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Occurrence, Ecotoxicology, and Treatment of Anticancer Agents as Water Contaminants

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Abstract

Anticancer agents as water contaminants belong to a general class of pharmaceuticals and personal care products as pollutants (PPCPs) that are widely present in the environment. They are less studied compared to other PPCPs in the past two decades. However, the cytotoxicity, genotoxicity, and endocrine disruption of these agents mar more concerning adverse effects on the environment and human health. Here, we review different classes of anticancer agents as emerging water contaminants, their occurrence in various waterbodies, the ecotoxicology, and the strategies for their treatment.

Keywords: Anticancer; PPCPs; Ecotoxicology; Occurrence; Treatment; Water contaminants

Introduction

Pharmaceuticals and personal care products as pollutants (PPCPs) have been identified in the environment for decades. But until recently, these mainly manmade chemicals are first called as PPCPs, which comprise bioactive substances such as therapeutic drugs, diagnostic agents, fragrances, cosmetics, and sun screen products. The major concerns with the ecotoxicities of PPCPs come from prescription and over-the-counter medications due to their specific targets on living tissues. Antibiotics have long been studied for the development of antibiotic resistant bacteria in the environment [1]. Endocrine disrupting compounds are examples of PPCPs that influence the sexual behavior and reproduction of aquatic organisms [2].

Anticancer agents, according to their mechanisms of action, are classified into alkylating agents, antimetabolites, cytotoxic antibiotics, natural products, topoisomerase inhibitors, endocrine therapies, other antineoplastic agents as well as newly developed tyrosine kinase inhibitors, and biologics. All of these agents achieve the antitumor effects through direct DNA damage, inhibition of cell proliferation, mitosis, DNA synthesis or promotion of cell apoptosis. However, these agents, not surprisingly, can also attack normal fast-growing cells such as gastrointestinal epithelia and hematopoietic stem cells, which are the origin of side effects during chemotherapy. In addition, due to the mode of action, some anticancer agents are themselves carcinogens capable of damaging and transforming all eukaryotic cells, especially the teratogenicity at low concentration [3]. When released into the environment, these agents, although present at nanogram per liter concentration, can often accumulate in aquatic organisms due to their hydrophobicity [4].

Cancer is generally a disease of old people. With the increase of aging population, more and more anticancer agents are consumed and thus released into the environment. Taking capecitabine as an example, its consumption in France increased one fold from the year 2004 to 2008. Correspondingly, the predicted environmental concentration increased approximately one fold from 1.8 ng/L to 3.5 ng/L [5]. The total delivered amount of anticancer agents as pollutants comes from hospital sewage and municipal wastewater due to improper disposal by the patients [6]. A Swiss study using a mass flow analysis of cytostatic compounds demonstrated that only 1.1-3.7% of the excreted amount of these compounds was found in the hospital effluent [7]. This result is consistent with the input pathways for anticancer drugs in the aquatic environment based on the French data, where only 13.8% of the total amount of anticancer agents in urban wastewater treatment plants (WWTP) comes from hospital effluents; the rest is directly from the municipal wastewater system due to outpatient consumption [5].

For this review, we searched the relevant keywords in the English literature indexed in ISI Web of Knowledge, PubMed, and hazardous substances data bank from U.S. national library of medicine [8]. We also browsed through US EPA bibliographic database of publications relevant to PPCPs [9]. In the following sections, we focus on the properties of common anticancer agents, their occurrence in the aquatic environment, ecotoxicology, and reported strategies for their inactivation and removal. Analytical methods developed for the measurement of anticancer agents as pollutants in various waterbodies are thoroughly reviewed elsewhere [10], thus not included in this review.

Classical Alkylating Agents (Nitrogen Mustards): Cyclophosphamide, Ifosfamide, Chlorambucil, Melphalan

Cyclophosphamide, ifosfamide, chlorambucil, melphalan are cytotoxic alkylating agents, structurally similar to mustard gas. (Table 1) Chemically, they all have the bis (2-chloroethyl) amine group, which is able to form aziridinium through intramolecular displacement of chloride by the nitrogen. The aziridinium group nonspecifically alkylates the N^7 of the guanine bases to form interstrand crosslinks in DNA [11]. This type of DNA damage blocks DNA replication and transcription, thus is highly cytotoxic. Clinically, this group of alkylating agents is widely used as the chemotherapy backbones for lymphoma, leukemia, and several solid tumors.

Alkylating agents in this class are polar compounds with small octanol-water partition coefficient (K_{ow}) and large solubility in water

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as shown in table 1 [8]. Thereby, they are less likely to be absorbed by sewage sludge or sediments as determined using LC/tandem MS and GC/MS. The concentration of both cyclophosphamide and ifosfamide in the WWTP sludge is less than 20 ng/g [12]. Thus these alkylating agents pass unchanged through WWTP to the surface water. On the other hand, cyclophosphamide and ifosfamide have limited biodegradability, which was demonstrated by Kümmerer et al. [13] using modified Zahn-Wellens test and a test simulating biological sewage treatment. When human metabolites are not counted, an average sized hospital can produce 1-10 µg/L cyclophosphamide or ifosfamide [14]. This number goes down to < 43 ng/L in WWTP effluent [13]. Steger-Hartmann et al. [15] using similar methods reported that the concentration of cyclophosphamide in hospital effluent ranged from 19 ng/L to 4.5 µg/L depending on when the sample was collected. As expected, the concentration went down to < 17 ng/L in WWTP effluent. Buerge et al. [16] studied the occurrence and fate of cyclophosphamide and ifosfamide in surface waters using solid-phase extraction and tandem LC/MS. They did not observe the direct photolysis of these compounds in natural conditions of surface water. But hydroxyl radical formed from photochemical processes can degrade them to some extent. The concentration of cyclophosphamide and ifosfamide is < 0.2 ng/L.

