Comportament de citostàtics a l'ambient: presència, degradament i avaluació de riscos

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Comportament de citostàtics a l'ambient: presència, degradabilitat i avaluació de riscos

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RESUM

Durant els darrers anys la incidència de càncer ha augmentat de forma gradual, fet que ha comportat l'administració d'un gran nombre de fàrmacs i un consum global que arriba a les tones anuals. Aquest compostos són els anomenats citostàtics i estan recollits en el codi L (agents antineoplàstics i immunomuduladors) segons la classificació de la Organització Mundial de la Salut. Després de ser administrats, aquests fàrmacs es metabolitzen parcialment, s'excreten i arriben a les aigües residuals. Aquestes aigües es tracten a les depuradores, que no sempre els poden eliminar completament, i s'acaben alliberant a les aigües superficials.

Per aquest motiu, l'objectiu principal d'aquesta tesi ha estat determinar l'impacte que tenen els fàrmacs citostàtics administrats a Catalunya i Espanya per al medi ambient.

Per conèixer el consum de citostàtics s'han recopilat les dades d'administració de tots els compostos amb codi L, en farmàcies i hospitals a Catalunya (2010-2012) i en farmàcies a Espanya (2010-2015). Aquestes dades, juntament amb els valors d'excreció i el percentatge de degradació en les depuradores ha permès calcular les concentracions que s'esperen detectar en aigües superficials, els anomenats PECs (*predicted environmental concentrations*). Dels més de 100 compostos administrats a Catalunya, l'àcid micofenòlic, la hidroxicarbamida i la capecitabina van tenir els PECs més elevats, mentre que a Espanya els consums totals van ser majors i els compostos prioritzats coincideixen majoritàriament amb els just esmentats. Els PECs també es van calcular per a cadascuna de les conques hidrogràfiques més importants de la península Ibèrica, i es va determinar que al riu Tajo s'esperen concentracions més elevades que a la resta.

El comportament dels citostàtics des de la seva excreció fins a l'arribada al medi no està ben definit, ja que hi ha poques dades experimentals per als diferents processos que poden tenir lloc al llarg d'aquest recorregut. Així, es va avaluar la degradació d'un grup de 16 citostàtics en diferents processos (hidròlisi, biodegradació, UV i UV-H_2O_2) i es van determinar les constants de degradació. Es va observar que la vinblastina, la vincristina, la doxorubicina, l'irinotecà, el clorambucil i el melfalan s'hidrolitzen en 24h, mentre que la biodegradació, l'UV i la llum solar no van poder degradar tots els citostàtics estudiats, fent necessari l'ús de tractaments avançats com l'UV-H_2O_2 per completar l'eliminació dels més persistents (com la ciclofosfamida i la ifosfamida). Per aquests compostos també s'espera la seva presència al medi.

Per confirmar aquests resultats i per comparar els PECs amb concentracions reals es va seleccionar un grup de citostàtics que es van monitoritzar al riu Besòs. Les mostres es van extreure mitjançant extracció en fase sòlida i es van analitzar per cromatografia de líquids acoblada a l'espectrometria de masses d'alta resolució. Al llarg de la conca del Besòs es va detectar la presència de set citostàtics entre 0,5 i 656 ng/L, amb major presència prop de la desembocadura. Entre els fàrmacs estudiats l'àcid micofenòlic va ser el que va donar valors de

PEC més elevats i el que es va trobar a concentracions més altes al riu. Amb els PECs i les concentracions reals, juntament amb els valors de toxicitat, es va dur a terme una avaluació de risc, que va determinar que aquestes concentracions no suposen un risc immediat per als organismes aquàtics.

També es va estudiar la presència d'àcid micofenòlic en el punt de captació d'aigua d'una planta potabilitzadora, que es va detectar a 17-56 ng/L, i es va fer el seguiment al llarg dels diferents punts del tractament. No obstant, es va observar que el diòxid de clor elimina aquest compost i que no arriba a l'aigua de consum. En estudiar la cinètica de degradació es va determinar que l'àcid micofenòlic s'elimina en pocs segons en presència de ClO_2, però no es van observar productes de degradació majoritaris.

Per tal de millorar la presa de mostra es van dissenyar i caracteritzar dos tipus de mostrejadors passius. El seu ús proporciona informació dels contaminants presents a l'aigua durant un interval de temps definit. El primer mostrejador va consistir en un cilindre ceràmic porós reblert de material adsorbent que es va calibrar per 16 citostàtics en condicions de laboratori, però es va observar que aquells més inestables no s'adsorbien adequadament. Per als citostàtics més estables es van obtenir coeficients de difusió de 1,08E-07 a 1,78E-07 cm^2/s. Aquests dispositius no van permetre l'anàlisi de citostàtics en l'influent d'una depuradora, degut a la matèria en suspensió que va obturar els mostrejadors, però si que van ser eficaços a la sortida d'aquesta, on les concentracions detectades van ser comparables a la presa de mostra manual. El segon mostrejador va ser un DGT (*diffusive gradients in thin films*), pel que també es va fer el calibratge al laboratori pels citostàtics més estables i es van obtenir coeficients de difusió de 2,05E-06 a 1,02E-05 cm^2/s. També es va determinar que les variacions de pH i força iònica del medi no afecten a la capacitat d'adsorció dels analits als mostrejadors.

Els resultats obtinguts demostren que l'alt consum de citostàtics pot produir la contaminació de les aigües superficials. L'arribada continua d'aquests fàrmacs al medi, conjuntament amb el seu ús creixent i les seves propietats citotòxiques, fan necessària la seva avaluació per tal d'assegurar que no hi ha risc per als organismes aquàtics ni per la salut humana.

OBJECTIUS I ESTRUCTURA

L'objectiu global d'aquesta tesi consisteix en avaluar la presència de fàrmacs citostàtics al medi ambient, estudiar-ne el seu comportament i determinar si la seva presència pot ser perjudicial per als organismes aquàtics.

Per aquest motiu, els objectius específics són els següents:

1. Determinar el consum dels fàrmacs citostàtics que s'utilitzen a Catalunya i Espanya, calcular les concentracions teòriques (PECs) i prioritzar aquells que s'esperin trobar en les aigües superficials i residuals.
2. Estudiar els processos de degradació que afecten als fàrmacs citostàtics al llarg del cicle de l'aigua.
3. Desenvolupar un mètode analític basat en la cromatografia de líquids acoblada a l'espectrometria de masses en tàndem (LC-MS/MS) o Orbitrap-MS i un mètode d'extracció per aquells citostàtics prioritzats segons els PECs.
4. Determinar la presència de fàrmacs citostàtics en aigua superficial i avaluar el risc a partir de les dades toxicològiques.
5. Determinar la presència de fàrmacs citostàtics en aigües de captació i al llarg del procés de potabilització d'una planta de tractament d'aigua potable.
6. Dissenyar i posar a punt sistemes de mostreig passiu per a l'anàlisi de citostàtics en aigües.

La memòria d'aquesta tesi s'ha estructurat en quatre capítols principals:

- En la introducció es classifiquen els fàrmacs citostàtics utilitzats en els diversos tractaments del càncer, es descriuen els seus consums a Europa i es resumeixen els processos pels quals els fàrmacs arriben al medi. També es recullen els nivells detectats, els valors de toxicitat i els mètodes emprats pel seu anàlisi.

- El segon capítol està enfocat a la descripció del consum de citostàtics a Catalunya i Espanya, i al càlcul de les seves concentracions previstes al medi. En aquest capítol s'inclouen els resultats de l'article científic I, *Predicting concentrations of cytostatic drugs in sewage effluents and surface waters of Catalonia (NE Spain)*,i de l'article II, *Anticancer drugs: consumption trends in Spain, prediction of environmental concentrations and potential risks*, i la seva discussió.

- El tercer capítol se centra en la presència d'aquesta família de compostos al medi ambient. En primer lloc s'ha avaluat la seva degradabilitat degut a diferents processos que tenen lloc al medi i en segon lloc s'ha determinat la seva presència en aigües superficials.En aquest capítol s'inclouen els resultats dels articles científics III, *Biological and photochemical degradation of cytostatic drugs under laboratory conditions*, IV, *Determination of cytostatic drugs in Besòs River (NE Spain) and comparison with predicted environmental concentrations*, i V,*Do cytostatic drugs reach drinking water? The case of mycophenolic acid*, i la seva discussió. També s'han inclòs altres aspectes del treball experimental que formen part dels objectius d'aquesta tesi, malgrat no s'hagin assolit.

- El quart capítol s'ha dedicat a l'avaluació dels mostrejadors passius per a l'anàlisi de citostàtics. En primer lloc, es descriuen els tipus de mostrejadors més habituals per a l'anàlisi de compostos orgànics. En el treball experimental s'han posat a punt dos tipus de mostrejadors diferents, que es presenten en l'article VI, *Development of a macroporous ceramic passive sampler for the monitoring of cytostatic drugs in water*, i l'article VII,*Laboratory calobration of o-DGT for analysis of cytostatic drugs*, i es discuteixen els resultats obtinguts per cadascun.

- Finalment, s'inclouen les conclusions obtingudes, així com la bibliografia i altre fonts consultades, seguit del llistat d'acrònims utilitzats en aquesta memòria.

1. INTRODUCCIÓ

El càncer és un creixement tumoral dels teixits, de caràcter maligne i pertorbador de les funcions biològiques (IEC, 2017). En condicions normals, el procés de divisió cel·lular està regulat per uns mecanismes de control que marquen quan les cèl·lules s'han de dividir i quan s'han de mantenir estables. En canvi, les cèl·lules cancerígenes tenen aquest mecanisme alterat i poden dividir-se sense control (AECC, 2017). A més, també poden desplaçar-se a través de la sang i el sistema limfàtic i afectar tant els òrgans propers com els més llunyans (NIH, 2017a).

Actualment, es desconeix amb precisió quines són les causes del càncer. Tot i això, s'han determinat diversos elements, anomenats factors de risc, que augmenten les possibilitats de desenvolupar-lo, encara que no en són la causa directa (NIH, 2017a). Alguns d'aquests factors no es poden controlar ja que depenen de l'envelliment i la genètica, però n'hi ha d'altres que si es poden controlar, com el consum de tabac i alcohol o l'exposició al sol i a substàncies químiques sense la protecció adequada, entre d'altres (Generalitat de Catalunya, 2017b). Els virus, com el del papil·loma humà, l'hepatitis o el virus de la immunodeficiència humana (VIH) entre d'altres, també poden augmenten el risc de patir determinats tipus de càncer (Generalitat de Catalunya, 2017b).

La incidència del càncer és el nombre de casos nous que es produeixen en una població durant un període determinat. A Catalunya, es van diagnosticar 48.171 casos anuals durant el període 2008-2012, dels quals 20.819 casos van ser detectats en dones i 27.352 en homes. Segons les últimes dades publicades pel Pla Director d'Oncologia de Catalunya (Generalitat de Catalunya, 2017a), els tipus de càncer més freqüents en els homes ha estat el de pròstata (21,6%), el de colon i recte (16,6%) i el de pulmó (14,9%) i en canvi, per les dones ha estat el de mama (28,6%), el de colon i recte (16,0%) i el de coll d'úter (5,5%). Així i tot, en els darrers anys s'ha vist un increment dels càncers associats al tabaquisme (de pulmó i cavitat oral-faringe) en les dones, un tipus de tumors que ha disminuït o s'ha estabilitzat pels homes (Generalitat de Catalunya, 2013). A Espanya, com que la població és més gran, la incidència de càncer en nombres absoluts també ho és: l'any 2012 es van estimar uns 86.984 casos nous en dones i 128.550 en homes. Els tumors amb més incidència coincideixen amb els descrits per Catalunya per ambdós sexes (SEOM, 2016). Comparat amb altres països europeus, a Catalunya, la incidència de càncer en la població masculina es troba en 11a posició, per sobre la mitjana europea, mentre que per les dones es troba per sota (Figura 1.1). No obstant, la mortalitat es troba al mateix nivell o per sota la mitjana europea per ambdós sexes.

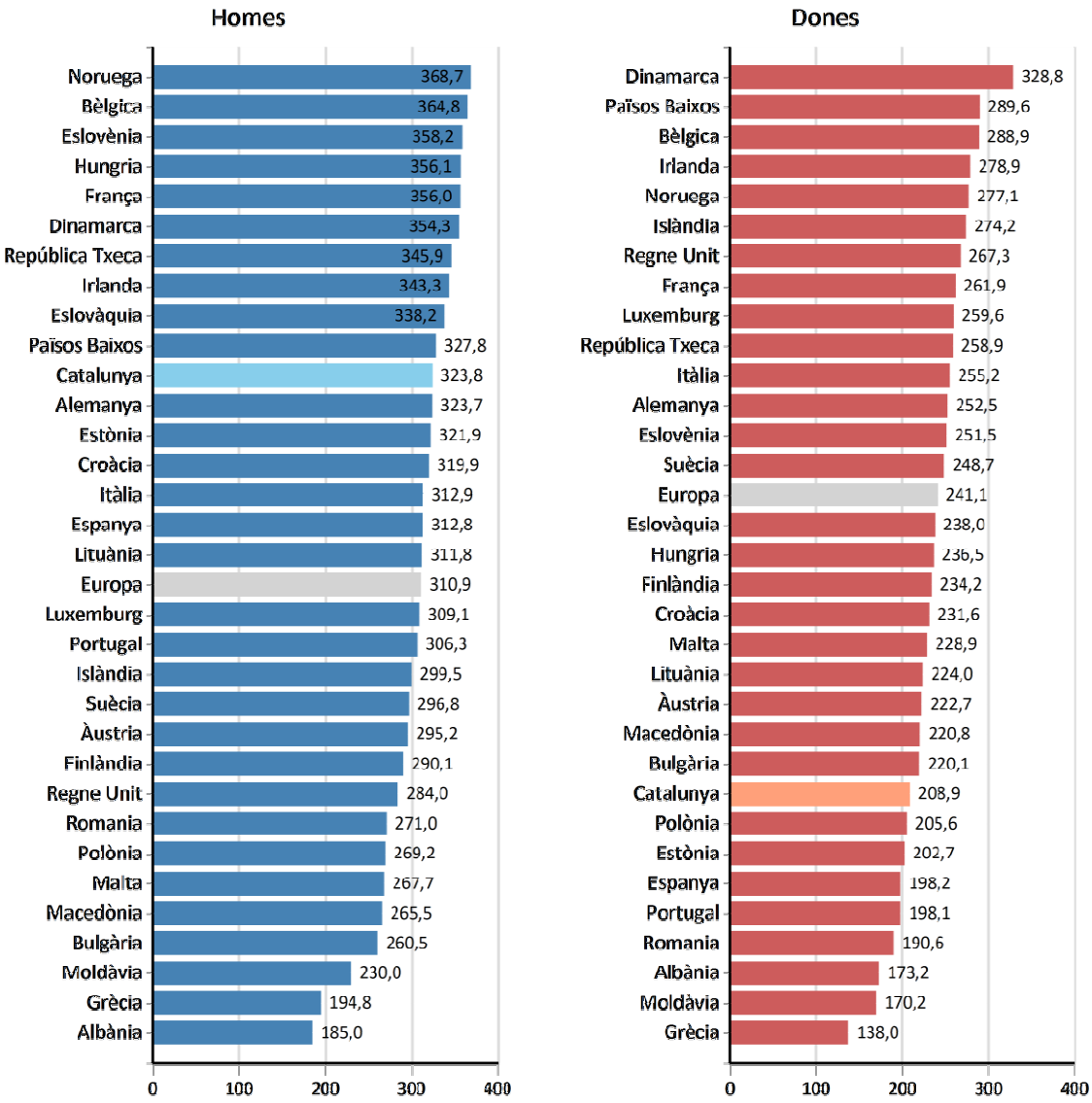

Figura 1.1. Incidència de del càncer en homes i dones a Europa. Taxa ajustada x10^5 persones/any.(Generalitat de Catalunya, 2017a)

El recull de dades dels darrers anys permet fer previsions de la incidència del càncer de cara l'any 2020, que en pronostica una estabilització (Generalitat de Catalunya, 2013). Tot i així, per alguns tumors en concret, la tendència és en augment degut al creixement de la població i al seu envelliment. Això provoca una major exposició a factors de risc i una disminució dels mecanismes de reparació cel·lular. A més, les tècniques i programes de detecció precoç fan que se'n detectin més casos en els seus estadis inicials. Aquest augment però, va acompanyat d'una disminució de la mortalitat degut a la detecció temprana de la malaltia i a una major eficàcia dels tractaments.

1.1. Fàrmacs citostàtics i classificació

Per tal de lluitar contra el càncer, hi ha diferents tractaments que es poden aplicar i que dependran del tipus de tumor o la seva extensió i de la salut de la persona a tractar, entre altres factors (Generalitat de Catalunya, 2017b). La majoria d'aquests tractaments implica l'administració de medicaments, anomenats fàrmacs antineoplàstics o citostàtics, de manera individual o en combinacions de dos o més. El conjunt de fàrmacs citostàtics està format per un gran nombre de compostos que seran l'objecte d'estudi en aquesta tesi.

Per tal de classificar aquests fàrmacs, un dels sistemes més utilitzats és el codi ATC (*AnatomicalTherapeuticChemical*) proposat per la Organització Mundial de la Salut (OMS). Aquesta classificació dóna un codi de cinc nivells a cada substància segons: el grup anatòmic (1r nivell), el grup terapèutic (2n nivell) i les propietats farmacològiques i químiques (3r i 4t nivell). El 5è nivell designa la pròpia substància (WHO, 2017). D'acord amb aquest codi, els fàrmacs citostàtics es troben en el grup anomenat agents antineoplàstics i immunomoduladors, designat amb la lletra L. El conjunt del grup L conté 300 principis actius, però és una llista que es revisa periòdicament i on s'inclouen fàrmacs nous cada any. Concretament, es preveu l'entrada de 18 compostos nous durant l'any 2017. A partir d'aquí, el grup L se subdivideix en els L01 (agents antineoplàstics), L02 (teràpia endocrina), L03 (immunostimulants) i L04 (immunosupressors), subgrups que es descriuen a continuació, i que també se subdivideixen en altres grups tal com es mostra a l'esquema de la Figura 1.2.

