In-depth chemical analysis and assessment of a broad range of liquid waste from *in-vitro* diagnostic instruments

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Abstract

Liquid waste from *in-vitro* diagnostic equipment for clinical chemistry and histology has been analyzed. A large set of environmentally relevant parameters was obtained including those commonly used in water analysis, such as pH, total organic carbon, metals, and nitrogen compounds. Additionally, a substantial set of other parameters was analyzed, i.e. parameters which are of particular interest either because they are typically used in *in-vitro* diagnostics or because they are of public concern for e.g. those substances listed on the European Chemical Agency's "Candidate List" as "Substances of very high concern". The obtained data can serve as a solid base for a comprehensive environmental risk assessment for chemicals used in *in-vitro* diagnostics. The objective of this paper is not to make advanced judgment on the ongoing environmental risk assessment. We however already compared the obtained measured concentrations with established reference values. Based on these results, it was found that the analyzed parameters are mostly below the available thresholds of concern.

Introduction

In-vitro diagnostic tests are essential in modern healthcare (Ref 1), necessary for the right choice of treatment and in particular for personalized medicine (Ref 2, 3). *In-vitro* tests are performed outside of the living body in a laboratory, for instance, for checking urine for the presence of glucose, or blood for signs of infections. Such *in-vitro* diagnostic tests are usually subsumed under the term "clinical chemistry". Other *in-vitro* techniques are used to examine tissue samples (tissue sections, "slides") for histological examination. Such tests we call hereinafter "histology".

Typically, the tests are run on professional instruments in laboratories. In order to minimize costs or for other reasons, the testing is often centralized in larger facilities. In some countries this is even required by legislation, for instance, in France (Ref 4).

As all *in-vitro* tests unavoidably involve biological material, the disposal of the latter is an intrinsic problem of the technology. Accordingly, the treatment of the biological waste from diagnostic methods has been a subject of technological development and regulatory measures. Biosafety issues of waste from *in-vitro* diagnostics are not dealt with in the present paper. Another aspect of the waste from *in-vitro* diagnostics, namely the presence of chemical contaminants in waste water, has only recently come into focus. Essentially all *in-vitro* methods require chemical reagents, which become part of the liquid waste upon performing their task. Normally, these chemical reagents and their transformation products still enter the waste water stream, if no separate collection system is established. Due to rinsing and cleaning routines during the normal operating cycles of the instruments, a dilution of the reactive reagents already takes place within the laboratory. This dilution is however also accompanied with the addition of certain other chemicals (e.g. surfactants) which are present in the rinsing and cleaning liquids.

Until now, essentially only calculation methods were applied to assess the environmental risks from the chemical reagents in liquid waste from diagnostic laboratories. These calculations were time consuming, laborious and delivered only approximate worst-case estimates.

To our best knowledge, this is the first time that an in-depth chemical analyses of liquid waste from a broad spectrum of *in-vitro* diagnostic methods was carried out to test a large set of ecologically relevant parameters. The measured parameters can now serve as a proper base for a comprehensive risk assessment of chemicals used in *in-vitro* diagnostics.

We are aware that in a regulatory context the term "liquid waste" is sometimes used for material which is separately collected and treated, e.g. incinerated. In the present publication, we, however, use the term for all liquids generated from the *in-vitro* diagnostic instrumentation in focus of our investigation. These liquids, at least the aqueous phase thereof, could also be called "waste water".

Methods

Sampling

Liquid waste generated by various diagnostic equipment (for details see below) was collected from different diagnostic facilities, such as labs in hospitals. The testing facilities (clinical and/or histological laboratories) were asked to deliver representative samples (ideally 7 liters) to be transported under cool conditions (4°C). The testing facilities were also asked to record the samples' physical aspect (e.g. color and homogeneity).

Special procedures of sample preparation for samples from histology

Samples from some of the histological instruments were biphasic and required therefore special pretreatment according to the specified analytical method. The organic phase was separated and not analyzed, because in practice the organic phase is sometimes collected separately from the aqueous

phase for incineration and thus not always of interest for an assessment of the risk for the aquatic environment

Analytical methods

Two sets of parameters were defined:

- "Standard" parameters: Parameters that are commonly used in water analysis (Ref 5, Ref 6, and Ref 7), such as pH, total organic carbon, metals, and nitrogen compounds.
- "Specific" parameters:
  - either hazardous chemicals used in the applied reagents,
  - or substances of general interest, for which the key selection criterion was that they are listed on the "Candidate List" of "Substances of Very High Concern" (SVHC) of the European Chemical Agency (Ref 8).

