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Ecotoxicological risk assessment of hospital wastewater: a proposed framework for raw effluents discharging into urban sewer network

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Abstract

In hospitals a large variety of substances are in use for medical purposes such as diagnostics and research. After application, diagnostic agents, disinfectants and excreted non-metabolized pharmaceuticals by patients, reach the wastewater. This form of elimination may generate risks for aquatic organisms. The aim of this study was to present: (i) the steps of an ecological risk assessment and management framework related to hospital effluents evacuating into wastewater treatment plant (WWTP) without preliminary treatment; and (ii) the results of its application on wastewater from an infectious and tropical diseases department of a hospital of a large city in southeastern France. The characterization of effects has been made under two assumptions, which were related to: (a) the effects of hospital wastewater on biological treatment process of WWTP, particularly on the community of organisms in charge of the biological decomposition of the organic matter; (b) the effects on aquatic organisms. COD and BOD_5 have been measured for studying global organic pollution. Assessment of halogenated organic compounds was made using halogenated organic compounds absorbable on activated carbon (AOX) concentrations. Heavy metals (arsenic, cadmium, chrome, copper, mercury, nickel, lead and zinc) were measured. Low most probable number (MPP) for faecal coliforms has been considered as an indirect detection of antibiotics and disinfectants presence. For toxicity assessment, bioluminescence assay using *Vibrio fischeri* photobacteria, 72-h EC_{50} algae growth *Pseudokirchneriella subcapitata* and 24-h EC_{50} on *Daphnia magna* were used. The scenario allows to a semi-quantitative risk characterization. It needs to be improved on some aspects, particularly those linked to: long term toxicity assessment on target organisms (bioaccumulation of pollutants, genotoxicity, etc.); ecotoxicological interactions between pharmaceuticals, disinfectants used both in diagnostics and in cleaning of surfaces, and detergents used in cleaning of surfaces; the interactions into the sewage network, between the hospital effluents and the aquatic ecosystem.
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1. Introduction

Hospitals use a variety of chemical substances such as pharmaceuticals, radionuclides, solvents and disinfectants for medical purposes as diagnostics, disinfections and re-

search [1–3]. After application, some of these substances and excreted non-metabolized drugs by the patients enter into the hospital effluents [4,5], which generally reach, as well as the urban wastewater (Fig. 1), the municipal sewer network without preliminary treatment [6]. Unused medications sometimes are also disposed in hospital drains [5]. Pollutants from hospital were measured in the effluents of WWTP, and in surface water [7]. Due to laboratory and research activities or medicine excretion into wastewater, hospitals may repre-

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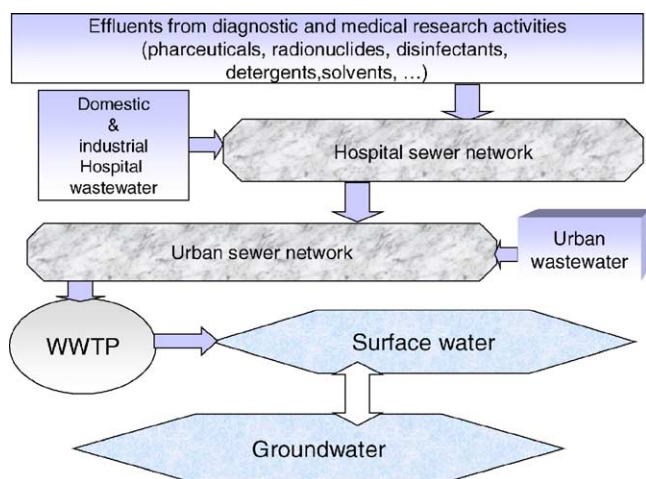*E-mail address:* evemm1@yahoo.fr (E. Emmanuel).

Fig. 1. Problems of hospital effluents and their impacts on WWTP and natural environments.

sent an incontestable release source of many toxic substances in the aquatic environment [8].

The contact of hospital pollutants with aquatic ecosystems leads to a risk directly related to the existence of hazardous substances which could have potential negative effects on biological balance of natural environments. Risk is the probability of appearance of toxic effects after organism's exposure to hazardous substances [9]. In the context of hospital wastewater discharge into the aquatic ecosystem, the exposure to hazardous substances, particularly disinfectants, non-metabolized pharmaceuticals and radionuclides, requires to consider possible risks for aquatic organisms. The fate of pharmaceuticals in the aquatic environment has been reported in different reviews of the literature [3,4,7,10]. The ecological risk of glutaraldehyde, a dialdehyde usually recommended as the disinfectant of choice for reusable fiber-optic endoscopes, has been also treated in other study [8]. However, few studies treat the total risk resulting from the simultaneous exposure to various pollutants present in the hospital effluents.