The major concern with this type of alkylating agents is its ecotoxicity and genotoxicity. However, previous studies indicated that cyclophosphamide alone posed minimal risk to aquatic organisms and human health. Zounková et al. [17] assessed the ecotoxicity of cyclophosphamide using bacterial growth inhibition assay, algal growth inhibition assay, and *D. magna* acute immobilization assay. They also assessed genotoxicity using SOS-chromo test and GreenScreen assay. The 50% effective concentration (EC50) of cyclophosphamide in these assays was at or above mg/L magnitude. It was significantly less toxic compared to other cytotoxic compounds such as 5-flurouracil in the same study, which was consistent with the findings from a previous report using ecotoxicological structural activity relationship screening [18]. This conclusion was also supported by a study from Kümmerer et al. [19]. They estimated that the relative risk of secondary cancer due to a life time intake of cyclophosphamide in surface water was approximately 10^{-6} times less than the secondary cancer risk caused by the therapeutic intake of cyclophosphamide.

Several strategies have been reported to inactivate this class of alkylating agents. Chemical degradation methods in harsh conditions [20] such as HCl or $NaCO_3$ pretreatment followed by Ni-Al alloy in KOH were demonstrated to be successful. More recently, some milder chemical degradation conditions [21] such as NaOCl, H_2O_2, and Fenton reagent were compared [22]. All of these methods were able to effectively degrade cyclophosphamide, ifosfamide, and melphalan and completely remove their mutagenicity. NaOCl (5.25%) proved to be the most efficient and readily available approach. In another study [23], NaOCl was generated by electrolyzing 0.9% NaCl solution, which was more cost effective than the dilution method to treat wastewater. In addition, the oxidative degradation of cyclophosphamide was also studied by Garcia-Ac et al. using ozone [24]. Compared to methotrexate, ozone was less effective to remove cyclophosphamide. Additional oxidant concentration and contact time were required. In addition to chemical degradation, new technologies such as membrane bioreactor were experimented. However, it suffered from extracellular polymeric substance formation when cyclophosphamide was present in the wastewater [25,26]. The membrane bioreactor effluent was carried on for advanced treatment with nanofiltration and reverse osmosis membranes. Efficiency of cyclophosphamide removal by reverse osmosis membrane was much higher than that by nanofiltration membrane [27]. This finding was confirmed in the treatment of hospital effluent that contained multiple targeted pharmaceutical compounds [28].

Nonclassical Alkylating Agents (Platinum Compounds): Cisplatin, Carboplatin

Cisplatin and carboplatin are square planar Pt (II) complexes (Table 2). They are classified as non-classical alkylating agents because they do not have or form electrophilic alkyl groups such as those in nitrogen mustards. However, they have similar mechanism of action in DNA damage. After the replacement of chloride or carboxylate ligands by water molecules, the platinum ion preferably binds the guanine bases in DNA. The second chloride or carboxylate ligands are subsequently displaced to form interstrand DNA crosslinks, which severely interfere with DNA replication and transcription. These compromises lead to mitosis arrest and apoptosis [29]. Platinum compounds are among the oldest chemotherapeutic agents and are still the main components of treatment regimens for lung cancer and ovarian cancer.

Cisplatin, similar to cyclophosphamide, is a very polar compound with low log K_{ow} and easily soluble in water [8]. The platinum input to environment from hospital effluent is not the main source compared to catalytic converter and some manufacturing industries. Using adsorptive voltammetry, Kümmerer et al. [30] determined the platinum concentration in hospital effluent ranging from 38 to176 ng/L, which was consistent with the annual consumption data. Kiffmeyer et al. [6] estimated the biodegradability of cisplatinum using a standard 21 day

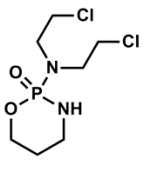
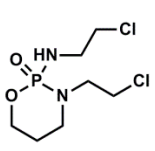
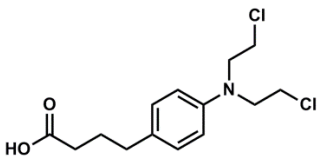
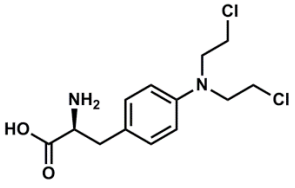
Features	Cyclophosphamide	Ifosfamide	Chlorambucil	Melphalan
Chemical structure				
Physical property [8]	log K_{ow}: 0.6, solubility: 4×10^4 mg/L	log K_{ow}: 0.9, solubility: 4×10^3 mg/L	log K_{ow}: 1.7, solubility: 1×10^4 mg/L	log K_{ow}: -0.5, solubility: 46 mg/L
Environmental occurrence [14-16]	hospital effluents: 10 µg/L, surface water: 0.2 ng/L	hospital effluents: 4.5 µg/L, surface water: 0.2 ng/L	---	---
Ecotoxicology [17]	EC50 > 1000 mg/L in various assays	---	---	---
Treatment strategy [20-23, 27]	Ni-Al in KOH, NaOCl, reverse osmosis membrane	Ni-Al in KOH, NaOCl, reverse osmosis membrane	Ni-Al in KOH	Ni-Al in KOH, NaOCl

Table 1: Summary of classical alkylating agents (nitrogen mustards).

(Organization for Economic Co-operation and Development) OECD screening test. Similar to cyclophosphamide, the biodegradation rate of cisplatin is close to zero.

The potential genotoxicity of cisplatin and carboplatin was evaluated by Ferk et al. [31] they used *Salmonella*/microsome assay and single-cell gel electrophoresis with rat hepatocytes to assess the induction of DNA damage caused by platinum compounds in hospital sewage. The results revealed that cisplatin and carboplatin only caused significant DNA damage in the single-cell gel electrophoresis assay when the concentration is > 1 mg/L.