All analyzed parameters are shown in Table 1 and 2.

For testing standard effluent parameters, the analytical methods used in this study were, whenever possible, applied according to the Advisory Leaflet M 115-2 of the German Association for Water, Wastewater and Waste (Ref 5). For some more specific parameters, HPLC-MS or GC-MS methods were developed. For more details see Tab. 1 and 2.

In-vitro diagnostic equipment monitored

The waste analyzed in the present study was generated by the following instruments manufactured by Roche Diagnostics:

a) Used in clinical chemistry

- Cobas® Integra 400plus/800
- Cobas® c6001/c8000 c501/c502
- Cobas® c6000/c8000 e601/e602
- Cobas® c6000/c8000 c701
- Cobas® 8800 and
- Cobas® 6800.

Cobas® equipment (Ref 9) allows the running of a large number of samples a day. Its application consolidates clinical chemistry including substrates, enzymes, electrolytes, specific proteins, therapeutic drug monitoring, drug abuse testing, etc.

b) Used in histology:

- BenchMark® Ultra ISH: Instrumentation for full automation of in-situ hybridization tissue stains (Ref 10, 11),
- BenchMark® Ultra IHC (immunohistochemistry): a fully automated staining equipment used in cancer diagnostic (Ref 10, 12, 13, 14).

The possible techniques are all slide preparation steps of immunehistochemistry (IHC), fluorescent, in-situ hybridization (ISH), Silver in-situ hybridization (SISH) dual stain and FITC (fluorescence) slide processing and titration.

Evaluation of the obtained results

In our result section the obtained measured values were contrasted to certain reference values. The reference values for the standard parameters were taken from the Advisory Leaflet M 115-2 of the German Association for Water, Wastewater and Waste (Ref 5). The reference values of specific parameters were obtained from the substance registration dossiers published on the webpage of the European Chemicals Agency. We found there either a No-Observed-Effect Concentration (NOEC) or a Predicted No Effect Concentration (PNEC). No reference values for specific parameters were found for the few substances not yet registered.

Table 1: "Standard" parameters analyzed in the present study with a reference to the applied method as described in the text

"Standard" parameter	**Test method**
Total organic carbon (TOC)	EN-1484
Biochemical oxygen demand (BOD)	EN-1899-1
Chemical oxygen demand (COD)	EN-6633
pH	DIN 38404-5
Settleable solids	DIN 38409-9
Semi volatile lipophilic substances	DIN 38409-56
Hydrocarbon index	ISO 9377-2 (mineral Oil)
Adsorbable organically bound halogens AOX	ISO 9562
Volatile halogenated hydrocarbons	ISO 10301
Phenol index, steam volatile	DIN 38404
Halogen free organic solvents	DIN 38407
Antimony	ISO-15587-1
Arsenic	ISO 17294-2
Lead	ISO 17294-2
Cadmium	ISO 17294-2
Chromium	ISO 17294-2
Chromium VI	ISO 17294-2
Cobalt	ISO 17294-2
Copper	ISO 17294-2
Nickel	ISO 17294-2
Mercury	ISO 17294-2
Silver	ICP-MS
Tin	ISO-16772
Zinc	ISO 17294-2
Nitrogen produced from ammonia and ammonium	ISO-11732
Nitrogen produced from nitrite	ISO-10304-2
Easily released cyanides	ISO-14403-1
Sulfate	ISO-10304-2
Easily released sulfide	USEPA1 Methylene blue
Fluoride solved	ISO-10304-2
Total phosphorous (ICP)	ISO-11885

[1] Excluding 1,2-Dichloroethane

Results

We defined a set of parameters that are commonly used in water analysis (Ref 5, 6), among these are typical water parameters such as pH, total organic carbon, metals, and nitrogen compounds. These parameters are called "standard" parameters in this paper. We also identified "specific" parameters. The latter are chemicals found in the applied reagents and are considered as substances of specific interest in terms of their

potential adverse environmental impact. Other parameters in the used reagents were excluded from the analysis, because they are easily biodegradable and/or not critical for the environment such as for instance monoclonal antibodies.