French legislation fixes the conditions for the connection of hospital wastewater system into the urban sewer network [11]. In the Directive No. 793/93, on the human and ecosystem exposures to the classified toxic substances, the European Commission [12] requires to all member states to carry out a sanitary and ecological risk assessment for substances such as: drugs, disinfectants and radioactive substances. These regulations fall under the context of the risk management concerning human health, and also the management of those concerning the biological balance of the natural ecosystems. In a very general way, the risk management always passes, formally or not, by the preliminary phases of risk assessment [13]. The aim of this study was to present: (i) an implemented framework for hospital wastewater management, which includes two steps: a 'light' step based on the hazard assessment related to hospital effluents and, if proof of hazard existence occurred, the execution of a 'heavy' step would take place. This step was based on an ecotoxicological risk assessment

of hospital wastewater discharging into the urban sewer network, then into the natural environment, (ii) detailed elaborated procedures for the steps of 'hazard assessment' and 'risk assessment', (iii) the results of their application on the effluents of an infectious and tropical diseases department (ITDD) of a hospital of a big city in southeastern France.

2. Effects of hospital wastewater on aquatic ecosystems

Hospitals consume an important volume of water per day. The minimal domestic water consumption is 100 L/person/day [14], whereas the value demand for the hospitals generally varies from 400 to 1200 L/bed/day [6]. In the United States of America, the hospital average water consumption is 968 L/bed/day [15]. In France, the water average need of university hospital facilities is estimated at 750 L/bed/day [6]. In the developing countries, this consumption seems to be around 500 L/bed/day [16]. This important water consumption in hospitals gives significant volumes of wastewater. Results of toxicity studies using the bacteria bioluminescence and *Daphnia magna* have revealed the important toxic activities of hospital wastewater on aquatic organisms [17].

The most frequent contaminants in hospital wastewater are: viruses and pathogenic bacteria (some of them are antibacterial resistant characters) [6], molecules from unused and excreted non-metabolized pharmaceuticals [4], organohalogen compounds, such as the halogenated organic compounds adsorbable on activated carbon (AOX) [5], radioisotopes [1,18].

Results on the microbiological characterization of hospital wastewater [6] reported these effluents have bacteria concentrations lower than 10^8/100 mL generally present in the municipal sewage system [19]. The low most probable number (MPN) detected for fecal bacteria in hospital is probably due to the presence of disinfectants and antibiotics [6]. Markers of viral pollution of water, such as enterovirus and other viruses have been identified in the hospital effluents [6]. Studies on the bacteria flora of hospital wastewater into WWTP have shown that bacteria acquired resistant character [20]. Antibacterial resistancy is a threat to the efficacy of antibacterial substances. The development of resistance to antimicrobial agents by many bacterial pathogens has compromised traditional therapeutic regimens, making treatment of infections more difficult [4]. Three factors have contributed to the development and spread of resistance: mutation in common genes that extend their spectrum of resistance, transfer of resistance genes among diverse microorganisms, and increase in selective pressures that enhance the development of resistant organisms [4,20–24].

Hospital effluents reveal the presence of organochlorine compounds in high concentrations [6]. AOX up to 10 mg/L were proved in the effluents of the hospitalization services of a university hospital center [25]. The major mass carri-

ers for the AOX in hospital effluents are most likely iodized X-ray contrast media, solvents, disinfectants, cleaners and drugs containing chlorine. Brominated organic compounds are negligible for the AOX in the hospital effluents [5]. In general, the maximum contribution of drugs to the AOX is not above 11% [26]. Beyond that it is also known that the AOX concentration in the urine of patients not treated with drugs is very low. It is normally between 0.001 and 0.2 mg/L [27]. Due to the dilution effect, no substantial contribution from this source is consequently expected [5]. The assessment of AOX shows that those non-conventional pollutants have a bad biodegradability and a bad behavior of adsorption [7].

3. Theoretical aspects of the ecological risk assessment

The ecotoxicological risk assessment is a subset of the ecological risk assessment and can thus, for this reason, be treated according to an approach of the same type. Ecological risk assessment is a process that evaluates the likelihood of one or more stressors [28]. This process is based on two major elements: characterization of effects and characterization of exposure, these provide the focus for conducting the three phases of risk assessment: problem formulation, analysis phase and risk characterization phase [29].

3.1. Problem formulation

The step is a process for generating and evaluating hypotheses about why ecological effects have occurred, or may occur, from human activities [29]. It provides the foundation for the entire ecological risk assessment. Problem formulation results in three products [29]: (1) assessment endpoints that adequately reflect management goals and the ecosystem they represent, (2) conceptual models that describe key relationships between a stressor and assessment endpoint or between several stressors and assessment endpoints, and (3) an analysis plan.

3.2. Analysis phase

Analysis is a process that examines the two primary components of risk, exposure and effects, and their relationships between each other and ecosystem characteristics [29].

3.3. Risk characterization phase

This operation is the final phase of ecological risk assessment and is the culmination of the planning, problem formulation, and analysis of predicted or observed adverse ecological effects related to the assessment endpoints [34]. There is a range of possible methods, of variable complexity [13]. The choice will depend on the operational constraints and the available data. Riviere [9] note 'the ecological risk can

be expressed in various manners: qualitative (absence or not of risk), semi-quantitative (weak, average and high risk), in probabilistic terms (the risk is x%)'.