Benvenuto et al. [21] investigated several chemical degradation methods for various antineoplastic agents. They found that the mutagenicity of cisplatin and carboplatin was completely removed by sodium diethyldithiocarbamate. More recently, Hirose et al. [32] applied an electrolytic treatment using two platinum electrodes at 100 mA to cisplatin. After two hours' electrolysis, the 50% cytotoxicity concentration against human lymphoblastoid cells reduced approximately 80%. Lenz et al. [33] subjected cisplatin and carboplatin in hospital wastewater to a membrane bioreactor system simulating WWTP and achieved 51-63% elimination efficiency. Similarly, membrane filtration method provided a 62-77% reduction in genotoxicity of cisplatin and carboplatin [31].

Other Alkylating Agents (Nitrosoureas, Methylhydrazines, and Tetrazines): Carmustine, Lomustine, Procarbazine, Dacarbazine, Temozolomide

Carmustine and lomustine are dialkylating agents (Table 3). Similar to cyclophosphamide and cisplatin, they are able to damage DNA by generating interstrand crosslinks between N^1 guanine and N^3 cytosine. Both procarbazine and dacarbazine are alkylating agents that induce DNA damage and subsequent cell apoptosis. However, the mechanism of action is not fully elucidated. Temozolomide is a prodrug of monomethyl triazeno imidazole carboxamide, which can readily methylate N^7 or O^6 positions of guanine residues and lead to tumor cell apoptosis [34]. Carmustine, lomustine, and temozolomide are hydrophobic and able to cross the blood brain barrier. Thus they are often used to treat brain tumors such as medulloblastoma and glioma [35]. Dacarbazine and procarbazine are traditionally used for the treatment of melanoma and glioma, respectively. However, their uses have been gradually replaced by newer regimens with better efficacy and toxicity profiles. Procarbazine is still an important component of combination therapy for Hodgkin's lymphoma [36].

Carmustine and lomustine are less polar than procarbazine, dacarbazine, and temozolomide. There is scarce information available for the concentration of these compounds in natural waterbodies except procarbazine. Its concentration is < 5 ng/L in hospital effluent collected from a number of hospitals in China [37]. In addition, no report so far indicated the effective removal of temozolomide from wastewater by sludge adsorption [38]. The estimated concentration of temozolomide in WWTP effluent was less than 0.4 ng/L based on the maximum annual temozolomide consumption and excretion [3]. The bioconcentration factor of lomustine was estimated to be 34 in fish, which suggested its moderate accumulation in aquatic organisms [39].

Besides the apparent mutagenicity present at therapeutic concentration, scare information for their ecotoxicology is available in the literature. However, some chemical degradation methods were developed for their removal. Reductive degradation of dacarbazine and procarbazine using Ni-Al alloy in KOH was superior to $KMnO_4$ or photolysis [40]. Conversely, the best chemical degradation conditions for carmustine and lomustine are acidic conditions with HBr in acetic acid [20]. Acidic $KMnO_4$ can also eliminate the chemical integrity of carmustine and lomustine. But the mutagenicity were still present [21]. As to temozolomide, the only reported method of degradation was 0.5 mol/L NaOH hydrolysis and oxidation with 10% H_2O_2 [38].

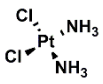
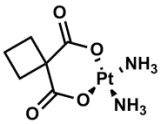
Features	Cisplatin	Carboplatin
Chemical structure		
Physical property [8]	log K_{ow}: -2.2, solubility: 3×10^3 mg/L	solubility: soluble in water
Environmental occurrence [30]	hospital effluents: 38-176 ng/L	---
Ecotoxicology [31]	no mutagenicity at the concentration in natural water	---
Treatment strategy [21, 31-33]	sodium diethyldithiocarbamate, electrolysis, membrane bioreactor, membrane filtration	sodium diethyldithiocarbamate, membrane bioreactor, membrane filtration

Table 2: Summary of nonclassical alkylating agents (platinum compounds).

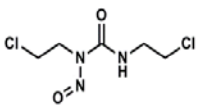
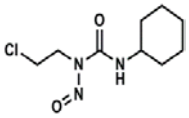
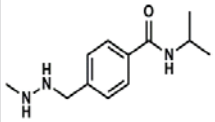
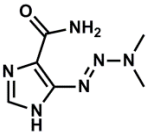
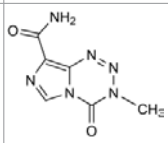
Features	Carmustine	Lomustine	Procarbazine	Dacarbazine	Temozolomide
Chemical structure					
Physical property [8]	log K_{ow}: 1.5, solubility: 4×10^3 mg/L	log K_{ow}: 2.8, solubility: 111 mg/L	log K_{ow}: 0.1, solubility: 1.4×10^3 mg/L	log K_{ow}: -0.2, solubility: 1×10^3 mg/L	log K_{ow}: -0.2, solubility: 5×10^3 mg/L
Environmental occurrence [3, 36]	---	---	hospital effluent: < 5ng/L	---	WWTP effluent: < 0.4 ng/L*
Ecotoxicology	---	---	---	---	---
Treatment strategy [20, 38, 38]	HBr in acetic acid	HBr in acetic acid	Ni-Al in KOH	Ni-Al in KOH	NaOH, H_2O_2

Table 3: Summary of other alkylating agents (nitrosoureas, methylhydrazines, and tetrazines).

Antimetabolites (Pyrimidines): Cytarabine, Gemcitabine, 5-Fluorouracil, Capecitabine

Cytarabine, gemcitabine, 5-fluorouracil, and capecitabine are pyrimidine analogues (Table 4). They are structurally similar to the pyrimidine bases in DNA. Thus they interfere with DNA synthesis in the S phase of cell cycle. In addition, they inhibit certain enzymes that are crucial for DNA replication. Cytarabine inhibits both DNA and RNA polymerase and nucleotide reductase. Gemcitabine targets ribonucleotide reductase larger subunit irreversibly [41]. 5-Fluorouracil and capecitabine as a prodrug of 5-fluorouracil are shown to inhibit exosome complex [42]. This group of antimetabolites is widely used for the chemotherapy of leukemia, colorectal, pancreatic, and lung cancer.

