June 2014

Fish exposure studies

Monitoring studies in the recipient of UPM pulp mill

April 2014

Innovation by experience

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1 INTRODUCTION

The UPM mill initiated its start-up process during the first half of November 2007 after the permit procedure was finalised and has since that been in operation. This study on fish exposure is part of the environmental monitoring program that the mill is performing yearly. The program is accepted by DINAMA and studies are performed at three different areas, one upstream and two downstream the mill site, and according to the same methodology as the preceding baseline studies during years 2005 – 2007 (Tana 2007). Fish exposure studies are part of a large monitoring of fish community and species diversity in the recipient of UPM kraft pulp mill in Fray Bentos (Tana 2014).

One of the best documented ways of establishing exposure levels of biota to pulp mill effluents is the analysis of conjugated phenolic compounds and resin acids in the bile liquid of fish. These low-molecular compounds are readily taken up from the water by fish through their gills or via food. The liver of fish and of other vertebrates is the centre where detoxification of foreign substances is taking place. Fat soluble substances are in the liver converted into more water soluble forms so that they can be excreted from the fish via the bile liquid.

The reason for analysing conjugated compounds from fish bile is two-fold: Firstly, it is known that such compounds are toxic to fish. Secondly, the conjugation and successive storage of conjugates in the bile of fish at concentrations in the order of 10^5 of the surrounding water concentration, enables accurate analysis from small volumes. The presence of conjugated compounds is also a sign of, that the compounds have been processed through the detoxification system of the fish, since conjugation of lipophilic compounds means that the compound has been made water soluble and it is on its way of being extracted by the fish (Oikari & Holmbom 1986, Grahn et al. 1991, Tana et al. 1994, Johnssen et al. 1995, Tana 2004).

The baseline studies from April 2005 to April 2007 have given information from Rio Uruguay on the background levels, both in river water and fish, of the pulp mill effluent related substances such as chlorophenolic compounds, resin acids and phytosterols. Baseline studies showed the exposure level of fish to be similar on all three study areas both upstream and downstream the future mill effluent discharge point. The muscle concentrations of dioxins, furans and PCBs were below the Total Daily Intake recommendations. Monitoring studies after the mill start up have indicated that the concentrations of chlorophenolic compounds, resin acids and phytosterols are within the variation limits as observed during the baseline studies and there are no indications of changes in the concentration levels caused by the effluent discharged from the UPM pulp mill. Also based on the observed concentrations and international recommendations there would be no limitations to human consumption of the studied fish.

2 MATERIAL AND METHODS

2.1 MILL DESCRIPTION AND EFFLUENT QUALITY

The Mill

The annual production capacity of the mill is about 1.1 million tons of bleached eucalyptus kraft pulp. The mill applies the Best Available Techniques (BAT) as presented in the BREF prepared by the European Commission (Anon 2001). Logs are debarked at the plantations when harvested, and chipped at the mill. Cooking applies downflow Lo-Solids® technology, in a continuous digester of 3 200 m^3. Delignification continues by means of a two-stage treatment with oxygen. Afterwards the pulp is bleached using an ECF "light" sequence (A/D EOP D P) in which the main bleaching chemicals are chlorine dioxide, sodium hydroxide (and/or oxidized white liquor), sulfuric acid and hydrogen peroxide. This bleaching method is considered as BAT according to European Union. The process has been designed to obtain a final product with 89-92 % ISO brightness. Pulp is dried in two equal parallel lines, and after baling it is transported by barge to the port of Nueva Palmira, 55 Km downstream in the river Uruguay, from where it is shipped to paper mills in other continents.
The recovery line consists of an evaporation plant of 7-effects with falling film type evaporator units, a recovery boiler with a design capacity of 4 450 tons of dry solids per day, and a recausticising line to produce the white liquor used to cook the wood chips. Odorous gases generated in the process are extensively collected and burnt in the recovery boiler; two back-up boilers are installed for burning those gases in case of disturbances.

Raw water is taken from Uruguay River at an average rate of about 900 L/s and treated for use in the process by means of a conventional water treatment plant. The mill effluent is discharged into the Uruguay River through a 200 m diffuser, at an average rate of about 700 L/s.

The effluent treatment plant consists of a primary treatment (sedimentation) followed by an activated sludge treatment (biological treatment). After primary treatment the effluent is led through a system of safety and equalization basins, with a total volume of 75 000 m^3, designed and operated to avoid disturbances in the quality of the effluent that is fed to the biological treatment. The activated sludge system has a total volume of 150 000 m^3, in two lines, with a hydraulic residence time of about 48 hours.

The annual average effluent flow between January – April 2014 was 17.1 m^3/ADT (air-dried ton of pulp). During the present study period in April (23.4-3.5.2014) the daily effluent flow was 16.7 m^3/ADT. The average river flow during the present study period was around 4 000 m^3/s giving an effluent dilution factor of over 6 000 as the average effluent flow is below 611 l/s.

The Effluent Quality

The physical-chemical characteristics of the treated whole mill effluent are given in Table 1. Table 1 describes the average values between January – April 2014 as well as the effluent characteristics during the present test fishing period in April 2014. Table 1 includes also the permit values and BAT values given in the BREF prepared by the European Commission (Anon 2001).

Table 1. Characteristics of the outgoing effluent from UPM pulp mill in Fray Bentos. The table includes the average discharge values (kg/Adt) between January-April 2013, the discharge during the study period in April 2014, the permit limits and the BAT values.

Kg / ADT	COD (Kg/ADT)*	BOD_5 (Kg/ADT)	AOX (Kg/ADT)	N total (Kg/ADT)	P total (Kg/ADT)	SST (Kg/ADT)	Effluent flow (m3/ADT)
BAT	23	1.5	0.25	0.25	0.030	1.5	50
Permit limit#	15	0.7	0.15	0.2	0.02	1.0	
Jan--April 2014	5.5	0.19	0.04	0.06	0.022	0.28	17.1
Test fishing period April 2014	4.4	0.13	0.03	0.10	0.022	0.33	16.7

*Adt = Air dried ton pulp
Annual limits

The effluent discharge values are low and clearly below the permit values both during the present study period and also as average during the first four months of the year 2014.

2.2 STUDY PERIOD AND AREAS

This fish exposure study as part of the overall monitoring investigation of fish and fisheries was performed between April 23rd and May 3rd in 2014. During the study, samples from river water and fish bile and muscle were sampled. The sampling sites are shown in Figure 1. Area B is close to the pulp mill effluent discharge site and is considered as the near recipient. Area C is situated outside the village of Las Cañas and can be considered as a remote recipient area. The reference area (Area A) was outside Nuevo Berlin some 10-15 km upstream the river. The sampling sites have been the same since the start up of the baseline studies in April 2005.