The method known as 'the quotient' is the most widespread method for the semi-quantitative characterization of risks. This method consists in calculating the ratio (or quotient) which is expressed as a 'probable exposure concentration (PEC)' divided by a 'probable non concentration effect (PNEC)' [29]. This 'probable concentration without effect' can be estimated starting from the available data in the literature for the pure substances, and using experimental measurements (bioassays) for the mixture such as the hospital effluents. Although the toxicity of a chemical mixture may be greater or lesser than predicted from toxicities of individual constituents of the mixture, a quotient addition approach assumes that toxicities are additive or approximately additive [29]. This assumption may be most applicable when the modes of actions of chemicals in a mixture are similar, but there is evidence that even with chemicals having dissimilar modes of action, additive or near-additive interactions are common [29–31].

When the quotient value 'Q' is greater than 1, the risk is considered as significant, and all the more extremely as the quotient is large. Conversely, more the quotient is lower than 1, more the risk is regarded as weak. The 'probable concentration without effect' on the organism is, in practice, generally represented by a EC_{10}, or a EC_{20}, or a NOEC, divided by a safety factor (10 for example). In the absence of a EC_{10} or of a NOEC, the EC_{50} is sometimes used with a rated-up safety factor [13].

4. Methodological approach for the ecotoxicological risk assessment of hospital wastewater

4.1. Hazard assessment

The conceptual framework for hazard assessment of hospital wastewater (Fig. 2), is based on a characterization of the hospital effluents in function of: (i) their chemical composition (measurement of global parameters and mineral and organic pollutants); (ii) their microbiological characterization; and (iii) of their intrinsic ecotoxicity.

The selected parameters (stressors and assessment endpoints) for these characterizations were: (1) COD and the BOD_5 for the measurement of the total organic load; (2) the organohalogen compounds adsorbable on activated carbon (AOX) for the evaluation of the contained organohalogen compounds; (3) heavy metals (arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc) for the mineral pollution characterization; (4) the most probable number of fecal bacteria for the microbiological characterization (this parameter was considered in this study like an indirect detection of the massive presence of disinfectants and/or antibiotics); (5) the measurement of EC_{50} of hospital wastewater on bacterial luminescence (*Vibrio fischeri*), on the algae growth (*Pseu-

Fig. 2. Conceptual framework for ecotoxicological hazard assessment of hospital wastewater.

Fig. 3. Presentation of the studied scenario.

dokirchneriella subcapitata) and on the mobility of *D. magna* for the characterization of the intrinsic ecotoxicity of the effluents.

The obtained results for these parameters have been compared with threshold values which were established in the following way: (1) global parameters, French regulations on effluents discharge [11]; (2) ecotoxicological parameters, the value of two Toxic Units (UT), proposed by French water agencies for industrial wastewater discharges [32], has been adopted as threshold value the selected bioassays; (3) microbiological parameter, a threshold value fixed of 1×10^8 fecal coliforms for 100 mL, corresponding to the average content of these fecal bacteria in the conventional urban sewer network [19] was adopted.

For any ratio $P_c/V_t > 1$ (P_c: pollutant concentration in the hospital effluents; V_t: threshold values) and for any number in fecal bacteria lower than 1×10^8 NPP for 100 mL, the framework recommends to pass at the following step: 'the ecotoxicological risk assessment of hospital wastewater'.

4.2. Description of the context of ecotoxicological risk assessment

This description, whose aim was to apprehend as comprehensive as possible the ecosystem exposure to the hospital effluents, was carried out for a management scenario of hospital wastewater usually observed in industrialized countries. This scenario envisages the connection of the hospital sewer network to the urban sewer network, as well as the biological WWTP which discharge its own effluents into the natural environment (Fig. 3). Since hospital pollutants have been detected in natural ecosystem [7], which may be interpreted as a deficiency of the WWTP, and since the effluents of the treatment plant are discharged in a river, the proposed scenario takes also into account the possible effects of hospital wastewater on the river ecosystem. Two types of exposed ecosystems to the hospital wastewater pollutants have been

considered in the studied scenario: (1) artificial ecosystems represented by the WWTP and (2) natural ecosystems represented by air, soils, surface water and ground water. The susceptible elements of these ecosystems, which could be affected by the exposure to hospital effluents, are summarized in Table 1.

4.3. Development of the conceptual model and choice of the parameters of evaluation

Within the framework of this evaluation, the WWTP, the fresh surface water and the species at the two first levels of food chains have been considered as the targets (Fig. 4). The fact, that the other ecosystems and the other species have not been considered, does not mean for instance that those are less important in the ecological level, but simply they were not taken into account in this first stage of the methodology development.

For the characterization of the effects, two assumptions were elaborated. They have been related to the ecological values to be protected: (a) 'the discharge of hospital pollutants into the WWTP will not affect the biological treatment process of WWTP, with possible adverse effects particularly