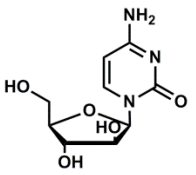
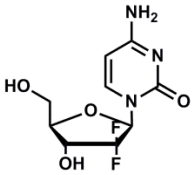
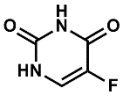
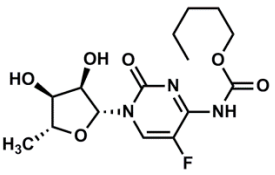
As shown in table 4, all these pyrimidine analogues have low log K_{ow}, which means they are polar compounds and readily soluble in water. Therefore, they are less likely to be adsorbed onto sewage sludge and sediments [43,44]. They often pass through the WWTP and are released unchanged to the surface water [43]. The higher polarity and water solubility pose a challenge to the analysis of low concentrations of these compounds in wastewater. Kovalova et al. [45] developed a method combining solid phase extraction and HPLC-MS/MS for the hospital effluent samples. The concentration of gemcitabine and 5-fluorouracil ranged from < 0.9 ng/L to 38 ng/L and from < 5 ng/L to 27 ng/L, respectively. Tauxe-Wuersch et al. [46] applied a similar analytical method to measure the concentration of 5-fluorouracil in municipal and WWTP effluent, which turned out to be less than the detection limit of 6-15 ng/L. In general, pyrimidine antimetabolites have very good biodegradability. Kümmerer et al. [47] using the closed bottle test and Zahn-Wellens test found that the biodegradation for gemcitabine was 42%. The initial biodegradation of cytarabine was only 50%, which increased to 80% after additional 40 days under test conditions. Surprisingly, 5-fluorouracil was not biodegradable in both tests, which was likely inhibited by antibiotics present in hospital wastewaters. An improved method similar to OECD 309 used by Yu et al. [48] demonstrated an approximately 50% biodegradation of 5-fluorouracil. This finding was further supported by Mahnik et al. [43] adopting a method using membrane bioreactor and radio-labelled substances. In addition, as a prodrug, capecitabine can be enzymatically converted to 5-fluorouracil in the cell. Its biodegradation profile was proved to be similar to 5-fluorouracil [44].

The combined risk assessment for 5-fluorouracil and capecitabine by Straub [44] using the calculated predicted environmental concentrations and measured environmental concentrations data concluded that no significant risk for 5-fluorouracil and capecitabine was present for the environment. Concerns with the ecotoxicity of 5-flurouracil and capecitabine were demonstrated in several studies where the concentration of them is much higher than that in natural waterbodies. Backhaus et al. [49] used a long-term bioluminescence inhibition assay with *V. fischeri* and found that the EC50 for 5-fluorouracil is 0.12 mg/L. DeYoung et al. [50] demonstrated that the minimal concentration for 5-fluorouracil to inhibit growth of fathead minnow *P. promelas* was as high as 20 mg/L. In addition, the genotoxicity study [31] of 5-fluorouracil on eukaryotic yeast revealed that the minimal genotoxic concentration was 0.02 mg/L, which was six orders of magnitude higher than the concentration detected in hospital effluent [31]. Similar studies on potential ecotoxicity have been done for other pyrimidine antimetabolites. Capecitabine inhibited crustacean *D. magna* reproduction with an EC50 >850 mg/L [44]. Cytarabine had a similar effect on *D. magna* with a minimal concentration of 3.7 mg/L [51]. Gemcitabine inhibited *D. magna* reproduction and mobilization at a minimal concentration > 1 mg/L.[51] Its 50% lethal concentration for *P. promelas* and *O. mykiss* is > 1000 mg/L [52]. In summary, pyrimidine antimetabolites at the concentration < 40 ng/L in natural waterbodies are unlikely to cause acute ecological adverse effects [3].

Both 5-fluorouracil and cytarabine can be degraded by photolysis in the presence of hydroxyl radical. In the case of 5-fluorouracil, direct photolysis is not effective [8], while this process was accelerated by the treatment of ozone to generate hydroxyl radicals in the aqueous medium [53]. In a similar fashion, UV radiation [54] or gamma radiation [55] alone cannot effectively remove cytarabine from wastewater samples. However, when they are treated with H_2O_2 or $K_2S_2O_8$, the generation of hydroxyl radical and sulfate radical anion significantly accelerated the removal rate of cytarabine. Other methods such as membrane bioreactor systems [43] were also tested and proved to be effective to eliminate 5-fluorouracil in hospital wastewater.

Antimetabolites (Folate Acid Analogues and Purines): Methotrexate, Azathioprine

Methotrexate is structurally similar to folic acid, but has approximately 10^3 times higher affinity to dihydrofolate reductase (Table 5). As a competitive inhibitor, methotrexate blocks the *de novo* pathway of thymidine synthesis, which is crucial for DNA synthesis [56]. Methotrexate is most widely used in the chemotherapy of leukemia and lymphoma. Azathioprine, a prodrug of 6-mercaptopurine, inhibits

Features	Cytarabine	Gemcitabine	5-Fluorouracil	Capecitabine
Chemical structure				
Physical property [8]	log K_{ow}: -2.5, solubility: 2×10^5 mg/L	log K_{ow}: -1.2, solubility: 5×10^4 mg/L	log K_{ow}: -1, solubility: 1×10^4 mg/L	log K_{ow}: 0.6, solubility: 3×10^4 mg/L
Environmental occurrence [43, 44]	---	hospital effluent: 38 ng/L	hospital effluent: 27 ng/L, WWTP effluent: < 15 ng/L	---
Ecotoxicology [31, 42, 47-50]	EC50 > 1 mg/L in various assays	EC50 > 1 mg/L in various assays	EC50 > 0.1 mg/L in various assays	EC50 > 1 mg/L in various assays
Treatment strategy [41, 51-53]	oxidative radiolysis	---	oxidative photolysis, membrane reactor	---

*predicted concentration in wastewater treatment plant effluent

Table 4: Summary of antimetabolites (pyrimidines).

normal purine synthesis in the cell. Lymphocytes are mostly affected by the inhibition of purine synthesis. Thus azathioprine is often used in the treatment of lymphoma, leukemia, certain autoimmune diseases as well as post-transplant immunosuppression [57].

