Risks of cytostatics in the aquatic environment - a Dutch case study

Eri van Heijnsbergen and Heike Schmitt, University of Utrecht, Institute for Risk Assessment Sciences

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A literature based environmental risk assessment of cytostatics for the aquatic environment was made. Cytostatics were detected in hospital effluents in a concentration of 122 µg/l. They were also detected in the influent and effluent of sewage treatment plants (STP's). However, in the Netherlands cytostatics have not been demonstrated in surface waters. Data on effects on organisms by prolonged exposure are scarce. Based on these limited toxicity data and the maximum measured and estimated concentrations in surface water, a risk to aquatic organisms for three of four selected cytostatics (cisplatin, cyclophosphamide, methotrexate) is not expected. A low margin exists between the expected concentration of 5-fluorouracil in the surface water and the effect concentrations in chronic tests with algae and bacteria. Therefore, depending on the breakdown during sewage treatment, chronic effects in surface water cannot be excluded for this cytostatic.

In the Netherlands and other countries there is a growing interest for the effects that medicines/ pharmaceuticals may have on the environment. Knowledge about the occurrence of pharmaceuticals in water is important from the viewpoint of the possible negative effects on the ecology of surface water in the short and long term. Studies, mainly from Germany[15] but also from the Netherlands[14], have shown that pharmaceuticals and their transformation products occur in low concentrations in sewage, surface and even in drinking water. Especially aquatic organisms, being exposed to (combinations of) these and other industrial substances during their entire lifespan, could be affected.
Commissioned by Stichting Huize Aarde the Knowledge Point Beta-Sciences of the University of Utrecht approached the Institute of Risk Assessment Sciences (IRAS) for a literature based environmental risk assessment of cytostatics[5]. Cytostatics are substances used for chemotherapy. Generally a cytostatic impedes partition of cells by acting on the chemical reactions in the cell involved in the cell division. Because of their specific mechanisms, and because a number of cytostatics are known low-biodegradables, these pharmaceuticals deserve attention from the environmental point of view.
In the EU there are no legal standards for the presence of human pharmaceuticals in surface water, groundwater and drinking water. Ecotoxicological aspects of pharmaceuticals are addressed when approving new substances, and in the case of existing substances when these will be more widely used[2].
Based on the occurrence of cytostatics in the environment and available ecotoxicological data, a risk assessment is made for the cytostatics cisplatin, cyclophosphamide, 5-fluorouracil and methotrexate in the aquatic environment.

Emission and occurrence of cytostatics

Cytostatics and their transformation products reach the sewer almost exclusively through excretion by the patient. Unlike the reduction of occupational exposure to cytostatics, no measures are taken to prevent emission of cytostatics into the environment.
Concentration measurements in the environment are done, in particular, in Germany. In Dutch studies, only the cytostatics cyclophosphamide and ifosfamide are analysed, while the latter is not commonly used in the Netherlands[1]. Cyclophosphamide and ifosfamide are only found in two of the six Dutch studies[12,14], namely in hospital effluents to a concentration of 9.9 µg/l. Research from other countries also considered the cytostatic drugs cisplatin, 5-fluorouracil and methotrexate. In general the cytostatic concentrations are decreasing gradually - as expected - following the sequence hospital effluent, STP influent, STP effluent and surface water (see table). Because methotrexate is biodegradable, it is assumed that this substance can be removed in STPs[8]. By their low biological degradation, the cytostatics ifosfamide, cyclophosphamide and cisplatin more likely remain unchanged passing STPs[10]. The degree of biological degradation of 5-fluorouracil is unclear.

Measurements of cytostatics in the environment often disregard the metabolisation of cytostatics. For example, for cyclophosphamide only the original substance is analysed, while cyclophosphamide itself is inactive and in the body converted to active metabolites. These metabolites may reach the environment, as does cyclophosphamide.

Maximum concentrations measured, classified into concentration classes (ng/l), per environmental compartment and type of cytostatic.

Environmental compartment	Maximum > 1000	Maximum > 100	Maximum > 10	< Detection limit
Hospital effluent	cyclophosphamide (NL,D) 5-fluorouracil (A) methotrexate (GB) ifosfamide (NL,D) cisplatin (A,D)			
Influent STP		cyclophosphamide (D)	ifosfamide (D)	5-fluorouracil (US)
Effluent STP	ifosfamide (D)		cyclophosphamide (D)	5-fluorouracil (US)
Surface water				cyclophosphamide (D) ifosfamide (NL,D,B) methotrexate (GB)

Estimates of concentrations

Because cytostatics have not been demonstrated in Dutch surface waters, two calculations are used to predict the environmental concentration (PEC) in Dutch surface waters of four cytostatics (cisplatin, cyclophosphamide, 5-fluorouracil, methotrexate). The first calculation (PEC_{EMEA}) is used for the legal environmental assessment of pharmaceuticals and is derived from the maximum daily dosage of cytostatics consumed per capita (mg/c/d), the rate of market penetration (assuming that one percent of the population is treated daily with the substance), and the production of waste water per capita per day, taking into account a dilution of waste water by surface water[2].

