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# A Preliminary Study on the Occurrence of Cytostatic Drugs in Hospital Effluents in Beijing, China

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**Abstract** Cytostatic drugs are used in cancer therapy. They can enter hospital wastewater due to excretion by patients undergoing chemotherapy. Little attention has been paid to these drugs in China even though their usage is high. The effluents of 21 hospitals of different size in Beijing, China, were investigated on 1–7 different days. Nine cytostatic compounds (methotrexate, azathioprine, doxorubicin, doxorubicinol, vincristine, ifosfamide, cyclophosphamide, etoposide, and procarbazine) were tested. Of the 65 effluent samples analyzed, the median concentrations for methotrexate, azathioprine, ifosfamide, cyclophosphamide and etoposide were 17, 15, 151, 100 and 42 ng/L, respectively. Doxorubicin, doxorubicinol, vincristine and procarbazine were not detected in this study. These results suggested that the hospital effluents are an important source of certain cytostatic drugs in aqueous environment.

**Keywords** Cytostatic drugs · Occurrence · Hospital effluents · UPLC-ESI–MS/MS

Recent studies have demonstrated that cytostatic drugs can exert carcinogenic, mutagenic and/or teratogenic effects in experimental animals and humans (Falck et al. 1979; DeMéo et al. 1995; Santos and Pacheco 1995; Jackson et al. 1996; Zhiyi and Haowen 2004; IARC 2008). Such adverse effects, in principle, may also be expected to occur in aquatic organisms such as fish.

The use of these drugs in hospitals for cancer therapy has increased considerably in the past decades due to the steady increase in the number of cancer patients worldwide. After administration, most of these agents are incompletely metabolized in the body. They can therefore enter hospital wastewater in their active forms via the urine and feces of patients undergoing chemotherapy (Turci et al. 2003). Discharges of effluents from hospitals usually reach the municipal sewage system after simple disinfection. Hospitals should therefore be considered to be the most important point sources of cytostatic drugs in the aqueous environment. The occurrence of these drugs in hospital effluents can serve as a starting point to monitor their fate in the environment.

The occurrence of cytostatic drugs in hospital effluents in Europe has been documented (Steger-Hartmann et al. 1996; Kümmerer 2000; Mahnik et al. 2007). Several studies examined the residues of some cytostatic agents in sewage treatment plant (STP) effluents and surface waters. Methotrexate, cyclophosphamide and ifosfamide were shown to be present in STP effluents at the level of nanograms per liter (Kümmerer et al. 1997; Steger-Hartmann et al. 1997; Castiglioni et al. 2005; Buerge et al. 2006). Cyclophosphamide was also found in lake water at a concentration of 0.07 ng/L (Buerge et al. 2006). These exposure concentrations are several orders of magnitude lower than the concentrations at which acute toxicological effects have been reported for aquatic organisms (Santos and Pacheco 1995; Matsumoto and Cólus 2000). However, studies on the chronic effects of these drugs on aquatic organisms are lacking. These compounds have a very potent mechanism of action (inhibiting the growth and division of cells) and often exhibit carcinogenic, mutagenic

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or teratogenic properties (IARC 2008), so their presence in the aquatic environment should not be ignored.

According to the Chinese Ministry of Health (MOH), 2.1 million cancer cases were estimated for the year 2000, and this figure was expected to increase by 14.6% by 2005 (Chinese Ministry of Health 2002). Therefore, the consumption of cytostatic drugs in China should be very large. However, most of the surveys charting the occurrence of these pharmaceuticals were done in Europe; little work has been carried out in China. The pattern and level of use of these pharmaceuticals varies between countries, so the levels of these drugs in hospital effluents may be different in China. A preliminary survey based on an established ultra-high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI–MS/MS) method for determination of nine cytostatic compounds in hospital effluents in Beijing, China, is presented. This is the first survey on the occurrence of cytostatic drugs in the aqueous environment in China.