Table 2: "Specific" parameters analyzed in the present study with a reference to the applied method and the reference value as described in the text.

"Specific" parameters		Test method	Reference value	Comment
4-Nonylphenol and homologues		HPLC-MS-MS	Between 0.1 and 10 µg/l	NOEC (*Crassostrea gigas* 48-72 h) supporting document for the candidate list
Octylphenol ethoxylate and homologues		HPLC-MS-MS	0.1 µg/l of Octylphenol in surface freshwater	Internal memo of F. Hoffmann-La Roche, Ltd.
Phthalates	DEHP CAS Nr. 117-81-7	HPLC-MS-MS	NOEC (90 d) ≥ 0.502 mg/l, freshwater, *Oncorhynchus mykiss*	Only those phthalates were selected which are already registered according to REACH
	Diisobutyl phthalate CAS Nr. 84-69-5		PNEC (freshwater) = 0.001 mg/l	
	BBP CAS Nr. 85-68-7		PNEC (freshwater) = 7.5 µg/l	
	DBP CAS Nr. 84-74-2		PNEC (freshwater) = 10 µg/l	
Formamide		HPLC-MS	125 mg/l	NOEC (72 h, *Desmodesmus subspicatus*)
Formaldehyde		DNPH derivatization and LC-UV	0.47 mg/l	PNEC (freshwater)
Acrylamide		HPLC-MS	60 mg/l	NOEC (48 h, *Daphnia magna*)
Ethylenediaminetetraacetid acid and salts		GC-MS	2.2 mg/l	PNEC (freshwater)
1-Methylpyrrolidin-2-on		HPLC-MS	500 mg/l	NOEC (96 h, *Oncorhynchus mykiss*)
Sodium azide		Ion chromatography	5 mg/l	NOEC (24 h, *Ptychocheilus oregonensis*)
Basic violet 3		HPLC-MS		No value available
Congo red		HPLC-MS		No value available
2-Anthracenesulfonic acid, 4-amino-9,10-dihydro-1,3-dihydroxy-9,10-dioxo-, monosodium salt		HPLC-MS		No value available

Another selection criterion for the "specific" substances was whether they are listed on the Candidate List of Substances of Very High Concern (SVHC) of the European Chemical Agency (Ref 8). All analyzed parameters are shown in Table 1 and 2. Biological activity and radioactivity were not analyzed. The former because it was beyond the scope of this study, the latter because radioactive material or reagents are not used in the assays which produced the waste analyzed in this study. Some of the substances (standard or specific) were included into the analysis although we did not expect them to be present based on the chemical composition of the used reagents. We included them anyway because they are (scientifically justified or not) frequently inquired by the authorities or by concerned users of the *in-vitro* diagnostic equipment.

The results of these measurements are shown in Table 3 and 4. The spreadsheet displays all single values, the maximal observed concentration, and, if available or applicable, a reference value.

Due to the difference in chemistry, the waste samples from histology differ significantly from those generated in clinical chemistry. The most striking difference is the high total organic carbon (TOC) value, which does not per se constitute an environmental problem, as the total volume of the generated liquid waste is low. The higher TOC content of the histology samples also manifests in a higher content of semi volatile lipophilic substances and a higher hydrocarbon index. Expectedly, in both types of samples, histological and clinical, no elevated levels of volatile halogenated substances were detected, as these substances are not used as reagents. In

none of the clinical samples were settleable solids detected whereas all samples from the histology had a small content of settleable substances.
It is important to understand that the composition of the liquid waste does not only depend on the type of the instrument used, but also on the operation modus (working regimen) applied. Therefore, we tried to cover a range of instruments and regimens, so that substance-specific worst cases could be identified.