Table 1
Concerned ecosystems

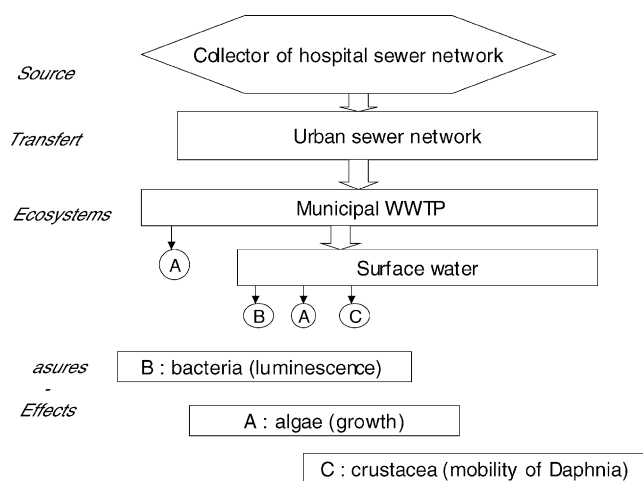| Ecosystems		Susceptible elements to be affected
Artificial	WWTP	Bacteria, algae and protozoa (in case where the biological treatment units have reactors of decomposition functioning under the 'aerobic' mode)
Natural	Air	The birds and the insects
	Soil	Microorganisms of the soils; wildlife of soils (insects, earth worms, ...); and soil vegetables
	Surface water	The primary producers (phytoplankton) of which unicellular and pluricellular green algae; the primary (invertebrate) in particular of the crustaceans; and secondary consumers of which fish and water birds
	Ground water	Protection of fresh water resources

Fig. 4. Conceptual model of the studied scenario.

on the algae community, which participates in the biological decomposition of the organic matter'; (b) 'the WWTP effluents will not have toxicological effects on the living species (particularly the: bacteria, algae and crustacean) of the natural aquatic environment'.

The characterization of the ecological effects of hospital pollutants on the bacteria, the algae growth and the crustacean survival, was carried out using French standardized bioassays. The bacteria were represented by 'V. fischeri', the species constituting the primary producers (phytoplankton) were represented by the algae 'P. subcapitata', and the fresh water crustaceans 'Daphnia magna Strauss' ensured the representation of the primary consumers. In the context of the proposed scenario, the results of toxicity test on bacteria and crustaceans were considered to apprehend the possible effects of hospital wastewater on the river ecosystem, while the EC_{50} value from algae test have been retained to study the effects of the studied samples on both WWTP and the river.

5. Materials and methods

5.1. Sampling and pH measurements

Wastewater from a hospital of a large city in southeastern France were used for the realization of the experimental phase of this study. It is a hospital of 750 beds approximately. Water consumption is estimated at 750 m^3/day. The effluents from the various departments are discharged into the hospital network sewer. This network consists of several collectors broken down by service or group of related services. The institution has a combined sewage system. The existence of such network could increase the concentration of the nitrogen substances during the first raining days and a dilution of all the pollutants during the other raining days [33]. This network could also increase the concentration of certain heavy metals, particularly zinc.

Two campaigns of sampling (2001 and 2002) were realized on the effluents originating from the infectious and tropical diseases department (ITDD), with a capacity of 144 beds, of the hospital. The ITDD represents 19.2% of the entire capacity of the hospital. During the sampling periods, this department had a percentage of occupied beds of 100%. Wastewater was collected before entering into the entire hospital sewer network, which discharges the total volume of effluents from the various departments into the urban wastewater network without pre-treatment. This ITDD collector does not receive effluents containing iodized X-ray contrast media from radiography department, substances which mainly contribute to AOX formation in hospital wastewater [8]. Water samples were collected by means of a telescopic perch in a 1-L glass flask. pH was measured directly on site after sampling with a pH meter HI 8417 (accuracy pH ± 0.01; mV ± 0.2, ± 1; °C ± 0.4). All the water samples and the mixture were kept at 4 °C until analysis.

5.2. Physicochemical analysis

BOD_5 concentrations in the 2001 and 2002 samples were carried out by following European and French standard NF EN 1899-1. COD concentrations in 2001 samples was measured by potassium dichromate method using HACH spectrophotometer 2010 and test procedure provided by the supplier. French standard NF T90-001 had been followed in the determination of COD concentrations in 2002 samples. AOX were measured according to European standard EN 1485.

Heavy metals have been determined according to ISO 11 885 protocol on filtered sample (0.45 μm) and acidified using nitric acid (pH < 2) and using inductively coupled plasma-atom emission spectroscopy (ICP-AES).

Beside the selected assessment endpoints, other physicochemical such as: total organic carbon, chlorides, total suspended solid and ammonia were carried out in order to compare the hospital wastewater composition with some physical and chemical constituents of conventional urban wastewater.

Total organic carbon (TOC) measurements were carried out on samples filtered at 0.45 μm and pre-treated with orthophosphoric acid (H_3PO_4). French standard T90-102 was applied by using a SPECTRA France carbon analyzer, LABTOC model, with potassium persulfate ($K_2S_2O_8$) as a reagent and UV oxidation. Chlorides were determined by conforming to European standard NF EN ISO 10304-1 on diluted and filtered samples at 0.45 μm by using a DIONEX DX-100 ion chromatograph with suppressed conductivity detection from 0.0 to 1000 μS. An Ionpac AS14 4 mm × 250 mm analytical column (P/N 046124) was used for chloride sample analysis.

Total suspended solid (TSS) concentrations were determined in conformity with European standard NF EN 872 after filtration through a 1.2-μm membrane and dewatering at 105 °C. French standard NF T90-015 had been followed in

the determination of ammonia concentrations in 2002 samples.

5.3. Microbiological analysis and toxicity test procedures

Fecal bacteria have been studied using French standard NF T 90–433 microplaque. For the study of assessment endpoints, three standardized bioassays were carried out. Results of EC_{50} for all these bioassays, with their confidence interval, are expressed in percentage of sample dilution in toxic unit TU (1 TU = 100/EC_{50}).