## Materials and Methods

Twenty-one hospitals in Beijing were chosen to assess the occurrence of target pharmaceuticals in their effluents. Detailed information on hospital accommodations, outflow rates, and the type of each hospital is shown in Table 1. Grab sampling at the same time each day was the only way to collect effluent samples because composite sampling during a certain period was not permitted by the hospitals investigated. Effluent samples were collected in glass bottles at 16:00 h on 1–7 different days in December 2008 and January 2009. Glass bottles were pre-rinsed several times with ultrapure water in the laboratory, and rinsed with sample water on site. On arrival in the laboratory, samples were filtered through a GF/A glass fiber membrane (1.6 μm; Whatman, Maidstone, Kent, UN) immediately for purification. Water samples were extracted within 24 h to avoid degradation.

We chose eight cytostatic drugs based on their frequent use in hospitals and ease of analyses: methotrexate, azathioprine, doxorubicin, vincristine, ifosfamide, cyclophosphamide, etoposide and procarbazine. Doxorubicinol, the toxic metabolite of doxorubicin, was also studied. These pharmaceuticals were purchased from Sigma–Aldrich (St. Louis, MO, USA) at the highest available purity (>99%). A stock standard solution of methotrexate was prepared in methanol–water (50:50, v/v) containing 0.01 M hydrochloric acid. Standard stock solutions of the other drugs were prepared in methanol. Common dosages of these drugs and their estimated excretion rates in humans are listed in Table 2.

Acetonitrile and methanol were of high-performance liquid chromatography (HPLC) grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ultra-pure water was obtained by using an in-house Milli-Q® ultrapure water system (Millipore, Bedford, MA, USA) at a resistivity of 18.2 MΩ/cm. HPLC-grade formic acid (HCOOC, 99%) was purchased from Acros Organics (Bridgewater, NJ, USA). Sodium hydroxide (NaOH), hydrochloric acid (36% HCl) and ammonia (25–28% $NH_3$) were of analytical grade and obtained from Beijing Chemical Company (Beijing, China). Glassware was rinsed with HPLC-grade methanol and ultrapure water. It was then baked in a muffle furnace at 400°C for 4 h before use to avoid contamination.

The procedure for sample preparation was the one previously established by our research team (*submitted to Journal of Chromatographic Science, accepted*). Briefly, sample extraction was done with an Oasis HLB (200 mg, 6 mL) cartridge (Waters, Milford, MA, USA). The cartridge was pre-conditioned with 6 mL methanol and 6 mL ultrapure water (pH 2). One-hundred milliliters of acidified sewage (pH = 2) was passed through a HLB cartridge at 5 mL/min under vacuum. An additional 5 mL of acidified water (pH = 2) was applied to wash the cartridge wall, which was then washed with 3 mL methanol–water (30:70, v/v). The drugs were eluted from the cartridge by 6 mL methanol–water (80:20, v/v). The eluate was dried under a gentle stream of nitrogen. The residual was reconstituted in 500 μL methanol, and diluted to 2 mL with water. For further purification, this extract was applied to an Oasis WAX cartridge (150 mg, 6 mL) conditioned with 6 mL methanol and 6 mL water. Target compounds were eluted consecutively with 7 mL methanol–water (60:40, v/v) and 5 mL methanol–water (40:60, v/v) containing 0.1% formic acid. The elution solvent was evaporated to dryness with nitrogen and dissolved in 500 μL methanol–water (50:50, v/v).

Pharmaceuticals were separated on an ACQUITY UPLC™ BEH C18 column (2.1 mm × 150 mm, 1.7 μm) using an ACQUITY Ultra Performance LC system from Waters Company. LC analyses were done with 0.01% formic acid in ultrapure water as eluent A and acetonitrile as eluent B at a flow rate of 0.3 mL/min. Gradient conditions were as follows: 0–2.50 min, linear from 5 to 35% B; 2.50–4.20 min, linear from 35 to 70% B; 4.20–4.50 min, linear from 70 to 100% B; 4.50–6.50 min, isocratic 100% B; 6.50–7.00 min, linear from 100 to 5% B; 7.00–10.00 min, isocratic 5% B. The oven temperature of the column was 40°C. An injection volume of 10 μL was used.