In the spreadsheets 3 and 4 the maximal measured value are opposed to reference values, which were taken from Advisory Leaflet M 115-2 ("Merkblatt DWA-M 115-2") (Ref 5) of the German Association for Water, Wastewater and Waste. These reference values serve as a measure to determine whether a discharge of non-domestic effluents into a public sewage system is of concern. Unfortunately, for quite a number of the measured substances, the Advisory Leaflet has no reference values. Therefore, we consulted the REACH registrations (REACH is the European regulation (EC) No 1907/2006.) of those substances, if the substance had already been registered. The registration dossiers contain among other things results from eco-toxicological studies and are published on the internet page of the European Chemical Agency (ECHA) (Ref 15). Due to regulatory and other reasons, not all substances of interest in the context of the present paper have already a REACH registration and not all registration have exactly the same data-set. For that reason, we sometimes had to use the PNEC (predicted no effect concentration) and in other cases the NOEC (No Observed Effect Concentration). For some of the substances, we could not find a reference value.

Discussion

To our best knowledge, this is the first time that liquid waste which is generated by *in-vitro* diagnostic equipment has been analyzed in depth and is being made available to the public. We are also aware about some weaknesses of the presented data. Above all, the aim of the present study is to reveal the composition of liquid waste from instruments of only one (although the largest) manufacturer of *in-vitro* diagnostics. Some of the features might be common for most of modern *in-vitro* diagnostics, other features might be not. It is in the individual responsibility of each manufacturer to ensure that the intended use of the substances in the supplied instruments will not cause harm to the environment.

We are also well aware that our selection of the analyzed parameters especially those, which we called "specific", has been somewhat arbitrary. On the other hand, the selection is based on the SVHC list which is based on verifiable scientific and other criteria (Ref 16). Some of the substances were measured not because we expected them to be present in relevant amounts, but for the purpose to document the absence of them. Such documentation helps to satisfy enquiries from customers or authorities which are not related to the specific product, but are triggered by general concerns. The composition of the liquid waste depends to some degree on the performed tests during the cycles. However this variability for discharges has always stayed within the limits of the substances in question.

The analyzed liquid waste differs strongly depending on whether the sample originates from clinical chemistry or histology techniques, which are based on completely different chemistries. The main difference (from the ecological point of view) is that in histology an organic phase occurs which leads to a significantly higher TOC, which per se and in particular in the small volumes generated is not an ecological problem.

The liquid waste generated by histological equipment is sometimes collected and treated for hydrocarbons removal and pH balance. The waste producing sites may choose to collect and separate waste while others will choose to dispose of the effluent in its entirety. Review of the waste profile will inform the decision at each laboratory installation site.

Obviously, also the composition of both waste types differences greatly especially if we consider the semi volatile lipophilic substances (over 47'000 mg/l for histological samples compared to max. 420 mg/l in clinical-chemistry samples) and the hydrocarbon index (about 14900 mg/l for histological samples against max. 7.9 mg/l in clinical-chemistry samples). Due to the typical biochemical reactions and the other typical features of clinical chemistry, most of the studied chemicals are in small amounts in the different samples generated by clinical chemistry equipment (Cobas® instruments).

We are aware that from the comparison of the measured maximal concentration of a substance with the indicated reference value it cannot necessarily be concluded that the use of this substance is safe for the aquatic environment or not. Reference values can differ depending on various national or local standards or requirements and it is up to the operator of the *in-vitro* diagnostic equipment to establish which legal requirements are applicable at the specific use site. Furthermore, only a risk assessment for the specific local setting can prove whether a use of a chemical results in an acceptable risk or not. The concentration from the majority of the substances investigated were below or close to the reference values and this can serve as a first indication that their use in *in-vitro* diagnostics is unlikely to pose an unacceptable risk to the aquatic environment. Since clinical chemistry laboratories and even more so the histological laboratories add only a very minor contribution to the waste water stream as the used substances are significantly diluted, so that concentrations fall with a comfortable margin of safety below the biological effect concentrations.

We are aware that sometimes (a) disinfectant(s) is/are added into the collected liquid waste. The environmental risk of such substances can directly be calculated from the amount added,

if a suitable environmental exposure scenario is available for the facility.

Currently, we are developing environmental exposure scenarios for *in-vitro* diagnostic techniques (Ref. 17). The present analytical results can then be used to directly assess the risk of using this technology for the aquatic environment.