The bioassay on bacteria luminescence was carried out with a LUMIStox system (Dr Lange GmbH, Duesseldorf, Germany) following the procedure of European standard NF EN ISO 11348-3. Tests were performed using Gram negative marine bioluminescent bacteria of the species *V. fischeri* NRRL-B-11177 of the *Vibrionaceae* family. In order to prevent TSS interferences on bacteria luminescence, samples were filtered using a 0.45-μm pore size membrane. The samples were treated with NaCl solution of 20 g/L and brought to 50 mS/cm conductivity before analysis. Starting from the concentration of the sample, eight consecutive dilutions were tested (dilution factor 1:2); the inhibition of bioluminescence was measured at a wavelength of 490 nm, with readings after 5 and 15 min of incubation at 15 °C. The EC_{50} values were calculated as reported by Bulich [34].

The 72-h EC_{50} algae growth toxicity test was monitored using French standard NF T90-375. Assays were carried out with the green algae inoculums *P. subcapitata* (formerly *Selenastrum capricornutum*) resulting from laboratory culture in exponential growth phases (POLDEN of the National Institute of Applied Sciences of Lyon–INSA de Lyon). The sensibility of the laboratory species was controlled by regular tests with potassium dichromate. Standard diluted medium was used with 0.1 mg of EDTA per liter of assay solution. In order to avoid the interferences of suspended solids and other microorganisms on algae growth during the realization of the assay, experimental solutions were filtered at 0.45 μm. Experimental solutions were maintained at 4 ± 3 °C. A set of five concentrations of experimental solution samples in the reference medium and a control were examined in each test. Assays were carried out in glass cups containing 25 mL of samples, with three replicates by concentration. The assay is static, under magnetic agitator and under constant luminosity, at 23 ± 2 °C. Algae concentration were measured all the 24 h using Malassez cell and optic microscope.

Determining the inhibition of *D. magna* mobility is an acute toxicity assay. Its objective is to identify the initial concentration of a pollutant in solution or an aqueous mixture that may immobilize 50% of the *Daphnia* exposed to a polluted source within 24 and 48 h. In conformity with European standard NF EN ISO 6341, the different assays were carried out on *Daphnia* sp. maintained in a parthenogenetic culture in the laboratory (POLDEN of the National Institute of Applied Sciences of Lyon–INSA de Lyon). The sensitivity

of the laboratory species was controlled by regular tests with potassium dichromate. Only young female *Daphnia* aged less than 24 h were used. The normal medium, without EDTA, was also used. The assays were carried out at 20 ± 2 °C in darkness. All the assays were carried out within 6–48 h after sampling.

Since hospital wastewater is considered toxic for aquatic environments, a volume of 250 mL unfiltered samples was taken for each assay. The three conditions required for the validity of the assays were observed: (i) the concentration of dissolved oxygen (DO) in the control group was ≥2 mg/L at the end of each assay; (ii) the percentage of immobilization observed in the control group vessels was ≤10%; (iii) EC_{50} 24 h for potassium dichromate was from 0.6 to 1.7 mg/L.

5.4. Risk assessment

The PEC/PNEC ratio was used to evaluate the environmental risk generated by hospital wastewater on aquatic ecosystem. Since the experimental results of bioassays were only in EC_{50} short-term toxicity, and since hospital wastewater is a mixture of pollutants, PNEC has been estimated by dividing lower short-term EC_{50} by an assessment factor [12]. PEC was expressed in percentage of dilution of the pollutant concentrations.

6. Results and discussion

6.1. Results of the physicochemical analysis

The highest concentrations obtained for the physicochemical characterization of the hospital wastewater from ITDD are summarized in Table 2 . In all studied samples of the two campaigns (2001 and 2002), pH was always in an alkaline range (7.7–8.8) with a variation lower than 1 pH.

Studies on hospital wastewater reported that these effluents are essentially domestic (i.e. sanitary wastewater from residential and commercial sources) and are characterized by pollutant concentrations of BOD_5 (ranged from 50 to 400 mg/L), COD (150 to 800 mg/L), TSS (60 to 200 mg/L) and TOC (50 to 300 mg/L) [15]. In the effluent samples BOD_5 concentrations ranged from 200 to 1559 mg/L were greater than values obtained for hospital wastewater [15]. The same observation was made on the studied hospital wastewater samples for COD (ranged from 362 to 2664 mg/L), TSS (155 to 298 mg/L) and TOC (160 to 3095 mg/L). The obtained values for these parameters were also greater than the values proposed by Metcalf and Eddy [19] for domestic wastewater.

The COD, BOD_5 and AOX threshold values for industrial wastewater that must be reached in the sewer network are given by French regulations, namely 125 mg/L for COD, 30 mg/L for BOD_5 and 1 mg/L for AOX [11]. In the effluent samples COD, BOD_5 and AOX concentrations have exceeded those discharge standards.

