Monitoring, removal and risk assessment of cytostatic drugs in hospital wastewater


*Umweltbundesamt Vienna, Spittelauer Lände 5, 1090 Vienna, Austria
**Medical University of Vienna, Department of Medicine I, Währinger Gürtel 18-20, 1090 Vienna, Austria
***BOKU University of Natural Resources and Applied Life Sciences Vienna (Project management)
Muthgasse 33, 1190 Vienna, Austria (E-mail: maria.fuerhacker@boku.ac.at)

Abstract Cytostatic agents are applied in cancer therapy and subsequently excreted into hospital wastewater. As these substances are known to be carcinogenic, mutagenic and toxic for reproduction, they should be removed from wastewater at their source of origin.

In this study the fate and effects of the cancerostatic platinum compounds (CPC) cisplatin, carboplatin, oxaliplatin, 5-fluorouracil (5-FU) and the anthracyclines doxorubicin, daunorubicin and epirubicin were investigated in hospital wastewater. Wastewater from the in-patient treatment ward of a hospital in Vienna was collected and monitored for the occurrence of the selected drugs. A calculation model was established to spot the correlation between administered dosage and measured concentrations. To investigate the fate of the selected substances during wastewater treatment, the oncologic wastewater was treated in a pilot membrane bioreactor system (MBR) and in downstream advanced wastewater treatment processes (adsorption to activated carbon and UV-treatment). Genotoxic effects of the oncologic wastewater were assessed before and after wastewater treatment followed by a risk assessment.

Monitoring concentrations of the selected cytostatics in the oncologic wastewater were in line with calculated concentrations. Due to different mechanisms (adsorption, biodegradation) in the MBR-system 5-FU and the anthracyclines were removed ~ LOD, whereas CPC were removed by 60%. In parallel, genotoxic effects could be reduced significantly by the MBR-system. The risk for humans, the aquatic and terrestrial environment by hospital wastewater containing cytostatic drugs was classified as small in a preliminary risk assessment.

Keywords Advanced wastewater treatment processes; cytostatic agents; hospital wastewater; membrane bioreactor system (MBR); risk assessment

Introduction

In the last decade the growing use of cytostatic agents, which are used in cancer therapy, has become a new environmental problem. As these substances act by either inhibiting cell growth or directly killing cells, several cytostatics are categorized as CMR-drugs (carcinogenic, mutagenic and reprotoxic). Different sources like emissions from production sites or direct disposal of pharmaceuticals in households contribute to the potential pollution of the environment, but the main source of cytostatic compounds in wastewater or in the environment are excretions (urine and faeces) of patients under medical treatment. As the separate collection of the excretions of cancer patients is regarded as intractable and dangerous (WHO, 1999), cytostatic agents and their metabolites are continuously disposed into hospital or municipal wastewater. The dilution of the excreted concentrations of cytostatic agents implicates the dispersion of the substances in the wastewater system and aggravates their removal in wastewater treatment plants. For some of the substances the incomplete elimination in wastewater treatment plants has already been investigated by Kuemmerer (1998).

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Although a high percentage of cancer therapy is predominantly done in out-patient treatment wards (e.g. in the investigated hospital 80%), relevant amounts of cytostatics can be individualised in the wastewater of in-patients treatment wards. The selective collection and treatment of this highly loaded wastewater stream in hospitals would facilitate an elimination of cytostatic agents at their source of origin and reduce the amount of cytostatics in the environment. This possible treatment gains even more importance as the European Community bans the discharge of chemicals and metabolites with carcinogenic or mutagenic potential into the wastewater system (Council Directive (80/68/EWG), 1991; Council Directive (76/464/EWG), 2000).

The main objectives of the project “Chemical analysis, risk assessment and elimination of selected cytostatic agents from hospital wastewater” focused on fate and effects of selected cytostatic agents in the wastewater stream, from their source of origin to the aquatic environment. The project included a calculation and chemical monitoring of exposure, biological effect monitoring (genotoxicity assays) and finally, a risk assessment for the cancerostatic platinum compounds (CPC) cisplatin, carboplatin, oxaliplatin, 5-fluorouracil (5-FU) and the anthracyclines doxorubicin, daunorubicin and epirubicin originating from a hospital in Vienna. The substances were selected for investigation due to their consumption and their hazardous properties.