Under the LC conditions above, column effluent was monitored by a Waters Quattro Premier XE™ triple quadrupole mass spectrometer equipped with an ESI interface operated in the positive mode. Nitrogen gas was used as the cone and desolvation gas at the flow rates of 50 and 650 L/h, respectively. Source temperature and

**Table 1** Hospital characteristics

| Hospital | Accommodation (number of beds) | Outflow rate ($m^3$/day) | Hospital type |
|---|---|---|---|
| A | 1008 | N.A. | General |
| B | 550 | N.A. | General |
| C | 150 | N.A. | Tibetan and ethnic medicine |
| D | 1200 | N.A. | General |
| E | 1315 | 1748 | General |
| F | 1100 | 1980 | General |
| G | 1800 | 3312 | General |
| H | 1062 | 1423 | General |
| I | 500 | N.A. | Chinese medicine |
| J | 575 | N.A. | Chinese medicine |
| K | 400 | 560 | General |
| L | 700 | 560 | General |
| M | 1420 | N.A. | General |
| N | 400 | N.A. | Pediatric |
| O | 600 | N.A. | Obstetrics and gynecology |
| P | 1200 | N.A. | Cancer |
| Q | 545 | 615 | Cancer |
| R | 139 | N.A. | General |
| S | 350 | N.A. | Chinese medicine |
| T | 250 | 300 | General |
| U | 970 | 1183 | Pediatric |

*N.A.* not available

**Table 2** Dosages and excretion in urine of unchanged cytostatic drugs

| Substances | Dosages (mg/$m^2$ body surface per day)[a] | Excretion (percentage of dose)[a] |
|---|---|---|
| Methotrexate | 3.3–30.0 | 60–95 |
| Azathioprine | 50–100 | Almost none |
| Doxorubicin | 30–75 | 5–20[b] |
| Vincristine | 1.5–2.0 | N.A. |
| Cyclophosphamide | 700–2800 | 5–25 |
| Ifosfamide | 1000–5000 | 12–61 (dose dependent) |
| Etoposide | 35–100 | 25 |
| Procarbazine | 50–100 | Almost none |

[a] Taken from rxlist (www.rxlist.com)

[b] Taken from the literature (McVie and Schwartsmann 1995)

*N.A.* not available

desolvation temperature were 100 and 450°C, respectively. The capillary voltage was 3.0 KV. During tandem mass spectrometric analysis, argon was the collision gas, and the pressure of the collision chamber was $3.3 \times 10^{-3}$ mbar. Sample acquisition was made in multiple-reaction monitoring mode, with dwell time varying between 50 and 500 ms depending on the compound. The selection of precursor and production ions, cone voltage, and collision energy for each compound was optimized individually to achieve maximal sensitivity. For each drug, two transitions were selected for identification but only one was used for quantification.

Absolute detection limits were in the low ng/L range. Performance of the entire analytical procedure was estimated using spiking samples of all target compounds, with recoveries ranging from 51 to 105% and a relative standard deviation of 2–20%. Detection limits were estimated to be 2 ng/L for cyclophosphamide, ifosfamide and methotrexate; 5 ng/L for etoposide, azathioprine and procarbazine; 10 ng/L for doxorubicin and doxorubicinol; and 20 ng/L for vincristine.

## Results and Discussion

The concentrations of nine cytostatic compounds in 65 sewage samples were determined over several days to provide a preliminary overview of the occurrence of these compounds in the effluents of 21 hospitals in Beijing, China. Methotrexate, azathioprine, cyclophosphamide,

ifosfamide and etoposide were found in the water samples of at least one hospital, whereas the other drugs (doxorubicin, doxorubicinol, vincristine, and procarbazine) were not detectable in any of these samples. Detected pharmaceuticals entered hospital effluents at levels of 4–10647 ng/L (Table 3). Figure 1 shows the LC–MS/MS chromatograms of five drugs found in effluent samples.

Cyclophosphamide and ifosfamide were the most abundant pharmaceuticals. They were present in most of the hospital effluents investigated in the present study. Cyclophosphamide was present in 47 out of 65 effluent samples, and the concentrations ranged from 6 to 2000 ng/L (median, 100 ng/L). Ifosfamide was detected in 38 of 65 samples with concentrations ranging from 4 to 10647 ng/L (median, 151 ng/L). These concentration values are comparable with those described by Steger-Hartmann and Kümmerer in Germany (Steger-Hartmann et al. 1996; Kümmerer 2000), who reported concentrations of 20–4500 ng/L for