Table 2
Physicochemical and microbiological characterization of hospital wastewater from ITDD

Parameters	Units	Highest concentrations		Threshold	
		2001	2002	Values	Reference
pH	U	8.8	8.2	–	
Chlorides	mg/L	359	127.1	–	
AOX	mg/L	1.24	1.61	1	[11]
TSS	mg/L	298	236	–	
BOD_5	mg/L	1559	1530	30	[11]
COD	mg/L	2516	2664	125	[11]
TOC	mg/L	350	3095	–	
NH_4^+	mg/L	ND	68	–	
Arsenic	mg/L	ND	0.011	–	
Cadmium	mg/L	ND	<0.007	–	
Chromium	mg/L	ND	<0.004	0.5	[11]
Copper	mg/L	ND	0.112	0.5	[11]
Lead	mg/L	ND	<0.0035	0.5	[11]
Mercury	mg/L	<0.0005	ND	–	
Nickel	mg/L	ND	<0.0007	0.5	[11]
Zinc	mg/L	ND	0.536	2	[11]
Fecal bacteria	NPP/100mL	2×10^3	1×10^6	1×10^8	[22]

[*]ND: non determined.

In all the effluents samples TSS concentrations, ranged from 155 to 298 mg/L, were lower than the values (100–350 mg/L) proposed for domestic wastewater [19]. Chloride concentrations from 47 to 359 were detected in the studied samples. The measured chloride values were greater than proposed concentrations for conventional urban wastewater. This difference may be due to the important quantity of chlorine disinfectant used in hospitals.

Studies on the presence of AOX in wastewater explained the formation of this 'non-conventional' pollutant by the presence of organochlorine compounds [35] or by the oxidation of iodized X-ray contrast media [36]. Brominated organic compounds are negligible for AOX in hospital effluents [5]. The results generated by the studied hospital wastewater samples for AOX (0.17–1.61 mg/L) were lower than the concentration of 10 mg/L determined [7] in hospital wastewater containing iodized contrast media. The sampling conditions chosen for this study, i.e. choice of a wastewater collector that does not receive iodized X-ray contrast media effluents from the radiography department, could explain this significant difference. Chloride could be attributed to the total presence of AOX.

6.2. Microbiological characterization

Low concentrations of bacteria flora were detected for the hospital effluents (Table 2). Previous studies on the microbiological characterization of hospital wastewater [6] reported that the bacteria concentrations of these effluents are lower than the $10^8/100$ mL generally present in the municipal sewage system [19]. Fecal coliform populations of hospital wastewater were affected because of the presence of disinfectants and probably antibiotics. Although hospitals use and discharge (into the sewer network) large amounts of water [6] thereby diluting high pollutant concentrations to lower ones, it seems necessary to monitor the behavior of the microbial populations of urban wastewater treatment plants that receive these hospital effluents containing higher chloride and AOX concentration.

6.3. Ecotoxicological characterization of ITDD wastewater

The obtained results for the bioassays are synthesized in Table 3. The results of toxicity test on *V. fischeri* obtained for the year 2001, lead to EC_{50} (5 min) greater than 50% of effluent for all the samples, i.e. with an ecotoxicity, expressed in UT, always lower than 2 UT. These results showed that 5 min assay can be considered as no toxic. However, significant differences were observed between EC_{50} (5 min) and obtained results for EC_{50} (15 and 30 min). In addition, there exist very little differences between the obtained results for 15 and 30 min assays. This report can be correlated with the contact time of 20 min contact required by chlorinated disinfectants to inactivate bacteria [37]. The results of 15 and 30 min greater than 2 TU. The maximal concentrations ranged from 4.2 to 4.6 showed that the hospital wastewater toxicity on *V. fischeri* is similar to domestic wastewater toxicity.

An acute toxicity of hospital wastewater on crustaceans has been demonstrated in all the studied samples. All obtained CE_{50} from *D. magna* bioassays were greater than the value of 2 TU proposed by French water agencies for industrial wastewater discharges [32]. The results of the toxicity tests of hospital wastewater on *D. magna* indicated potential toxicity. Values ranged from 9 to 56 TU were obtained for the EC_{50} on algae. Thus those effluents can alter the biological

Table 3
Ecotoxicological characterizations of hospital wastewater

Parameters	Units	Highest effective concentrations (HEC_{50})		Variations of EC_{50} (2001–2002)			
		2001	2002	Means	Minima	S.D.	n
EC_{50} 5 min *Vibrio fischeri*	UT	1.54	2.5	–	<1.3	–	9
EC_{50} 15 min *Vibrio fischeri*	UT	4.15	4.2	–	<1.3	–	9
EC_{50} 30 min *Vibrio fischeri*	UT	ND	4.6	–	<1.3	–	5
EC_{50} 72 h *Pseudokirchneriella subcapitata*	UT	ND	56	32	9	18	5
EC_{50} 24 h *Daphnia*	UT	117	62	43	10	27	13
EC_{50} 48 h *Daphnia*	UT	ND	71	58	52	9	4

[*]ND: non determined.

process of the WWTP. The toxicity of hospital wastewater on aquatic organisms could be attributed to the important NH_4^+ values (28–68 mg/L) detected in the samples. Ammonia nitrogen is well known as toxic to aquatic organisms [38]. Aquatic communities should be adversely affected by ammonia at ≥ 1.04 mg total NH_3/L or 0.01 unionized NH_3/L [39]. In this study, the value of NH_3/L was not measured. Theoretically, ammonia nitrogen exists in aqueous solution as either the ammonium ion or ammonia, depending on the pH of the solution, in accordance with the following equilibrium reaction [19]:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \qquad (1)$$

At pH levels above 7, the equilibrium is displaced to the left, at levels below pH 7, the ammonium ion is predominant [19]. Since in all the studied samples, pH was always in an alkaline range (7.7–8.8) above 7, the displacement of the equilibrium may allow to the existence of NH_3 in concentrations probably greater than 1.04 mg total NH_3/L or 0.01 unionized NH_3/L. Based on the equilibrium reaction criteria and on samples pH, ammonia has an important contribution in the adverse observed effects of hospital wastewater on aquatic organisms.