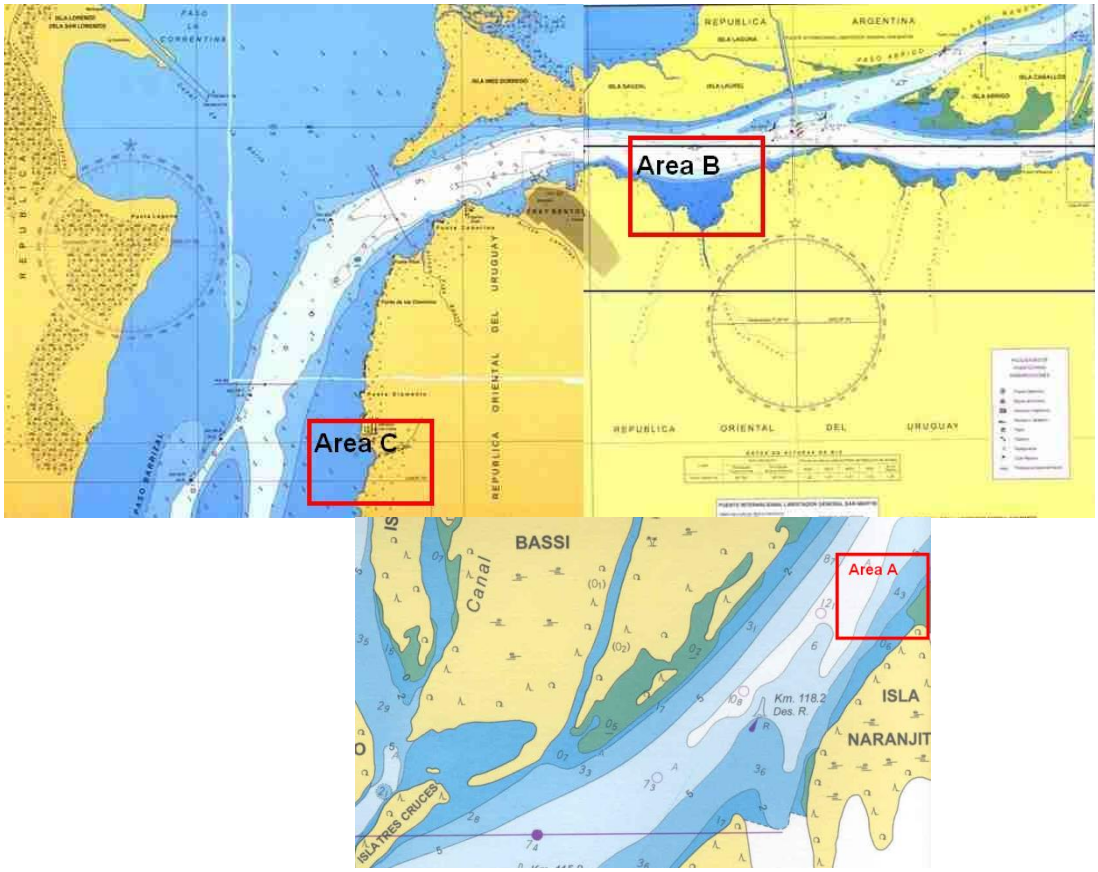

Figure 1. Sampling sites. Area B is at the effluent discharge site and Area C outside Las Cañas. Area A is the reference area outside Nuevo Berlin.

The composition of fish community and fish species diversity was studied simultaneously (Tana 2013) at the same areas. Water quality is studied within a regular monitoring program also from sites A, B and C.

2.3 RIVER WATER

The river water samples from all sampling areas were taken with a water sampler at three depths (surface, middle and bottom) within the fishing area. The samples at each station were united to form one composite sample of the river water.

The parameters analysed consisted of AOX (Adsorbable Organic Halogens), chlorophenolic compounds, resin acids, phytosterols, dioxins and PCB. The water samples were sampled into 0.25 l, 0.75 l and 1.5 l polyethylene bottles. After sampling the samples were brought to a freezer and kept in frozen state until analysis. AOX was analysed in the accredited laboratory of Eurofins, dioxins and PCBs in The National Institute of Health and Welfare in Finland and chlorinated phenolics, resin acids and phytosterols in The Institute of Environmental Chemistry of the University of Åbo Akademi, Finland.

2.4 FISH BILE AND MUSCLE

Fish from the three different areas (A, B and C) were collected between April 23rd and May 3rd during the test fishing. The fish were caught immediately before sampling. In total, bile from 30 fish was sampled (Table 2).

Table 2. Number and species of fish sampled for bile analysis.

Area	Species	Number of fish
A, Nuevo Berlin	Bagre trompudo*#	10
B1, Yaguarete estuary	Bagre trompudo*#	10
C, Las Cañas	Bagre trompudo*#	10

* muscle sample for dioxin and PCB analysis,
# muscle sample for Hg and Pb analysis

During sampling each fish was stunned with a blow on the head, the body cavity opened and the gall bladder emptied of its bile content by disposable syringe. The bile sample was emptied into a 5 ml septum glass bottle equipped with a Teflon membrane. Bile was collected as a pooled sample from 10 Bagre trompudo individuals at each sampling area.The bile samples together with the water samples were transported to Finland and they did not face temperatures above 0 ºC.

One composite sample of muscle from 10 Bagre trompudo individuals was collected from each area A, B and C for analysis of dioxins, PCBs, Hg and Pb (see Table 2).

The muscle samples were taken from the middle part of the fish as a cross section cut and a muscle piece of about 200 g was stored in aluminium foil and frozen. The muscle samples were transported as frozen to Finland for dioxin and PCB analysis in The National Institute of Health and Welfare, Department of Environmental Health Chemical Exposure Unit. Hg and Pb analysis were made in the accredited laboratory of Eurofins.

This study included also EOX analysis from mussels caught at the three study areas (See chapter 3.5.).

3 RESULTS

3.1 RIVER WATER

The river water concentrations of AOX, chlorophenolic compounds, resin acids and phytosterols are presented in Table 3.

Table 3. The concentration of AOX, chlorophenolic compounds (Cl-P), resin acids (RA), phytosterols in the river water at different sampling areas during fish studies in April 2014.

Area	AOX*	Cl-P	RA#	Phytosterols#
Unit	µg/l	µg/l	µg/l	µg/l
A	10	0.044	193	109
B	10	0.049	158	0
C	10	0.045	89	41

*detection limit 5 µg/l
detection limit 1-3 µg/l

During the present sampling period in April 2014 the AOX concentration (Table 3, Appendix 1) at all the sampling areas was 10 µg/l and the same level as previously observed in the river. The AOX analysis from the water of Rio Uruguay during the operation period of the mill (November 2007 – December 2013) have shown, that the AOX concentration has varied between 5 and 20 µg/l at the area of the effluent discharge site (area B in this study). During baseline studies prior to mill operation the AOX concentration was observed to vary between 6-12 µg/l at the same area.

According to this study the concentration of chlorophenolic compounds is very low in Rio Uruguay, only at nanogram (ng/l) level (ppb) and during April 2014 sampling the concentrations were at the same level in the recipient areas B and C as compared to the reference area (area A). The chlorophenolic compounds observed consisted only of trichlorophenols (2,3,6-trichlorophenol)

(Appendix 1/2). The concentrations of chlorophenolic compounds observed during the present study were at the lower level of chlorophenolic compound concentrations as observed during the baseline studies from all study areas.

The results from a study by CARU in 2002 in Rio Uruguay (Anon 2002) show that the concentration of all chlorophenolic compounds analysed were below detection limit, which was on a microgram level (µg/l). As compared to these concentrations the levels observed in April 2013 were very low. The limit values given in the government regulation 253/79 for di- and trichlorinated phenols are max 4 µg/l and 10 µg/l respectively, and for 2,4,6-trichlorophenol the limit value is max 2 µg/l.

Resin acids could be detected in relatively high concentrations from the river water samples taken during the present monitoring period at all study areas. During previous studies (Tana 2012) the concentrations at area A were observed to be 4-224 µg/l, at area B 5-183 µg/l and at area C 3-202 µg/l. Background concentrations in Scandinavian watercourses have been observed to be at a level of 1 - 10 µg/l (Soimasuo 1997, Karels 2000). In rivers from the tropic region resin acid concentrations of more than 1 000 µg/l have been observed at areas with no pulp or paper industry (Tana, unpublished data). One of the reasons for such high concentrations was considered to be the runoff waters from the surrounding areas and tributaries from swamp areas. Based on the results of the baseline and monitoring studies there seems to be a natural variation in river concentrations of resin acids connected to the river flow. However no clear correlation has been observed between the resin acid concentrations and the river flow.

During the present sampling period, the concentration of phytosterols analysed from the water samples taken were exceptionally high at the reference area and the far recipient area (Table 3). The detection limit for phytosterols is 1-3 µg/l. The concentration of individual phytosterols as well as resin acids and chlorophenolic compounds are presented in Appendix 1/2 and 3/2. Reason for the exceptionally higher phytosterol concentrations presently observed in the Rio Uruguay river water are for the time being not known.

The results from the river water samples together with the results from the analysis of the outgoing effluent indicate that the contribution of the effluent to the concentrations of chlorinated phenols, resin acids, phytosterols and AOX is insignificant.