L01- Agents antineoplàstics: Els fàrmacs inclosos dins aquest grup actuen seguint diferents mecanismes d'acció amb l'objectiu d'inhibir o alterar la transcripció de l'ADN o interaccionar amb proteïnes que regulen els processos biològics de les cèl·lules per tal de destruir o controlar el creixement de les cèl·lules cancerígenes (Besse et al., 2012). Aquests s'apliquen en els tractaments de quimioteràpia i arriben a gairebé tots els teixits de l'organisme on actuen tant sobre les cèl·lules malignes com les sanes, provocant també efectes no desitjats.

L02- Teràpia endocrina: Com el seu nom indica, aquests fàrmacs s'utilitzen en teràpia endocrina, també anomenada teràpia hormonal, i s'encarreguen d'inhibir la síntesi d'hormones o els seus receptors (Besse et al., 2012). S'utilitzen en casos com el càncer de pròstata o mama, tumors hormonosensibles, que es desenvolupen degut a la influència dels estrògens o la testosterona.

L03- Immunostimulants: Són substàncies que augmenten la capacitat del sistema immunològic per lluitar contra malalties o infeccions i s'utilitzen en les teràpies biològiques,

també anomenades teràpies dirigides. Aquests tractaments tenen menys efectes secundaris ja que ataquen en menor mesura sobre les cèl·lules sanes (NIH, 2017a).

L04- Immunosupressors: Comunament, els immunosupressors s'utilitzen per suprimir el sistema immunitari després d'un transplantament, i per tant, dificulten la detecció de cèl·lules canceroses i poden fer augmentar el risc de desenvolupar un determinat càncer (NIH, 2017b). Tot i això, després de determinats tractaments com el transplantament de medul·la òssia contra la leucèmia se solen utilitzar fàrmacs del grup L04 en combinació amb diferents fàrmacs d'altres subgrups L. Per alguns d'aquests L04, a més del seu efecte immunosupressor, s'ha vist que poden tenir un efecte anticancerigen i s'estan aplicant o estudiant amb aquest objectiu. En són alguns exemples l'àcid micofenòlic (Carter et al., 1969; Dun et al., 2013; Majd et al., 2014), el sirolimus (Law, 2005) o la lenalidomida (NIH, 2017a).

Figura 1.2. Classificació dels fàrmacs L (agents antineoplàstics i immunomoduladors) segons el codi ATC.

- L01 Agentsantineoplàstics
  - L01A Agents alquilants
    - L01AA Anàlegs de la mostassa nitrogenada
    - L01AB Alquilsulfonats
    - L01AC Etilenimines
    - L01AD Nitrosourees
    - L01AG Epòxids
    - L01AX Altres agents alquilants
  - L01B Antimetabòlits
    - L01BA Anàlegs de l'àcid fòlic
    - L01BB Anàlegs de les purines
    - L01BC Anàlegs de les pirimidines
  - L01C Alcaloides de plantes i altres productes naturals
    - L01CA Alcaloides de la vinca i anàlegs
    - L01CB Derivats de la podofilotoxina
    - L01CC Derivats de la colquicina
    - L01CD Taxans
    - L01CX Altres alcaloides de plantes i productes naturals
  - L01D Antibiòtics citotòxics i altres substàncies relacionades
    - L01DA Actinomicines
    - L01DB Antraciclines i substàncies relacionades
    - L01DC Altres antibiòtics citotòxics

Figura 1.2. (continuació) Classificació dels fàrmacs L (agents antineoplàstics i immunomoduladors) segons el codi ATC.

- L01X Altres agents antineoplàstics
  - L01XA Compostos del platí
  - L01XB Metilhidrazines
  - L01XC Anticossos monoclonals
  - L01XD Sensibilitzadors usats en teràpia fotodinàmica i radiació
  - L01XE Inhibidors directes de la proteïna-cinasa
  - L01XX Altres agents antineoplàstics
  - L01XY Combinacions d'agents antineoplàstics
- L02 Teràpia endocrina
  - L02A Hormones i agents relacionats
    - L02AA EstròGens
    - L02AB Progestàgens
    - L02AE Anàlegs de l'hormona alliberadora de gonadotrofines
    - L02AX Altres hormones
  - L02B Antagonistes d'hormones i agents relacionats
    - L02BA AntiestròGens
    - L02BB AntiandròGens
    - L02BG Inhibidors de l'aromatasa
    - L02BX Altres antagonistes d'hormones i substàncies relacionades
- L03 Immunostimulants
  - L03A Immunostimulants
    - L03AA Factors estimulants de colònies
    - L03AB Interferons
    - L03AC Interleukines
    - L03AX Altres immunostimulants
- L04 Immunosupressors
  - L04A Immunosupressors
    - L04AA Immunosupressors selectius
    - L04AB Inhibidors del factor de necrosi tumoral alfa
    - L04AC Inhibidors de la interleucina
    - L04AD Inhibidors de la carcineurina
    - L04AX Altres immunosupressors

A més dels fàrmacs del grup L, altres compostos també s'administren en tractaments contra el càncer, com són alguns fàrmacs del grup G03 (hormones sexuals i moduladors del sistema genital) i els H02 (corticosteroides per a ús sistèmic). Concretament, alguns antiandròGens (G03H) com la ciproterona s'administren en el tractament del càncer de pròstata, i els glucocorticoides (H02A) com la prednisona, s'utilitzen en el tractament de la leucèmia, entre d'altres tipus de càncers (NIH, 2017a).

Respecte l'administració d'aquests fàrmacs, diferents estudis publicats coincideixen en que la quantitat dispensada en farmàcies és major que l'administrada en hospitals (en unitats de massa de principi actiu). Concretament a Alemanya, l'administració farmacèutica va ser d'un 78,9% respecte un 20,8% d'administració hospitalària (Kümmerer et al., 2016) i a França, durant el període 2004-2008, l'administració hospitalària va disminuir des d'un 82% a un 35%, produint un augment en l'administració farmacèutica (Besse et al., 2012). A més, part dels pacients tractats a l'hospital tornen a casa després del tractament (Weissbrodt et al., 2009). Per tant, determinar l'abast del consum d'aquests compostos i conèixer-ne les vies d'administració permetrà fer una avaluació del destí d'aquests fàrmacs i de les implicacions que poden tenir pel medi ambient.

1.2. Consum de fàrmacs citostàtics

Degut a l'alta incidència del càncer el consum de fàrmacs citostàtics en els països europeus se situa en l'ordre de tones anuals(Besse et al., 2012). El consum de fàrmacs individuals però, pot variar entre diferents països malgrat que la incidència dels tipus de càncer sigui similar. Les publicacions que descriuen el consum global de citostàtics a nivell nacional per alguns països de la Unió Europea són pocs i en alguns casos es considera només el consum hospitalari i en d'altres, s'hi inclou l'administració en farmàcies. Tot i així, s'observen diferències geogràfiques per alguns dels fàrmacs descrits a la bibliografia, tal i com s'indica a continuació.

Concretament a França, l'any 2004, es van administrar 13 tones corresponents a tots els fàrmacs amb codi L01 i L02 en farmàcies i hospitals, consum que va incrementar l'any 2008 fins a 17,5 tones(Besse et al., 2012). A Alemanya en canvi, només el consum de fàrmacs L01 administrats en farmàcies i hospitals ja va ser de 20,7 tones l'any 2012 (Kümmerer et al., 2016). Aquests valors corresponen a un consum de 235 i 257 mg anuals per habitant a França (valor mitjà) i Alemanya respectivament.

A nivell individual, els fàrmacs més consumits es troben a la Taula 1.1, on es recull el seu consum per càpita (µg/dia/habitant) en diferents països de la Unió Europea. La major part d'articles publicats se centren en un nombre reduït de compostos, que pertanyen majoritàriament al grup L01 de la classificació de l'ATC, i a nivell europeu només hi ha dades de consum per a quatre citostàtics (la ciclofosfamida, el 5-fluorouracil, la capecitabina i el carboplatí). A la taula també s'indica si les dades recollides corresponen a l'administració en farmàcia, en hospitals o ambdós segons cada estudi. Així, dels 90 fàrmacs recollits a la

bibliografia, els de més consum van ser la capecitabina (L01BC06), la hidroxicarbamida (L01XX05) i el 5-fluorouracil (L01BC02).

La capecitabina s'utilitza per al tractament de diferents càncers, incloent els de mama, intestí, estómac i esòfag (Cancer Research (UK), 2012). El seu consum s'ha recollit en 12 països, amb valors de 79 μg/dia/habitant a Anglaterra (Johnson et al., 2008) i fins a 494 μg/dia/habitant a la República Txeca, amb una mitjana Europea de 258 μg/dia/habitant (Johnson et al., 2013). La hidroxicarbamida s'utilitza per tractar diferents tipus de leucèmia o els tumors al coll de l'úter entre d'altres, i en combinació amb radioteràpia (Cancer Research (UK), 2012). Per aquest fàrmac, només hi ha dades a nivell nacional per França i Alemanya, on es van registrar consums d'entre 284 i 231 μg/dia/habitant respectivament. A Anglaterra també es va recollir el seu consum en hospitals del nord-oest del país, que va ser de només 33 μg/dia/habitant (Booker et al., 2014). El 5-fluorouracil s'aplica en diversos tractaments, que inclouen el càncer de mama, colon, recte, estómac i pell, de manera individual o en combinació amb altres fàrmacs (Cancer Research (UK), 2012). En aquest cas s'han registrat consums de 2,6-72 μg/dia/habitant, amb una mitjana europea de 30 μg/dia/habitant (Besse et al., 2012; Johnson et al., 2013). Altres fàrmacs, amb un consum menor, presenten diferències menys acusades entre països. En són un exemple el tamoxifè, amb consums d'entre 15,0 i 15,6 μg/dia/habitant a França i Espanya (Besse et al., 2012; Coetsier et al., 2009; Ortiz de García et al., 2013) i l'imatinib, amb 48-36 μg/dia/habitant a França i Alemanya (Besse et al., 2012; Kümmerer et al., 2016).

Excepte per França i Alemanya, es desconeix amb precisió quins són tots els fàrmacs utilitzats per al tractament del càncer en els països europeus. La manca d'informació sobre consums de citostàtics reflecteix que es tracta de compostos poc estudiats en relació a altres fàrmacs, com els antibiòtics. Les dades de consum permeten en primera instància determinar aquells compostos més utilitzats i per tant que poden tenir un impacte ambiental elevat. Així mateix, permeten centrar els estudis de monitorització ambiental en aquells compostos amb més probabilitat de detectar-se a l'ambient. Per tant, la capacitat de disposar d'aquestes dades en un país o zona determinada esdevé clau a l'hora de realitzar estudis d'impacte ambiental.

Taula 1.1: Consum de fàrmacs citostàtics (µg/dia/hab) a diferents països d'Europa, en farmàcies (f) i hospitals (h).

		França		Alemanya	Anglaterra			Espanya		Suïssa
		Besse 2012	Coetsier 2009	Kummerer 2016	Rowney 2009	Booker 2014	Johnson 2008	Ortiz de Garcia 2013	Martín 2013	Buerge 2006
Codi ATC	Nom	f+h	f+h	f+h	f+h	h	f+h	f+h	1 hospital	nd
L01AA01	Ciclofosfamida	12,69	-	11,78	20,64	40,00	-	74,6	4,7	20,64
L01AA02	Clorambucil	0,34	-	0,04	-	-	-	-	-	-
L01AA03	Melfalan	0,20	-	0,08	-	-	-	-	-	-
L01AA06	Ifosfamida	4,28	5,3	4,73	-	0,99	-	20,5	3,6	4,50
L01AA09	Bendamustina	-	-	0,65	-	-	-	-	-	-
L01AB01	Busulfan	0,001	-	0,02	-	-	-	-	-	-
L01AC01	Tiotepa	-	-	0,01	-	-	-	-	-	-
L01AD02	Lomustina	0,13	-	0,03	-	-	-	-	-	-
L01AD04	Estreptozocina	0,35	-	-	-	-	-	-	-	-
L01AD05	Fotemustina	0,05	-	0,001	-	-	-	-	-	-
L01AX03	Temozolomida	2,22	-	2,82	0,84	0,99	-	-	-	-
L01AX04	Dacarbazina	1,22	-	0,82	-	-	-	-	-	-
L01BA01	Metotrexat	3,10	-	9,90	-	1,00	-	-	1,5	-
L01BA03	Raltitrexed	0,001	-	-	-	-	-	-	-	-
L01BA04	Pemetrexed	1,55	-	1,63	-	0,99	-	-	-	-
L01BB02	Mercaptopurina	3,94	-	2,48	-	-	-	-	-	-
L01BB03	Tioguanina	0,09	-	0,13	-	-	-	-	-	-
L01BB04	Cladribina	0,001	-	0,001	-	-	-	-	-	-
L01BB05	Fludarabina	0,23	-	0,06	0,2	-	-	-	-	-
L01BB06	Clofarabina	0,01	-	-	-	-	-	-	-	-
L01BB07	Nelarabina	-	-	0,02	-	-	-	-	-	-
L01BC01	Citarabina	5,55	-	4,56	-	0,99	-	-	2,2	-
L01BC02	Fluorouracil	71,95	-	66,47	-	12,00	46	-	45,4	-
L01BC03	Tegafur	1,55	-	0,11	-	-	-	-	-	-
L01BC05	Gemcitabina	15,74	-	16,27	14,2	6,99	-	51,2	6,7	-
L01BC06	Capecitabina	213,16	-	204,07	311,8* (+5FU)	182,99	79	-	-	-
L01BC07	Azacitidina	-	-	0,37	-	-	-	-	-	-
L01CA01	Vinblastina	0,03	-	0,003	-	-	-	-	-	-
L01CA02	Vincristina	0,01	-	0,003	-	-	-	-	-	-
L01CA03	Vindesina	-	-	0,0003	-	-	-	-	-	-
L01CA04	Vinorelbina	0,54	-	0,31	-	-	-	-	0,12	-
L01CA05	Vinflunina	-	-	0,06	-	-	-	-	-	-
L01CB01	Etopòsid	1,71	-	1,19	-	0,99	-	-	0,40	-

		França		Alemanya	Anglaterra			Espanya		Suïssa
		Besse 2012	Coetsier 2009	Kummerer 2016	Rowney 2009	Booker 2014	Johnson 2008	Ortiz de Garcia 2013	Martín 2013	Buerge 2006
Codi ATC	Nom	f+h	f+h	f+h	f+h	h	f+h?	f+h	1 hospital	nd
L01CD01	Paclitaxel	1,61	-	1,50	-	-	-	-	0,80	-
L01CD02	Docetaxel	1,14	-	0,65	-	-	-	-	0,29	-
L01CD04	Cabazitaxel	-	-	0,01	-	-	-	-	-	-
L01CX01	Trabectedina	0,001	-	0,0003	-	-	-	-	-	-
L01DB01	Doxorubicina	0,70	-	0,34	1,52	-	-	-	0,16	-
L01DB03	Epirubicina	0,73	-	0,54	0,9	-	-	-	0,25	-
L01DB06	Idarubicina	0,01	-	0,003	-	-	-	-	-	-
L01DB07	Mitoxantrona	0,01	-	0,01	-	-	-	-	-	-
L01DC01	Bleomicina	0,04	-	0,03	-	-	-	-	-	-
L01DC03	Mitomicina	0,12	-	0,13	-	-	-	0,776	0,094	-
L01XA01	Cisplatí	0,94	-	0,65	1,090	-	-	-	-	-
L01XA02	Carboplatí	3,47	-	3,23	8,008	3,00	-	-	-	-
L01XA03	Oxaliplatí	1,39	-	0,85	0,430	0,99	-	-	-	-
L01XB01	Procarbazina	-	-	0,44	-	-	-	-	-	-
L01XC02	Rituximab	3,02	-	3,85	-	-	-	-	-	-
L01XC03	Trastuzumab	2,33	-	3,30	-	-	-	-	-	-
L01XC04	Alemtuzumab	0,03	-	0,01	-	-	-	-	-	-
L01XC06	Cetuximab	2,28	-	1,77	-	-	-	-	-	-
L01XC07	Bevacizumab	3,62	-	3,91	-	-	-	-	-	-
L01XC08	Panitumumab	0,05	-	0,29	-	-	-	-	-	-
L01XD03	Aminolevulinat de metil	0,10	-	0,11	-	-	-	-	-	-
L01XD04	Àcid aminolevulínic	-	-	0,03	-	-	-	-	-	-
L01XE01	Imatinib	36,28	-	48,19	-	10,00	-	-	-	-
L01XE02	Gefitinib	-	-	1,94	-	-	-	-	-	-
L01XE03	Erlotinib	6,18	-	4,49	-	2,00	-	-	-	-
L01XE04	Sunitinib	0,83	-	1,16	-	-	-	-	-	-
L01XE05	Sorafenib	-	-	13,75	-	5,00	-	-	-	-
L01XE06	Dasatinib	-	-	0,85	-	-	-	-	-	-
L01XE07	Lapatinib	4,82	-	10,48	-	1,92	-	-	-	-
L01XE08	Nilotinib	2,44	-	11,10	-	-	-	-	-	-
L01XE09	Temsirolimus	0,05	-	0,02	-	-	-	-	-	-
L01XE10	Everolimus	-	-	0,13	-	-	-	-	-	-
L01XE11	Pazopanib	-	-	7,08	-	-	-	-	-	-
L01XX05	Hidroxicarbamida	283,88	-	230,82	-	32,99	-	-	-	-
L01XX08	Pentostatina	0,001	-	0,0002	-	-	-	-	-	-