Materials and methods

Experimental setup

For the calculation of concentrations of anticancer agents in the wastewater of the in-patient treatment ward of the investigated hospital, an input-output model calculation was established based on the parameters drug consumption, water consumption and renal human excretion rates. For the consumption of the cytostatic drugs, data from the central cytostatics pharmacy of the hospital were compiled and for the excretion rates, medical data were used. By this, minimum and maximum average concentrations in the sewer of the oncologic in-patient treatment ward were calculated for the administered cytostatics (Mahnik et al., 2003).

Excretion of the selected cytostatic agents into hospital wastewater as well as their fate in wastewater treatment plants were investigated in a pilot membrane bioreactor system (MBR) (Weissenbacher et al., 2005), which was fed with wastewater from the oncologic in-patient treatment ward of the university affiliated hospital. The wastewater collection system of the oncologic ward of the hospital was reconstructed in a way to allow the collection of the full amount of wastewater from 18 in-patients (75% of the oncologic ward of the hospital) over 24 h. The wastewater was stored in two 1,000 L tanks at the technical service level of the hospital and treated in a pilot MBR system. The system consisted of a 150 L aeration tank and a subsequent tubular ultrafiltration membrane (cross-flow mode, MOLSEP®, Nadir Filtration GmbH) with an active area of 1 m² and a nominal cut-off of 100 kDa. The bioreactor system was run with a hydraulic average load of 260 L d⁻¹. The concentration of suspended solids in the aeration tank amounted to 11.8–15.2 g L⁻¹.

Over a time period of 18 months four monitoring periods (covering 98 sampling days in total) were conducted. In the first monitoring period only the occurrence of the substances in wastewater was investigated. Daily sampling of the influent, the effluent and the activated sludge of the MBR-system from the second to the fourth monitoring allowed first conclusions on the elimination rates of the selected compounds in wastewater treatment.

In order to investigate the performance of advanced wastewater treatment processes two additional treatment methods were established after the MBR and sampled in the
frame of the fourth monitoring period. The effluent of the MBR was divided with one part of the effluent exposed to adsorption to activated carbon and the other part exposed to UV-radiation and subsequent adsorption to activated carbon. Adsorption to activated carbon was established by glass columns (36.7 cm filter length and 19.6 cm² filter width) filled with 500 g of granular activated carbon (Chemviron F200) and fed in up-flow mode (flow rate 7.6 L h⁻¹). UV-radiation at 254 nm was provided by a low pressure mercury lamp (UWZ 48 UVAUDES⁶) with an energy input of 900 W m⁻². The average radiation time of 2 min. resulted in an energy input of 110 kJ m⁻².

For the monitoring of biological effects of the oncologic wastewater, samples were taken from the influent and the effluent of the MBR-system as well as behind the advanced wastewater treatment processes during two test series.

Eco-toxicological risk assessment was carried out according to the EU-guidelines TGD-RA (2003) and EMEA (2005). As data on ecotoxicological profiles of the selected cytostatic agents was not sufficient, only a preliminary risk assessment focusing on priorities of possible measures on risk management could be done.

**Analytical methods**

The total amount of platinum in all investigated samples was measured using a quadrupole based inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC II, PE SCIEX). The limit of detection (LOD) of platinum was 0.01 µg L⁻¹. For CPC speciation online coupling of an inert high performance liquid chromatography (HPLC) gradient system (Rheos 2000, Flux Instruments AG) to ICP-MS was used (Hann et al., 2005). The LOD was 0.09 µg L⁻¹ for cisplatin, 0.10 µg L⁻¹ for carboplatin and 0.15 µg L⁻¹ for oxaliplatin. Total Pt-concentrations adsorbed to activated sludge were analysed after digestion according to DIN 38414-S7 (DEV, 2003; Lenz et al., 2007).