One composite sample of river water was collected at sampling areas A, B and C for determination of polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF) and PCBs. The results of PCDD/F analysis presented in Table 4 and Appendix 3 are expressed both as single congeners as well as Toxic Equivalent (WHO-TEQ) because the toxicity of individual dioxins and furans vary widely. Results of PCB analysis are presented in Table 5 and Appendix 3. By multiplying the known toxicity equivalency factors TEFs, (see also Appendix 3) for single congeners by

individual concentrations of PCDD/PCDF compounds in a sample yields weighted sum that expresses overall toxicity potential. This weighted sum is called the toxicity equivalent or TEQ.

Table 4. Concentration of polychlorinated dibenzo-dioxins and –furans (PCDD/PCDF) in the river water from sampling areas A, B and C (see Fig 1) in Rio Uruguay in April 2014.

	Area A	Area B	Area C
	concentration pg/l	concentration pg/l	concentration pg/l
2378-TCDD	<0.15	<0.13	<0.17
12378-PeCDD	<0.19	<0.16	<0.21
123478HxCDD	<0.38	<0.24	<0.30
123678-HxCDD	<0.36	<0.22	<0.28
123789-HxCDD	<0.33	<0.20	<0.26
1234678-HpCDD	<0.36	<0.12	0.71
OCDD	<0.54	<0.54	2.1
2378-TCDF	<1.7	<1.7	<1.7
12378-PeCDF	<0.14	<0.095	<0.095
23478-PeCDF	<0.24	<0.24	<0.24
123478-HxCDF	<0.14	<0.13	<0.13
123678-HxCDF	<0.11	<0.11	<0.11
234678-HxCDF	<0.19	<0.16	<0.16
123789-HxCDF	<0.30	<0.21	<0.21
1234678-HpCDF	<0.19	<0.19	<0.19
1234789-HpCDF	<0.28	<0.14	<0.15
OCDF	<0.45	<0.45	<0.45
SUM	6.0	5.0	7.5
In total WHO$_{2005}$ TEQ	0.77	0.66	0.78

The results, presented in Table 4 show, that at areas A, B and C none of the congeners had concentrations above the limits of quantitation except 1234678-HpCDD at area C which had a concentration of 0.71 pg/l. During the baseline studies PCDD/F concentrations above quantitation limit were observed at area A in April 2005 (Sum 1.04 pg/l) and at area C in December 2005 (Sum 49.8 pg/l). At area B, concentrations above quantitation limit have been measured in December 2006

(Sum 0.99 pg/l). As a comparison a mean concentration of dioxins in 40 surface waters used for raw water in Japan was 56.4 pg/l (Kim et al. 2002).

In this study the limits of quantitation for individual PCDD/PCDF congeners ranged between 0.095 -1.7 pg/l. The limit of quantification of individual PCB congeners ranged between 0.1 – 140 pg/l. According to the analysing laboratory, the uncertainty of results is as follows:
when the WHO-TEQ of PCDD/PCDF is

<1 pg/l the uncertainty of analysis is ± 50%
1-5 pg/l the uncertainty of analysis is ± 40%
> 5 pg/l the uncertainty of analysis is ± 30%.

when the WHO-TEQ of PCBs is:

< 1pg/l, the uncertainty of analysis is ± 50%
>1 pg/l, the uncertainty of analysis is ± 35 %

The concentration of PCB in the river water can be considered low (Table 5). For example in British Columbia in Canada it is recommended that the total PCB concentration in water should not exceed 0.1 ng/l for the protection of freshwater life and consumers of fish and shellfish (Anon 2002).

According to the Regionally Based Assessment of Persistent Toxic Substances report from eastern and western South-America (UNEP 2002) the following has been reported: "*The majority of information on levels of persistent toxic substances (PTS) comes from major rivers of the region. The data base is not completely representative for the whole area since it contains information mostly from Argentina and Brazil and to a lesser extent, Uruguay, Ecuador and Chile. Most date correspond to chlorinated pesticides (>90%) and PAHs (8%) with a few reports of PCBs (1%). PCBs within a range of 7-39 ng/l have only been reported from the Uruguay River, Paraiba River (Rio de Janeiro), Rio de la Plata (Argentina) and the Biobio River in Chile. In the Uruguay River, concentrations of 7 ng/l have been reported (DINAMA-SOHMA, SHN 1998) and are above the recommended Argentinean limit of 1 ng/l. USEPA recommended guideline value of 14 ng/l. In the Rio de la Plata , PCB levels in contaminated sediments close to Buenos Aires are even higher than this more permissive guideline. In the Biobio river PCB concentrations are also reported to be higher (22 ng/l) than recommended guidelines. In Brazil Telles (2001) showed that concentrations of PCBs and organochlorine compounds in surface water samples collected in the State of Pernambuco were below the detection limit. Another important survey carried out in Brazil by UFFSCar/UNICAMP/CETESB showed non-detectable levels of PCBs in the water column and interstitial waters in the Tiete River*"

As compared to the reported values the presently detected concentrations of PCBs in samples collected from Rio Uruguay can be considered low and below all guideline values.

Table 5. Concentration of PCB in the river water from sampling areas A, B and C (see Fig 1) in Rio Uruguay in April 2014.

	Area A concentration ng/l	Area B concentration ng/l	Area C concentration ng/l
PCB 18	<0.11	<0.11	<0.11
PCB 28/31	<0.14	<0.14	<0.14
PCB 33	<0.11	<0.11	<0.11
PCB 47	<0.11	<0.11	<0.11
PCB 49	<0.057	<0.057	<0.057
PCB 51	<0.024	<0.024	<0.024
PCB 52	<0.12	<0.12	<0.12
PCB 60	<0.071	<0.071	<0.071
PCB 66	<0.11	<0.11	<0.11
PCB 74	<0.049	<0.049	<0.049
PCB 99	<0.018	<0.018	<0.018
PCB 101	<0.078	<0.078	<0.078
PCB 105	<0.015	<0.015	<0.015
PCB 110	<0.12	<0.12	<0.12
PCB 114	<0.0023	<0.0028	<0.0021
PCB 118	<0.042	<0.042	<0.042
PCB 122	<0.0023	<0.0029	<0.0022
PCB 123	<0.0026	<0.0034	<0.0027
PCB 128	<0.012	<0.012	<0.012
PCB 138	<0.082	<0.082	<0.082
PCB 141	<0.023	<0.023	<0.023
PCB 153	<0.091	<0.091	<0.091
PCB 156	<0.0060	<0.0060	<0.0060
PCB 157	<0.0015	<0.0018	0.0015
PCB 167	<0.0037	<0.0037	<0.0037
PCB 170	<0.025	<0.025	<0.025
PCB 180	<0.045	<0.045	<0.045
PCB 183	<0.013	<0.013	<0.013
PCB 187	<0.021	<0.021	<0.021
PCB 189	<0.0021	<0.0021	<0.0021
PCB 194	<0.0066	<0.0066	<0.0066
PCB 206	<0.0049	<0.0053	<0.0045
PCB 209	<0.0043	<0.0044	<0.0048
Sum of all	1.5	1.5	1.5
In total pg WHO$_{2005}$ TEQ/	0.017	0.017	0.020

3.2 FISH BILE

In this monitoring study bile samples were only taken from the indicator species Bagre trompudo caught from all study areas. Consistent with the small amounts of chlorophenolic compounds in the river water, the concentration of chlorophenolic compounds in the bile samples were also small (Fig. 2). Figure 2 shows the average concentration of chlorophenolic compounds in pooled bile samples from fish caught at different sampling areas (Table 2). Observe that the concentrations are given in nanograms per gram bile dry weight (ng/g d.w.). The main part of the chlorophenols were 2,3,6-trichlorophenols with lower amounts of tetrachlorophenols and in samples analysed from fish caught at each study area (Appendix 2). Chlorocatecoles were observed in the bile of fish caught from areas A and B. Small amounts of chloroguajacoles were present in samples collected from area A and trchlorosyringoles were this time present in all bile samples. The concentrations measured in the fish bile did not, however, correlate with the concentrations observed in the river water, which were very low and consisted only of 2,3,6-trichlorophenols.