6.4. Hazard assessment

According to the proposed framework, the hazard assessment of hospital effluents to the aquatic ecosystems consists of comparing the obtained results for physicochemical, microbiological and ecotoxicological characterizations with the threshold values presented in Tables 2 and 3 for the different parameters. Table 4 showed the results of ratios obtained from this comparison.

With the exception of the heavy metals, all the ratios P_c/V_t carried out for the other physicochemical parameters were greater than 1. The same observation was made for the bioassays ratios. In addition, the results of genotoxicity tests on

hospital wastewater using AMES and HAMSTER, reported in the literature, indicated that the effluents from clinical services and hospital laboratories have presented a genotoxicity character [25].

The ratio, by dividing the MPN/100 mL of fecal bacteria from hospital wastewater with the average of those usually found in the urban effluents, was largely lower than 1, that could, at least partially being related to the presence of disinfectants and/or antibiotics in the effluents.

All of the results confirm the existence of hazardous substances in the studied hospital effluents, and thus the need for continuing the approach by the setting of the ecotoxicological risk assessment of hospital wastewater for the concerned aquatic ecosystems (WWTP and natural environment).

6.5. Ecotoxicological risk assessment

In the absence of the hospital pollution control practices for wastewater, or of its own WWTP, all the contained pollutants into the ITDD effluents as those of the whole hospital are evacuated towards the municipal WWTP. In the proposed scenario, an artificial ecosystem 'the WWTP' as well as the natural aquatic ecosystem were retained as targets, by restricting the study to the species of the two first levels of aquatic food chains.

6.5.1. Impacts on the WWTP

Assumptions: "the discharge of hospital pollutants into the WWTP will not affect the biological treatment process of WWTP, with possible adverse effects on the community of organisms in charge of the biological decomposition of the organic matter".

The preservation of the biological efficiency of a WWTP can, in a first approach, being evaluated by means of the biodegradability studies of inflow pollutants. The biodegradability of organic substances is a measure of the speed and

Table 4
Comparison of the highest concentrations with the threshold values

Parameters	Units	Highest measured concentrations	Threshold values	Ratio (P_c/V_t)
Physicochemical				
BOD$_5$	mg/L	1559	30	>1
COD	mg/L	2664	125	>1
AOX	mg/L	1.61	1	>1
Chromium	mg/L	<0.004	0.5	<1
Copper	mg/L	0.112	0.5	<1
Nickel	mg/L	<0.0007	0.5	<1
Lead	mg/L	<0.0035	0.5	<1
Zinc	mg/L	0.536	2	<1
Microbiological				
Fecal bacteria	NPP/100 mL	1×10^6	1×10^8	<1
Ecotoxicological				
EC$_{50}$ 30 min *Vibrio fischeri*	UT	4.6	2	>1
EC$_{50}$ 72 h *Pseudokirchneriella subcapitata*	UT	56	2	>1
EC$_{50}$ 24 h *Daphnia*	UT	117	2	>1
EC$_{50}$ 48 h *Daphnia*	UT	71	2	>1

completeness of its biodegradability by microorganisms [40], and therefore the BOD_5/COD and COD/TOC ratios could be used to analyze the difficulty or not for organic substances to be degraded [35].

Fresenius et al. [41] reported with a $BOD_5/COD \geq 0.5$, the biological degradation starts immediately and runs rapidly. However, with a $BOD_5/COD < 0.5$, there is a possibility for chemical substances which have a bad biodegradability to slacken or to delay the biological process. Based on these criteria a threshold value of 0.5 has been retained to study the biodegradability of organic substances into the ITDD hospital wastewater. For the 2002 campaign, the BOD_5/COD ratio oscillated between 0.38 and 0.57, which indicate that the pollutants would be sometimes difficult to degrade, which describes a potential impact on the WWTP efficiency.

The information reported in the literature gives a COD/TOC of ratio 3 frequently found in many wastewaters [35]. A semi-empirical equation to determine the ratio between COD expressed in mg O_2/L and TOC in mg C/L ($COD = 2.67$ TOC) is also reported in the literature [42]. The COD/TOC ratios found in hospital wastewater, for 2001 campaign, ranged from 2.01 to 4.26. For COD/TOC values ranged from 2.01 to 3.00, the degradation of organic substances by microorganisms would occur without difficulty; however, for COD/TOC from 3.01 to 4.26, the substances would be difficult to degrade.