**Table 3** Concentrations of selected cytostatic drugs in effluent samples taken from 21 hospitals on 1–7 days

| Hospital | Sampling date | Concentrations (ng/L)[a] Cyclophosphamide | Ifosfamide | Methotrexate | Etoposide | Azathioprine |
|---|---|---|---|---|---|---|
| A | 14 Jan 2009 | 6 | <2 | <2 | 51 | <5 |
| | 15 Jan 2009 | 432 | 4 | <2 | <5 | <5 |
| | 16 Jan 2009 | 36 | 17 | <2 | <5 | <5 |
| B | 14 Jan 2009 | 53 | 384 | <2 | <5 | <5 |
| | 15 Jan 2009 | <2 | 9 | <2 | <5 | <5 |
| | 16 Jan 2009 | 9 | 25 | <2 | <5 | <5 |
| C | 14 Jan 2009 | 1116 | <2 | 19 | <5 | <5 |
| | 15 Jan 2009 | 827 | <2 | <2 | <5 | <5 |
| | 16 Jan 2009 | 800 | <2 | <2 | <5 | <5 |
| D | 14 Jan 2009 | 45 | <2 | 6 | <5 | <5 |
| | 15 Jan 2009 | 62 | <2 | <2 | <5 | <5 |
| | 16 Jan 2009 | 38 | <2 | 4 | <5 | <5 |
| E | 14 Jan 2009 | 62 | 447 | <2 | <5 | <5 |
| | 15 Jan 2009 | 225 | 195 | <2 | 6 | <5 |
| | 16 Jan 2009 | 18 | <2 | <2 | <5 | <5 |
| F | 14 Jan 2009 | 119 | 39 | <2 | 42 | <5 |
| | 15 Jan 2009 | 71 | <2 | 10 | 120 | <5 |
| | 16 Jan 2009 | 1717 | 1053 | <2 | <5 | <5 |
| | 17 Jan 2009 | 247 | 44 | <2 | <5 | <5 |
| G | 11 Dec 2008 | 2000 | 268 | 11 | <5 | <5 |
| | 14 Jan 2009 | 291 | 164 | <2 | <5 | <5 |
| | 15 Jan 2009 | 242 | 192 | <2 | <5 | <5 |
| | 16 Jan 2009 | 95 | 281 | <2 | <5 | <5 |
| H | 14 Jan 2009 | 36 | 10 | <2 | <5 | <5 |
| | 15 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| | 16 Jan 2009 | 23 | 17 | <2 | <5 | <5 |
| I | 14 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| | 15 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| | 16 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| J | 14 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| | 15 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| | 16 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| K | 14 Jan 2009 | 41 | 7 | <2 | <5 | <5 |
| | 15 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| | 16 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| L | 14 Jan 2009 | 65 | 124 | <2 | 57 | 15 |
| | 15 Jan 2009 | 57 | 46 | <2 | 40 | 9 |
| | 16 Jan 2009 | 55 | 67 | <2 | 37 | 32 |

**Table 3** continued

| Hospital | Sampling date | Concentrations (ng/L)[a] Cyclophosphamide | Ifosfamide | Methotrexate | Etoposide | Azathioprine |
|---|---|---|---|---|---|---|
| M | 14 Jan 2009 | 127 | <2 | <2 | <5 | <5 |
| | 15 Jan 2009 | 368 | 70 | <2 | 18 | <5 |
| | 16 Jan 2009 | 79 | 138 | <2 | 62 | <5 |
| N | 14 Jan 2009 | 1011 | 5 | 4689 | 49 | <5 |
| | 15 Jan 2009 | 51 | 59 | 91 | 56 | <5 |
| O | 14 Jan 2009 | <2 | 40 | 46 | <5 | <5 |
| | 15 Jan 2009 | 76 | 4 | 4 | <5 | <5 |
| | 16 Jan 2009 | <2 | 350 | <2 | 34 | <5 |
| P | 8 Dec 2008 | 100 | 1400 | <2 | <5 | <5 |
| | 11 Dec 2008 | 680 | 1329 | <2 | <5 | <5 |
| | 16 Dec 2008 | 27 | 1300 | <2 | <5 | <5 |
| | 18 Dec 2008 | 430 | 2500 | <2 | <5 | <5 |
| | 14 Jan 2009 | 369 | 3400 | <2 | <5 | <5 |
| | 15 Jan 2009 | 271 | 1894 | 7 | <5 | <5 |
| | 16 Jan 2009 | 138 | 2594 | <2 | <5 | <5 |
| Q | 13 Dec 2008 | 1900 | 10647 | 3000 | 380 | <5 |
| | 15 Jan 2009 | 1872 | 1188 | 29 | <5 | <5 |
| | 16 Jan 2009 | 1817 | 1663 | <2 | <5 | <5 |
| R | 15 Jan 2009 | 46 | 112 | 14 | 29 | <5 |
| | 16 Jan 2009 | 12 | <2 | <2 | <5 | <5 |
| S | 14 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| | 15 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| | 16 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| T | 14 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| | 15 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| | 16 Jan 2009 | <2 | <2 | <2 | <5 | <5 |
| U | 16 Jan 2009 | 253 | <2 | 245 | 33 | <5 |