For a more realistic approach, the second calculation (PEC_{JV}) is based on the annual consumption of a substance and not on the market penetration[10]. The annual consumption in the Netherlands can be derived from the number of daily doses used in the Netherlands per year. These data were obtained from the GIP databank at the Dutch National Health Insurance Organisation. The PEC_{JV} calculation also takes into account the metabolism of substances in the human body. For 5-fluorouracil the consumption of capecitabine, which in the body is changed into 5-fluorouracil, was included as well.

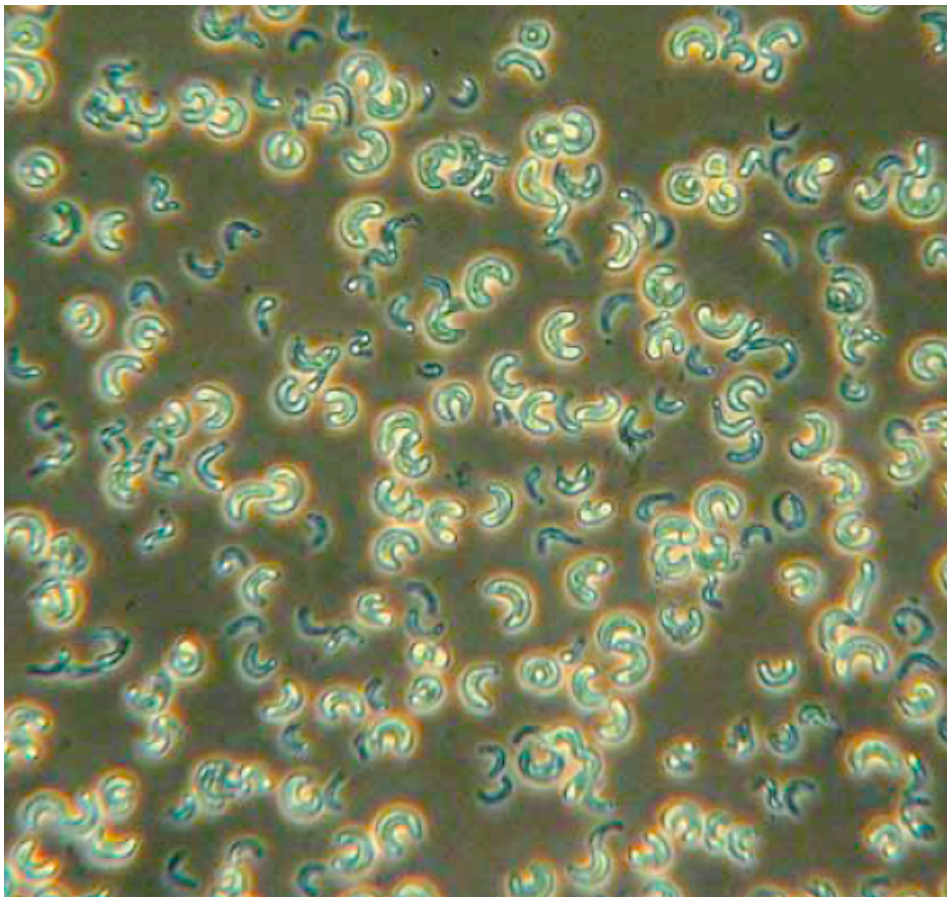
The calculated expected concentrations in surface waters using the PEC_{JV} calculation are in the order of a few nanograms (cyclophosphamide, methotrexate) to tens of nanograms (5-fluorouracil) per litre. The calculated PEC_{JV} for cisplatin was extremely low ($3.3*10^{-6}$ ng/l), because the national consumption in the Netherlands does not exceed one gram per year. The PEC_{EMEA} calculated concentrations are higher, from a concentration of 0.3 µg/l for cisplatin up to 5.5 µg/l for fluorouracil.

As for the above-mentioned PEC_{JV} concentrations realistic consumption values are used, these calculations are probably closer to the actual concentrations of cytostatics in the surface water. Moreover, concentrations of micrograms (PEC_{EMEA}) are shown in the various studies involving measurements of cytostatics in surface water (detection limits: 6.2 and 10 ng/l[15]). The found absence of cytostatics in surface water can be explained by the fact that the environmental concentrations are below the detection limits. Several studies have also made estimates of the occurrence of cytostatics in hospital effluent[4]. These estimates coincide for most substances fairly well with the measurements made (difference up to a factor 10).

Effects on aquatic organisms

Aquatic organisms are exposed over a longer period, perhaps throughout their lifetime. Therefore, to make a risk assessment of the degree of harmfulness of cytostatics for the aquatic environment, chronic toxicity data, in particular, are of interest. As cytostatics intervene in cell division, chronic toxicity testing that considers growth and reproduction, is of great importance.