		França		Alemanya	Anglaterra			Espanya		Suïssa
		Besse 2012	Coetsier 2009	Kummerer 2016	Rowney 2009	Booker 2014	Johnson 2008	Ortiz de Garcia 2013	Martín 2013	Buerge 2006
Codi ATC	Nom	f+h	f+h	f+h	f+h	h	f+h?	f+h	1 hospital	nd
L01XX09	Miltefosina	0,01	-	0,02	-	-	-	-	-	-
L01XX11	Estramustina	11,94	-	5,24	-	-	-	-	-	-
L01XX14	Tretinoina	0,14	-	0,10	-	-	-	-	-	-
L01XX17	Topotecà	0,01	-	0,01	-	-	-	-	-	-
L01XX19	Irinotecà	1,93	-	1,29	-	-	-	-	0,34	-
L01XX23	Mitotà	9,70	-	5,21	-	1,92	-	-	-	-
L01XX25	Bexarotè	0,98	-	0,99	-	-	-	-	-	-
L01XX27	Triòxid d'arsènic	0,00	-	-	-	-	-	-	-	-
L01XX32	Bortezomib	0,01	-	0,01	-	-	-	-	-	-
L01XX35	Anagrelida	0,05	-	-	-	-	-	-	-	-
L02AB01	Megestrol	-	-	-	-	-	-	79,32	-	-
L02AE01	Buserelina	0,002	-	-	-	-	-	-	-	-
L02AE02	Leuprorelina	0,13	-	-	-	-	-	-	-	-
L02AE03	Goserelina	0,05	-	-	-	-	-	-	-	-
L02AE04	Triptorelina	0,08	-	-	-	-	-	-	-	-
L02BA01	Tamoxifè	15,65	15,11	-	-	-	-	14,98	-	-
L02BA02	Toremifè	0,04	-	-	-	-	-	-	-	-
L02BA03	Fulvestrant	0,28	-	-	-	-	-	-	-	-
L02BB01	Flutamida	21,63	-	-	-	-	-	115,85	-	-
L02BB03	Bicalutamida	35,82	-	-	-	-	-	-	-	-
L02BG03	Anastrozole	1,32	-	-	-	-	-	-	-	-
L02BG04	Letrozole	1,42	-	-	-	-	-	-	-	-
L02BG06	Exemestà	7,56	-	-	-	-	-	-	-	-

	Europa (Johnson 2013)	Mitjana Europea	Alemanya	Anglaterra	Itàlia	Holanda	Àustrica	Dinamarca	Suïssa	Suècia	Noruega	Finlàndia	Rep, Txeca
L01AA01	Ciclofosfamida	10,4	8,4	-	3,4	2,7	13,4	5,7	12,7	35,7	9,2	2,3	10,2
L01BC02	Fluorouracil	29,7	51,9	-	3,2	18,5	41	2,6	30,3	30,2	27	-	19,8
L01BC06	Capecitabina	258,5	183,8	133,5	147,6	393,8	-	388,2	170,1	226,7	116,7	372,8	493,7
L01XA02	Carboplatí	3	3,4	2,1	-	-	1	1	-	1,5	-	-	8,4

nd: no descrit; -: dades no disponibles; 5FU: 5-fluorouracil.

1.3. Presència al medi

Els fàrmacs citostàtics són consumits pels pacients que reben un tractament de quimioteràpia i, després de ser administrats, part del principi actiu pot arribar al medi ambient seguint el circuit que es mostra en la Figura 1.3.Un cop consumit, una part del fàrmac es metabolitza i la resta s'excreta en la seva forma original a través de l'orina o la femta, que arriba a les aigües residuals. Aquestes aigües es tracten a les depuradores, on segueixen diferents processos que poden no ser suficients per eliminar aquests fàrmacs. Així, serien alliberats a les aigües superficials on podrien afectar els organismes aquàtics. A més, aquestes aigües poden ser captades per les estacions de tractament d'aigua potable (ETAP) per ser destinades a aigua de consum i distribuir-se a la xarxa. Per tant, si no s'eliminen, els citostàtics podrien arribar a l'aigua de beguda.

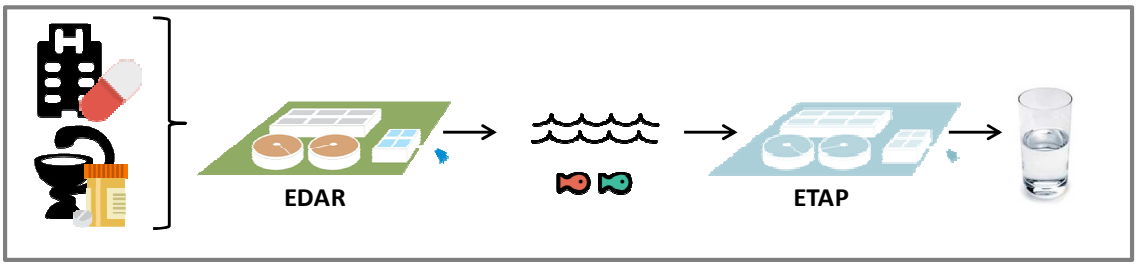

Figura 1.3. Circuit d'entrada dels fàrmacs citostàtics al medi ambient. EDAR: estació depuradora d'aigües residuals; ETAP: estació de tractament d'aigua potable.

Com s'ha descrit a l'apartat 1.1, la major part d'aquests fàrmacs s'administren en farmàcies i, a més, un alt percentatge de pacients tractats en hospitals són pacients externs, que tornen a casa després del tractament (Weissbrodt et al., 2009) on excretaran part del fàrmac administrat. Per tant, la via d'arribada d'aquests compostos al medi no és només a través de les aigües residuals dels hospitals sinó que, majoritàriament, arriba a través de les aigües residuals domèstiques.

El percentatge excretat en forma del fàrmac original pot variar en un ampli rang, que no depèn només de les propietats del principi actiu sinó també de l'edat i la salut del pacient (Johnson et al., 2013). Per exemple, es donen percentatges d'excreció entre el 5 i el 25% per la ciclofosfamida (Drugs Information Database, 2014), o entre el 56-78% pel megestrol (Provincial Health Services Authority, 2013). De manera similar, l'eliminació en depuradores pot variar entre el 0 i el 100% en funció de la degradabilitat de cada fàrmac. També es donen variacions naturals en l'eficàcia del tractament secundari de les EDAR que resulta en diferents valors d'eliminació

per un mateix compost (Johnson et al., 2013). Així, per exemple, es donen eliminacions del15% (Besse et al., 2012) i del 50% (Johnson et al., 2013)per la capecitabina. Per tant, la fracció de fàrmac original que arriba al medi pot variar en funció dels paràmetres esmentats.

En els següents apartats es descriu la presència de citostàtics al llarg del cicle de l'aigua, incloent-hi els efluents d'hospitals, els influents i efluents de depuradora, les aigües superficials i subterrànies i l'aigua potable. Per seguir un mateix criteri, primer s'han descrit els citostàtics que s'han detectat a concentracions més elevades i que s'han estudiat més extensament a la bibliografia, seguit d'aquells que només s'han analitzat de manera puntual.La Taula 1.2 mostra els nivells de fàrmacs detectats en la bibliografia per les diferents matrius.

1.3.1. Efluents d'hospital

Tot i que s'ha vist que el consum d'aquests fàrmacs en hospitals és menor que l'administració farmacèutica, la presència de citostàtics en els efluents d'hospitals ha estat estudiada i confirmada per diversos autors (Taula 1.2). De 31 citostàtics descrits a la bibliografia en els efluents d'hospital, 19 s'han trobat per sobre els límits de quantificació. Entre ells, el 5-fluorouracil és el que s'ha estudiat en un major nombre de publicacions i el que també s'ha detectat a una concentració més elevada. Concretament, Mahnik et al. (2007) van detectar la seva presència a l'efluent de l'ala oncològica de l'Hospital Universitari de Viena a concentracions de fins a 123.500 ng/L, durant un mostreig de dos anys. En aquest mateix estudi també es va detectar la doxorubicina a 1.350 ng/L, però no es va detectar l'epirubicina ni la daunorubicina. Altres autors també han analitzat la presència de 5-fluorouracil en efluents d'hospital. Mullot et al. (2009), van analitzar els efluents d'un hospital durant una setmana, fent dues campanyes de mostreig a l'hivern i una a l'estiu. Durant aquest període, es van detectar concentracions entre 90 i 4.000 ng/L, amb els valors més alts a l'hivern. Kosjek et al. (2013) en van trobar concentracions a Eslovènia de 35-92 ng/L a la sortida de l'ala oncològica i per sota el límit de detecció (<LOD) a la sortida d'un altre hospital on es feien tractaments de quimioteràpia però no amb 5-fluorouracil. A Suïssa, se'n van detectar <5-27 ng/L, juntament amb <0,9-38 ng/L de gemcitabina (Weissbrodt et al., 2009). En canvi, en un altre mostreig realitzat a l'efluent de l'hospital universitari de Lausana, també a Suïssa, no en van detectar la seva presència (Tauxe-Wuersch et al., 2006). Kovalova et al. (2009) també van estudiar el 5-fluorouracil i la gemcitabina, que van ser detectats només en els dies que s'administrava el tractament oncològic, a concentracions màximes de 27 i 38 ng/L respectivament. Finalment, Isidori et al. (2016), van estudiar el 5-fluorouracil, juntament amb 9 citostàtics més i alguns metabòlits, en dos hospitals de Ljubljana i dos hospitals de Barcelona. Específicament, a Barcelona, es va

detectar la presència de la ciclofosfamida (32 ng/L), el 5-fluorouracil (2,1 ng/L), el metotrexat (29 ng/L)i el tamoxifè (7,4 ng/L) en un dels hospitals, i de l'erlotinib (2,4-5,5 ng/L) en ambdós. D'altra banda, els compostos de platí, la ifosfamida, la gemcitabina, l'irinotecà, la capecitabina i els seus metabòlits van estar per sota els límits de detecció o de quantificació. En canvi, a Ljubljana, es van detectar tots els compostos analitzats a concentracions més elevades que a Barcelona, amb nivells de ciclofosfamida de 1.080-22.100 ng/L o de metotrexat a 19-3920 ng/L.

La ciclofosfamida i la ifosfamida són també dos dels compostos més àmpliament estudiats, i s'analitzen juntament amb altres citostàtics o fàrmacs d'altres famílies. Yin et al. (2009) van estudiar la presència de 7 citostàtics en 21 hospitals a Pequín, dels quals en van detectar 5. La ciclofosfamida i la ifosfamida van ser els citostàtic amb major freqüència de detecció i a concentracions entre <2-2.000 ng/L i <2-10.647 ng/L respectivament. En aquest mateix estudi també es va detectar amb menys freqüència el metotrexat (<2-4.689 ng/L), l'etopòsid (<5-380 ng/L) i l'azatioprina (<5-32 ng/L), i no van detectar la vincristina ni la doxorubicina. A Catalunya, Gómez-Canela et al. (2012), van detectar la ciclofosfamida entre 3,1 i 5.730 ng/L, però no l'epirubicina (<MDL). En un treball posterior, a Barcelona, es van analitzar 26 fàrmacs anticancerigens i se'n van poder detectar 8 (Gómez-Canela et al., 2014). Concretament, el compost més freqüent i a més alta concentració (<6-86.200 ng/L) va ser la ifosfamida, seguida de la ciclofosfamida (<4-4.720 ng/L), el megestrol (<3-1.260 ng/L), l'irinotecà (<4-730), la capecitabina (<15-490 ng/L), la goserelina (<16-350 ng/L), la prednisona (<12-210 ng/L) i l'epirubicina (<45-60 ng/L). En canvi, no es va detectar la presència de la ciproterona, el clorambucil, el melfalan, la fludarabina, la citarabina, la gemcitabina, la vinblastina, la vincristina, l'etopòsid, el paclitaxel, el docetaxel, la daunorubicina, l'imatinib, l'erlotinib, la leuprolida, el tamoxifè i l'aminoglutetimida. També a Barcelona, Negreira et al. (2014a), van analitzar 13 citostàtics i llurs metabòlits als efluents d'un hospital. Durant una setmana de mostreig es va detectar la ciclofosfamida (5,9-100 ng/L), la ifosfamida (7,1-19,4 ng/L) i el metotrexat (2,0-19,4 ng/L). Tampoc en cap de les mostres es va detectar la temozolomida, la gemcitabina, la capecitabina, l'etopòsid, el paclitaxel, la doxorubicina, l'imatinib, l'erlotinib, l'irinotecà i el tamoxifè. Ferrando-Climent et al. (2013) van analitzar les aigües en 4 hospitals de Coïmbra (Portugal), València i Girona, on la ciclofosfamida es va detectar en tres d'ells (35,9-200,7 ng/L), i la ifosfamida només en dos (31,5-227,9 ng/L). En aquest estudi també es va detectar l'azatioprina (14,5-187,9 ng/L) i el tamoxifè (26,3-133,4 ng/L) en els quatre hospitals, l'etopòsid en dos (97,5-406,0 ng/L), el docetaxel (97,7 ng/L), el paclitaxel (99,7 ng/L) i la vincristina (49,1 ng/L) en un hospital, i no es va detectar el metotrexat (<MDL).

De tots els citostàtics estudiats en els efluents d'hospital, la ciclofosfamida i la ifosfamida són els que s'han detectat amb més freqüència perquè també s'han inclòs en més estudis. En alguns treballs s'ha vist que el moment del mostreig pot afectar el nombre de fàrmacs detectats i llurs concentracions, fent que siguin més elevades si coincideixen amb els tractaments oncològics administrats.

1.3.2. Estacions depuradores d'aigües residuals

Seguint el circuit de l'aigua segons mostra la Figura 1.3, els efluents d'hospital, juntament amb les aigües residuals domèstiques arriben a les estacions depuradores d'aigües residuals (EDAR). Els influents i efluents d'EDAR han estat típicament caracteritzats per determinar la presència de citostàtics ja que aquests efluents s'han identificat com una font de citostàtics cap al medi.

El 5-fluorouracil va ser el fàrmac detectat a concentracions més altes en els efluents d'hospital (Mahnik et al., 2007), però no tots els autors n'estudien la seva presència en les EDAR. Kosjek et al. (2013) van analitzar les plantes que recullen les aigües procedents de dos hospitals oncològics, on un administrava 5-florouracil i l'altre no. Aquest fàrmac només es va detectar a l'entrada que recull les aigües del primer (4,7-14 ng/L), a nivells més baixos que l'efluent de l'hospital, i no es va detectar a la sortida (<LOD). Isidori et al. (2016) van analitzar diversos citostàtics en les EDAR que recullen els efluents de diferents hospitals a Ljubljana i Barcelona. Els compostos que s'havien detectat als hospitals, es van detectar també en els influents de l'EDAR, tals com el 5-fluorouracil (3,1-3,5 ng/L), el metotrexat (8,3-303 ng/L), l'irinotecà (49 ng/L), la capecitabina (158 ng/L), els compostos de platí (23-27 ng/L), el tamoxifè (6,7-61 ng/L), l'erlotinib (3,5-8,1 ng/L) i la ciclofosfamida (6,0-27 ng/L). Aquests tres darrers es van detectar també als efluents de l'EDAR a concentracions similars que als influents (7,1, 3 i 17 ng/L respectivament). Contràriament, altres autors també han analitzat el 5-fluorouracil en influents i efluents d'EDAR, però no l'han detectat. Concretament a Suïssa juntament amb el tamoxifè, que tampoc no es va detectar(Tauxe-Wuersch et al., 2006), a Baltimore (EEUU) juntament amb altres fàrmacs (Yu et al., 2006), i a Sevilla (Martín et al., 2011, 2014). En aquest darrer estudi, tampoc no es va detectar el metotrexat, la vinorelbina, el paclitaxel, el docetaxel, l'epirubicina, la mitomicina, l'irinotecà ni la ciclofosfamida (Martín et al., 2011). En canvi es va detectar la presència de la citarabina (9,2 ng/L), la doxorubicina (4,5 ng/L), l'etopòsid (15 ng/L), la gemcitabina (9,3 ng/L) i la ifosfamida (3,5 ng/L), que, excepte la doxorubicina, es van detectar també a la sortida de l'EDAR a concentracions més baixes (1,2-14 ng/L) (Martín et al., 2011). En un mostreig més exhaustiu en quatre EDARs de Sevilla, es van detectar puntualment concentracions més elevades d'etopòsid (15,1-46,8 ng/L), gemcitabina (39,3–52,1 ng/L) i ifosfamida (6,49–19,1 ng/L), es va detectar

també el metotrexat (7,30–55,8 ng/L) i la citarabina (en totes les mostres, a 44,4–464 ng/L) i en canvi no es va detectar la doxorubicina, la ciclofosfamida, la vinorelbina, el paclitaxel, el docetaxel, l'epirubicina, la mitomicina, i l'irinotecà (Martín et al., 2014). En el mateix estudi, a la sortida de les EDAR es va tornar a detectar la ifosfamida (4,14–15,6 ng/L), la citarabina (9,90–190 ng/L) i la gemcitabina (64,6–88,4 ng/L) juntament amb la vinorelbina (44,1–170 ng/L), el paclitaxel (1,40–2,19) i la doxorubicina (20,3–42,4 ng/L), que no s'havien detectat a l'entrada. Els autors, però, no donen una explicació per aquest fet.

