	<310
2110	Dalarna	Falun	Främby STP	Effluent	2013-01-15	Composite sample, 24h		ng/L	25	<6	<14	<310
2111	Dalarna	Falun	Främby STP	Sludge	2013-01-14	Grab sample/Batch sample	26	µg/kg dw	270	<30	*	*
2112	Dalarna	Falun	Främby STP	Influent from industry	2013-01-14/16	Water from 5 rinses, Industrial		ng/L	<200	<600	<1400	<31000
2076	Kronoberg	Alvesta	Alvesta STP	Effluent	2013-01-08	Composite sample, 24h		ng/L	39	<6	<14	<310
2077	Kronoberg	Alvesta	Alvesta STP	Sludge	2013-01-08	Grab sample	34	µg/kg dw	47	<30	*	*
1992	Kronoberg	Lessebo	Lessebo STP	Effluent	2012-11-27	Composite sample, 24h		ng/L	77	<6	<14	<310
1993	Kronoberg	Lessebo	Lessebo STP	Sludge	2012-11-27	Grab sample	23	µg/kg dw	71	<30	*	*
1977	Kronoberg	Ljungby	Ljungby STP	Effluent	2012-11-21	Composite sample, 24h		ng/L	59	<6	<14	<310
1978	Kronoberg	Ljungby	Ljungby STP	Sludge	2012-11-21	Grab sample	20	µg/kg dw	100	<30	*	*
2128	Kronoberg	Markaryd	Ribersdals STP	Effluent	2013-01-30	Composite sample, 24h		ng/L	330	<6	<14	<310
2129	Kronoberg	Markaryd	Ribersdals STP	Sludge	2013-01-30	Grab sample	22	µg/kg dw	42	<30	*	*
2069	Kronoberg	Tingsryd	Tingsryds STP	Effluent	2013-01-08	Composite sample, 24h		ng/L	150	<6	<14	<310
2070	Kronoberg	Tingsryd	Tingsryds STP	Sludge	2013-01-08	Grab sample	21	µg/kg dw	46	<30	*	*
2050	Kronoberg	Uppvidinge	Åseda STP	Effluent	2012-12-17	Grab sample		ng/L	22	<6	<14	<310
2051	Kronoberg	Uppvidinge	Åseda STP	Sludge	2012-12-17	Grab sample	19	µg/kg dw	110	<30	*	*
2023	Kronoberg	Växjö	Sundet STP	Influent	2012-12-04	Grab sample		ng/L	130	17	<14	<310
2024	Kronoberg	Växjö	Sundet STP	Effluent	2012-12-04	Grab sample		ng/L	33	<6	<14	<310
2025	Kronoberg	Växjö	Sundet STP	Sludge	2012-12-04	Grab sample	4	µg/kg dw	57	<30	*	*
1990	Kronoberg	Älmhult	Älmhult STP	Effluent	2012-11-28	Composite sample, 24h		ng/L	25	<6	<14	<310
1991	Kronoberg	Älmhult	Älmhult STP	Sludge	2012-11-28	Sludge from reed, Digested, Grab sample	9	µg/kg dw	25	<30	*	*
2073	Norrbotten	Luleå	Uddebo STP	Influent	2013-01-08	Composite sample, 24h		ng/L	390	6	<14	<310
2074	Norrbotten	Luleå	Uddebo STP	Effluent	2013-01-08	Composite sample, 24h		ng/L	130	<6	<14	<310
2075	Norrbotten	Luleå	Uddebo STP	Sludge	2013-01-08	Grab sample, Digested, Dewatered	21	µg/kg dw	190	<30	*	*
2026	Norrbotten	Piteå	Sandholmens STP	Influent	2012-11-30	Composite sample, 24h		ng/L	480	<6	<14	<310
2027	Norrbotten	Piteå	Sandholmens STP	Effluent	2012-11-30	Composite sample, 24h		ng/L	82	<6	<14	<310
2028	Norrbotten	Piteå	Sandholmens STP	Sludge	2012-11-30	Grab sample	28	µg/kg dw	280	<30	*	*
2056	Norrbotten	Gällivare	Kavahedens STP	Influent	2012-12-11	Composite sample, 24h		ng/L	78	<6	<14	<310
2057	Norrbotten	Gällivare	Kavahedens STP	Effluent	2012-12-11	Composite sample, 24h		ng/L	120	<6	<14	<310

MR nr	County	Municipality	Site	Matrix	Sampling date	Sample Information	dw (%)	Unit	PSA	ZnPT and/or CuPT	PS_2	Total Pyrithione
2058	Norrbotten	Gällivare	Kavahedens STP	Sludge	2012-12	Grab sample	17	µg/kg dw	140	<30	*	*
2068	Västernorrland	Sollefteå	Hågesta STP	Effluent	2013-01-07	Grab sample		ng/L	4	40	<14	<310
2039	Västernorrland	Örnsköldsvik	Knorthems STP	Effluent	2012-12-11	Composite sample, 24h		ng/L	140	<6	<14	<310
2060	Västernorrland	Sundsvall	Tivoliverket	Effluent	2012-12-13	Composite sample, 24h		ng/L	12	<6	<14	<310
2059	Västernorrland	Sundsvall	Fillanverket	Effluent	2012-12-13	Composite sample, 24h		ng/L	40	<6	<14	<310
2038	Västernorrland	Härnösand	Kattastrand STP	Effluent	2012-12-10	Grab sample		ng/L	200	<6	<14	<310
1979	Jönköping	Gnosjö	Gnosjö STP	Effluent	2012-11-22	Composite sample, 24h		ng/L	230	23	<14	<310
1969	Jönköping	Gislaved	Gislaveds STP	Effluent	2012-11-21	Composite sample, 24h		ng/L	74	<6	<14	<310
1988	Jönköping	Vetlanda	Vetlanda STP	Effluent	2012-11-26	Composite sample, 24h		ng/L	27	<6	<14	<310