Methotrexate is a weak acid with pKa 4.7. It is present in an ionic form in natural waterbodies. Thus it is unlikely to be adsorbed by wastewater sludge or sediments. Aherne et al. using a radioimmunoassay measured the concentration of methotrexate in hospital effluent as 1 µg/L and in river water as < 6.25 ng/L [58]. The concentration of methotrexate in WWTP effluent was 12.6 ng/L using solid phase extraction and HPLC/MS [59]. The apparent concentration gradient of methotrexate from hospital wastewater to surface water could be explained by its high biodegradability and direct degradation by photolysis [8]. In contrast to methotrexate, azathioprine is insoluble in water and is largely adsorbed by sewage sludge or sediments. The azathioprine in hospital effluent is only 15 ng/L [37].

Henschel et al. [60] did extensive assessment of the potential ecotoxicological effect for methotrexate. They found that the EC50 in a bioluminescence inhibition test and *D. magna* mobilization test was above 1000 mg/L. The EC50s of methotrexate in other assays including *S. subspicatus*, *T. pyriformis*, and *B. rerio* growth and survival tests were all above 10 mg/L. Considering the concentration of methotrexate in river water < 6.25 ng/L, methotrexate is unlikely to pose acute ecotoxicological effects on the environment.

Chemical degradation strategies were first adopted to eliminate methotrexate in hospital wastewater. Oxidation with $KMnO_4$ or 5.25% NaOCl completely degraded methotrexate and removed its mutagenicity [21] The disposal process could be effectively monitored by HPLC or a bioluminescence assay developed by Wren et al.[61] Alternative to chlorine-based treatment, ozone was also utilized to efficiently remove methotrexate in drinking water [24]. Compared to chemical degradation methods, electrolysis using two platinum electrodes at 100 mA current was able to eliminate 99% of the cytotoxicity from methotrexate in 2 hours [32]. Regarding the degradation of azathioprine, Barek et al. [62] compared 5% NaOCl, 30% H_2O_2, and Fenton reagent. They found that both NaOCl and Fenton reagent effectively eliminated 99% of azathioprine and its mutagenicity.

Natural Products (Vinca Alkaloids and Taxanes): Vinblastine, Vincristine, Paclitaxel

Vinblastine and vincristine are vinca alkaloids that bind to tubulin dimers and thus inhibit the assembly of microtubules, essential components of mitotic spindle and kinetochore (Table 6). The direct consequence of this inhibition is the inability for cells to undergo mitosis; instead they either stay in G phase or undergo apoptosis [63]. Conversely, paclitaxel, a member of taxane family, stabilizes the microtubule polymer and stops it from disassembly. The direct effect on cell is the same with vinca alkaloids. Cells are unable to undergo chromosomal segregation and cell division [64]. Vincristine and vinblastine are the backbone of combination chemotherapy

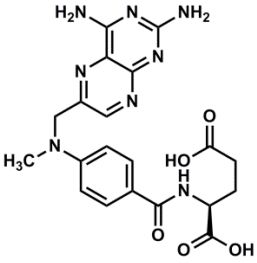
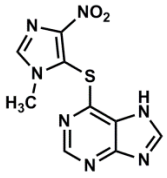
Features	Methotrexate	Azathioprine
Chemical structure		
Physical property [8]	pKa: 4.7, log K_{ow}: -1.8, solubility: 3×10^3 mg/L	log K_{ow}: 0.1, solubility: insoluble in water
Environmental occurrence [36, 56]	hospital effluent: 1 µg/L, river water: < 6.2 ng/L	hospital effluent: 15 ng/L
Ecotoxicology [58]	EC50 > 10 mg/L in various assays	---
Treatment strategy [21, 24, 32, 59, 60]	$KMnO_4$, NaOCl, O_3, electrolysis	NaOCl, Fenton reagent

Table 5: Summary of antimetabolites (folate acid analogues and purines).

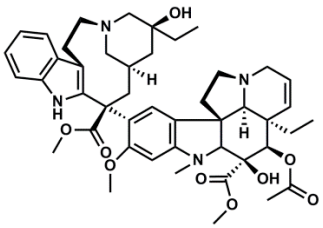
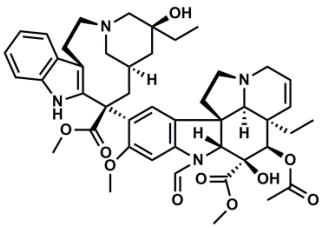
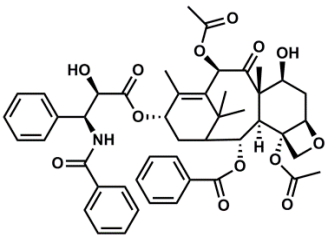
Features	Vinblastine	Vincristine	Paclitaxel
Chemical structure			
Physical property [8]	log K_{ow}: 3.7, solubility: 0.04 mg/L	log K_{ow}: 2.8, solubility: 2.3 mg/L	solubility: insoluble in water
Environmental occurrence [36]	---	hospital effluent: < 20 ng/L	---
Ecotoxicology [64]	---	---	EC50 > 0.74 mg/L in *D. magna* immobilization assay
Treatment strategy [32]	electrolysis	electrolysis	electrolysis

Table 6: Summary of natural products (vinca alkaloids and taxanes).

for lymphoma. Paclitaxel is often used for the treatment of lung and ovarian cancer.