[a] The following pharmaceutical compounds in all samples were below the limit of detection: procarbazine <5 ng/L, doxorubicin <10 ng/L, doxorubicinol <10 ng/L, vincristine <20 ng/L

cyclophosphamide and 6–1900 ng/L for ifosfamide. The relatively high occurrence and concentrations of these two cytostatic agents in hospital effluents may be attributed to their frequent use in the chemotherapy of various cancers (bronchial, breast, ovarian; lymphoma, leukemia), treatment of autoimmune disease, and as immunosuppressants after organ transplantations (Zhang et al. 2005). The administered doses of the two oxazaphosphorines are usually much higher than the others (Table 2).

Methotrexate, etoposide and azathioprine were detectable in a few effluents, but they were not as widespread as cyclophosphamide and ifosfamide. Methotrexate was observed in only 21.5% of samples at a maximal level of 4689 ng/L, which was the second-highest concentration observed in this study, but the 80-percentile value was already decreased to 245 ng/L. Etoposdie was detected in 15 effluent samples with concentrations ranging from 6 to 380 ng/L (median, 42 ng/L). Azathioprine was detected in three samples collected from the same hospital on three different days at low ng/L level. Azathioprine is extensively converted to 6-mercaptopurine in the blood and not detectable in urine after 8 h (Van Os et al. 1996), so its appearance in effluents is probably due to improper disposal of unused medications in the drains of that hospital. The occurrence of these three cytostatic compounds in hospital effluents has not been reported before.

Doxorubicin in the effluents from a cancer hospital was investigated, and concentrations ranging from 0.26 to 1.35 μg/L were observed (Mahnik et al. 2007). Neither doxorubicin nor its toxic metabolite doxorubicinol was detectable in any of the samples collected in the present study. Vincristine and procarbazine were also not observed. Their absence may be attributed to the low usage of these drugs in the hospitals investigated and/or the low excretion rates of unchanged drugs (particularly procarbazine, see Table 2). Most of the wastewater from these hospitals was treated with