Altres estudis també posen de manifest la presència de citostàtics als influents de les EDARs degut que recullen les aigües residuals procedents d'efluents d'hospital. A Girona i Tolosa (França) es va detectar en almenys un dels influents estudiats la presència de ciclofosfamida (25,5 ng/L), ifosfamida (130,1 ng/L), metotrexat (23,0 ng/L), vincristina (22,9 ng/L), etopòsid (83,0 ng/L), docetaxel (175,1 ng/L), tamoxifè (30,0-58,3 ng/L) i azatioprina (18,2-19,1 ng/L), on les concentracions van ser en general menors que als hospitals (Ferrando-Climent et al., 2013). El paclitaxel va ser l'únic que no es va detectar en cap mostra, tot i que s'havia identificat en un hospital. Gómez-Canela et al. (2012) van analitzar la ciclofosfamida i l'epirubicina, i només van detectar ciclofosfamida en una de les tres EDARs estudiades de Catalunya, a 13.100 ng/L, però sense residus a la sortida (<MDL). En canvi, en un altre estudi es van analitzar 26 citostàtics i es va detectar puntualment la ciclofosfamida (<4-10 ng/L) i el megestrol (<3-220) als influents, que es van tornar a detectar a la sortida tot i que a concentracions molt més baixes (<4-5 i <3-20 ng/L respectivament) (Gómez-Canela et al., 2014). Tots els altres citostàtics analitzats van estar per sota els límits de detecció tant als influents com als efluents. En un mostreig preliminar, com a validació del mètode d'anàlisi, Negreira et al. (2013a) van analitzar l'influent d'una depuradora de Catalunya. En aquest estudi van detectar el metotrexat en les 8 mostres analitzades (2,1-20,1 ng/L) i en menor freqüència també la capecitabina (8,2-27,0 ng/L), la ifosfamida (7,3-43,3 ng/L) i el tamoxifè (3,5-17,2 ng/L). No es va detectar la ciclofosfamida, l'etopòsid, el paclitaxel, la doxorubicina ni l'irinotecà. En un mostreig posterior més exhaustiu, es van analitzar 12 EDARs, incloent-hi els seus influents i efluents (Negreira et al., 2014a). Dels 13 citostàtics estudiats, només es va detectar la ifosfamida (2,2-27,9 ng/L), la ciclofosfamida (2,4-43,8 ng/L) i la capecitabina (5,1-52,6 ng/L), generalment a l'entrada i a la sortida de la mateixa EDAR. De manera puntual també es va detectar el metotrexat (2,6-18,1 ng/L) i la doxorubicina (2,5-2,7 ng/L), i en canvi la temozolomida, la gemcitabina, l'etopòsid, el paclitaxel, l'imatinib i l'erlotinib, l'irinotecà i el tamoxifè no van ser identificats.

En un estudi realitzat a Zurich(Suïssa) es va constatar que les EDAR tenen una capacitat limitada per eliminar citostàtics. La ifosfamida i la ciclofosfamida es van detectar a l'entrada a 2-

11 i <0,3-5 ng/L respectivament, mentre que a la sortida les concentracions van ser de 2-10 i 1,7-6 ng/L respectivament (Buerge et al., 2006). En canvi, al Regne Unit no es va detectar ifosfamida a la sortida d'una EDAR de però si ciclofosfamida (0,19-3,5 ng/L) en les 3 mostres analitzades (Llewellyn et al., 2011). A Montreal (Canadà), també van detectar la ciclofosfamida i el metotrexat en un dels dos influents analitzats (9 i 59 ng/L), però cap dels dos citostàtics es van detectar a l'efluent (<LOD)(Garcia-Ac et al., 2009a). La presència d'aquests dos fàrmacs també va ser estudiada per Castiglioni et al. (2005) en efluents d'EDARs de diferents ciutats d'Itàlia. En aquest estudi la ciclofosfamida només es va detectar en dues de les vuit mostres (2,1-9,0 ng/L) i el metotrexat en una (12,6 ng/L).

Altres autors que han determinat la presència de diferents famílies de fàrmacs han inclòs alguns citostàtics. Concretament la ifosfamida i el tamoxifè es van estudiar en l'efluent de l'EDAR d'Alès, França durant els mesos de juliol, agost i febrer (Coetsier et al., 2009). La ifosfamida no es va poder detectar en cap de les mostres, però el tamoxifè es va detectar a <5,8-102 ng/L durant els mesos de juliol. El megestrol va ser analitzat juntament amb altres hormones per Chang et al. (2011), que el van detectar a l'entrada de 7 EDARs a Pequín (1,9-9,3 ng/L), però només puntualment es va identificar a la sortida (0,1-0,7 ng/L).

Com s'ha vist en algun treball les concentracions d'alguns fàrmacs a l'efluent són més altes que les detectades a l'influent. Aquest fet es dóna puntualment per la vinorelbina, el paclitaxel i la doxorubicina(Martín et al., 2014), la gemcitabina (Martín et al., 2011) i l'epirubicina (Gómez-Canela et al., 2012). Malgrat que molts estudis utilitzen mostres compostes, no s'ha considerat el temps de retenció hidràulic i per tant, en el moment de mostreig l'aigua de la sortida no és la mateixa que s'ha agafat a l'entrada. Cal tenir en compte també que alguns fàrmacs arriben a les depuradores en forma conjugada i, degut als tractaments fisicoquímics i biològics, es poden desconjugar i donar lloc al fàrmac original, fent que es pugui detectar a concentracions més elevades a la sortida.

No obstant, les baixes concentracions en els influents de les EDAR en relació als efluents d'hospital posa de manifest el procés de dilució i degradació durant el transport al llarg de la xarxa de clavegueram. Les concentracions als efluents de les EDAR solen ser també menors que les de l'influent, ja que els diferents processos que tenen lloc per al tractament de les aigües poden eliminar-los, com és el cas del 5-fluorouracil, el metotrexat, la doxorubicina o l'epirubicina. Tot i així, compostos com la ciclofosfamida, la ifosfamida, el tamoxifè o la capecitabina són més recalcitrants a la biodegradació ja que es detecten de manera recurrent i a concentracions similars en influents i efluents d'una mateixa planta. Això fa pensar que aquests seran més susceptibles de trobar-se en aigües superficials.

1.3.3 Aigües superficials, subterrànies i potables

Després del tractament a la depuradora, les aigües són retornades al medi, concretament al mar o al riu. Sobretot en les zones urbanes d'interior d'ambients mediterranis l'aigua dels rius prové majoritàriament d'efluents on, enlloc de diluir-se, la càrrega de citostàtics podria acumular-se. El nombre de publicacions que avaluen els nivells de citostàtics en aigües superficials, així com el nombre de fàrmacs analitzats, és menor que en hospitals i depuradores però tot i així s'ha confirmat la seva presència en diferents rius del món.

Les concentracions més elevades s'han detectat a Bangkok (Tailàndia), concretament al riu Chao Phraya, on s'ha determinat la ciclofosfamida (1907 ng/L), el 5-fluorouracil (578 ng/L) i la hidroxicarbamida (788 ng/L) (Usawanuwat et al., 2014). La qualitat de l'aigua d'aquest riu va ser catalogada com a "pobre"al 2015, la categoria més baixa atorgada a la qualitat de les aigües superficials pel Departament de Control Ambiental de Tailàndia (Pollution Control Department, 2015). Les causes a la mala qualitat de l'aigua són atribuïdes a l'abocament d'aigua residual directament al riu i a la poca efectivitat de les plantes de tractament d'aigua, raons que poden ser també la causa de la presència de citostàtics.

També es van detectar altes concentracions a Polònia, on es van monitorar la presència d'àcid micofenòlic i tacrolimus durant gairebé dos anys en els rius Vístula i Utrata, situats al nord i a l'oest de la ciutat de Varsòvia, respectivament (Giebułtowicz and Nałęcz-Jawecki, 2016). Tot i ser rius de cabals molt diferents (255-2026 m^3/s per al Vístula i aproximadament 1 m^3/s a l'Utrata) ambdós creuen nuclis de població i reben els efluents de les depuradores. Durant el període de mostreig el tacrolimus no va ser detectat, però l'àcid micofenòlic es va detectar al riu Vístula en la majoria de punts mostrejats, entre 0,5 i 140 ng/L, i al riu Utrata només es va detectar a la primavera i a l'estiu, entre 4,7 i 130 ng/L. En ambdós rius les concentracions més altes es van detectar a la primavera, que es va atribuir a una menor eficiènciadel tractament de les depuradores durant aquesta estació.

Degut a la poca capacitat de les EDAR per eliminar la ciclofosfamida i la ifosfamida, aquests compostos s'han detectat de forma recurrent en diversos rius europeus. Buerge et al. (2006) van analitzar aquests dos citostàtics en aigües del llac Zurich i a diferents punts del riu Limmat, a Suïssa. La ciclofosfamida es va detectar a 0,05-0,17 ng/L en els diferents punts de mostreig, i la ifosfamida només al riu Limmat (0,08-0,14 ng/L) i al punt localitzat després de l'abocament de l'EDAR.Valcárcel et al. (2011) van analitzar aquests dos citostàtics, juntament amb altres fàrmacs, als principals rius de la comunitat de Madrid, 100 m després de la sortida de les 10 EDAR seleccionades. En tot el mostreig, la ciclofosfamida es va trobar per sota els límits de detecció (<3 ng/L) i la ifosfamida només es va detectar al riu Guadarrama a 41 ng/L. A prop de

Windsor, Canadà, es va detectar ciclofosfamida de manera puntual (Metcalfe et al., 2008), concretament a 5 ng/L al *Little River*, un canal que rep els efluents d'una EDAR i desemboca al riu Detroit, però no en els rius principals (Otonabee i Detroit) ni al port de Hamilton. A la ciutat de Montreal(Canadà), la ciclofosfamida i el metotrexat no es van detectar al riu St. Llorenç (Garcia-Ac et al., 2009a; Garcia-Ac et al., 2009b), ni als rius *Thousand Islands* i *Des Praiaries*(Garcia-Ac et al., 2009b) que constitueixen fonts d'aigua potable per la regió de Montreal. Aquests resultats eren d'esperar, ja que tampoc s'havien trobat a la sortida de l'EDAR. Tampoc es va detectar ciclofosfamida a Itàlia, al riu Po, que recull aigua de zones industrialitzades, i a l'Adda, que recull l'aigua de petits pobles de muntanya (Zuccato et al., 2000). En aquest mateix estudi però, si que es va identificar en el riu Lambro, que recull les aigües de Milà, a 2,2-10,1 ng/L. La ifosfamida tampoc va ser detectada al riu Allier prop de la ciutat de Clermont-Ferrand, a França (Celle-Jeanton et al., 2014), que està connectat a l'aqüífer que abasteix d'aigua potable la població. Les baixes concentracions poden atribuir-se als processos de dilució en rius d'elevats cabals.

Com altres compostos, el tamoxifè s'aboca al medi a través d'efluents d'EDAR i cal esperar que es trobi en aigües superficials. La seva presència, juntament amb la ifosfamida, va ser analitzada al riu Gard a l'alçada de la ciutat d'Alès (França), 10 m després de l'emissari de l'EDAR (Coetsier et al., 2009). La concentració de tamoxifè oscil·lava entre 22-25 ng/L mentre que la ifosfamida no va ser detectada (<LOD). A Catalunya, el tamoxifè tampoc va ser detectat al riu Llobregat (López-Serna et al., 2010) però si al llarg del riu Ebre, des del naixement a la desembocadura, a concentracions entre 12,4 i 26,8 ng/L (López-Serna et al., 2012). Les concentracions detectades al llarg del curs del riu van ser del mateix ordre, atribuït a que la dilució o degradació natural està contrarestada per la contínua aportació des de les aigües residuals. Contràriament, el tamoxifè tampoc no es va detectar al nord d'Escòcia (Nebot et al., 2007)ni al centre d'Anglaterra, tot i fer el mostreig en el moment de baix cabal del riu i major descàrrega de l'efluent de l'EDAR(Ashton et al., 2004).

El 5-fluorouracil, malgrat ser el citostàtic més analitzat en efluents d'hospital, la seva presència en aigües superficials gairebé no ha estat estudiada, probablement degut que a les EDAR gairebé no s'ha detectat. Kosjek et al. (2013) van analitzar les aigües on desembocava una de les EDAR situades a Eslovènia, i no va ser identificat.

La incidència de 14 citostàtics ha estat analitzada perMartín et al. (2011) en aigües del riu Guadalquivir, a Sevilla. Dels citostàtics que s'havien detectat en l'efluent de l'EDAR van tornar a detectar la gemcitabina (2,4 ng/L) i la citarabina (13 ng/L), atribuït a la descàrrega de la planta, i no es va detectar la vinorelbina, la ifosfamida ni l'etopòsid. La resta de compostos analitzats

(ciclofosfamida, metotrexat, 5-fluorouracil, paclitaxel, docetaxel, doxorubicina, epirubicina, mitomicina, i irinotecà) no van ser detectats (<MDL).

Els compostos citostàtics són molt polars i teòricament podrien lixiviar cap a les aigües subterrànies. Aquestes masses d'aigua es troben al subsòl i s'utilitzen en l'abastament d'aigua potable i subministrament d'aigua a la indústria i l'agricultura (ACA, 2017). Sacher et al. (2001) van analitzar un llistat de 60 fàrmacsen 105 pous d'aigua subterrània a l'estat de Baden-Württemberg, Alemanya, on s'hi incloïa ifosfamida i ciclofosfamida. Es coneixia que alguns d'aquests pous estaven influenciats per aigua residual, industrial o urbana, però en cap d'ells no es van detectar citostàtics. A Barcelona, López-Serna et al. (2013) van estudiar 95 fàrmacs de diferents famílies i metabòlits en les aigües subterrànies, on es va detectar tamoxifè a 9,3-223 ng/L. La seva presència, juntament amb altes concentracions d'antibiòtics i analgèsics, va ser atribuïda a l'aigua que percola provinent del sistema de clavegueram i de les infiltracions provinents del riu. No obstant, les aigües subterrànies de Barcelona no s'utilitzen per al consum, sinó en usos on no es requereix una qualitat d'aigua molt estricta (reg dels parcs, neteja de carrers o cotxes, refrigeració en edificis, etc.) (López-Serna et al., 2013).

Com s'ha descrit a l'inici de l'apartat 1.3, les aigües del riu poden captar-se per tal de ser potabilitzades i distribuir-se a la xarxa pública com a aigua de consum. Per tant, la presència de citostàtics en l'aigua de beguda podria posar en perill la salut humana. L'aigua de l'aixeta, presa en domicilis privats durant 7 dies consecutius, va ser analitzada en quatre poblacions de la comunitat de Madrid (Mendoza et al., 2016). D'un total de 13 citostàtics estudiats (ciclofosfamida, ifosfamida, temozolomida, capecitabina, gemcitabina, metotrexat, etopòsid, paclitaxel, doxorubicina, erlotinib, imatinib, irinotecà i tamoxifè) i 4 metabòlits, no es van detectar en cap de les mostres. Un altre estudi fet a Madrid va analitzar aigua d'aixeta procedent de residències privades i fonts públiques on, malgrat es van detectar altres substàncies estimulants, no es va identificar ni la ciclofosfamida ni la ifosfamida (Valcárcel et al., 2011). Aquests resultats coincideixen amb els estudis realitzats arreu del món. A Montreal no es va detectar la ciclofosfamida ni el metotrexat en aigua d'aixeta recollida en residències privades de tres barris diferents (Garcia-Ac et al., 2009b). L'àcid micofenòlic i el tacrolimus tampoc van ser detectats en aigua de l'aixeta a Varsòvia recollida en 10 mostres en intervals de tres mesos(Giebułtowicz and Nałęcz-Jawecki, 2016). Així doncs, es posa en evidència que els citostàtics no estan presents en aigua d'aixeta corresponent a països europeus i el Canadà. Això es deu a que els processos de potabilització són eficaços per eliminar la càrrega d'aquests fàrmacs procedents dels rius.

Al final del seu recorregut, les aigües dels rius desemboquen al mar. Les concentracions detectades fins ara en aigües superficials, sovint baixes i properes als límits de detecció, i l'alta dilució que te lloc entre el riu i el mar fa pensar que no hi haurà presència de citostàtics en aquest medi. Malgrat tot, si la càrrega en el riu és alta, com en el cas de Bangkok (Usawanuwat et al., 2014) es podria detectar la seva presència en zones properes a la costa. Malauradament, fins ara només Nebot et al. (2007) han analitzat el tamoxifè en aigua de mar, procedent del nord d'Escòcia, on no va ser detectat.

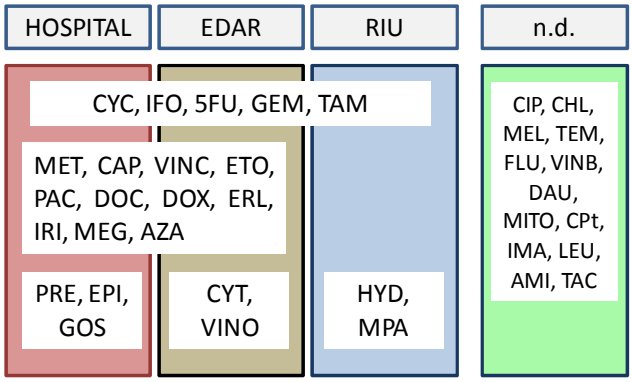

Figura 1.4. Citostàtics detectats en aigües.
n.d: no detectat. AMI: aminoglutetimida, AZA: azatioprina, CAP: capecitabina, CHL: clorambucil, CIP: ciproterona,CPt: cisplatí,CYC: ciclofosfamida, CYT: citarabina, DAU: daunorubicina, DOC: docetaxel,DOX: doxorubicina,EPI: epirubicina, ERL: erlotinib ETO: etopòsid, FLU: fludarabina,5FU: 5-fluorouracil, GEM: gemcitabina, GOS: goserelina,HYD: hidroxicarbamida,IFO: ifosfamida, IMA: imatinib, IRI: irinotecà, LEU: leuprolida, MEG: megestrol, MEL: melfalan, MET: metotrexat, MITO: mitomicina, MPA: àcid micofenòlic, PAC: paclitaxel, PRE: prednisona, TAC: tacrolimus, TAM: tamoxifè, TEM: temozolomida, VINB: vinblastinaVINC: vincristinaVINO: vinorelbina.