In conclusion, one should also be aware that the discharge of the liquid waste into the drain is a worst case scenario, as many laboratories and hospitals have separate collections systems for this type of liquid waste.

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Table 3: Analytical results of clinical chemistry waste samples

"Standard" parameter	Unit	Clinical chemistry 1	Clinical chemistry 2	Clinical chemistry 3	Clinical chemistry 4	Clinical chemistry 5	Clinical chemistry 6	Clinical chemistry 7	Clinical chemistry 8	Maximal observed value	Limit of quantification	Reference Value
Aspect		odorless, colorless solution	odorless, colorless solution	odorless, colorless solution	colorless solution, smell of ammonia	odorless, greenish, solution	milky w hite foamy suspension, smell like DMSO	colorless solution smell of ammonia	colorless/w hitish emulsion smell of ammonia			
Total organic carbon	mg/l	12	3	28	530	18	7600	15	12000	12000	0.1	
Biochemical oxygen demand	mg O2/L	3	1.4	9.9	190	18	4300	1.9	13200	13200	1	
Chemical oxygen demand	mg O2/L	25	<10	72	1830	44	22600	35	33200	33200	5	6.5-10
pH	pH	6.8	6.9	10.8	8.6	7.1	5.6	6.9	5.3	10.8		
Settleable solids	mg/L	<2	<2	<2	<2	<2	617	<2	384	617	1	
Semi volatile lipophilic substances	mg/L	2	2	2	27	<2	420	<10	9.1	420	1	300
Hydrocarbon index	mg/L	<1.0	<1.0	<1.0	7.9	<1.0	1.63	0.08	0.5	7.9	1	100
Adsorbable organically bound halogens AOX	mg/L	0.016	<0.010	<0.010	<0.010	<0.010	<0.20	0.012	<0.10	<0.20	0.1	1
Volatile halogenated hydrocarbons	mg/L	0.001	<0.01	<0.01	<0.01	<0.01	0.11	<0.01	<1	<1	0.01	0.5
Phenol index, steam volatile	mg/L	<0.05	<0.010	<0.010	<0.010	0.055	<0.05	<0.01	80	80	5	100
Halogen free organic solvents	mg/L	2	<2	<2	<2	<2	0.62	<2	<1	2	<2	10000
Antimony	mg/L	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.025	<0.025	<0.025	<0.025	0.005	0.5
Arsenic	mg/L	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0050	<0.0050	<0.010	<0.010	0.0005	0.5
Lead	mg/L	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.025	<0.025	<0.025	<0.025	0.005	1
Boron	mg/L	0.045	0.02	0.02	0.02	0.053	0.24	<0.05	0.1	0.24	0.015	
Cadmium	mg/L	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.025	<0.025	<0.025	<0.025	0.002	0.5
Chromium	mg/L	<0.020	<0.020	<0.020	<0.020	<0.020	<0.10	<0.10	<0.1	<0.1	0.020	1
Chromium VI	mg/L	<0.02	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	can not be determined	<0.005	0.050	0.2
Cobalt	mg/L	<0.0020	<0.0020	<0.0020	<0.0020	<0.0020	<0.010	<0.010	<0.01	<0.01	0.002	2
Copper	mg/L	<0.010	<0.010	<0.010	<0.010	<0.010	<0.050	<0.050	<0.050	<0.050	0.010	1
Nickel	mg/L	<0.020	<0.020	<0.020	<0.020	<0.020	<0.10	<0.10	<0.1	<0.1	0.020	1