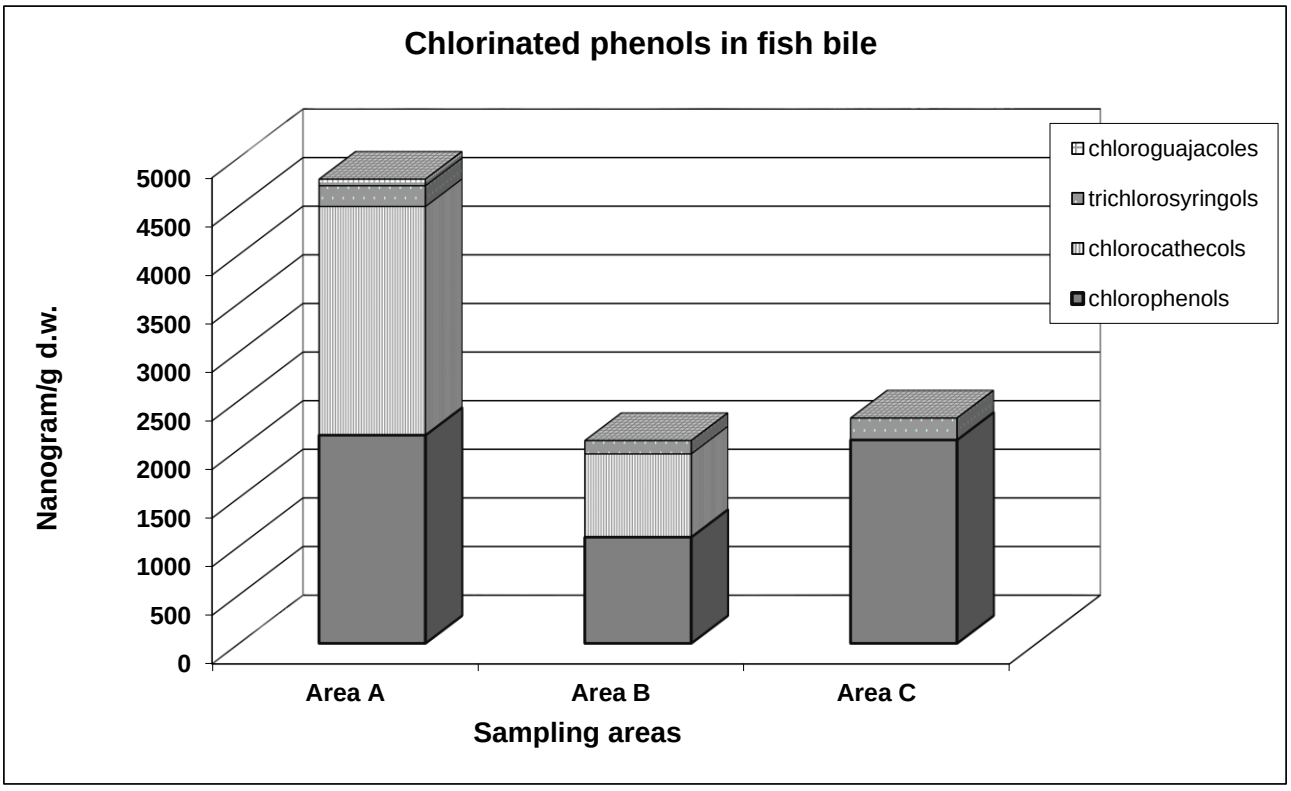

Figure 2. The average concentration of chlorophenolic compounds in the pooled bile samples of Bagre trompudo caught at the different sampling areas in April 2014.

The natural background levels of chlorinated phenolic substances in fish bile in Nordic waters is about 1-10 µg/g dry weight of bile. Based on this information, the concentrations (ng/g) of chlorophenolic compounds in the bile of fish obtained from all sampling areas in Rio Uruguay were at the same Nordic background levels as 1000 ng is the same as 1 µg. The highest concentration of chlorophenols was analysed from Bagre trompudos caught from the reference area (Fig. 2).

During the present study recin acids could be detected in all pooled bile samples analysed from Bagre trompudos caught from all study areas (Fig.3).

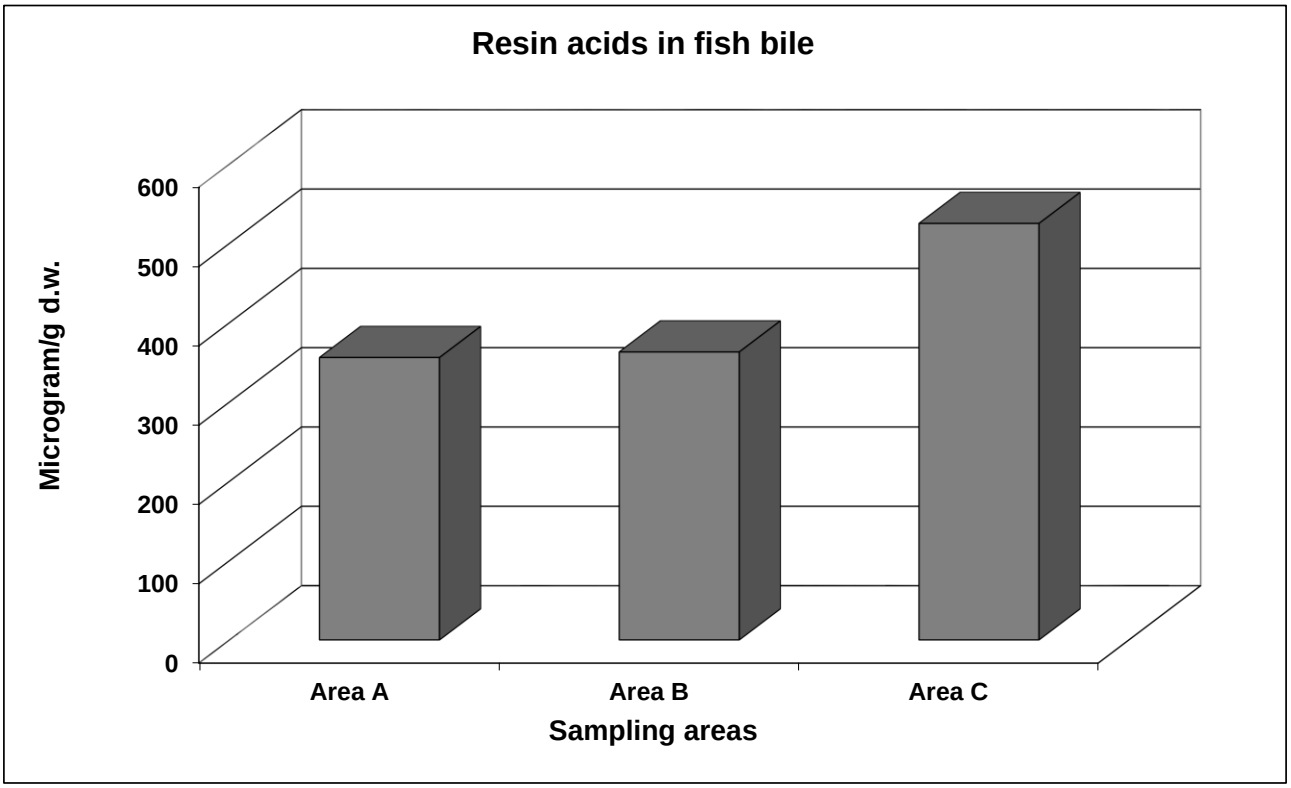

Figure 3. The average concentration of resin acids in the pooled samples of Bagre trompudo caught at different study areas in April 2014.

Most of the resin acids observed in the fish bile from all study areas were abietic and dehydroabietic acids (Appendix 2). Dehydroabietic acid was also analysed from the river water at the different sampling sites but there was no correlation between the concentrations of river water and fish bile. At area C where the river water concentration on recin acids was the lowest (Table 3) the fish bile concentration was highest. In previous monitoring studies the bile concentrations of recin acids analysed from fish have varied between few micrograms to more than 3 000 micrograms (Tana 2012).