A la Figura 1.4 es recullen els citostàtics detectats en les diferents matrius estudiades (efluents d'hospital, EDARs i rius) on alguns compostos s'han detectat de manera reiterada.D'altres, en canvi, només s'han detectat o s'han estudiat en algun d'aquests tipus d'aigua. En general, les concentracions detectades són més baixes en aigües superficials, de l'ordre de ng/L, però s'ha vist que l'alliberament d'aquests fàrmacs és continu i per tant pot representar un risc pels organismes aquàtics.

Taula 1.2: Presència de citostàtics al medi ambient (ng/L): efluents d'hospital, influents i efluents d'EDAR, riu, mar, aigua subterrània i aigua potable.

Codi ATC	Nom	Efluent hospital	Influent EDAR	Efluent EDAR	Riu	Mar	Subterrània	Potable	Referència
G03HA01	Ciproterona	nd	nd	nd					Gomez-Canela 2014
H02AB07	Prednisona	<12-210	<12	nd					Gomez-Canela 2014
L01AA01	Ciclofosfamida				nd-10				Zuccato 2000
							Nd		Sacher 2001
				nd-8	nd-5				Metcalfe 2003
				nd-9,0					Castiglioni 2005
			2-11	2-10	0,05-0,17				Buerge 2006
			<LOD-9	<LOD	<LOD				Garcia-Ac 2009
					<MDL			<MDL	Garcia-Ac 2009-online
		<2-2.000							Yin 2010
				0,19-3,5					Llewellyn 2011
			<MDL	<MDL	<MDL				Martín 2011
					<3			nd	Valcarcel 2011
		<MDL-5.730	<MDL-13.100	<MDL					Gomez-Canela 2012
		<MQL- 200,7	nd-25,5						Ferrando-Climent 2013
			nd						Negreira 2013
		<4–4.720	<4-10	<4-5					Gomez-Canela 2014
			<LOD	<LOD					Martin 2014
		nd-100	nd-43,8	nd-25,0					Negreira 2014
					1.907				Usawanuwat 2014
		<LOD-22.100	<LOD-27	<LOD-17					Isidori 2016
								<LOD	Mendoza 2016
L01AA02	Clorambucil	nd	nd	nd					Gomez-Canela 2014
L01AA03	Melfalan	nd	nd	nd					Gomez-Canela 2014
L01AA06	Ifosfamida						nd		Sacher 2001
			<0,3-5	1,7-6	<0,05-0,14				Buerge 2006
				<3,8	<3,8				Coetsier 2009
		<2-10.647							Yin 2010
				nd					Llewellyn 2011

Codi ATC	Nom	Efluent hospital	Influent EDAR	Efluent EDAR	Riu	Mar	Subterrània	Potable	Referència
			3,5	1,2	<MDL				Martin 2011
					<1-41			nd	Valcarcel 2011
		nd-227,9	nd-130,1						Ferrando-Climent 2013
			nq-43,3						Negreira 2013
					nd				Celle-Jeanton 2014
		<6–86.200	<6	nd					Gomez-Canela 2014
			6,49–19,1	4,14–15,6					Martin 2014
		nd-19,4	nd-27,9	nd-15,9					Negreira 2014
		<LOD-48	<LOD	<LOD					Isidori 2016
								<LOD	Mendoza 2016
L01AX03	Temozolomida	nd	nd	nd					Negreira 2014
								<LOD	Mendoza 2016
L01BA01	Metotrexat			nd-12,6					Castiglioni 2005
			<LOD-59	<LOD	<LOD				Garcia-Ac 2009
					<MDL			<MDL	Garcia-Ac 2009-online
		<2-4.689							Yin 2010
			<MDL	<MDL	<MDL				Martin 2011
		nd - <MQL	<MQL-23,0						Ferrando-Climent 2013
			2,1-20,1						Negreira 2013
			7,30–55,8	<LOD					Martin 2014
		nd-19,4	nd-18,1	nd					Negreira 2014
		<LOD-3.920	8,3-303	<LOD					Isidori 2016
								<LOD	Mendoza 2016
L01BB05	Fludarabina	nd	nd	nd					Gomez-Canela 2014
L01BC01	Citarabina		9,2	14	13				Martin 2011
		nd	nd	nd					Gomez-Canela 2014
			44,4–464	9,90–190					Martin 2014
L01BC02	5-fluorouracil	nd	nd	nd					Tauxe-Wuersch 2006
			nd	nd					Yu 2006
		<8.600-123.500							Mahnik 2007

Codi ATC	Nom	Efluent hospital	Influent EDAR	Efluent EDAR	Riu	Mar	Subterrània	Potable	Referència
		<LOQ-27							Kovalova 2009
		90-4.000							Mullot 2009
		<5-27							Weissbrodt 2009
			<MDL	<MDL	<MDL				Martin 2011
		<LOD-92	<LOD-14	<LOD	<LOD				Kosjek 2013
			<LOD	<LOD					Martin 2014
					578				Usawanuwat 2014
		<LOD-6.9	<LOD-3,5	<LOD					Isidori 2016
L01BC05	Gemcitabina	<LOQ-38							Kovalova 2009
		<0,9-38							Weissbrodt 2009
			9,3	7	2,4				Martin 2011
		nd	nd	nd					Gomez-Canela 2014
			39,3–52,1	64,6–88,4					Martin 2014
		nd	nd	nd					Negreira 2014
		<LOD	<LOD-61	<LOD					Isidori 2016
								<LOD	Mendoza2016
L01BC06	Capecitabina		nq-27,0						Negreira 2013
		<15–490	<15	nd					Gomez-Canela 2014
		nd	<LOD-72,6	nd-36,0					Negreira 2014
		<LOD-106	<LOD-158	<LOD					Isidori 2016
								<LOD	Mendoza2016
L01CA01	Vinblastina	nd	nd	nd					Gomez-Canela 2014
L01CA02	Vincristina	nd							Yin 2010
		nd-49,1	nd-22,9						Ferrando-Climent 2013
		nd	nd	nd					Gomez-Canela 2014
L01CA04	Vinorelbina		<MDL	9,1	<MDL				Martin 2011
			<LOD	44,1–170					Martin 2014
L01CB01	Etopòsid	<5-380							Yin 2010
			15	3,4	<MDL				Martin 2011
		nd-406,0	nd-83,0						Ferrando-Climent 2013
			nd						Negreira 2013

Codi ATC	Nom	Efluent hospital	Influent EDAR	Efluent EDAR	Riu	Mar	Subterrània	Potable	Referència
		nd	nd	nd					Gomez-Canela 2014
			15,1-46,8	<LOD					Martin 2014
		nd	nd	nd					Negreira 2014
								<LOD	Mendoza2016
L01CD01	Paclitaxel		<MDL	<MDL	<MDL				Martin 2011
		nd-99,7	nd						Ferrando-Climent 2013
			nd						Negreira 2013
		nd	nd	nd					Gomez-Canela 2014
			<LOD	1,40–2,19					Martin 2014
		nd	nd	nd					Negreira 2014
								<LOD	Mendoza2016
L01CD02	Docetaxel		<MDL	<MDL	<MDL				Martin 2011
		nd-97,7	nd-175,1						Ferrando-Climent 2013
		nd	nd	nd					Gomez-Canela 2014
			<LOD	<LOD					Martin 2014
L01DB01	Doxorubicin	100-1.400							Mahnik 2006
		<260-1.350							Mahnik 2007
		nd							Yin 2010
			4,5	<MDL	<MDL				Martin 2011
			nd						Negreira 2013
		nd	nd	nd					Gomez-Canela 2014
			<LOD	20,3–42,4					Martin 2014
		nd	nd-2,7	nd					Negreira 2014
								<LOD	Mendoza 2016
dauno	Daunorubicina	nd							Mahnik 2006
		nd	nd	nd					Gomez-Canela 2014
L01DB03	Epirubicina	100-500							Mahnik 2006
			<MDL	<MDL	<MDL				Martin 2011
		<MDL	<MDL	<MDL					Gomez-Canela 2012
		<45–60	<45	nd					Gomez-Canela 2014
			<LOD	<LOD					Martin 2014

Codi ATC	Nom	Efluent hospital	Influent EDAR	Efluent EDAR	Riu	Mar	Subterrània	Potable	Referència
L01DC03	Mitomicin C		<MDL	<MDL	<MDL				Martin 2011
			<LOD	<LOD					Martin 2014
L01XA01	Cisplatí	<LOD	<LOD	<LOD					Isidori 2016
L01XE01	Imatinib	nd	nd	nd					Gomez-Canela 2014
		nd	nd	nd					Negreira 2014
								<LOD	Mendoza 2016
L01XE03	Erlotinib	nd	nd	nd					Gomez-Canela 2014
		nd	nd	nd					Negreira 2014
		2,0-5,5	3,5-8,1	3,3-3,8					Isidori 2016
								<LOD	Mendoza 2016
L01XX05	Hidroxicarbamida				788				Usawanuwat 2014
L01XX19	Irinotecà		<MDL	<MDL	<MDL				Martin 2011
			nd						Negreira 2013
		<4–730	<4	nd					Gomez-Canela 2014
			<LOD	<LOD					Martin 2014
		nd	nd-21,3	nd-16,8					Negreira 2014
		<LOD-9,2	<LOD-49	<LOD					Isidori 2016
								<LOD	Mendoza 2016
L02AB01	Megestrol		1,9-9,3	nd-0,7					Chang 2011
		<3-1.260	<3-220	<3-20					Gomez-Canela 2014
L02AE02	Leuprolida	nd	nd	nd					Gomez-Canela 2014
L02AE03	Goserelina	<16-350	<16	nd					Gomez-Canela 2014
L02BA01	Tamoxifè			<10	<10				Ashton 2004
		LOD-LOQ	LOD-LOQ	LOD-LOQ					Tauxe-Wuersch 2006
				nd	nd	Nd		nd	Nebot 2007
				<5,8-102	<5,8-25				Coetsier 2009
					nd				Lopez-Serna 2010
					12,4-26,8				Lopez-Serna 2012
		26,3-133,4	30,0-58,3						Ferrando-Climent 2013
							9,25-223		Lopez-Serna 2013

Codi ATC	Nom	Efluent hospital	Influent EDAR	Efluent EDAR	Riu	Mar	Subterrània	Potable	Referència
			nq-17,2						Negreira 2013
		nd	nd	nd					Gomez-Canela 2014
		nd	nd-180,6	nd-147,0					Negreira 2014
		<LOD-10	6,7-61	<LOD-7,1					Isidori 2016
								<LOD	Mendoza 2016
L02BG01	Aminoglutetimida	nd	nd	nd					Gomez-Canela 2014
L04AA06	Àcid micofenòlic				nd-141			nd	Giebultowicz 2016
L04AD02	Tacrolimus				nd				Giebultowicz 2016
L04AX01	Azatioprina	<5-32							Yin 2010
		14,5-187,9	18,2-19,1						Ferrando-Climent 2013

nd: no detectat; nq: no quantificable; LOD: limit de detecció; MDL: límit de detecció metodològic

1.4. Toxicitat i regulacions

L'acció dels fàrmacs citostàtics té com a objectiu evitar la proliferació de les cèl·lules cancerígenes, a través de diferents mecanismes segons la seva naturalesa. Com s'ha esmentat a l'apartat 1.1, el grup L01 interacciona amb l'ADN per alterar la seva transcripció. Per exemple, un dels mecanismes d'acció dels agents alquilants (grup L01A) és addicionar grups alquil a les bases de l'ADN i així, els enzims el fragmenten en intentar reparar-lo (Drug Bank Database, 2013). El mecanisme d'altres citostàtics consisteix en interaccionar amb els processos metabòlics de les cèl·lules, i poden també alterar el metabolisme de les cèl·lules sanes. Per tant, aquests fàrmacs estan classificats com a mutàgens, ja que en interaccionar amb l'ADN incrementen la ràtio de mutacions genètiques, i carcinògens, ja que poden provocar l'aparició de nous tumors. Per aquest motiu, l'alliberament d'aquests fàrmacs al medi aquàtic pot ser perjudicial per als organismes que hi habiten.

Per tal d'avaluar la toxicitat dels fàrmacs de consum humà en els organismes aquàtics, organitzacions com l'OECD (*Organisation for Economic Co-operation and Development*) han desenvolupat i proposatmètodes que avaluen la toxicitat aguda i crònica en espècies de diferents nivells tròfics (OECD, 2016). Els resultats de les proves de toxicitat donen la concentració a la que un determinat fàrmac produeix un efecte concret (*endpoint*), segons diferents criteris: el NOEC és la concentració que no produeix efectes observables; el LOEC és la concentració mínima que te efectes sobre els organismes exposats; l'EC_{50} és la concentració que produeix efectes negatius en el 50% de la població exposada; i l'LC_{50} és la concentració que causa la mort a la meitat de la població exposada.

En el cas dels citostàtics, els estudis que s'han dut a terme se centren majoritàriament en proves de toxicitat aguda per alguns dels compostos administrats. Com es tracta de fàrmacs amb propietats fisicoquímiques diverses, els valors publicats en articles científics i en els fulls de seguretat dels principis actius varien en un rang molt ampli en funció del fàrmac i l'espècie.

La toxicitat aguda en algues s'avalua exposant aquestes a una solució que contingui els analits i observant la inhibició que produeixen en el seu creixement a diferents temps (16h, 24h, 48h, 3 dies...) segons la prova. Així, s'han publicat valors d'EC_{50} per la ciclofosfamida de 930 mg/L (96h d'exposició en l'alga *P. Subcapitata*) (Zounková et al., 2007), de 31 mg/L per la prednisona (3 dies en *P.subcapitata*) (Cunningham et al., 2006) o 13 mg/L per la doxorubicina (96h en *P. Subcapitata*) (Zounková et al., 2007), concentracions molt més altes que les que es troben al medi. En canvi, l'EC_{50}de la tioguanina va ser de 0,0034 mg/L (72h en alga verda)(GSK, 2014), que

amb 4 ordres de magnitud per sota el compostos anteriorment esmentats és el valor més tòxic descrit a la bibliografia.

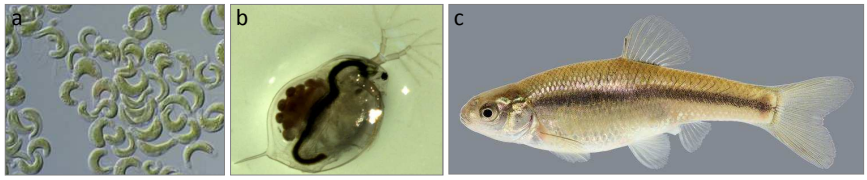

Figura 1.5. Exemples d'organismes utilitzats en estudis de toxicitat:
a) *P. subcapitata*, b) *D. magna*, c) *P. promelas*

L'organisme més comú en els estudis de toxicitat és el crustaci *Daphnia magna*(Figura 1.5b). Per avaluar la toxicitat aguda es te en compte la concentració que causa la immobilització o mort d'aquests organismes en 24 o 48h d'exposició. Per la prednisona es va determinar un NOEC de 99,9 mg/L, resultant menys tòxica per la dàfnia que per les algues (DellaGreca et al., 2003). En canvi la doxorubicina va donar un NOEC de 0,01 mg/L i un EC_{50} de 2 mg/L (Zounková et al., 2007), concentracions més baixes que en les algues i per tant més tòxiques. Per a la toxicitat crònica generalment s'avalua la capacitat de reproducció d'aquests organismes en exposicions de 21 dies, i els resultats solen ser concentracions més baixes. Per exemple, la capecitabina te un EC_{50} agut de 224 mg/L (immobilització a 48h, *D. magna*) i un EC_{50} crònic de 20,6 mg/L (reproducció a 21 dies, *D. magna*)(Parrella et al., 2014).

La toxicitat en peixos representa un nivell tròfic més elevat, però hi ha molt pocs estudis per a citostàtics. Per a l'etopòsid s'ha determinat un LC_{50} de 12.900 mg/L en *O. mykiss* a 96h (Pfizer, 2012) i per la bicalutamida un NOEC de 4,4 mg/L en *B. sunfish* a 96h (AstraZeneca, 2006). Els estudis de toxicitat aguda avaluen la mortalitat normalment a 14 dies d'exposició, el creixement de les larves o llur fecunditat. Per al tamoxifè s'ha determinat un EC_{50} de 0,00008 mg/L pel creixement de les larves F1 de *P. promelas* en 28 dies, sent el fàrmac amb una toxicitat aguda més elevada (Besse et al., 2012). Altres citostàtics tenen toxicitats més baixes, com el letrozol, amb un LOEC de 0,005 mg/L per a la fecunditat de l'*O. latipes* a 21 dies (Besse et al., 2012) o la flutamida, amb un LC_{50} per sobre de 1000 mg/L per al *P. promelas* a 14 dies (Cunningham et al., 2006).

Actualment no hi ha cap normativa que reguli les concentracions màximes permeses de fàrmacs citostàtics en el medi ambient. La EMA (*European Medicines Agency*) és l'únic organisme que dóna una guia sobre com avaluar els seus riscos potencials en les aigües (EMA, 2006), i que consisteix en primer lloc a determinar l'exposició del medi a aquests compostos. Si

els valors que es preveu detectar són iguals o superiors a 10 ng/L se suggereix fer un anàlisi dels seus efectes basat en els valors de toxicitat.