Mercury	mg/L	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0050	<0.0050	0.0001	0.1
Silver	mg/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.50	<0.50	<0.5	<0.5	0.005	
Tin	mg/L	<0.020	<0.020	<0.020	<0.020	<0.020	<0.10	<0.10	<0.1	<0.1	0.02	5
Zinc	mg/L	<0.020	<0.020	<0.020	<0.020	<0.020	<0.10	<0.10	<0.1	<0.1	0.02	5
Nitrogen produced from ammonia and ammonium (expressed as N)	mg/L	0.46	0.13	<0.100	<0.100	1.19	<0.1	0.46	<0.1	1.19	0.1	100
Nitrogen produced from nitrite (expressed as N)	mg/L	<0.050	<0.050	0.761	<0.050	<0.050	0.052	<0.050	<0.05	0.761	0.1	10
Easily released cyanides	mg/L	<0.003	<0.003	0.0063	<0.003	<0.003	1	0.004	Detetected but <5	<5	0.003	1
Sulfate	mg/L	1.5	<0.1	<0.1	<0.1	<0.1	3	3.6	5.8	5.8	1	600
Easily released sulfide	mg/L	<0.1	<0.1	<0.1	<0.1	<0.1	0.62	<0.1	can not be determined	0.62	0.1	2
Fluorid solved	mg/L	0.31	<0.1	<0.1	<0.1	<0.1	1.1	0.15	4.3	4.3	0.1	50
Total phosphorous (expressed as P)	mg/L	<0.100	<0.10	1.06	185	0.38	0.59	3.24	0.21	185	0.1	50
"Specific" parameter	**Unit**	**Clinical chemistry 1**	**Clinical chemistry 2**	**Clinical chemistry 3**	**Clinical chemistry 4**	**Clinical chemistry 5**	**Clinical chemistry 6**	**Clinical chemistry 7**	**Clinical chemistry 8**	**Maximal observed value**	**Limit of quantification**	**Reference Value**
4-Nonylphenoles and homologues	mg/L	<0.001	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.01	<0.02	0.001	0.0001
Ethoxylated octylphenols	mg/L	<0.03	<0.03	1.1	<0.03	<0.03	<0.03	0.2	<0.03	1.1	0.03	0.0001
Phthalates	mg/L	<0.05	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<10	<10	0.05	0.001
Formamide [75-12-7]	mg/L	<1	can not be determined	can not be determined	can not be determined	can not be determined	can not be determined	<5	can not be determined	<5	1	125
Formaldehyde [50-00-0]	mg/L	7.3	<0.1	<0.1	0.9	<0.1	<0.1	<0.1	<0.1	7.3	0.1	0.47
Acrylamide [79-06-1]	mg/L	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.05	0.02	0.01	60
Ethylenediaminetetraacetic acid [60-00-4]	mg/L	0.04	1.5	0.19	0.045	0.38	can not be determined	0.09	can not be determined	1.5	0.01	2.2
1-Methyl-2-pyrrolidone [872-50-4]	mg/L	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	500
Basic violet 3 [548-62-9]	mg/L	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	
Congo red [573-58-0]	mg/L	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	can not be determined	<0.01	0.01	
Nuclear fast red [6409-77-4]	mg/L	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	can not be determined	<0.01	0.01	
Sodium azide [26628-22-8]	mg/L	<0.05	<0.05	0.19	<0.05	0.13	12	0.2	52	52	0.05	5