For the analysis of 5-FU, the samples were concentrated by a factor 500 using solid-phase-extraction on ENV⁺. During analysis by capillary electrophoresis (CE, Agilent), 5-FU was monitored at 265 nm with a LOD of 1.7 µg L⁻¹ (Mahnik et al., 2004).

For the analysis of the anthracyclines, sample enrichment by a factor 100 was performed using solid phase extraction columns (C8 columns) followed by analysis using HPLC (Agilent, 1090). Substances were identified by their retention time in the fluorescence scan (fluorescence detection) and quantified by peak area (Mahnik et al., 2006). The LOD for epirubicin and doxorubicin was 0.05 µg L⁻¹, for daunorubicin it was 0.06 µg L⁻¹.

Monitoring of mutagenic and genotoxic effects was performed by two test procedures, the Salmonella/Microsoma test (Ames, 1983) using TA98 and TA100 as well as single cell gel electrophoresis test with primary rat liver hepatocytes (Ferk et al., 2007). For the assessment of acute toxicity, liver hepatocytes were incubated with wastewater samples for 60 min. and viability was detected by using Trypan blue exclusions technique. DNA-migration was either assessed after treatment with H₂O₂ (100 µM, 5 min.) or in untreated cells using electrophoresis (20 min., 40 min.).

**Results and discussion**

**CPC in hospital wastewater and their fate in wastewater treatment**

The four monitoring periods revealed similar concentrations of total Pt in the wastewater of the oncologic in-patient treatment ward (Table 1). Only 27%–34% of total administered Pt was found in the wastewater of the oncologic in-patient treatment ward which can be explained by a short in-patient stay of cancer patients in comparison to the biological half-life of CPC (Lenz et al., 2005). Measured concentrations were in good accordance with calculated ones considering minimal excretion rates. Adsorption to activated sludge was the mechanism of CPC elimination in the MBR (Lenz et al.,
2007). During the operation of the bioreactor over a time period of one year, Pt concentrations adsorbed to activated sludge were consequently increasing, ranging from 7.1 μg Pt g⁻¹ TSS after 30 days of wastewater treatment to 175 μg Pt g⁻¹ TSS after one year of operation. This indicates that the maximum adsorption capacity of the activated sludge had not been reached after the third monitoring. In spite of the high loading of the sludge, adsorption efficiencies in the membrane bioreactor did not significantly decrease. Elevated concentrations in the effluent of the membrane system originate from Pt concentration peaks in the influent of the reactor and not from saturation of the adsorption capacity of the activated sludge (see Figure 1). Low concentrations of Pt were always present in the effluent of the MBR-system. This might be due to the fact that the contact time of wastewater and activated sludge was too short for removal < LOD.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>1st monitoring</th>
<th>2nd monitoring</th>
<th>3rd monitoring</th>
<th>4th monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administered amount of Pt</td>
<td>19.5–590</td>
<td>26.2–651</td>
<td>23.5–450</td>
<td>105–532</td>
</tr>
<tr>
<td>Calculated concentration in the wastewater of the in-patient treatment ward (μg Pt L⁻¹)</td>
<td>min</td>
<td>2.0–199</td>
<td>2.7–230</td>
<td>2.5–144</td>
</tr>
<tr>
<td>Influent (μg L⁻¹ Pt)</td>
<td>max</td>
<td>6.6–303</td>
<td>8.8–343</td>
<td>7.9–232</td>
</tr>
<tr>
<td>mean</td>
<td>30.6</td>
<td>38.1</td>
<td>35.2</td>
<td>41.3</td>
</tr>
<tr>
<td>Recovery in the wastewater*</td>
<td>27% ± 12%</td>
<td>28% ± 12%</td>
<td>32% ± 9%</td>
<td>34%</td>
</tr>
<tr>
<td>Effluent (μg L⁻¹ Pt)</td>
<td>min/max</td>
<td>2.81–144</td>
<td>1.77–40.4</td>
<td>2.57–65.4</td>
</tr>
<tr>
<td>mean</td>
<td>18.7</td>
<td>14.9</td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td>Adsorption of Pt to activated sludge in the membrane bioreactor (%)</td>
<td>63% ± 17%</td>
<td>51% ± 17%</td>
<td>64% ± 3%</td>
<td></td>
</tr>
</tbody>
</table>