The average bile concentrations of phytosterols in fish caught from different study areas are presented in Figure 4.

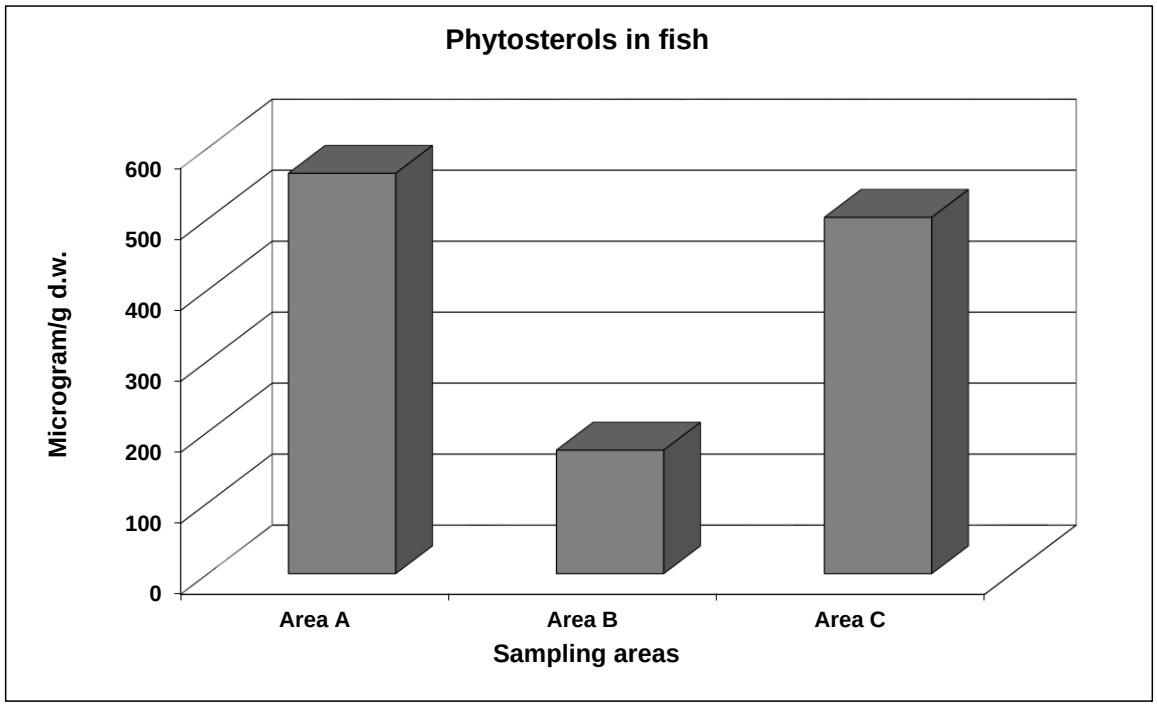

Figure 4. The concentration (µg/g dry weight, d.w.) of phytosterols in pooled bile sample of Bagre trompudo caught at different sampling areas in April 2014.

The highest average phytosterol concentrations were measured from fish caught from Area A (Fig 4.). At this time there was correlation between the bile and river water concentrations of phytosterols as the highest river water concentrations of phytosterols were also analysed from area A (Table 3). The main intake of phytosterols in fish, however, is via food as has been indicated in the previous study periods (Tana 2013).

3.3 DIOXINS AND PCBS IN FISH MUSCLE

The muscle samples for analysis of dioxins and PCB were taken from the same Bagre trompudos as bile samples were taken (Table 2). The analytes were extracted from dried solid sample Soxhlet extraction apparatus with toluene/ethanol (85/15 v/v). Solvent was exchanged to hexane and fat % was determined gravimetrically. Sample was purified on a silica gel column, an activated carbon column and an activated alumina column.

^{13}C-labelled PCDD/PCDFs (altogether 16 standards) were used as internal standards to quantitate the amount of PCDDs/PCDFs. ^{12}C PCB 30 and ^{13}C-labelled PCB congeners (PCB 80, 101, 105, 114, 118, 123, 128, 138, 153, 156, 157, 167, 170, 180, 189, 194 and 209) and ^{13}C-labelled non-*ortho*-(co-PCB) congeners (PCB 77, 81, 126 and 169) were used as internal standards for PCBs and co-planar PCBs. The quantification of PCDD/PCDF and PCB congeners was performed by selective ion recording using a HP6890/Autospec Ultima mass spectrometer (resolution 10 000) with column DB-5 MS (60 m, ID 0.25 nm, 0.25 µm). The analytical method is accredited.

Limits of quantification for individual PCDD/PCDF compounds were 0.16 – 3.2 pg/g fat. The limits of quantification of individual non-*ortho*-PCB congeners ranged between 0.41- 1700 pg/g fat.

Expanded measurement uncertainty of results:

When WHO-TEQ for PCDD/PCDFs is
< 0.5 pg/g fat the uncertainty of analysis is ± 50%
0.5-5 pg/g fat the uncertainty of analysis is ± 30%
>5 pg/g fat the uncertainty of analysis is ± 20%

When WHO-TEQ of PCBs is
<0.5 pg/g fat the uncertainty of the results is ± 50%
0.5-5 pg/g fat the uncertainty of the results is ± 30%
>5 pg/g fat the uncertainty of the results is ± 20%

The results are presented in Table 6 and Appendix 4. In all samples the total amount of dioxins was below 1 pg/g fresh weight at areas A and B and below 4 ng/g at area C, and the concentration of the most toxic dioxin 2378-TCDD was below 0.01 pg/g fresh weight in analysed fish from all areas. The PCB sum values varied between 2.3 and 4.2 ng/g fresh weight (f.w.) being lowest in fish from area B.

EU Council has set maximum levels for certain contaminants in foodstuffs in Council Regulation 1259/2011. Maximum level of PCDD/PCDF in fish and fish products was set to 3.5 pg WHO-PCDD/F-TEQ (2005)/g fresh weight. PCBs were added into maximum levels in this regulation and combined maximum level of PCDD/PCDF and PCB was set to 6.5 pg WHO-PCDD/F-PCB- TEQ

(2005)/g fresh weight. These values are based on upper bound results. So far there are no special limit values for dioxins and PCBs in Uruguay, but the WHO recommendations are followed.

All the studied samples had WHO-PCDD/F-TEQ and combined WHO-PCDD/F-PCB-TEQ upper bound values below the maximum limit values set in the Council Regulation 1259/2011. The recommended Total Daily Intake (TDI) by European Union is 1-4 pg/kg body weight and this includes about 10-fold safety margin. Based on this recommendation there would be no limitation to human consumption of the studied fish.

The dioxin concentration were at the same low level as previously observed during the baseline fish studies from April 2005 to April 2007 and the monitoring studies from December 2007 to December 2012 (Tana 2012). In all studies the observed dioxin concentrations have been far below the recommended limit values. So far these studies have also been the only ones available on dioxin concentrations in fish from Rio Uruguay. During this study there were no significant differences between muscle dioxin concentrations in Bagre trompudos caught from the three different study areas.

Table 6. Concentration of polychlorinated dibenzo-dioxins and – furans (WHO-TEQ pg/g, fresh weight) and PCB (WHO-TEQ pg/g, fresh weight) in the muscle of Bagre trompudo caught from areas A, B and C (see Fig 1) in Rio Uruguay in April 2014.

	Area A		**Area B**		**Area C**	
	Dioxins	**PCB**	**Dioxins**	**PCB**	**Dioxins**	**PCB**
Unit	WHO-TEQ, pg/g fw	WHO-TEQ, pg/g fw	WHO-TEQ, pg/g fw	WHO-TEQ, pg/g fw	WHO-TEQ, pg/g fw	WHO-TEQ, pg/g fw
Bagre trompudo	0.10	0.095	0.077	0.080	0.13	0.16

As a comparison to concentrations presented in Table 6, dioxin and PCB concentrations expressed as WHO-TEQ pg/g fresh weight have varied between 0.061-1.68 for dioxins and 0.051-1.46 for PCBs in different fish species from Finnish inland waters (Hallikainen et al. 2004).