A dia d'avui, el nombre de citostàtics estudiats és petit en comparació amb tots els que estan llistats en el codi L de l'ATC però la seva presència en el medi s'ha pogut confirmar per a molts d'ells. Degut que les concentracions detectades al medi són generalment baixes i per tal de complir amb la recomanació de la EMA calen mètodes d'anàlisi selectius i sensibles, que permetin detectar un gran nombre de fàrmacs en diferents matrius aquoses a baixes concentracions.

1.5. Metodologia analítica

Els fàrmacs citostàtics són molt nombrosos i tenen propietats fisicoquímiques molt diverses. Aquestes propietats es descriuen amb detall a l'apartat 3.1 per al grup de citostàtics analitzats en aquesta tesi, però per entendre el repte analític que comporta l'anàlisi d'aquesta família de fàrmacs, cal fer-ne un petit esment en aquesta secció. En general es tracta de compostos polars, força solubles en aigua amb un log Kow per sota de 4. El pes molecular oscil·la entre 130 pel 5-fluorouracil i 1269 per la goserelina, i el pKa varia entre 1 i 18. Per tant, a l'hora de desenvolupar un mètode d'anàlisi, les mateixes condicions d'extracció no seran adequades per a tots els compostos.

Existeixen a la bibliografia diferents mètodes per l'anàlisi de citostàtics, la majoria dels quals se centren en estudiar la presència d'aquests fàrmacs en efluents d'hospitals, en depuradores (influents i efluents) i en aigües superficials, però també hi ha alguns autors que inclouen les aigües subterrànies, l'aigua potable i l'aigua de mar. Les diferents metodologies optimitzades tenen però un mateix objectiu: aconseguir una alta preconcentració dels analits i obtenir unes bones recuperacions i una gran selectivitat, per tal d'identificar els compostos de manera inequívoca i poder-los quantificar tot i ser presents a baixes concentracions. Aherne et al. (1985) van descriure per primera vegada la presència del citostàtics al medi, mitjançant la liofilització de les mostres procedent dels efluents d'un hospital, però no va ser fins al voltant de l'any 2000 que la preocupació per la presència d'aquests compostos a les aigües va augmentar significativament i conseqüentment, el nombre d'estudis publicats. A la Taula 1.3 es recullen els mètodes més destacats publicats a la literatura per a l'anàlisi de fàrmacs citostàtics en aquestes matrius. En primer lloc es descriuen en detall els mètodes d'extracció, senyalant els tipus de cartutx i eluents emprats en cada un i els paràmetres de qualitat obtinguts, i en segon lloc

s'enumeren els instruments d'anàlisi, senyalant les columnes analítiques i les fases mòbils utilitzades.

1.5.1. Extracció

Els mètodes desenvolupats analitzen diversos citostàtics o els inclouen entre fàrmacs d'altres famílies. La majoria d'autors coincideixen en utilitzar l'extracció en fase sòlida (SPE), tot i que els volums de mostra, cartutxos i solvents utilitzats són molt diversos.

Els cartutxos més emprats han estat els polimèrics de fase reversa, concretament els Oasis HLB, amb un grup hidrofílic de N-vinilpirrolidona i un grup lipofílic de divinilbenzè, que s'han utilitzat per extreure un major nombre de citostàtics en un mateix mètode. Aquests cartutxos són aptes per a l'extracció de compostos lipofílics i hidrofílics, i poden treballar en un ampli rang de pH (0-14). El primer treball publicat on es van utilitzar aquests cartutxos va ser per extreure la ciclofosfamida, juntament amb altres fàrmacs, en 2 L d'aigua de riu (Metcalfe et al., 2003). Les mostres es van filtrar i ajustar a pH 7,5, i l'elució es va fer amb metanol (MeOH), l'eluent més habitual per aquests cartutxos, però no s'indica la recuperació que es va obtenir.Per altra banda,Martín et al. (2011) van treballar a pH 7 per l'extracció de 14 citostàtics (5-fluorouracil, gemcitabina, ciclofosfamida, ifosfamida, citarabina, metotrexat, paclitaxel, etopòsid, irinotecà, docetaxel, epirubicina doxorubicina, vinorelbina i mitomicina). L'elució es va fer amb 4 addicions consecutives d'1 mL de MeOH, i es van obtenir recuperacions del 75-105% i límits de detecció metodològics (MDL) de 0,1-5,3 ng/L i de 38 ng/L pel 5-fluorouracil. Prèvia a l'extracció, el pH de les mostres pot ajustar-se també a altres valors. Yin et al. (2009) van treballar a pH2 per les mostres procedents d'efluents d'hospital, on van analitzar metotrexat, azatioprina, doxorubicina, vincristina, ciclofosfamida, ifosfamida, etopòsid i procarbazina. L'elució es va fer amb MeOH:H_2O (80:20) i es van obtenir recuperacions de 51-105% i MDLs de 2-20 ng/L. De manera similar, Gómez-Canela et al. (2014)van optimitzar un mètode per 26 citostàtics (veure Taula 1.3) en aigua residual d'hospital i depuradora. Abans de filtrar i ajustar les mostres a pH2, aquestes es van centrifugar per eliminar les partícules sòlides més grans. L'extracció es va fer també amb cartutxos HLB, i utilitzant MeOH i MeOH amb àcid fòrmic (HCOOH)(95:5) per l'elució. Les recuperacions obtingudes van ser superiors al 69%, excepte per la citarabina, la gemcitabina, l'etopòsid, el docetaxel, l'imatinib, l'irinotecà, el tamoxifè i l'aminoglutetimida, que van tenir recuperacions inferiors al 50%. Els MDL van ser de 0,7-356 ng/L. L'ajust del pH no és sempre un pas necessari i altres autors han optat per treballar al pH natural de les mostres. Per l'anàlisi del 5-fluorouracil, la ciclofosfamida i la hidroxicarbamida les mostres es van extreure sense ajustar-se prèviament (Usawanuwat et al., 2014). En aquest cas es va fer una primera elució amb una

solució de MeOH:H_2O (50:50) amb 0,1% HCOOH seguit d'una solució aquosa al 5% d'hidròxid d'amoni. Per aquests tres citostàtics es van obtenir recuperacions del 77-108% i límits de detecció de 6-50 ng/L. Un altre procediment utilitzat per tal d'augmentar la recuperació dels analits ha estat addicionar EDTA a les mostres, que evita que els fàrmacs s'uneixin als metalls presents en la matriu o en el material de vidre. Aquest mètode ha estat aplicat per l'anàlisi de 9 citostàtics (ciclofosfamida, ifosfamida, docetaxel, paclitaxel, etopòsid, vincristina, tamoxifè, metotrexat i azatioprina) en aigües residuals d'hospital i influents de depuradora (Ferrando-Climent et al., 2013). En aquest mètode es van extreure 50 mL de mostra, prèviament filtrada i amb 0,1M d'EDTA, i l'elució del cartutx es va fer amb MeOH. Les recuperacions van ser del 60-129%, excepte per la ciproterona, l'etopòsid i el tamoxifè, que van tenir recuperacions lleugerament inferiors, i els MDL van ser de 0,8-24 ng/L. Com que els cartutxos HLB són molt universals, també s'han emprat per optimitzar l'extracció de fàrmacs de diverses famílies, on també s'ha inclòs algun citostàtic. Concretament,Martínez Bueno et al. (2010) van analitzar la ifosfamida i la ciclofosfamida en aigua de riu, en aquest cas fent l'ajust de les mostres a pH 8 i quatre elucions consecutives amb MeOH. Per aquests dos compostos les recuperacions van ser del 81-84% i els MDL de 0,4 i 1 ng/L. La ifosfamida també es va incloure en el mètode de Celle-Jeanton et al. (2014), per a l'anàlisi de mostres de riu. En aquest mètode no es va fer pretractament de la mostra, i l'elució es va fer amb tres addicions consecutives de MeOH:ACN (70:30). Així es va obtenir una recuperació del 117% i un límit de quantificació de 1 ng/L.

Els cartutxos Strata X també s'han utilitzat freqüentment en la optimització de mètodes d'extracció per citostàtics juntament amb altres fàrmacs. Aquests són cartutxos polimèrics, adequats per a l'extracció d'analits àcids, bàsics o neutres, amb característiques similars als HBL descrits prèviament ja que també tenen el grup *N*-vinilpirrolidona. Yu et al. (2006) els van utilitzar per a l'extracció de 5-fluorouracil en aigües residuals, ajustant la mostra a pH 2 i utilitzant ACN com a eluent, però no mostren els paràmetres de qualitat del mètode. De manera similar, Coetsier et al. (2009) van analitzar ifosfamida i tamoxifè, ajustant les mostres a pH2 però fent l'elució del cartutx amb una solució a diferents proporcions d'acetat d'etil, acetona i hidròxid d'amoni. Així van obtenir recuperacions de 71-94% i límits de detecció de 3-16 ng/L. En canvi, Nebot et al. (2007) van fer l'extracció del tamoxifè sense ajust del pH i eluint amb acetona i MeOH, però van obtenir recuperacions més baixes (del 15-29% per les diferents matrius estudiades). Finalment Llewellyn et al. (2011) van emprar els mateixos cartutxos fent l'elució amb MeOH i acetat d'etil. En aquest treball van afegir un pas addicional, que consistia en fer la neteja de l'extracte amb Florisil ®, un sorbent utilitzat per eliminar altres compostos polars de la

matriu que puguin provocar interferències. En aquest cas es van obtenir recuperacions del 57-70%.

Per l'extracció de citostàtics també s'han emprat els cartutxos Isolute ENV+, una resina polimèrica no polar que crea interaccions hidrofòbiques amb els analits, que han estat utilitzats sobretot per extreure el 5-fluorouracil d'aigües residuals. Un d'aquests mètodes va ser desenvolupat per Mahnik et al. (2004), que van centrifugar i filtrar la mostra prèviament a l'extracció i van fer l'elució amb MeOH a pH 9. Així van obtenir recuperacions de 79-96% en funció de la concentració addicionada. En canvi, un procediment similar amb l'elució amb MeOH sense ajust de pH va donar recuperacions de 53-93% (Kosjek et al., 2013). L'ajust del pH previ a l'extracció també ha donat bones recuperacions. En el mètode de Mullot et al. (2009), les mostres es van ajustar a pH 4, i fent també l'elució amb MeOH, van obtenir recuperacions del 95-101% a diferent nivells de fortificació. En canvi, ajustant les mostres a pH 5, i fent 4 elucions consecutives amb MeOH es van obtenir recuperacions del 73%, lleugerament més baixes (Tauxe-Wuersch et al., 2006). Juntament amb el 5-fluorouracil, també s'ha extret la citarabina, la gemcitabina i alguns dels seus metabòlits. En aquest cas es van acoblar els cartutxos d'extracció Isolute ENV+ amb els Speedisk H2O-philic SA-DVB, un adsorbent d'intercanvi aniònic. L'elució es va fer amb MeOH i MeOH:HCOOH (98:2) i es van obtenir recuperacions del 46-79% (Kovalova et al., 2009; Weissbrodt et al., 2009).

Un altre mecanisme de retenció dels analits en el cartutx és mitjançant l'intercanvi iònic. Per als citostàtics s'han utilitzat els Oasis MCX, que contenen un polímer mixt de fase reversa i intercanvi catiònic fort que és selectiu per a les bases. Aquests cartutx s'ha utilitzat per extreure el tamoxifè en aigües residuals, amb una extracció líquid-líquid (LLE) prèvia. Els extractes de LLE es van tornar a extreure per SPE i l'elució es va fer amb MeOH:NH_4OH (95:5), que va donar una recuperació del 81% (Tauxe-Wuersch et al., 2006). Castiglioni et al. (2005) van optimitzar un mètode per al metotrexat i la ciclofosfamida juntament amb altres fàrmacs. Pel metotrexat es van utilitzar els cartutxos d'intercanvi iònic, però la ciclofosfamida es va incloure en un segon mètode d'extracció amb cartutxos Lichrolut EN. En el primer mètode, les mostres es van ajustar a pH 2 i l'elució es va fer amb MeOH, MeOH amb 2% d'amoni i MeOH amb 0,2% d'NaOH, i es va obtenir un 76% de recuperació del metotrexat. En el mètode de la ciclofosfamida, les mostres es van ajustar a pH 7, i es va fer l'elució amb MeOH i acetat d'etil, i la recuperació obtingudes van ser del 106%. En canvi, Zuccato et al. (2000) van desenvolupar un mètode per analitzar 16 fàrmacs en aigua superficial i potable, on van acoblar els cartutxos Lichrolut EN i Oasis MCX per fer-hi passar la mostra consecutivament. Així també es va extreure la ciclofosfamida sense haver de fer dues preparatives de mostra diferents. Els dissolvents d'extracció van ser el MeOH amb

acetat d'etil, seguit de MeOH amb un 5% d'NH_4OH, i es va obtenir una recuperació superior al 70%.

De manera menys estesa, també s'han utilitzat altres cartutxos que han permès obtenir una bona retenció de diferents citostàtics. Els de C8 han estat emprats per a l'extracció de doxorubicina, epirubicina i daunorubicina, a partir de 10 mL d'aigua residual d'hospital. En aquest mètode es va utilitzar MeOH:cloroform (1:2) per a l'elució, i es van obtenir recuperacions del 80-91%. (Mahnik et al., 2006). D'altra banda, Buerge et al. (2006), van utilitzar columnes reutilitzables de 10 mL de poliestirè divinilbenzè (Bio-Beads SM-2) per la ciclofosfamida i la ifosfamida, que es van eluir amb diferents volums de diclorometà. Amb l'extracció d'1 L de mostra es van obtenir recuperacions de 74-102%, i límits de detecció de 0,02-2 ng/L.

En tots els mètodes que s'han descrit fins ara l'extracció de les mostres s'ha fet prèvia a l'instrument d'anàlisi, és a dir, amb mètodes *offline*. Altres autors han optat per fer l'extracció *online*, en línia amb l'equip d'anàlisi, on l'eluent del cartutx és la mateixa fase mòbil del cromatògraf de líquids. Aquests tipus d'extracció també es caracteritza per preconcentrar volums de mostra menors, entre 5 i 50 mL, mentre que amb l'SPE habitual s'extreuen fins a 2,5 L de mostra. Amb aquests mètodes s'han emprat dos tipus de cartutxos diferents, els Strata X i els PLRP-s. Els primers són cartutxos polimèrics que retenen analits àcids, bàsics i neutres, amb els quals s'ha extret ciclofosfamida i metotrexat juntament amb altres fàrmacs (Garcia-Ac et al., 2009a; Garcia-Ac et al., 2009b). En aquest cas, les mostres (10-50 mL) es van acidificar abans de ser extretes i es van obtenir recuperacions del 55-148%. Els cartutxos PLRPS-s són també polimèrics però estan empaquetats com una columna analítica. En aquest cas s'han aplicat en l'extracció de la gemcitabina, la temozolomida, el metotrexat, l'irinotecà, l'imatinib, la ifosfamida, la ciclofosfamida, l'erlotinib, l'etopòsid, la doxorubicina, la capecitabina, el tamoxifè, el paclitaxel i alguns dels seus metabòlits. Les mostres (5 mL) es van acidificar a pH 2, i es van obtenir recuperacions del 72-119% (Negreira et al., 2013a).

Si la concentració de citostàtics en les mostres és elevada, no és necessària una alta preconcentració. Per això alguns autors també han optat per la injecció directa de les mostres. La ciclofosfamida i l'epirubicina van ser analitzades en mostres procedents d'EDAR, i amb la injecció de 100 µL es van obtenir recuperacions del 44-107% i límits de detecció de 3,1 i 85 ng/L respectivament (Gómez-Canela et al., 2012). Els límits de detecció són més alts que els obtinguts amb una extracció prèvia, però permet obtenir resultats molt més ràpidament. El mètode d'injecció directa és més habitual en l'anàlisi dels compostos de platí, que s'analitzen per plasma d'acoblament inductiu (ICP) (Isidori et al., 2016). En aquest cas es va analitzar la mostra prèviament acidificada i es va obtenir un límit de detecció de 1 ng/L.

1.5.2. Anàlisi

La determinació de citostàtics en aigües s'ha dut a terme mitjançant diferents tècniques analítiques, amb predomini de la cromatografia de líquids (LC) acoblada a l'espectrometria de masses (MS) enfront a la cromatografia de gasos (GC) o l'electroforesi capil·lar (CE).

Els mètodes de LC es poden classificar com a mètodes d'HPLC (cromatografia de líquids d'elevada eficàcia) o d'UHPLC (cromatografia de líquids d'ultra elevada eficàcia). Els mètodes d'HPLC per a l'anàlisi de citostàtics recollits a la bibliografia utilitzen majoritàriament columnes de fase reversa, amb base de sílica i fases enllaçades de C18 o C8. Cada casa comercial però, desenvolupa les seves pròpies columnes amb determinades modificacions en la fase estacionària per tal d'obtenir una millor separació dels analits. Les mides d'aquestes es troben entre els 50-250 mm de longitud, els 2-10 mm de diàmetre intern i una mida de partícula de 3-10 µm, i han permès la separació des d'uns pocs compostos i fins a més de 20 citostàtics (Gómez-Canela et al., 2014). En algunes aplicacions també s'han utilitzat columnes de fase normal, tipus HILIC (*hydrophilic interaction líquid chromatography*), més adequades per la separació de compostos hidrofílics. Per exemple, aquestes s'han utilitzat per la separació del 5-fluorouracil, la citarabina, la gemcitabina i alguns metabòlits (Kovalova et al., 2009) o per al 5-fluorouracil i la gemcitabina (Weissbrodt et al., 2009). Els mètodes d'UHPLC empren columnes amb una mida de partícula més petita, per sota dels 2 µm, i permeten obtenir una millor resolució en la separació dels analits en temps d'anàlisi més baixos. En els mètodes d'UHPLC desenvolupats també s'han utilitzat columnes de fase reversa per a la separació de fins a 8 citostàtics (Celle-Jeanton et al., 2014; Ferrando-Climent et al., 2013; Llewellyn et al., 2011; Martín et al., 2011; Usawanuwat et al., 2014; Yin et al., 2009).