*Based on a weekly input/output budget

During the operation of the bioreactor over a time period of one year, Pt concentrations adsorbed to activated sludge were consequently increasing, ranging from 7.1 μg Pt g⁻¹ TSS after 30 days of wastewater treatment to 175 μg Pt g⁻¹ TSS after one year of operation. This indicates that the maximum adsorption capacity of the activated sludge had not been reached after the third monitoring. In spite of the high loading of the sludge, adsorption efficiencies in the membrane bioreactor did not significantly decrease. Elevated concentrations in the effluent of the membrane system originate from Pt concentration peaks in the influent of the reactor and not from saturation of the adsorption capacity of the activated sludge (see Figure 1). Low concentrations of Pt were always present in the effluent of the MBR-system. This might be due to the fact that the contact time of wastewater and activated sludge was too short for removal < LOD.
It can be concluded that elimination of CPC by wastewater treatment is due to both removal of the solid constituents of wastewater and adsorption of platinum compounds to activated sludge. In the MBR-system highly efficient removal of solid constituents is achieved by membrane filtration (<0.1 μm) leading to a low total solid and CPC concentration.

Species analysis revealed that Pt-concentrations in the wastewater originated mainly from carboplatin (0.56 μg L\(^{-1}\) Pt–65.1 μg L\(^{-1}\) Pt), while cisplatin was detected only once. Oxaliplatin was not identified at all, which is in line with the studies done by Hann et al. (2003).

Figure 2 shows the elimination of total Pt from hospital wastewater using the advanced wastewater treatment processes UV-radiation and adsorption to activated carbon during the fourth monitoring period. Oxidation of CPC by UV-treatment resulted in a negligible reduction of total Pt, as the substances are only transformed by oxidation, while the total amount of Pt stays constant; 60% of carboplatin, the CPC predominant in the MBR-effluent, were transformed with Pt(0) supposed to be the end product. Combining UV-radiation and adsorption to activated carbon resulted in lower elimination of total Pt than the use of adsorption only. This might be due to the fact that degradation products of CPC show lower affinity to activated carbon.

5-FU and anthracyclines in hospital wastewater and their fate in wastewater treatment

The four monitoring periods revealed homogenous concentrations for 5-FU and doxorubicin in the wastewater of the oncologic in-patient treatment ward (Table 2). Epirubicin and daunorubicin were administered infrequently and could not be detected in the sewer.

Similar to the results for CPC monitored concentrations of 5-FU and doxorubicin were in line with calculated concentrations, when considering minimal excretion rates. In total, 0.5–4.5% of the administered amount of 5-FU and 0.1–0.2% of the administered amount of doxorubicin were found in the wastewater of the oncologic in-patient treatment ward. This might be explained by different reasons like the fact that only 75% of the toilets of the oncologic ward were connected to the storage tanks, or that human excretion rates vary considerably (Mahnik et al., 2007).

The wastewater was stored for approximately 24 h before entering the MBR-system. Samples taken immediately before entering the MBR-system revealed 5-FU and doxorubicin concentrations < LOD. Mahnik et al. (2007) incubated 5-FU with activated sludge.
Table 2 Administered amounts of 5-FU and doxorubicin at the oncologic in-patient-treatment ward as well as calculated and analysed concentrations in the wastewater (Mahnik et al., 2007)

<table>
<thead>
<tr>
<th></th>
<th>5-FU</th>
<th>Doxorubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Administered amount</td>
<td>Administered amount</td>
</tr>
<tr>
<td></td>
<td>min.</td>
<td>max.</td>
</tr>
<tr>
<td>Monitoring 1</td>
<td>1800</td>
<td>6083</td>
</tr>
<tr>
<td>Monitoring 2</td>
<td>790</td>
<td>3555</td>
</tr>
<tr>
<td>Monitoring 3</td>
<td>1010</td>
<td>4905</td>
</tr>
<tr>
<td>Monitoring 4</td>
<td>648</td>
<td>2788</td>
</tr>
</tbody>
</table>

5-FU and doxorubicin concentrations were calculated and analysed with a 2% and a 0.5% excretion rate, respectively.
and showed that >99% were removed from the liquid phase within 24 hours due to biodegradation and metabolization. Incubation of anthracyclines with activated sludge yielded an elimination of >90% from the liquid phase with adsorption being the major elimination pathway.