Vinca alkaloids have a high log K_{ow} and low water solubility. The latter is true for paclitaxel as well. Therefore, they are easily adsorbed by sewage sludge and sediments. The concentration of vincristine in hospital effluent was < 20 ng/L [37]. Al-Ahmad et al. [65] studied the biodegradability of vinca alkaloids in the aqueous media and found that the biodegradability of these vinca alkaloids was < 30% even after a prolonged period of time, which was not considered as biodegradable. As to the ecotoxicity, a retrospective review of ecotoxicity data from FDA center for drug evaluation and research revealed that EC50 of paclitaxel to immobilize crustacean *D. magna* is > 0.74 mg/L [66]. Therefore, it is unlikely to cause any acute harm to aquatic organisms in natural waterbodies.

Vinca alkaloids are prone to direct photolysis in natural environment and this processes is accelerated by hydroxyl radicals [8]. Their structural integrity and cytotoxicity can be completely eliminated by 4-hour electrolysis using two platinum electrodes at a current of 100 mA. The same method also applied to paclitaxel for its complete degradation [32].

Topoisomerase Inhibitors: Etoposide, Irinotecan

Irinotecan is a topoisomerase I inhibitor to prevent DNA from unwinding. Etoposide is a topoisomerase II inhibitor and also binds DNA to form a complex (Table 7). This complex prevents DNA from relegation [67]. The interference with DNA replication by irinotecan and etoposide causes DNA damage and promotes cell apoptosis. Irinotecan is most often used as a component of combination therapy for colorectal cancer. Etoposide is used in combination for lymphoma and several solid tumors.

Etoposide is not readily water soluble. It is not considered as a biodegradable compound. However, it can undergo direct photolysis in natural waterbodies and indirect photolysis accelerated by hydroxyl radicals [8]. The concentration of etoposide in hospital effluent is approximately 42 ng/L [37] The ecotoxicological study on etoposide by Zounková et al. [17] revealed that the EC50 to inhibit the growth of *P. Putida* was 630 mg/L; the EC50 to inhibit the growth of *P. subcapitata* was 250 mg/L; the EC50 to inhibit the mobilization of *D. magna* was 30 mg/L. These results, when compared to the concentration in hospital effluent, indicate the unlikelihood of acute ecotoxicity from etoposide. Oxidative degradation using $KMnO_4$ or 5.25% NaOCl was effective for the complete removal of etoposide [21]. Hirose et al. [32] reported that 72% cytotoxicity of irinotecan was eliminated by electrolysis using two platinum electrodes with 100 mA electric current for 4 hours.

Cytotoxic Antibiotics (Anthracycline): Doxorubicin, Epirubicin, Daunorubicin

Anthracyclines are made up of the planar aromatic moiety and the daunosamine moiety (Table 8). The aromatic portion intercalates between two base pairs, while the daunosamine residue is able to form a complex with the adjacent base pairs at the minor groove [68]. This type of interaction between anthracyclines and DNA can effectively block DNA replication and prevent DNA religation by stabilizing topoisomerase II. Other mechanisms of action are also proposed such as disruption of cell membrane, plasma protein complexation, and free radical generation. All these effects inevitably lead to cell death. Anthracyclines have been widely used against human malignancies. They are essentially components of the induction therapy for acute leukemia and combination therapy for lymphoma. In addition, they also play important role in solid tumor chemotherapy.

Anthracyclines are relatively nonpolar molecules with log K_{ow} greater than 1. In addition, compared to other antineoplastic agents, their solubility in water is quite low. Therefore, anthracyclines in wastewater are easily adsorbed onto sewage sludge and sediments [69]. Mahnik et al. [43] studied the fate of anthracyclines in hospital wastewater using a membrane bioreactor system and radio-labelled substances. They found that 90% of anthracyclines were removed from aqueous phase and the radioactivity was detected in the suspended solid phase. In contrast to 5-fluorouracil with radioactivity found in the gaseous phase, anthracyclines have no biodegradability and are effectively eliminated by adsorption onto sewage sludge. The same group of investigators also developed an analytical method using reverse-phase-HPLC with fluorescence detection to determine the concentration of anthracyclines in hospital wastewater samples. They found that the concentration of doxorubicin and epirubicin ranged from 0.1 to 0.5 μg/L; the concentration of daunorubicin is < 0.1 μg/L [70].

Anthracyclines only demonstrated ecotoxicity and genotoxicity at relatively high concentrations in a spectrum of species. The EC50 of doxorubicin in *P. putida* bacterial growth inhibition test, *P. subscapitata* inhibition test, and crustacean *D. magna* immobilization test was > 1000 mg/L, 13 mg/L, and 30 mg/L, respectively. In genotoxicity evaluation,

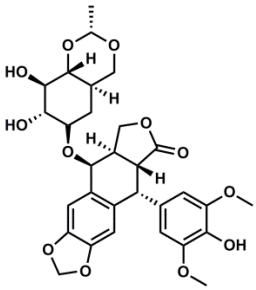
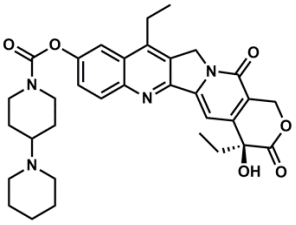
Features	Etoposide	Irinotecan
Chemical structure		
Physical property [8]	log K_{ow}: 0.6, solubility: 59 mg/L	---
Environmental occurrence [36]	hospital effluent: 42 ng/L	---
Ecotoxicology [17]	EC50 > 10 mg/L in various assays	---
Treatment strategy [21, 32]	$KMnO_4$, NaOCl	electrolysis

Table 7: Summary of topoisomerase inhibitors.

the minimum genotoxic concentration of doxorubicin in bacterial SOS-chromotest and yeast GreenScreen assay was approximately 0.1 mg/L and 2.8 mg/L, respectively [17].