3.4 ANALYSIS OF MERCURY (HG) AND LEAD (PB) IN FISH MUSCLE

Mercury (Hg) and lead (Pb) concentrations were analysed from the muscle of Bagre trompudos caught from all study areas (Table 2). The composite samples were from the same fish individuals as from where the samples for dioxin and PCB analysis were collected. The samples were transported frozen to accredited laboratory of Eurofins for analysis of lead and mercury.

Heavy metals are analysed after microwave digestion. The sample preparation is made according to §64 LFGB L 00.00-19/1. Food stuff samples like fish muscle are mixed with 6 ml of nitric acid, followed by a microwave digestion (maximum temperature:235 °C) for 90 to 120 minutes in order to obtain a clear, liquid sample. After digestion samples will be diluted with water to obtain 25 ml.

Mercury was analysed by Atomic Absorption Spectrophotometer (AAS). Reduction of mercury compounds to mercury (Hg) with Tin(II)Chloride and then analysis of the mercury vapour with AAS according to LFGB L 00.00-19/4.

Lead was analysed by ICP-MS. The Analysis of liquid samples was made by mass spectrometry and ionisation by inductively coupled plasma (ICP-MS) according to EN 15763:2009.

The results are presented in Table 7 and Appendix 5.

Table 7. The concentration (mg/kg d.w., dry weight) of mercury (Hg) and lead (Pb) in the muscle of Bagre trompudo caught from the three study areas in April 2014.

	Area A		Area B		Area C	
	Mercury	**Lead**	**Mercury**	**Lead**	**Mercury**	**Lead**
Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Bagre trompudo	0.32	<0.05	0.28	<0.05	0.14	<0.05

The concentrations of mercury were low in all analysed samples and below the EU-limit values (0.5 mg/kg) (EU directive 466/2001). The concentrations of lead were also below the detection limit and below EU-limit values in all samples from all study areas.

3.5 EOX IN MUSSELS

In order to detect the possible amount of chloro-organic compounds measured as Extractable Organic Halogens (EOX) in benthos, samples have been collected from benthic fauna. For this purpose, *Limnoperna fortunei*, a mussel species rich in abundance and a dominant species in the benthic community was chosen as a sample species. The mussels were sampled during the test fishing period by collecting mussels attached to the stones within 1-2 m depth at the study areas. The mussel samples were frozen after sampling and kept frozen until analysis, which were made in the accredited laboratory of Eurofins. Determination of extractable organic halogens (EOX) was analysed by method DIN 38414 (S17). The concept of this method is to release the hydrohalogens out of the halocompounds by burning of the sample extracts. The determination is done by micro coulometric titration. The extraction to separate the organic halogens is done with a non-polar solvent (n-hexane) by reflux. After that the extract is brought into a high temperature oven. The build gases were absorbed in a coulometric cell and measured there.

The results, including the sampling time, are presented in Table 8 and Appendix 6.

Table 8. The concentration of Extractable Organic Halogens (EOX) in mussel samples collected from the fish study areas in Rio Uruguay in April 2014.

	EOX, µg/g, dw*	Sampling time
Method	DIN 38414 (S17)	
Sample area		
A	<1	26.4.2014
B	11	27.4.2014
C	8.7	27.4.2013

*dw = dry weight

As presented in the Table 8 the EOX concentrations were at the same level at the recipient areas (Area B and C) but higher as compared to the reference area (area B). In mussels collected from the reference area the EOX concentration was below detection limit. The limited information of EOX concentrations in the benthic fauna of Rio Uruguay restricts to analysis observations from the baseline and monitoring studies of UPM S.A pulp mill environmental monitoring program. In these studies the EOX concentrations measured from *Limnoperna fortunei* have varied between a concentration below the detection limit (1 µg/g) and 22 µg/g.

The EOX concentrations analysed during the present study were at the range observed within the previous studies from all study areas.

In a study from Ninety Mile Beach, Victoria, Australia mussels were studied to assess water column concentrations of extractable organohalogens (EOX). In this study the background tissue EOX concentrations in mussels ranged from 16 to 69 µg/g (Haynes et al. 1995). This is at a higher level than the EOX concentrations observed in the present study in mussels collected from Rio Uruguay.

4 DISCUSSION

The present study is the fourteenth monitoring study on fish exposure performed after the start up of the new UPM pulp mill in Fray Bentos. During 2005, 2006 and 2007 similar studies were performed as part of the baseline studies. One of the main objectives of the baseline studies, which were the first ones performed in Rio Uruguay, was to collect background data on pulp mill related substances such as chlorophenolic compounds, resin acids and phytosterols in river water and fish bile from different parts of Rio Uruguay at the affect area of the future mill. The studies also included analysis of dioxins and PCB from river water and fish muscle.

At present, when four (4) baseline study periods and eleven (13) monitoring studies have been completed, there is information of dioxin concentrations from six (6-7) different fish species and the information of bile concentrations include analysis from twelve (12) different fish species. The information is still limited as compared to more than hundred different fish species identified in the river, but gives good indications of the concentration levels and their yearly variation at the study areas. The information includes analysis from both local and migrating fish species, especially those fish species which commonly are present in the test fishing catches. There is at present also longer series (13 monitoring and 3 baseline periods samplings) of information of the concentrations analysed from the indicator species Bagre trompudo (*Iheringichthys labrosus*).

The river water concentrations of AOX, chlorophenolic compounds, resin acids, phytosterols and dioxins can in general be considered as low (Table 9). Table 9 presents the concentrations analysed during the present study and the variation of background concentrations observed during the baseline studies from years 2005-2007 prior to mill operation.

Table 9. The concentration of AOX, chlorophenolic compounds (Cl-P), resin acids (RA), phytosterols and dioxins (PCDD/PCDF) in river water sampled in April 2014 at different sampling areas and the concentration range observed during baseline studies 2005-2007.

Area	AOX*	Cl-P	RA#	Phytosterols#	PCDD/PCDF
Unit	µg/l	µg/l	µg/l	µg/l	WHO-TEQ pg/l
	April 2014 /background	April 2014 /background	April 2014 /background	April 2014 /background	April 2014 /background
A	**10** / 8-11	**0.044** /0.089-0.104	**193** /27-224	**109** /ND-22	**0.77** /0.00-0.60
B	**10** / 6-12	**0.049** /0.080-0.114	**158** /6-183	**0** /ND-2	**0.66** /0.00-0.89
C	**10** / <5-12	**0.045** /0.089-0.185	**89** /3-202	**41** /ND-8	**0.78** /0.00-0.96

*detection limit 5 µg/l
detection limit 1-3 µg/l

The AOX concentrations observed in April 2013 were at the same level as compared to concentrations analyzed within baseline. Usually the AOX concentrations at study areas have varied between 5 and 12 µg/l throughout the studies between April 2005 and December 2013. The higher AOX values during the last study periods in 2012 and 2013 and observed at all study areas were observed in the present study to be at normal average values previously analysed from Rio Uruguay.

The concentrations of chlorinated phenols (Cl-P) were very low in April 2014 at all study areas and lower as compared to baseline study levels. During monitoring studies (not showed in Table 9) since December 2007 the concentration of chlorinated phenols in river water have varied at area A between 0.161-1.052 µg/l, at area B between 0.120-0.688 µg/l and at area C between 0.103-0.417 µg/l. Thus the Cl-P concentrations of river water in April 2014 were at significantly lower level as compared to previous monitoring studies since November 2007.

The concentrations of phytosterols like sitosterol, campesterol and sitostanol were detected at all study areas in April 2014 and the levels were higher at areas A and C as compared to the results from baseline studies. Resin acid concentrations analysed from study areas during April 2014 were at the average level observed during the whole study periods since 2005.