To evaluate in a semi-quantitative way the risks of a term-source on the ecosystems in a specific context, it is possible in a first approach, to consider the dilution coefficients generated by the global system. Within the framework of this study, three assumptions of dilution were considered for the risk characterization of hospital wastewater on the WWTP: (i) the daily flow of water supply by bed per day is equal to the volume of wastewater generated by bed per day; (ii) the ITDD generates a volume of wastewater of 144 m^3/day. In absence of specific considerations on the interactions between the various pollutants inside the hospital sewer network, the contained organic pollutants in the effluents of the service will be diluted at least of four times in total volume, i.e. 750 m^3/day of wastewater on average are generated by the different services of the hospital, before entering the urban sewer network; (iii) the ITDD effluents are treated into the WWTP of the considered city, this plant receives on average a hydraulic daily load of 87,000 m^3, which ensures a dilution of the measured pollutant concentrations in the hospital effluents at least 600 times. However, this method of evaluation will not allow preventing the discharge into WWTP effluents of low biodegradable and toxic pollutants (like pharmaceutical residues and AOX) for the ecosystems.

6.5.2. Impacts on the natural aquatic ecosystems

Assumption: "the WWTP effluents will not have toxicological effects on the living species of the natural aquatic environments".

The PEC/PNEC ratio was used to evaluate the environmental risk generated by hospital wastewater on aquatic ecosystem. It was seen previously that the dilution of hospital effluents in WWTP was equal to 600. For this, it is necessary to add, in the studied case, a dilution by 1000 of the WWTP effluents in the river water bodies. That led in fine to a dilution of 6×10^5 of the hospital effluents to their arrival in the receiving receptor. A PEC estimated at 0.006% ((6×10^5)/100) was considered for the hospital pollutants in normal condition. However in particular cases, as drought or concentration peak, this PEC (or the factor of dilution) would be higher.

The PNEC was established using the different toxicity data from hospital wastewater (EC_{50} expressed as a percentage: *V. fischeri* = 21.7%, *P. subcapitata* = 1.78%, *D. magna* = 0.8%). According to European Commission [12], an assessment factor of 1000 is to be applied to the lower short-term EC_{50} value. Since: (i) different short-tests have been carried out with decomposer, producer and consumer, (ii) the hospital effluents are a mixture, and (iii) the toxicity of a mixture is higher than the sum of toxicity of the different substances which compose the mixture, as observed in studies on combined effects of pollutants on aquatic organism [31]; an assessment factor of 100 has been used in this study to estimate the PNEC value (0.008%) by using the EC_{50} 24-h (0.8%) from *D. magna* test the PEC/PNEC ratio was $0.75 < 1$. This risk seems to be acceptable, however it is not completely far away from the red line.

This very simplified and very operational first approach implies however assumptions which for some are rather pessimistic and, for others, relatively 'imperfect' and being able, so as to lead to an incomplete assessment of long-term impacts of the hospital effluents on the natural environments.

Concerning the 'pessimistic' aspects, the reasoning is led as if the pollutants in the hospital effluents were not degraded, and any volatilization process has been occurred during their transport in the urban sewer network, and during their passage in the WWTP. However, this interpretation is not completely aberrant in comparison with the characteristics of some pollutants such as the AOX, which are considered to be non-biodegradable with 90% by certain authors like Sprehe et al. [36]. If these assumptions had led in fine to a positive evaluation of the ecotoxicological risks, it would have been necessary to conduct a thorough study of the concerned phenomena. In the particular case, which we are concerned, savings of time and means (and thus 'effectiveness') were carried out on these points.

Concerning the aspects 'incomplete assessment', the approach based on standardized ecotoxicity test and the dilution of the effluents in the natural environment implies imperfections on several levels: (1) the battery of the selected bioassays is limited. Thus organisms such as fish, for example, were not taken into account, (2) the long-term effects of the pollutants in question on the ecosystems are complex and difficult to evaluate on the basis of mono-specific simple test of ecotoxicity. Thus phenomena such as the genotoxicity of the pollutants or the their bio-accumulation in the food chains or the sediments of the river (with delay effect) were not

treated, (3) the reasoning on the basis of dilution cannot be sufficient in term of decision for the environmental protection. Indeed, many other effluents are rejected into the same 'target' medium. It will be thus more judicious in the future, and for an enlightened decision-making of the managers, to reason rather in terms of contribution of the hospital effluents to the total risk generated by the discharge of all the industrial and urban effluents in the concerned river.

7. Conclusion

This study has demonstrated that it is possible to carry out the ecotoxicological risk assessment of hospital effluents by the use of standardized bioassays, global physicochemical parameters and the analysis of some targeted pollutants. The proposed scenario allows to a semi-quantitative risk characterization for the WWTP and the fresh surface water. The evaluation will need now to be improved on certain aspects, and will require in particular a better knowledge on the fates of pollutants in the urban sewer network and in the WWTP. This increase of knowledge will relate in particular to the study of chemical and ecotoxicological interactions between pharmaceuticals, disinfectants and surfactants. It seems necessary to characterize the ecotoxicological risk of the hospital effluents by experimental and fundamental studies on the fates of disinfectants, pharmaceuticals and surfactants present in the hospital effluents, while taking care to include, on the ecotoxicological plan, the transfers towards the food chains.

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