As suspended solids were present in the storage tanks and biomass production could not be inhibited, elimination during storage might be the reason, why neither 5-FU nor doxorubicin were detected immediately before entering the MBR-system. Accordingly, in the effluent of the MBR-system the concentrations of 5-FU and doxorubicin were < LOD.

Biological effect monitoring
The Salmonella/Microsoma test clearly showed that acute toxicity of the oncologic wastewater (bacteriocide and/or bacteriostatic effects) was reduced by wastewater treatment in the MBR-system. When applying single cell gel electrophoresis tests genotoxic effects of the oncologic wastewater (DNA single strands and double strands breaks) could be clearly identified in all oncologic wastewater samples (1.4–2.6 fold increase of DNA-migration compared to drinking water). The effects were dose-dependent; genotoxicity decreased with increasing dilution of the waste water. Behind the MBR-system genotoxicity of the wastewater decreased by 50% which means that biological wastewater treatment could significantly reduce the genotoxic activity of the wastewater. The advanced wastewater treatment processes resulted in no significant changes of the genotoxic effects of the wastewater.

Risk assessment
Biological effect monitoring revealed a low genotoxic potential of the wastewater of the oncologic in-patient treatment ward. Furthermore, genotoxic effects of the applied standard substances could be detected in the Ames test only in concentrations beyond typical wastewater concentrations. Considering a “worst-case”-scenario, the calculated maximum concentrations of the selected cytostatic agents (using maximum excretion rates found in literature) in the total wastewater of the hospital were opposed to the lowest effective dose (LED) and to the highest ineffective dose (HID) of the substances (IARC, 1987; Gebel et al., 1997). It could be shown that the maximum calculated concentrations for all substances were below LED (1–3 times the factor 10). Data on ecotoxicity of the cytostatic drugs are very scarce. According to the draft of the European Medicines Agency for a “Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use” it was concluded that the available data were not sufficient to perform a risk assessment and a preliminary risk assessment was performed in order to gain further information concerning priorities and the necessity of risk management measures. Based on the available data it was concluded that the risk derived from the cytostatic drugs in the hospital waste water was low, assuming the worst case scenario: maximum bioavailability and the presence of cisplatinum, which has the highest toxicity compared with other platinum compounds. However, additive effects, which had not explicitly been quantified in the present study have to be taken into account. An inactivation of the selected substances can only be achieved by wastewater treatment.

Conclusion
From the results of this study it can be concluded that the established model calculation was a suitable method to estimate the concentrations of selected cytostatic agents in hospital wastewater. 0.1%–34% of the selected cytostatic agents administered in the oncologic in-patient treatment ward could be analysed in the oncologic wastewater. Wastewater treatment by means of a MBR-system was able to remove the selected cytostatic drugs due to different mechanisms. Based on metabolization and biodegradation 5-FU was reduced in the liquid phase < LOD. Anthracyclines (< LOD) and CPC
(60% removal of total Pt) were removed from the liquid phase in the MBR due to adsorption processes and the efficient retention of suspended solids during membrane filtration. Adsorption to activated carbon resulted in a further removal of CPC.

The endangerment of humans, the aquatic and terrestrial environment by cytostatic drugs in hospital wastewater was preliminarily (due to limited data-base) classified as low. According to EU guideline 2000/60/EC, however, Member States have to adhere to the highest possible ecological and chemical status, given impacts that could not reasonably have been avoided due to the nature of the human activity or pollution. Therefore it is recommended to prevent the discharge of CMR-substances into sewer systems and subsequently into the environment. This can be achieved by advanced wastewater treatment methods to remove high concentrations at the source as successfully demonstrated within the framework of this project.

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References