Zounková et al[17] exposed the bacterium *Pseudomonas putida* to 5-fluorouracil. On the basis of change in the absorption by a bacterial culture it was investigated whether this cytostatic inhibits growth of this bacterium. The lowest concentration of 5-fluorouracil that had a statistically significant effect (LOEC: Lowest Observed Effect Concentration) in the study of Zounková et al was 10 µg/l. In a similar test with the alga *Pseudokirchneriella subcapitata* for 5-fluorouracil a LOEC was found of 10 µg/l as well.
Cisplatin also showed chronic toxicity at relatively low concentrations (*P. putida*: LOEC 0.1 mg/l and *P. subcapitata*: LOEC 1 mg/l[16]). Methotrexate and cyclophosphamide, however, were not or only slightly found to be toxic in various toxicity tests[3,6,17]. Genotoxicity tests in the case of cytostatics are also of importance, as some cytostatics are known to damage genetic material. Cisplatin and 5-fluorouracil showed genotoxicity at concentrations of some tens of µg/l and cyclophosphamide of 9.8 mg/l. However, other types of tests often did not demonstrate genotoxicity for cyclophosphamide. In other studies genotoxicity of 5-fluorouracil was limited to much higher concentrations. The question to be asked is, how problematic is it that aquatic organisms are exposed to genotoxic agents. Presumably, tumour development does not make a large impact on a population. The induction of mutations in gametes, on the other hand, is serious because it may directly affect the reproductive potential of a population[13]. In the long term mutagens in the environment could represent a risk for ecosystems.



5-Fluorouracil in relatively low concentrations inhibits the reproduction of the alga Pseudokirchneriella subspicata

Risk assessment

In order to obtain an indication of the environmental risk of cytostatics, the lowest chronic effect concentrations of the four cytostatics are compared with the measured and estimated maximum concentrations. For cisplatin, cyclophosphamide and methotrexate there is a large margin, ranging from a factor 28 to a factor 45,000 between effective and environmental concentrations. Therefore, no high risk to aquatic organisms is expected. It should be noted that the effect data for cyclophosphamide probably are biased, because only ecotoxicity tests have

been carried out for the inactive precursor of cyclophosphamide and not for the (active) metabolites[16].
The chronic effect concentrations for 5-fluorouracil are relatively low: 10 µg/l (no more observable effects at 1 µg/l). With a safety factor of ten[2], environmental concentrations above 0.1 µg/l should be avoided. 5-Fluorouracil was only detected in hospital effluent. The highest concentration there was 122 µg/l (effluent of an Austrian oncology department[11]). Estimates for hospital effluent are lower (2.03 µg/l[4]). However, wastewater from hospitals flows to STP's and concentrations are diluted. Perhaps the cytostatic is removed. In the study by Yu et al[16] 5-fluorouracil was not found in the influent and effluent of a local STP in the US. Because of contradictory research findings, it is difficult to make statements about the behaviour of 5-fluorouracil in STP's. In biodegradation tests, conducted by Kümmerer and Al-Ahmad[9] and Yu et al[16] 5-fluorouracil was not or to a very low degree degraded. However, the results of Kiffmeyer et al[8], showed that 5-fluorouracil is biodegradable.
No studies are found that searched for 5-fluorouracil in surface water. Therefore, it is unknown whether and in what concentrations this cytostatic appears here. The estimates vary strongly (PEC_{EMEA} 5.5 µg/l; PEC_{JV} 0.045 µg/l). Compared to the actual situation the PEC_{EMEA} is probably on the high side. But in model calculations on the rivers Aire and Calder in England locally up to eight times higher concentrations were found than were to be expected following the PEC_{JV}[7]. Consequently, this cytostatic would be a risk for aquatic organisms, if concentrations observed in hotspots (hospitals) were not sufficiently diluted during sewage water treatment, or reduced by degradation.

Discussion
Based on the literature found for three of the four selected cytostatics, no risks were expected for aquatic organisms. The cytostatic 5-fluorouracil may constitute a risk, depending on the reduction of the concentration of this cytostatic by degradation and dilution. It is important to note that there are very few toxicity data available. A lack of, in particular, chronic toxicity data is the primary obstacle for a proper risk assessment of pharmaceuticals in the environment[3]. Almost no testing is done with higher taxa. For that reason risks to these organisms could not be estimated at all. An additional problem is that the used toxicity tests and measurements of cytostatics in the environment do not take into account transformation products of the substances. It is also unclear whether cytostatics occur in surface waters, as the anticipated concentrations lie below the detection limits. Moreover, in the Netherlands only the occurrence of ifosfamide and cyclophosphamide was investigated. The calculated consumption per year of 5-fluorouracil in the Netherlands is the highest of the four selected cytostatics (306 kg). Further research on this cytostatic in the environment is advised.

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