Table 4: Analytical results of histology waste samples

"Standard" parameter	Unit	Histology sample 1	Histology sample 2	Histology sample 3	Histology sample 4	Histology sample 5	Maximal observed value	Limit of quantification	Reference Value
Aspect		slightly redish, foamy suspension, smell of glycol	slightly greenish, foamy suspension, smell of glycol	colorless solution, fishy smell	2 layers dark purple	2 layers dark purple			
Total organic carbon	mg/l	12000	18000	7400	160000	150000	160000	0.1	
Biochemical oxygen demand	mg O2/L	1700	3400	2800	240000	210000	240000	1	
Chemical oxygen demand	mg O2/L	33800	47300	22000	521000	497000	521000	5	
pH	pH	6.4	7.7	5.2	3.3	3.1	7.7		6.5-10
Settleable solids	mg/L	742	332	14	4	< 1	742	1	
Semi volatile lipophilic substances	mg/L	34000	47000	0.5	34800	10800	47000	1	300
Hydrocarbon index	mg/L	5900	5200	6	14900	12400	14900	1	100
Adsorbable organically bound halogens AOX	mg/L	<0.05	<0.05	0.023	7.8	8	8	0.1	1
Volatile halogenated hydrocarbons	mg/L	<0.1	<0.1	<0.5	<250	<250	<250	0.01	0.5
Phenol index, steam volatile	mg/L	<0.100	<0.100	0.02	0.15	0.19	0.19	5	100
Halogen free organic solvents	mg/L	<2	<2	3	<5000	<5000	<5000	<2	10000
Antimony	mg/L	0.012	0.012	<0.025	<0.025	<0.025	<0.025	0.005	0.5
Arsenic	mg/L	0.0074	0.0054	<0.0005	<0.0005	<0.0005	0.0074	0.0005	0.5
Lead	mg/L	0.012	0.012	<0.025	<0.025	<0.025	<0.025	0.005	1
Boron	mg/L	0.18	2.9	0.133	0.019	0.026	2.9	0.015	
Cadmium	mg/L	0.012	0.012	<0.025	<0.025	<0.025	<0.025	0.002	0.5
Chromium	mg/L	0.57	0.47	<0.1	<0.1	<0.1	0.57	0.020	1
Chromium VI	mg/L	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.050	0.2
Cobalt	mg/L	0.005	0.005	<0.01	<0.01	<0.01	<0.01	0.002	2
Copper	mg/L	0.73	1.6	<0.05	0.21	0.2	1.6	0.010	1
Nickel	mg/L	1.5	0.62	<0.1	<0.1	<0.1	1.5	0.020	1
Mercury	mg/L	<0.005	<0.005	<0.0002	<0.0002	<0.0002	<0.005	0.0001	0.1
Silver	mg/L	0.75	0.25	<0.5	<0.5	<0.5	0.75	0.005	
Tin	mg/L	0.26	0.23	<0.1	<0.1	<0.1	0.26	0.02	5
Zinc	mg/L	0.47	0.44	<0.1	0.14	0.2	0.47	0.02	5
Nitrogen produced from ammonia and ammonium (expressed as N)	mg/L	<0.1	<0.1	<0.1	90	96	96	0.1	100
Nitrogen produced from nitrite (expressed as N)	mg/L	<0.05	<0.05	<0.05	<1	<1	<1	0.1	10
Easily released cyanides	mg/L	<0.010	<0.010	4.6	<0.003	<0.003	4.6	0.003	1
Sulfate	mg/L	3.7	3.4	107	637	798	798	1	600
Easily released sulfide	mg/L	<0.5	<0.5	<10	can not be determined	can not be determined	<10	0.1	2
Fluorid solved	mg/L	0.1	0.27	1.4	<0.1	<0.1	1.4	0.1	50
Total phosphorous (expressed as P)	mg/L	1.07	1.38	0.11	<0.1	<0.1	1.38	0.1	50
"Specific" parameter	**Unit**	**Histology sample 1**	**Histology sample 2**	**Histology sample 3**	**Histology sample 4**	**Histology sample 5**	**Maximal observed value**	**Limit of quantification**	**Reference Value**
4-Nonylphenoles, and homologues	mg/L	<0.02	<0.02	<0.01	<0.01	<0.01	<0.02	0.001	0,0001
Ethoxylated octylphenols	mg/L	15	4	<0.03	<1	<1	15	0.03	0,0001
Phthalates	mg/L	<1	<1	<0.5	<500	<500	<500	0.05	0,001
Formamide [75-12.7]	mg/L	can not be determined	can not be determined	<0.1	can not be determined	can not be determined	<0.1	1	125
Formaldehyde [50-00-0]	mg/L	0.2	0.3	<0.1	4.5	4.7	4.7	0.1	0,47
Acrylamide [79-06-1]	mg/L	<0.01	<0.01	<1	<0.05	<0.05	<1	0.01	60
Ethylenediaminetetraacetic acid [60-00-4]	mg/L	can not be determined	can not be determined	<0.01	can not be determined	can not be determined	<0.01	0.01	2.2
1-Methyl-2-pyrrolidone [872-50-4]	mg/L	<0.01	<0.01	<0.01	<1	<1	<1	0.01	500
Basic violet 3 [548-82-9]	mg/L	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	
Congo red [573-58-0]	mg/L	<0.01	<0.01	<1	<0.01	<0.01	<1	0.01	
Nuclear fast red [6409-77-4]	mg/L	<0.01	<0.01	<1	<0.01	<0.01	<1	0.01	
Sodium azide [26628-22-8]	mg/L	<0.05	<0.01	12	<0.05	<0.05	12	0.05	5