As discussed earlier, anthracyclines can be effectively eliminated by membrane bioreactor system. In addition, Castegnaro et al. [71] compared several chemical degradation methods and measured the residual mutagenicity using Ames test. They found that 5.25% NaOCl was the most efficient and cost effective method for anthracycline degradation. Hirose et al. [32] applied their electrolysis method using platinum electrodes at 100 mA to the degradation of epirubicin. 100% of epirubicin and its cytotoxicity, mutagenicity and antibacterial activity were eliminated after 6-hour electrolysis.

Endocrine Therapy: Tamoxifen, Letrozole, Anastrozole, Flutamide

Endocrine disrupting compounds are chemicals structurally similar or dissimilar to natural hormones. They are known to interfere with endocrine system and cause disorders or defects in reproduction system. Their ecological adverse effects also extend to behavioral disorders as well as cancers [72]. Endocrine disrupting compounds have been extensively studied since the ear of dichlorodiphenyltrichloroethane (DDT) use. In this review, we will focus on the major endocrine modulatory compounds used for the treatment of breast cancer and prostate cancer. Tamoxifen is a prodrug of 4-hydroxytamoxifen and N-desmethyl-4-hydroxytamoxifen. They are competitive inhibitors of estrogen receptor and subsequently inhibit the transcription of estrogen responsive genes, which are responsible for the proliferation of estrogen receptor positive breast cancer cells [73]. Estrogen is produced through the conversion of androgens by aromatase in the peripheral tissues. Both letrozole and anastrozole are aromatase inhibitors, thus are able to block estrogen synthesis [74]. They are mostly used for the treatment of estrogen responsive breast cancer. Flutamide is a competitive androgen receptor inhibitor. It prevents prostate epithelial cells from being stimulated to proliferate by testosterone and dihydrotestosterone. Flutamide is used for the treatment of androgen responsive prostate cancer.

Tamoxifen, letrozole, anastrozole, and flutamide are lipophilic compounds. The first three as shown in table 9 have high log K_{ow} and low water solubility. These properties indicate that these endocrine therapeutic agents are likely to be absorbed by the sewage sludge and sediments. Liu et al. [75] systematically measured ten hormone antagonists in hospital and WWTP wastewater samples. The concentration of tamoxifen in hospital effluent, WWTP effluent, and surface water [76] was 0.2-8.2 ng/L, < 0.1 ng/L, and < 5.8 ng/L, respectively. The concentration of letrozole in hospital effluent and WWTP effluent was 0.2-2.4 ng/L and 0.3-0.6 ng/L, respectively. The concentration of anastrozole in hospital effluent and WWTP effluent was 0.3-3.7 ng/L and 0.3 ng/L, respectively.

Ecotoxicology of these endocrine disrupting and highly lipophilic compounds has been extensively studied, especially considering the possibility of bioaccumulation. Williams et al. found that the lowest observed effect concentration of tamoxifen was 5.6 µg/L on the changes of histology, reproduction, and growth of fathead minnows [77]. Andersen et al. revealed that the EC50 of tamoxifen to inhibit naupliar development was 49 µg/L [78]. Fertilized eggs of Japanese medaka were exposed to tamoxifen for 14 days. Adverse effects on hatchability

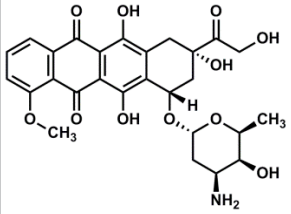
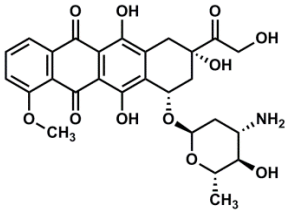
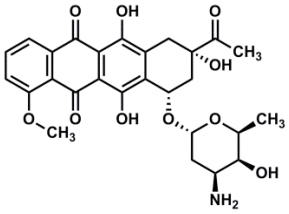
Features	Doxorubicin	Epirubicin	Daunorubicin
Chemical structure			
Physical property [8]	log K_{ow}: 1.3, solubility: 93 mg/L	log K_{ow}: 1.8, solubility: 93 mg/L	log K_{ow}: 1.8, solubility: 39 mg/L
Environmental occurrence [68]	hospital effluent: 0.5 µg/L	hospital effluent: 0.5 µg/L	hospital effluent: 0.1 µg/L
Ecotoxicology [17]	EC50 > 0.1 mg/L in various assays	---	---
Treatment strategy [32, 41, 69]	membrane bioreactor, 5.25% NaOCl, electrolysis	membrane bioreactor, 5.25% NaOCl	membrane bioreactor, 5.25% NaOCl

Table 8: Summary of cytotoxic antibiotics (anthracycline).

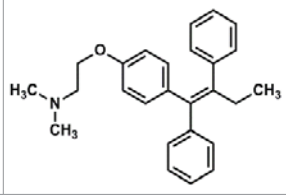
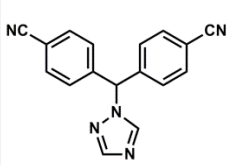
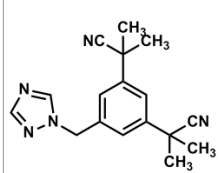
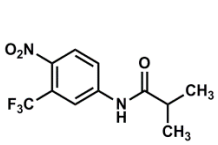
Features	Tamoxifen	Letrozole	Anastrozole	Flutamide
Chemical structure				
Physical property [8]	log K_{ow}: 6.3, solubility: 17 mg/L	log K_{ow}: 2.2, solubility: 102 mg/L	log K_{ow}: 2.4, solubility: 500 mg/L	---
Environmental occurrence [73, 74]	hospital effluent: 8.2 ng/L, surface water: < 5.8 ng/L	hospital effluent: 2.4 ng/L	hospital effluent: 3.7 ng/L	---
Ecotoxicology [2, 75-80]	EC50 > 5 µg/L in various assays	---	---	minimal effective concentration > 1 µg/L in various assays
Treatment strategy	---	---	---	---

Table 9 Summary of endocrine therapy.