The concentration of polychloro dibenzo-p-dioxins (PCDD) and polychloro dibenzofurans (PCDF) observed in this study from April 2014 were at the same level at all study areas and at the same level as compared to the baseline studies. The dioxin congeners measured above the detection limit have mostly been octachloro dibenzo-p-dioxins or heptachloro dibenzo-p-dioxins, which are the least toxic of the different dioxin congeners. The most toxic dioxin congener, 2,3,7,8-tetrachloro dibenzo-p-dioxin (TCDD) have been measured only once above the detection limit (0.16 pg/l) and this was at

the reference area (Area A) prior to mill operation (April 2005). PCB concentrations analysed from the river water were all at a low level.

The amount of chlorophenolic compounds, resin acids and phytosterols in fish bile is presented as average concentrations of different fish species in order to give a general picture of the situation at each sampling area (Fig. 5). However, it must be kept in mind, that there is a great variation in bile concentrations between different fish species both in time and space, and depending on the feeding and living habits of each species.

The fish bile concentrations of chlorophenolic compounds (Cl-P) can be considered low and consist mainly of chlorophenols. In this group the most abundant have been 2,4-dichlorophenols, 2,4,6-trichlorophenols and 2,3,6-trichlorophenols, which have been demonstrated to be ubiquitous in humus rich waters and formed by the action of micro-organisms (Grimvall et al. 1994). The average concentration of chlorinated phenols measured from fish bile in April 2014 was significantly higher especially at the reference area (Area A) as compared to the previous monitoring periods and even higher as the average level observed during the baseline studies. Concentrations of chlorophenolic substances in bile of fish (Bagre trompudo) caught from recipient areas (Areas B and C) were within the limit values observed during the baseline studies.

Generally the concentrations of chlorinated phenols during these monitoring studies have stayed within the levels measured in the baseline studies and the variation between study periods can be considered of natural bases. The concentration of the chlorinated phenols in the bile of fish during the whole monitoring study period between November 2007 and April 2014 have been on levels where no toxic effects are expected. Chlorinated phenols dominating in the bile and river water can also be considered as natural origin in humus rich waters.

The resin acid concentrations have varied at a level which in Scandinavian water courses is considered as natural background levels. During the fourteen monitoring studies since the start up of the UPM pulp mill the concentrations of resin acids analysed from the bile of fish caught from Rio Uruguay have been observed to be at the same level. The present study showed continuously low levels and the concentrations were below the detection limit.

The measured concentrations have also been within the variation limits observed during the baseline studies prior to the mill operation (Fig 5.) with the exception of December 2009 with extremely exceptional flow conditions in the river. As the concentrations in December 2009 were higher in fish from all study areas the source must have been diffuse rather than a point source. The mill is using eucalypt as raw material and resin acids have not been detected in this tree species (Hillis 1991) indicating that the mill effluent would not be a source of resin acids

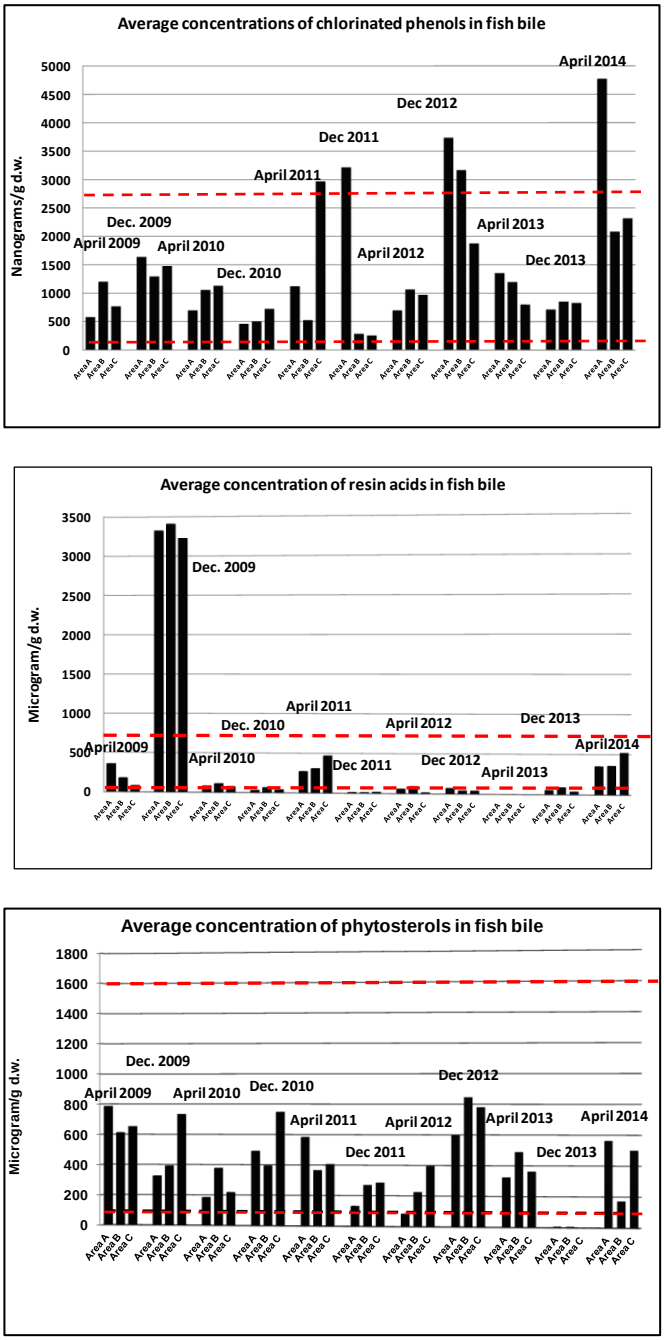

Figure 5 Average concentration of chlorophenolic compounds, resin acids and phytosterols in bile of fish caught from different sampling areas during monitoring studies. The dotted lines describe the variation observed in baseline studies.

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The phytosterol concentrations in fish bile observed during April 2014 studies were at the average level as compared to the previous monitoring studies. The higher water concentrations (Table 3) of phytosterols indicate the first time that there could be a correlation between the water and bile concentration, but this cannot be verified based on one study result. Otherwise the feeding habit of the sampled fish plays a significant role in the phytosterol intake and the fish will get the phytosterols via food. This hypothesis is strengthened by the fact the bottom living fish species generally show higher bile concentrations of phytosterols. The phenomena have also been observed in the monitoring studies.

Table 10. The concentration of polychlorinated dibenzo-dioxins and –furans (WHO-TEQ pg/g fresh weight) and PCB (WHO-TEQ pg/g fresh weight) in the muscle of Bagre trompudo (*Iheringichthys labrosus*) caught from study areas in April 2013. Numbers within parenthesis describe the background levels observed in fish during the baseline studies in 2005 – 2007.

Area/Fish species	**PCDD/F**	**PCB**
Bagra trompudo (*Iheringichthys labrosus*)	**WHO_{2005} TEQ pg/g f.w.**	**WHO_{2005} TEQ pg/g f.w.**
Area A	**0.10** (0.11-0.39)	**0.095** (0.19-0.22)
Area B	**0.077** (0.08-1.01)	**0.080** (0.28-2.1)
Area C	**0.13** (0.19-0.49)	**0.16** (0.28-0.51)

The concentrations of dioxins and PCBs analysed from muscle of Bagre trompudos during the April 2014 sampling period were low and at the lower concentration level as observed in fish muscles during the baseline studies in 2005-2007 prior to the start-up of the UPM pulp mill (Table 10). The results indicate also that the concentration of dioxins and PCB are significantly below the recommended international guideline values and maximum levels set for certain contaminants in foodstuff.