Les fases mòbils utilitzades en ambdós modes de cromatografia líquida consisteixen en acetonitril o metanol, per a la fase orgànica, i aigua amb addició d'àcid fòrmic (Buerge et al., 2006), formiat d'amoni (Celle-Jeanton et al., 2014) o acetat d'amoni (Sacher et al., 2001)per a la fase aquosa. L'addició d'aquests reactius a les fases mòbils manté els analits en la seva forma neutra i ajuda a millorar la forma del pic cromatogràfic.

L'espectrometria de masses en tàndem (MS/MS) és el mode més utilitzat si es treballa amb LC. Gairebé en totes les publicacions s'ha treballat amb un triple quadrupol en mode MRM (*multiple reaction monitoring*), seleccionant un ió precursor i dos ions producte per a la identificació dels analits. La ionització típicament emprada és amb electrosprai (ESI) en mode positiu o negatiu en funció dels analits escollits. De manera menys generalitzada, també s'ha utilitzat l'espectrometria de masses d'alta resolució, amb equips Orbitrap. Concretament s'ha utilitzat per la determinació de ciclofosfamida i epirubicina en aigües residuals utilitzant la

injecció directa (Gómez-Canela et al., 2012) i per a un conjunt de més de 20 citostàtics prèvia extracció per SPE (Gómez-Canela et al., 2014). L'us de l'Orbitrap va donar una sensibilitat 100 vegades major que la mateixa analítica realitzada amb un triple quadrupol (Gómez-Canela et al., 2013b). Tot i no ser un mètode habitual per a la determinació d'aquests fàrmacs, també s'ha publicat un article on s'ha utilitzat un detector de fluorescència per la doxorubicina, l'epirubicina i la daunorubicina (Mahnik et al., 2006). En aquest cas en va treballar amb una longitud d'excitació de 480 nm i una longitud d'emissió de 560 nm, i és una tècnica menys sensible.

Per a la determinació de citostàtics mitjançant GC, la majoria de compostos requereixen una derivatització prèvia ja que no són volàtils. Com a agents derivatitzants se sol utilitzar l'àcid trifluoroacètic anhidre (TFAA) per la ifosfamida i la ciclofosfamida (Isidori et al., 2016) o el bromur de pentafluorobenzil (PFBBr) per al 5-fluorouracil(Mullot et al., 2009; Tauxe-Wuersch et al., 2006; Yu et al., 2006). En canvi, el tamoxifè s'ha analitzat sense derivatitzar (Tauxe-Wuersch et al., 2006). Després de la separació cromatogràfica mitjançant GC, se sol utilitzar l'espectrometria de masses amb una font d'ionització d'ionització electrònica (EI) i analitzador de quadrupol simple o de trampa d'ions.

Finalment, una altra de les tècniques analítiques que s'han utilitzat ha estat l'electroforesi capil·lar, concretament per a la determinació de 5-fluorouracil (Mahnik et al., 2004). En aquest estudi es va utilitzar un capil·lar de 56 cm i 75 µm de diàmetre, amb borat de sodi 160 mM (pH 9):ACN (80:20) com a electròlit de separació. La detecció es va fer mitjançant un detector de feix de díodes (DAD), i es va obtenir un MDL de 1.700 ng/L, més alt que per espectrometria de masses.

Taula 1.3. Mètodes d'extracció i anàlisi

			Extracció				Anàlisi				Paràmetres de qualitat	
Ref.	**Analits****	**Matriu**	**Volum de mostra**	**Pre.***	**Cartutx**	**Elució**		**Columna**	**Fase mòbil**	**Ionització**	**LOD (ng/L)**	**% ****
Buerge 2006	CIC, IFO	EDAR; super-ficial	1 L	--	Bio-Beads SM-2, 20-50 mesh,	5+10+5 mL DCM	LC	XTerra RP18 (50x2,1 mm, 3,5 µm)	H_2O (0,1% HCOOH): MeOH (0,1% HCOOH)	ESI (+)	LOD: 0,02-2	74-102
Castiglioni 2005	MET (+ altres)[X]	efluent EDAR	500 mL	filtrar, pH 2	Oasis MCX	2mL MeOH + 2mL 2% amoni en MeOH + 2 mL 0,2% NaOH en MeOH	LC	Luna C8 (50 × 2mm, 3 µm)	H_2O (0,1% HCOOH): ACN	ESI (+/-)	LOQ: 1,9	76
	CIC	efluent EDAR	500 mL	filtrar, pH 7	Lichrolut EN	3 mL MeOH + 3 mL acetat d'etil	LC	Luna C8 (50 × 2mm, 3 µm)	H_2O (0,1% HCOOH): ACN		LOQ: 0,83	106
Celle-Jeanton 2014	IFO (+ altres)[X]	riu	1 L	--	Oasis HLB	0,5 mL MeOH:ACN (70:30) (x3)	LC	C18 Acquity HSS T3 (100 × 2,1 mm, 1.8 µm)	H_2O (5 mM formiat d'amoni+ 0,1% HCOOH): MeOH (5 mM formiat d'amoni + 0,1% HCOOH)	ESI (+)	LOQ: 1	117
Coetsier 2009	IFO, TAM (+ altres)[X]	efluent EDAR	500 mL	filtrar, pH 2	Strata X	5 mL acetat d'etil + 5 mL acetat d'etil:acetona (50:50) + 5 mL acetat d'etil:acetona: NH_4OH (49:49:2)	LC	Ascentis C18 (50 ×2,1 mm, 3 µm)	H_2O (0,1% HCOOH):ACN	ESI	LOD: 2,8-5,8	71-94

			Extracció				Anàlisi				Paràmetres de qualitat	
Ref.	**Analits****	**Matriu**	**Volum de mostra**	**Pre.***	**Cartutx**	**Elució**		**Columna**	**Fase mòbil**	**Ionització**	**LOD (ng/L)**	**% ****
Ferrando-Climent 2013	CIC, IFO, DOC, PAC, ETO, VINC, TAM, MET, AZA	hospital; influent EDAR	50 mL	filtrar, 0,1 M EDTA	Oasis HLB	5 mL MeOH (x2)	LC	Acquity HSS T3 (50 ×2,1 mm, 1,7 µm)	H_2O (0,1 % HCOOH): ACN	ESI (+)	MDL: 0,8-24	46-115
Garcia-Ac 2009	CIC, MET (+altres)[X]	EDAR; riu	50 mL	filtrar, pH 2,8	Online Strata X	fase mòbil	LC	Synergi Max RP-C12 (75 x 2,0 mm, 4 µm)	H_2O (0,2% HCOOH):ACN	ESI (+)	LOD: 3-16	55-148
Garcia-Ac 2009	CIC, MET (+altres)[X]	riu; potable	10 mL	filtrar, acidificar	Online Strata X	fase mòbil	LC	Synergi Fusion RP (50 × 2 mm, 4 µm)	H_2O (0,1% HCOOH): MeOH (0,1% HCOOH): ACN(0,1% HCOOH)	ESI (+)	MDL: 1-2	60-109
Gomez-Canela 2012	CIC, EPI	EDAR	100 µL	centrifugar	(injecció directa)	--	LC	LiChroCART 55×2 mm Purospher STAR RP-18 endcapped, 3 µm	H_2O (0,1%HCOOH): MeOH (0,1%HCOOH)	ESI (+)	LOD: 3,1-85	44-107

			Extracció				Anàlisi				Paràmetres de qualitat	
Ref.	**Analits****	**Matriu**	**Volum de mostra**	**Pre.***	**Cartutx**	**Elució**		**Columna**	**Fase mòbil**	**Ionització**	**LOD (ng/L)**	**% ****
Gomez-Canela 2014	CIC, CHL, MEL, IFO, FLU, CIT, GEM, CAP, VINB, VINC, ETO, PAC, DOC, DOX, DAU, EPI, IMA, ERL, IRI, LEU, GOS, TAM, AMI, MEG, CYP, PRE	hopital; EDAR	100 mL	centrigufar, filtrar, pH2	Oasis HLB	6mL MeOH + 6mL MeOH:HCOOH (95:5)	LC	Luna C18 (150 ×2 mm, 5 µm)	H_2O (0,1%HCOOH): ACN (0,1%HCOOH)	ESI (+)	MDL: 0,7-356	40-133
Isidori 2016	CAP, DOX, ERL, ETO, GEM IMA, IRI, MET, PAC, TAM, TMZ (+ metabolits)	hospital ; EDAR	5 mL	filtrar, pH 2	Online PLRP-s	fase mobil	LC	Purospher STAR RP-18e (125 × 2 mm, 5 µm)	H_2O (0,1%HCOOH): MeOH (0,1%HCOOH)	ESI(+)	LOD: 0,4-54	
	IFO, CIC	hospital ; EDAR	100 mL	Filtrar	Oasis HLB	5% acid acètic en acetat d'etil	GC			EI	LOD: 2,3-4,8	
	5-FU	hospital ; EDAR	100 mL	Filtrar	Isolute ENV+	2 mL MeOH (x3)	GC			EI	LOD: 0,5	
	Pt	hospital ; EDAR	10 mL	filtrar, acidificar	--	--	ICP	--	--	(MS)	LOD: 1,0	
Kosjek 2013	5-FU	hospital ; EDAR	100 mL	Filtrar	Isolute ENV+	2 mL MeOH (x3)	GC	DB-5 MS 30 m × 0,25 mm × 0,25 m		EI	LOD: 0,16-0,48	53-93
Kovalova 2009	5-FU, CIT, GEM, + metabolits	hospital	50 mL	filtrar, pH 11,2	Isolute ENV+/Speedisk H_2O -philic SA-DVB	MeOH + 3mL MeOH: HCOOH (98:2)	LC	ZIC-HILIC (150 × 2,1 mm)	30mM acetat d'amoni i ACN (2:3) : ACN	ESI (+/-)	LOQ: 0,9-5	46-79

			Extracció				Anàlisi				Paràmetres de qualitat	
Ref.	**Analits****	**Matriu**	**Volum de mostra**	**Pre.***	**Cartutx**	**Elució**		**Columna**	**Fase mòbil**	**Ionització**	**LOD (ng/L)**	**% ****
Mullot 2009	5-FU	hospital	100 mL	filtrar, pH 4	Isolute ENV+	5 mL MeOH	GC	Factor Four 5 (30 m × 0,25 mm, 0,25 mm)			LOD: 12	95-101
Nebot 2007	TAM (+altres) [X]	efluent EDAR; riu; potable	2 L	Filtrar	Strata X	5 mL acetona + 5 mL MeOH (x2)	LC	Luna C18 (250 ×10 mm, 10 µm)	H2O: MeOH: 10 mM acetat d'amoni: 0,87 M HCOOH	ESI	LOD: 0,03	15-29
Negreira 2013	GEM, TMZ, MET, IRI, IMA, IFO, CIC, ERL, ETO, DOX, CAP, TAM, PAC (+metabolits)	EDAR; riu; sub-terrània	5 mL	filtrar, pH 2	Online PLRP-s	fase mòbil	LC	Purospher STAR RP-18e (125 × 2 mm, 5 µm)	H2O (0,1% HCOOH): MeOH (0,1% HCOOH)	ESI (+)	LOD: 0,2-1,6	72-119
Sacher 2001	CIC, IFO (+ altres) [X]	Sub-terrània	1 L	pH 7	PPL Bond-Elut	5 mL MeOH	LC	Nucleosil 120-3-C18 (250 x 2 mm, 3 µm)	20 mM acetat d'amoni en H2O (pH 6.8):20 mM acetat d'amoni en ACN/MeOH (2:1)	ESI (+)	LOD: 4,2-10	71-102
Tauxe-Wuersch 2006	TAM	hospital ; EDAR	1 L		LLE amb DCM + Oasis MCX	3mL MeOH:NH_4OH (95:5)	GC	RTX-5 (60 m x 0,25mm; 0,25 mm)		EI	LOD: 1	81
	5-FU	hospital ; EDAR	150 mL	pH 5	Isolute ENV+	3mL MeOH (x4)	GC	RTX-5 (60 m x 0,25mm; 0,25 mm)		NCI	LOD: 15	73
Usawanu-wat 2014	5-FU, CIC, HID	efluent EDAR; riu	2,5 L	Filtrar	Oasis HLB	1mL 0,1% HCOOH en MeOH/H_2O (50:50)+ 1mL 5% NH_4OH	LC	Zorbax Eclipse Plus XDB C18 (100x2,1 mm, 1,8 µm)	H_2O (0,1% HCOOH): MeOH (0,1% HCOOH)	ESI (+/-)	LOD: 6-50	77-108

			Extracció				Anàlisi				Paràmetres de qualitat	
Ref.	**Analits****	**Matriu**	**Volum de mostra**	**Pre.***	**Cartutx**	**Elució**		**Columna**	**Fase mòbil**	**Ionització**	**LOD (ng/L)**	**% ****
Llewellyn 2011	CIC, IFO	efluent EDAR	500 mL	Filtrar	Strata X +Florisil	MeOH amb acetat d'etil	LC	Hypersil GOLD C18 (50 × 2,1 mm, 1,9 µm)	H2O (0,1%HCOOH): MeOH (0,1%HCOOH)	ESI (+)	LOD: 0,03-0,12	57-70
Mahnik 2004	5-FU	hospital	50 mL	Centrifudar, filtrar	Isolute ENV+	8 mL MeOH (pH 9)	CE	Capil·lar 56cm, 75 µm	160 mM borat de sodi (pH 9,5) : ACN (80:20)	DAD	MDL: 1700	79-96
Mahnik 2006	DOX, EPI, DAU	hospital	10 mL	centrifugar, filtrar, pH 7,5	C8	1,5 ml MeOH/CHCl3 (1:2).	LC	Hypersil ODS (C18, 200 x 2,1 mm, 5µm)	10 mM buffer dihidrogenfosfat, pH2: ACN	Fluorescencia	LOD: 50-60	79,8-90,8
Martin et al 2011	5-FU, GEM CIC, IFO, CIT, MET, PAC, ETO, IRI, DOC, EPI, DOX, VINO, MIT	EDAR; riu	250 mL	pH 7	Oasis HLB	1 mL MeOH (x4)	LC	Zorbax Eclipse XDBC18 Rapid Resolution HT (50x4,6 mm; 1,8 µm)	15mM formiat d'amoni (0,1% HCOOH): ACN (0,1% HCOOH)	ESI (+/-)	MDL: 0,1-38	75-105
Martin 2014	CIT, CIC, DOC, DOX, EPI, ETO, 5-FU, GEM, IFO, IRI, MET, MITO, PAC, VINO	EDAR	250 mL	pH 7	Oasis HLB	1 mL MeOH (x4)	LC	Zorbax Eclipse XDB-C18 Rapid Resolution HT (50x4.6 mm; 1,8 µm)	15mM formiat d'amoni (0,1% HCOOH): ACN (0,1% HCOOH)	ESI (+/-)	LOD: 0,08-5,18	11-105
Martinez-Bueno 2010	CIC, IFO (+ altres) [X]	riu	400 mL	pH 8	Oasis HLB	4 mL MeOH (x2)	LC	C18 ZORBAX SB,(250x 3,0 mm, 5 µm)	H2O (0,1% HCOOH): ACN (0,1% HCOOH)	ESI (+/-)	MDL: 0,4-1	81-84
Metcalfe 2003	CIC (+ altres) [X]	riu	2 L	Filtrar, pH 7,5	Oasis HLB	3 mL MeOH (x3)	LC	Genesis C18 (150 x 2,1 mm, 4 µm)	20 mM acetat d'amoni (0.1% HCOOH): ACN	ESI	LOD: 1-20	nd

			Extracció				Anàlisi				Paràmetres de qualitat	
Ref.	**Analits****	**Matriu**	**Volum de mostra**	**Pre.***	**Cartutx**	**Elució**		**Columna**	**Fase mòbil**	**Ionització**	**LOD (ng/L)**	**% ****
Weissbrodt 2009	5-FU, GEM (+ altres)[X]	hospital	50 mL		IsoluteENV+ i SA-DVB	MeOH + 3mL MeOH:HCOOH (98:2)	LC	ZIC-HILIC (150 x 2,1 mm, 4,5 µm)	H_2O (1% ACN + 0,5% HCOOH + 0,1% formiat d'amoni 5M): ACN	ESI (+/-)	LOQ: 0,9-5	46-79
Yin 2010	MET, AZA, DOX, VINC, CIC, IFO, ETO, PRO	hospital	100 mL	filtrar, pH 2	Oasis HLB	6 mL MeOH:H_2O (80:20)	LC	ACQUITY UPLC TM BEH C18 (2.1 x 150 mm, 1,7 µm)	H_2O (0,1% HCOOH): ACN	ESI (+)	LOD: 2-20	51-105%
Yu 2006	5-FU (+ altres)[X]	EDAR	250/ 500 mL	filtrar, pH 2	Strata X	7 mL ACN	GC	DB-5MS (30m x 0,25mm; 0,25 µm)		EI	nd	nd
Zuccato 2000	CIC (+ altres)[X]	riu; potable	1 L		Lichrolut EN + Oasis MCX	acetat d'etil+MeOH/5% NH_4OH en MeOH		Inertsil ODS-2	nd	Nd	LOD: 0,02	>70%

* Pretractament ; ** % de recuperació; [X]També s'inclouen al mètode altres fàrmacs (no citostàtics); LC: cromatografia de líquids; GC: cromatografia de gasos; CE: electroforesi capil·lar; ESI: electroesprai; EI: impacte electrònic; NCI: ionització química negativa; DAD: detector de feix de díodes; nd: no s'especifica.