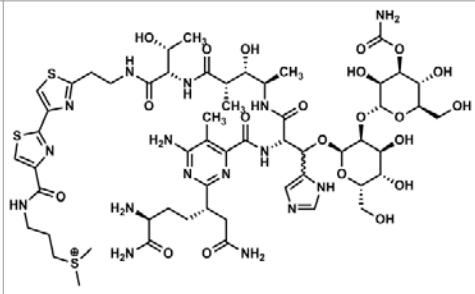
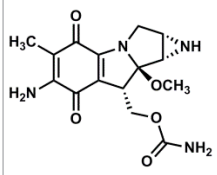
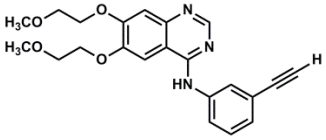
Features	Bleomycin	Mitomycin	Erlotinib
Chemical structure			
Physical property [8]	freely soluble in water	log K_{ow}: -0.4, soluble in water	---
Environmental occurrence [87]	WWTP effluent: 19 ng/L, river water: 17 ng/L	---	---
Ecotoxicology [50]	---	---	no observed effect concentration > 0.02 mg/L in various assays
Treatment strategy	---	electrolysis, $KMnO_4$, NaOCl	---

Table 10: Summary of other antineoplastics (cytotoxic antibiotics and tyrosine kinase inhibitors).

and the time required to hatch were only found at a concentration of at least 125 μg/L [79,80]. After released into the wastewater, tamoxifen is able to undergo photolysis after prolonged exposure to sunlight radiation. DellaGreca et al. evaluated the chronic toxicities of tamoxifen and photolysis derivatives on model aquatic organisms and found the EC50 > 0.1 mg/L [81]. In summary, the endocrine disrupting effect of tamoxifen is only apparent at a much higher concentration than that in natural waterbodies. The same conclusion is reached for the antiandrogen compound flutamide. Preston et al. [82] built a reproductive assay using freshwater rotifer *B. calyciflorus* and found that the inhibition effect of flutamide on fertilization was observed at a concentration of 1 μg/L. The behavioral changes of male stickleback including nest building and courting to the female occurred at 100 μg/L of flutamide [2].

Scare information is available for treatment strategies specifically for tamoxifen, letrozole, anastrozole, and flutamide. The likely reason of this gap is the extremely low concentration of these compounds that can be detected in natural waterbodies. However, many methods have been developed for the general treatment of other endocrine disrupting compounds. These methods include microfiltration and reverse osmosis systems [83], chemical degradations [84], aerobic granular biomass reactors [85], photolysis, and ultrasonic irradiation [86].

Other Antineoplastics (Cytotoxic Antibiotics and Tyrosine Kinase Inhibitor): Bleomycin, Mitomycin, Erlotinib

Bleomycin is a glycopeptide antibiotic able to form a complex with metal ions. In vivo, the complex of bleomycin and iron-containing enzymes generates hydroxyl radical and superoxide from a reaction with O_2. These reactive oxygen species not only damage cellular components but also break DNA [87]. Bleomycin is an important component of combination chemotherapy for Hodgkin's lymphoma and testicular cancer. Mitomycin is an aziridine-containing natural product, which can crosslink DNA bases by dialkylation in a similar fashion to the classical alkylating agents. Mitomycin is often used for certain upper gastrointestinal cancer treatment and the topical treatment of bladder cancer [88]. Erlotinib is a targeted cancer therapy inhibiting epidermal growth factor receptor (EGFR) tyrosine kinase. It blocks the growth signal of cells transducing through EGFR pathway. Erlotinib is mainly used for the treatment of EGFR positive non-small cell lung cancer.

Bleomycin and mitomycin are polar compounds and are freely soluble in water (Table 10). Aherne et al. [89] was able to enrich bleomycin in various water samples by lyophilization and determined that the concentration of bleomycin in WWTP effluent and river water is 11-19 ng/L and 5-17 ng/L, respectively. The ecotoxicological information for erlotinib is available from the Swedish medicine information engine [52]. The no observed effect concentration of erlotinib for *S. capricornutum* growth is 0.14 mg/L, for *D. manga* reproduction is 0.7 mg/L, and for *O. mykiss* is 0.02 mg/L. Regarding treatment strategies for these compound, mitomycin along with its cytotoxicity and mutagenicity can be removed completely by 6-hour electrolysis at 100 mA [32]. Oxidative degradation methods using $KMnO_4$ and 5.25% NaOCl are effective for 100% elimination of mitomycin [21].

Conclusion

In this review article, we introduced the mechanisms of action and physical properties of common antineoplastic agents. We focused on the discussion of environmental occurrence, ecotoxicology, and treatment strategies for these compounds. For the majority of these compounds, the concentration gradient is present in various waterbodies. The hospital effluent has higher amount of antineoplastic agents, while the concentration of these compounds is usually at nanogram per liter level in surface water. The ecotoxicological studies on these agents using various aquatic animal models are available. The results indicate no acute adverse effect on environment or human health because the concentration required for observing such effect is at least three orders of magnitude higher than the concentration of these agents present in natural waterbodies. Thus no special treatment of wastewater containing these compounds is routinely needed. However, data on chronic ecotoxicity of these antineoplastic agents are still scarce, which should be a direction of future research.

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