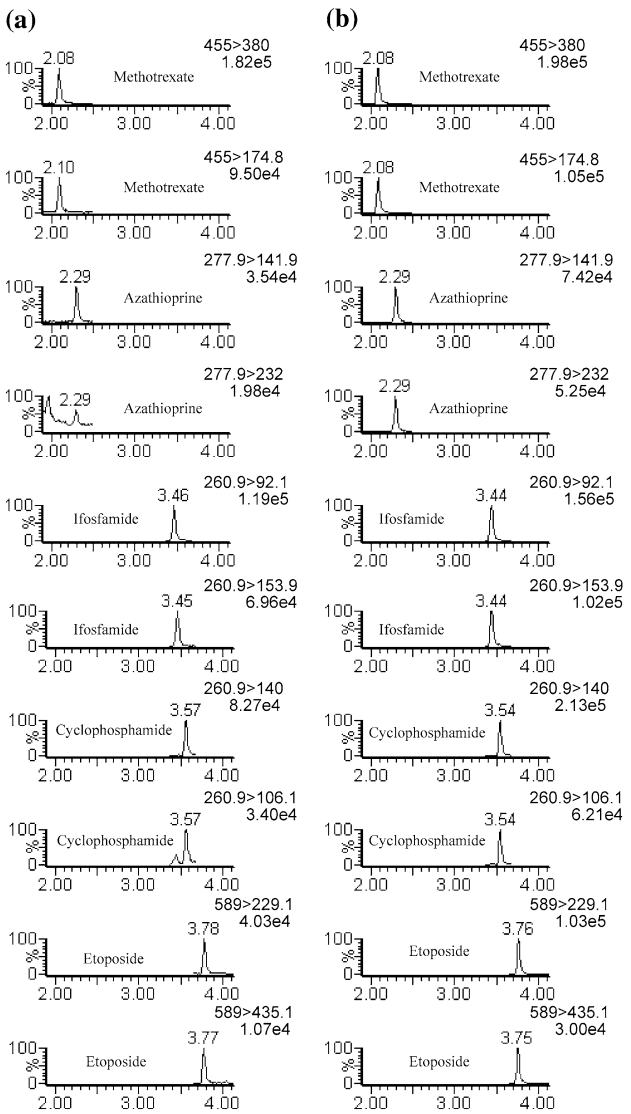


**Fig. 1** LC-MS–MS-MRM chromatograms of the detected drugs **a** methotrexate, azathioprine, cyclophosphamide, ifosfamide and etoposide in effluent samples at concentrations of 91, 32, 51, 59 and 56 ng/L, respectively, **b** a 10 μg/L standard

chlorine disinfection before discharge. These drugs may therefore be partially or completely transformed, and the concentrations in effluents below the limits of detection.

Table 3 shows the distribution of detected drugs in the effluents of different hospitals. The drugs tested were mostly detected in samples collected from large hospitals or cancer hospitals specializing in tumor treatment. For these hospitals, the total loads of cytostatic drugs released into wastewater were usually much greater, so the concentrations of the drugs in their effluents could be higher than smaller hospitals. In the samples of hospitals F, P and Q, cyclophosphamide and ifosfamide were observed up to μg/L level, much higher than those detected in the small hospitals K, R and T. There were some exceptions, such as hospitals C, D and H. In the case of hospitals D and H, their use of large amounts of drugs may be partially counteracted by their higher consumption of water compared with smaller hospitals, and the final concentrations of cyclophosphamide and ifosfamide in their effluents was not very high. The daily consumption of water of hospital C (only 150 beds) should be much less, so the unchanged cyclophosphamide reached the effluents with comparatively little dilution and its final concentrations were near or up to the μg/L level. Drug occurrence was also distinctly influenced by the patterns of pharmaceutical use, which varied considerably among hospitals. This could explain why the detected drugs, particularly the less prevailing ones (i.e., etoposide, methotrexate, azathioprine), were undetectable in any effluent samples. For the samples from hospitals I, J and S, none of the investigated drugs were detected, indicating their infrequent use in the hospitals investigated. Drug concentrations measured for the same hospital could differ between a low ng/L and μg/L range on different days (e.g., ifosfamide concentrations measured in samples from hospitals F and Q, and methotrexate concentrations measured in samples from hospitals N and Q). This phenomenon was similar to that reported by Mahnik et al. (2007), and was probably due to the high variability of the consumption of water and drug between hospitals. In the current study, the administered amounts of drugs on the sampling days were not accessible to us, and the mean rates of outflow were available only for a minority of hospitals. Predicted concentrations of the tested drugs in effluent samples could therefore not be calculated.

In conclusion, the present study demonstrates the occurrence of several cytostatic drugs (methotrexate, azathioprine, cyclophosphamide, ifosfamide and etoposide) in the effluents of hospitals located in Beijing, China. These drugs were subsequently discharged into a communal sewer, and then may be released into surface waters after treatment in STPs. Additional work should be carried out to elucidate levels of these drugs in STPs and surface waters. Determining the levels of other frequently used cytostatic drugs not tested in the current study would be beneficial.