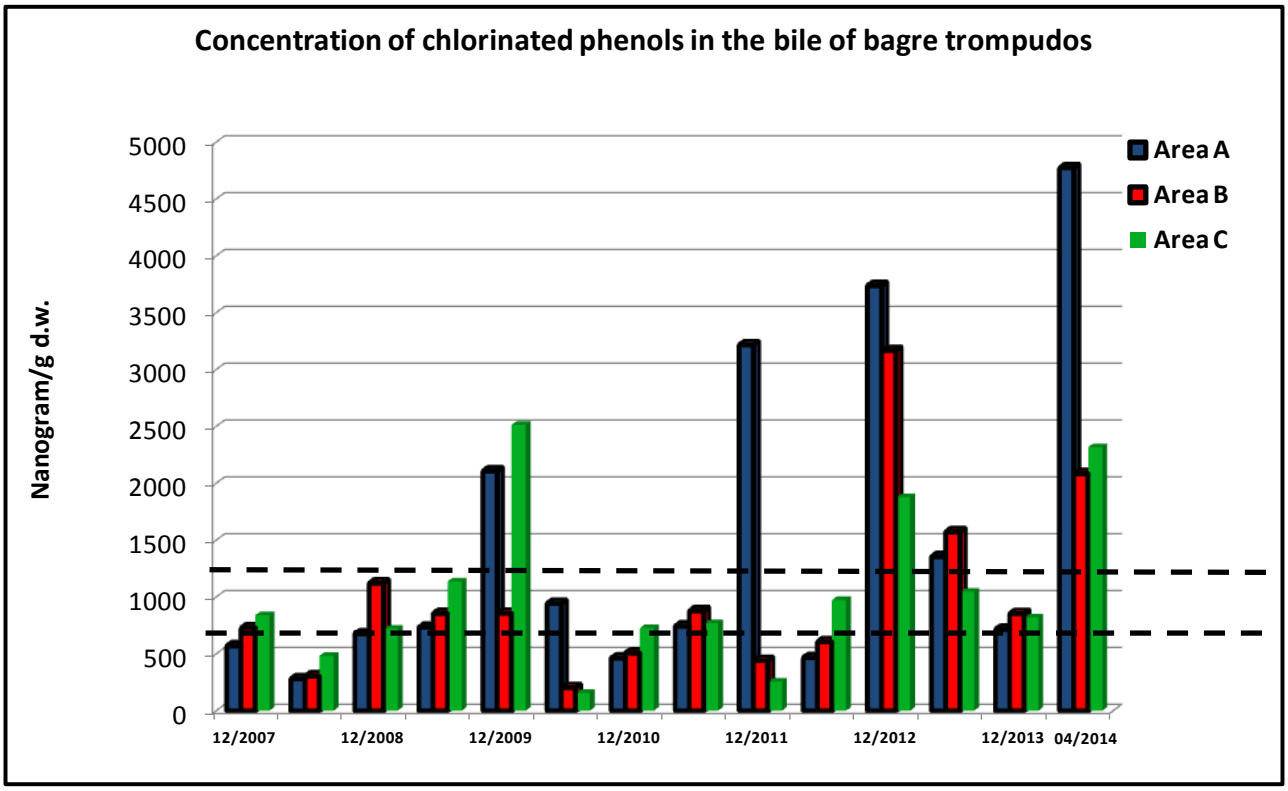

Figure 6. The concentration of chlorophenolic compounds analysed from the bile of Bagre trompudo (*Iheringichthys labrosus*) during the monitoring studies between December 2007 and April 2014. The dotted lines describe the variation observed in baseline studies in 2005 - 2007.

The concentration of chlorinated phenols in the bile of the indicator species Bagre trompudo (*Iheringichthys labrosus*) and analysed during the thirteen monitoring studies is presented in Figure 6. The dotted lines in Figure 6 describe the variation of chlorinated phenols analysed from the bile of Bagre trompudos during baseline studies. The results presented in Figure 6 show that the concentration of chlorinated phenols in the bile of Bagre trompudos caught from the reference and the near recipient areas (Areas A and B) were elevated in April 2014 an among the highest as compared to the previous monitoring periods since mill start up in December 2007. The chlorinated phenols analysed in the bile samples in April 2014 consisted both of 2,3,6-trichlorophenols and 2,3,4,6-tetrachlorophenols which have been shown to be of natural origin or degradation products of more chlorine atoms including substances. In April 2014 the chlorophenolic substances included also chlorocathecols and trichlorosyringoles, which previously have been more seldom in the fish bile samples. The presence of chlorocathecols and trichlorosyringoles in the bile samples is unknown for the time being and do not correlate with the chlorophenolic substances observed in the water samples of Rio Uruguay collected at the same areas as the fish were caught.

In all the monitoring studies no correlation between the river water concentration of chlorinated phenols and the concentration analysed from the bile of Bagre trompudos have been observed. Interesting observation has also been that higher concentrations of chlorophenolic compounds tend to be detected during the December sampling as compared to April sampling. However, the present sampling period makes an exception in this respect. Still, the detected chlorinated substances (chlorophenols, chloroguajacols, chlorocathecols) are not always the same.

5 CONCLUDING REMARKS

The present aquatic environmental evaluation was the fourteenth study after the start-up of the greenfield UPM pulp mill. Pulp mill effluent related substances such as chlorophenolic compounds, resin acids and phytosterols in the bile of fish captured from different parts of Rio Uruguay were analysed. In addition dioxin and PCB concentrations were studied from river water and fish muscle samples, mercury and lead from fish muscle and Extractable Organic Halogens (EOX) from mussels.

The concentrations of chlorinated phenols and dioxins observed during the April 2014 monitoring in the river water from all study areas were all at a low and same level as observed during the baseline studies. Altogether the concentrations of organochlorines in Rio Uruguay are low and far below all limit and regulation values, and mainly at the same level as natural background concentrations measured in Scandinavian watercourses.

The fish bile investigations indicate that the concentrations of 2,4-dichlorophenol and 2,3,6-trichlorophenols dominate the concentration of other chlorophenolic compounds in indicator species Bagre trompudo at all three study areas. Other chlorophenolic compounds, resin acids and phytosterols are within the variation limits as observed during the baseline studies and there are no indications of changes in the concentration levels, caused by the effluent discharged from the UPM pulp mill. This consideration is strengthen by the fact that most elevations and variations are observed from the reference area effluent from the pulp mill is not present. The resin acid concentrations analysed from the fish bile during this study period in April 2014 continued to stay at levels as previously observed within the baseline studies at all three study areas. The measured dioxin, furan and PCB concentrations in river water were all at very low level. Muscle concentrations of dioxins, furans and PCBs were below the Total Daily Intake recommendations and, based on the observed concentrations and international recommendations there would be no limitations to human consumption of the studied fish.

Mercury and lead concentrations in fish muscle samples collected from the study areas were below the recommended levels.

Concentrations of Extractable Organic Halogens (EOX) in April 2014 were higher in mussel samples collected from the recipient area at Yaguarete estuary and outside Las Cañas as compared to the reference area outside Nuevo Berlin. However, no elevation in the EOX levels could be observed as compared to previous monitoring studies.

The results indicate that the effluent discharges from the UPM mill, which are far below the permit limit concentrations, have not caused any impacts to the river water concentrations of measured parameters or to the exposure level of fish as compared to the situation prior to the mill operation.

6 RECOMMENDATIONS

This was the fourteenth time fish community and fish exposure was studied after the mill start up in November 2007. The mill is continuously monitoring its outgoing effluent and the results have shown that the discharges are far below the permit and international standard limits. The fish exposure studies, with analysis of effluent related substances from the fish bile, reported so far since the start up of the UPM mill have not indicated any permanent differences or changes as compared to the situation prior to mill operation. As mentioned in the conclusions and based on the monitoring results the mill effluent discharge have not had any impact on the exposure situation of fish in Rio Uruguay. Therefore, as already recommended in the last monitoring report from December 2012, a stepwise decrease in the monitoring performances of fish and fish community could be initiated. The next fish monitoring will be performed in December 2014. The production process used by the mill and the treatment processes to decrease the effluent discharge and air emissions have proved and accepted to be the Best Available Technology (BAT). The scientific research on effects of effluents from modern mills like UPM mill and a dilution factor in the recipient like in Fray Bentos have neither indicated significant effects on aquatic environment. Based on these facts and the information from several years of monitoring studies in Rio Uruguay it is recommended that the monitoring program should be re-evaluated and a discussion on the decrease of the monitoring activities of fish communities started.

Monitoring studies are performed to be able to know what is happening, to be able to mitigate if effects are found and to be able to inform of possible impacts. In this respect the performed monitoring studies between 2007 and 2014 have been able to fulfil that demand.

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