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## References

Buerge IJ, Buser HR, Poiger T, Muller MD (2006) Occurrence and fate of the cytostatic drugs cyclophosphamide and ifosfamide in wastewater and surface waters. Environ Sci Technol 40:7242–7250. doi:10.1021/es0609405

Castiglioni S, Bagnati R, Calamari D, Fanelli R, Zuccato E (2005) A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in

urban wastewaters. J Chromatogr A 1092:206–215. doi:10.1016/j.chroma.2005.07.012

Chinese Ministry of Health (2002) Statistical data of death causes. In: China health statistical yearbook 2000. http://www.moh.gov.cn/publicfiles/business/htmlfiles/zwgkzt/ptjty/200805/35308.htm. Accessed May 2002 (in Chinese)

DeMéo MP, Mérono S, DeBaille AD, Botta A, Laget M, Guiraud H, Duménil G (1995) Monitoring exposure of hospital personnel handling cytostatic drugs and contaminated materials. Int Arch Occup Environ Health 66:363–368. doi:10.1007/BF00383141

Falck K, Grohn P, Sorsa M, Vainio H, Heinonen E, Holsti LR (1979) Mutagenicity in urine of nurses handling cytostatic drugs. Lancet 313:1250–1251

IARC (2008) Overall evaluations of carcinogenicity to humans-list of all agents, mixtures and exposures evaluated to date. In: Complete list of agents evaluated and their classification. http://monographs.iarc.fr/ENG/Classification/index.php. Accessed 27 April 2009

Jackson MA, Stack HF, Waters MD (1996) Genetic activity profiles of anticancer drugs. Mutat Res 355:171–208

Kümmerer K (2000) Laboratory scale degradation of pharmaceuticals and emission control (Abbau von Arzneimitteln in Testsystemen und Möglichkeiten zur Emissionsreduktion). Schriftenreihe Wasserforschung 6:165–177

Kümmerer K, Steger-Hartmann T, Meyer M (1997) Biodegradability of the anti-tumour agent ifosfamide and its occurrence in hospital effluents and communal sewage. Water Res 31:2705–2710

Mahnik SN, Lenz K, Weissenbacher N, Mader RM, Fuerhacker M (2007) Fate of 5-fluorouracil, doxorubicin, epirubicin, and daunorubicin in hospital wastewater and their elimination by activated sludge and treatment in a membrane-bio-reactor system. Chemosphere 66:30–37. doi:10.1016/j.chemosphere.2006.05.051

Matsumoto FE, Cólus IMS (2000) Micronucleus frequencies in Astyanax bimaculatus (Characidae) treated with cyclophosphamide or vinblastine sulfate. Genet Mol Biol 23:489–492

McVie JG, Schwartsmann G (1995) Oxford textbook of oncology. Oxford University Press, Oxford

Santos MA, Pacheco M (1995) Mutagenicity of cyclophosphamide and kraft mill effluent and sediment on the eel Anguilla anguilla L. Sci Total Environ 171:127–130

Steger-Hartmann T, Kummerer K, Schecker J (1996) Trace analysis of the antineoplastics ifosfamide and cyclophosphamide in sewage water by two step solid-phase extraction and gas chromatography-mass spectrometry. J Chromatogr A 726:179–184. doi:10.1016/0021-9673(95)01063-7

Steger-Hartmann T, Kummerer K, Hartmann A (1997) Biological degradation of cyclophosphamide and its occurrence in sewage water. Ecotoxicol Environ Saf 36:174–179. doi:10.1006/eesa.1996.1506

Turci R, Sottani C, Spagnoli G, Minoia C (2003) Biological and environmental monitoring of hospital personnel exposed to antineoplastic agents: a review of analytical methods. J Chromatogr B 789:169–209. doi:10.1016/S1570-0232(03)00100-4

Van Os EC, McKinney JA, Zins BJ, Mays DC, Schriver ZH, Sandborn WJ, Lipsky JJ (1996) Simultaneous determination of azathioprine and 6-mercaptopurine by high-performance liquid chromatography. J Chromatogr B 679:147–154. doi:10.1016/0378-4347(96)00007-2

Zhang J, Tian Q, Yung Chan S, Chuen Li S, Zhou S, Duan W, Zhu YZ (2005) Metabolism and transport of oxazaphosphorines and the clinical implications. Drug Metab Rev 37:611–703. doi:10.1080/03602530500364023

Zhiyi R, Haowen Y (2004) A method for genotoxicity detection using random amplified polymorphism DNA with Danio rerio. Ecotox Environ Safe 58:96–103. doi:10.1016/j.ecoenv.2003.09.016