**AMI: aminoglutetimida, AZA: azatioprina, CAP: capecitabina, CHL: clorambucil, CIC: ciclofosfamida, CIT: citarabina,CYP: ciproterona , DAU: daunorubicina, DOC: docetaxel, DOX: doxorubicina, EPI: epirubicina, ERL: erlotinib, ETO: etopòsid, 5-FU: 5-fluorouracil, FLU: fludarabina, GEM: gemcitabina, GOS: goserelin, HID: hidroxicarbamida, IFO: ifosfamida, IMA: imatinib, IRI: irinotecà, LEU: leuprolida, MEG: megestrol, MEL: melfalan, MET: metotrexat, MIT: mitoxantrona, TAM: tamoxifè, TMZ: temozolomida, PAC: paclitaxel, PRE: prednisona, PRO: procarbazina, Pt: compostos de platí, VINB: vinblastina, VINC: vincristina, VINO: vinorelbina.

1.6. Consideracions de seguretat

Alguns fàrmacs citostàtics s'han qualificat com a mutàgens i carcinògens i per tant cal tenir-ho en compte a l'hora de manipular aquests compostos i les seves solucions i no posar en perill la salut del treballador.

Les guies de manipulació d'agents citostàtics estan dirigides a personal que prepara aquesta medicació o pot estar-hi en contacte al llarg de tota la jornada laboral. En aquests documents es descriu que la preparació dels productes s'ha de fer en cabines de seguretat biològica i que la persona que els manipula ha de dur guants, bata, mascareta, ulleres de seguretat i gorra d'un sol ús (MSSSI, 2013). També s'especifica com s'han de col·locar els guants i que aquests s'han de canviar cada mitja hora si es treballa contínuament amb citostàtics. Les preparacions injectables que s'utilitzen en els tractaments oncològics, d'acord amb el llistat de fàrmacs proporcionat per CatSalut, tenen una concentració que varia des dels 2.000 mg/L fins als 50.000 mg/L, i els comprimits contenen entre 5 i 500 mg de principi actiu. Per tant, com que les concentracions que es manipulen en ambients mèdics són molt elevades, les mesures de seguretat han de ser extremes.

Per contra, en un laboratori de recerca les quantitats de producte manipulades, així com la concentració de les solucions preparades són molt menors. En la preparació dels patrons de treball s'utilitzen uns pocs mg de principi actiu on ràpidament s'addiciona el dissolvent per obtenir solucions d'uns 1.000 mg/L. A partir d'aquestes, es preparen les solucions de treballa 10 mg/L, que s'utilitzen més habitualment per fortificar les mostres operpreparar les rectes de calibratge dels instruments d'anàlisi. Per altra banda, els citostàtics són molt poc volàtils i per tant el perill per inhalació a aquestes concentracions de treball és molt reduït. En aquest cas, les normes generals de seguretat al laboratori són suficients per treballar amb aquests productes: cal dur guants i bata, i s'ha de treballar dins la vitrina de flux laminar.

La seguretat és important a l'hora de treballar amb aquests tipus de fàrmacs, però també al manipular els dissolvents típics del treball al laboratori. Per això cal seguir les normes de seguretat i higiene bàsiques, destinades a mantenir un entorn segur per a la salut.

2. PRIORITZACIÓ DE FÀRMACS CITOSTÀTICS EN AIGUA RESIDUAL I SUPERFICIAL

2.1. Introducció

Com s'ha vist a l'apartat 1.1, el nombre de fàrmacs que s'utilitzen en els tractaments contra el càncer és elevat, com també el seu consum a Europa, degut a l'elevada incidència de càncer en la població. A Catalunya i Espanya el consum és de l'ordre de tones/any i s'utilitzen més de cent compostos. A l'hora d'avaluar la seva presència en el medi, no es poden monitoritzar tots ells ja que és inviable des del punt de vista analític i de costos associats. Per tant, és necessari trobar altres mètodes que permetin prioritzar aquells fàrmacs més importants per tal d'optimitzar temps i recursos. Alguns dels sistemes de priorització inclouen l'ús de models informàtics que combinen la informació geogràfica amb la informació ambiental, com el *GREAT-ER* o el *Low Flows 2000*(Johnson et al., 2008; Rowney et al., 2009), però que requereixen mapes de la zona objecte d'estudi o, en el seu defecte, coneixements de programació. Un mètode alternatiu i més accessible és el càlcul de les concentracions previstes en el medi (PEC, de *predicted environmental concentration*) proposat en la guia de la EMA (2006). Aquest document té com a objectiu avaluar els possibles riscos ambientals que comporten els medicaments d'ús humà, informació necessària per a l'autorització d'un nou producte al mercat. Per això proposa, en primer lloc, calcular l'exposició del medi a aquests fàrmacs mitjançant el càlcul dels PECs.Aquest sistema consisteix en calcular amb una simple fórmula la concentració que s'espera trobar en el medi a partir del consumd'un compost determinat, la fracció excretada i llur eliminació en depuradores. L'equació proposada per la EMA va ser adaptada i simplificada per Besse i Garric (2008), i és la següent:

$$PEC\ (mg/L) = \frac{consum \cdot\ Fexc \cdot\ Fstp}{WW \cdot\ \ hab \cdot\ DF \cdot 365} \qquad (2.1)$$

on el *consum* (mg/any) és la quantitat de principi actiu administrada d'un determinat fàrmac; *Fexc* és la fracció excretada; *Fstp* és la fracció emesa per l'efluent de les depuradores; *WW* (L/hab/dia) és el consum d'aigua per habitant; *hab* és el nombre d'habitants; *DF* és el factor de dilució des de la depuradora al riu i els 356 dies/any permet fer el canvi d'unitats. Segons si s'aplica o no el factor de dilució, es poden obtenir dos valors de PEC diferents: PEC_{wwtp}, que correspon a la concentració prevista en l'efluent de depuradora i un PEC_{riu}, amb el factor de dilució, que correspon a la concentració prevista en aigües superficials.

Per motius pràctics a l'hora de treballar amb l'equació, en el si d'aquesta tesi s'han modificat les unitats d'algun paràmetre i s'ha treballat amb la fórmula següent:

$$PEC\ (ng/L) = \frac{consum \cdot\ Fexc \cdot\ (1 - Fwwtp)}{WW \cdot\ \ hab \cdot\ DF} 10^9 \quad (2.2)$$

on el consum es troba en g/dia, *Fwwtp* és la fracció eliminada en les depuradores i s'aplica un factor de 10^9 per al canvi d'unitats, per tal d'expressar el valor de PEC en ng/L.

Amb algunes variacions en la fórmula, aquest sistema s'ha utilitzat per prioritzar diferents tipus de fàrmacs, incloent analgèsics, antibiòtics i hormones, entre altres famílies (Al-Khazrajy and Boxall, 2016; Mansour et al., 2016; Perazzolo et al., 2010). En el cas dels citostàtics és un mètode especialment adequat ja que tot el que s'administra als hospitals o es ven a les farmàcies serà consumit pel pacient i per tant, permet utilitzar les dades d' administració o venda, més fàcils d'obtenir, com a sinònim de consum. Sovint, els estudis de PECs se centren majoritàriament en alguns citostàticspre-seleccionats individualment(Gómez-Canela et al., 2014; Tauxe-Wuersch et al., 2006), i només alguns autors recullen els consum de tot un grup (L01, L02...) i en calculen els PECs amb l'objectiu de seleccionar i prioritzar els més importants (Booker et al., 2014; Kümmerer et al., 2016). Tot i haver-hi diversos treballs dedicats a l'estudi de PECs, els mètodes per obtenir les dades crues o els criteris per interpretar l'equació són lleugerament diferents. Per tant, de cara a una millor priorització és preferible conèixer el consum de tots els fàrmacs antineoplàstics administrats en la regió d'estudi i tenir en compte com poden afectar els paràmetres més influents de l'equació pel càlcul dels PECs: Fexc, Fwwtp i DF.

En el marc d'aquesta tesi, els consums que s'apliquen a l'equació s'han obtingut gràcies a les dades facilitades per organismes públics. En el primer estudi les dades es van sol·licitar a CatSalut, l'entitat que s'encarrega de gestionar l'atenció sanitària de Catalunya i manté un registre de tots els fàrmacs administrats en els hospitals i de les receptes emeses, sempre dins de l'àmbit públic. A nivell espanyol, el Ministeri de Sanitat, Serveis Socials i Igualtat és qui manté el registre de fàrmacs. En aquest cas només es comptabilitza l'administració farmacèutica ja que el control de la dispensació hospitalària encara no està centralitzat. Si l'obtenció directa d'aquestes dades no fos possible, també es poden estimar els consums mitjançant les dosis diàries definides per cada compost conjuntament amb la incidència de càncer per una població determinada (Ortiz de García et al., 2013). No obstant, les noves teràpies que s'administren en aquest camp van cada cop més dirigides a les necessitats específiques de cada pacient i per tant, una estimació dels consums en funció de la incidència general i l'ús de cada fàrmac pot ser menys acurada.

Els factors d'excreció i eliminació en depuradores (Fexc i Fwwtp respectivament) varien en un ampli marge, com s'ha comentat en l'apartat 1.3 de la introducció, fent que pugui variar també el resultat de PEC. A l'hora d'aplicar aquest valor en l'equació cal decidir quin serà més

adequat: un valor concret, la mitjana dels valors reportats a la bibliografia, etc. Un criteri sovint utilitzat ha estat escollir les condicions que suposarien una situació més desfavorable per al medi ambient ("*worst case scenario"*), és a dir, els valors d'excreció més alts i les eliminacions en depuradora més baixes. Així, el valor de PEC obtingut serà el més alt possible. Tanmateix, alguns autors han optat per no tenir en compte aquests factors, en funció de les dades disponibles. En general, s'apliquen ambdós factors (Coetsier et al., 2009) però si no hi ha valors suficients pels compostos que es vol estudiar, el factor d'eliminació o degradació pot no aplicar-se. Besse et al. (2012) calculen els PECs de tots els fàrmacs del grup L01 i L02 administrats a França però consideren que no hi ha eliminació en les depuradores ja que no hi ha prou dades publicades per molts dels fàrmacs llistats (*Fwwtp*=0). De manera similar, Ashton et al. (2004) donen valors de PEC per diferents famílies de fàrmacs administrats a Anglaterra sense tenir en compte el percentatge excretat (Fexc=1). Per tant, especificar quins factors s'han utilitzat és important per fer un càlcul adequat dels PECs i poder-los comparar amb la bibliografia.

El factor de dilució (DF) és també un altre paràmetre que pot donar molta variabilitat en el resultat. La EMA proposa un factor de 10per defecte, tot i que la ECHA (*European Chemicals Agency*) reconeix que aquest valor podria variar entre 1 (rius secs en època estival) i 1000 (ECHA, 2003). Degut a aquesta alta variabilitat, que pot arribar a ser més important que altres variacions en el comportament dels analits, Keller et al. (2014) van calcular el factor de dilució per a cada país del món. Els autors utilitzen un model que divideix la superfície terrestre en fraccions de 0,5ºx0,5º (equivalent a uns 55x55 Km a l'equador) i tenen en compte el cabal dels rius, el consum d'aigua i la població, per obtenir un DF mitjà de cada país. Els resultats obtinguts varien entre 0,0050 a Qatar i 94.463 al Surinam, i per Espanya donen un valor de 25,92, més gran que el valor per defecte de la EMA. L'ús d'aquest nou valor pot donar uns PECs i una avaluació del risc més acurats. Tot i així, dins un mateix país les variacions del cabal del riu poden ser també molt àmplies, especialment a les zones mediterrànies on el cabal i la freqüència de precipitacions són molt irregulars. Per tant, el càlcul del DF per una àrea més reduïda permetria ajustar els PECs a la conca hidrogràfica d'un determinat riu i veure quines són les zones més impactades.

La senzillesa de l'equació permet adaptar-la a les dades disponibles i a la informació que es vol obtenir (PEC_{wwtp} o PEC_{riu}) i si les dades utilitzades estan ben documentades, permet la comparació entre els valors publicats en els diferents estudis. Si se segueix un criteri uniforme,el càlcul de PECs permet prioritzar els fàrmacs que tenen majors consums i menor percentatge d'eliminació en les plantes de tractament i permet fer-ne una selecció d'aquells amb més probabilitat de ser detectats al medi ambient.

2.2. Resultats

En el marc d'aquesta tesi, l'estudi del consum i el destí del fàrmacs citostàtics a Catalunya i Espanya ha donat lloc a dues publicacions científiques. L'article científic I, titulat "*Predicting concentrations of cytostatic drugs in sewage effluents and surface waters of Catalonia (NE Spain)"* publicat a la revista*Environmental Research*138:161-172(2015) recull el consum de fàrmacs administrats en els tractaments contra el càncer a Catalunya, en farmàcies i hospitals, i en calcula el seu PEC. Les dades recollides en el període 2010-2012 s'han comparat amb els consums a nivell europeu. Amb els valors de PEC s'ha determinat quins són els citostàtics que es preveu trobar a concentracions més altes en els rius de Catalunya i s'han comparat els valors predits amb les dades dels valors mesurats (MECs) publicades a la bibliografia. Finalment, en funció dels PECs obtinguts s'ha fet un estudi de risc per determinar si aquests fàrmacs poden ser tòxics per als organismes aquàtics.

En l'article científic II, titulat "*Anticancer drugs: consumption trends in Spain, prediction of environmental concentrations and potential risks." Environmental Pollution (2017)* (acceptat), s'han recollit les dades de consum farmacèutic a nivell estatal (2010-2015) i s'han calculat els seus PECs. En aquest cas, degut que els cabals dels rius més importants de la península Ibèrica són molt variables, s'ha calculat el factor de dilució per a cadascuna de les principals conques hidrogràfiques. Amb aquest nou factor, s'han recalculat els PECs corresponents a cada conca, per tal d'obtenir uns valors més acurats. Finalment s'ha dut a terme una avaluació del risc per als organismes aquàtics, a nivell agut i crònic.

2.1.1. Article científic I:

*Predicting concentrations of cytostatic drugs in sewage effluents and surface waters of Catalonia (NE Spain).*Helena Franquet-Griell, Cristian Gómez-Canela , Francesc Ventura,Silvia Lacorte. Environmental Research (2015) 138: 161-172.

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Predicting concentrations of cytostatic drugs in sewage effluents and surface waters of Catalonia (NE Spain)

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ABSTRACT

Cytostatic drugs, used in chemotherapy, are excreted unchanged by urine and feces or modified as metabolites. Elimination of these drugs in wastewater treatment plants (WWTPs) is often incomplete and residues reach surface water. Their presence in the natural environment depends on consumption patterns, excretion fraction and the effectiveness of the wastewater treatment. This study compiled the total consumption of cytostatic drugs in Catalonia (NE Spain) and provides data on the occurrence and risk of anticancer drugs in the aquatic environment by calculating predicted environmental concentrations (PECs). PECs were estimated using publicly available consumption data in the period of 2010–2012, published or calculated excretion values and wastewater elimination rates for a suite of 132 compounds. This allows predicting the range of concentrations in effluent wastewaters and receiving waters. Out of the 132 cytostatics, mycophenolic acid and hydroxycarbamide had a PEC value higher than 10 ng L^{-1}. PECs were compared with MECs (measured environmental concentrations) to evaluate the reliability of the estimation. A risk assessment was conducted to determine the potential adverse effects of cytostatics in the environment. All the risk quotients calculated using EC_{50} in *Daphnia magna* were below 1, showing no significant risk.

1. Introduction

Since the 1970s, the occurrence, fate and risk of pharmaceuticals in the aquatic environment have been studied (Daughton and Ternes, 1999; Heberer, 2002; Kümmerer, 2001; Ternes, 1998). Pharmaceuticals are released to wastewaters after excretion and due to incomplete removal in WWTPs (Castiglioni et al., 2005; Gros et al., 2010; Heberer, 2002; Ternes, 1998) they are discharged to receiving streams affecting water quality. Thus, traces of pharmaceuticals have been detected in hospital effluents, influent/effluent wastewaters and in surface and groundwaters (Daughton, 2010; Hughes et al., 2013; Jones et al., 2005; Lapworth et al., 2012; Loos et al., 2010; Mompelat et al., 2009; Monteiro and Boxall, 2010; Verlicchi et al., 2012; Zhang et al., 2013).

Concern on the presence of cytostatics compounds has increased in recent years, as cancer incidence in the population is gradually increasing. In 2008, it caused 7.6 million deaths worldwide (around 13% of the total), of these, 56% of the cases and 64% of the deaths occurred in the economically developing world (World Health Organization-WHO, 2013). Cancer is treated with cytostatic drugs, also called anticancer or antineoplastic drugs, which are a broad group of chemotherapy compounds with different action modes. These drugs are classified in the Anatomical Therapeutic Chemical (ATC) by the WHO (www.whocc.no/atcddd) under class L which belongs to antineoplastic and immunomodulating agents. Four main groups are currently used: antineoplastic agents (L01), endocrine therapy (L02), immunostimulants (L03) and immunosuppressants (L04). The L01 group is subdivided into alkylating agents (L01A); antimetabolites (L01B); plant alkaloids and natural products (L01C); cytotoxic antibiotics and related substances (L01D) or other antineoplastic agents (L01X). The subgroups L02A and L02B are referred to hormone and hormone antagonists, respectively, whereas L03 and L04 comprise L03A and L04A subgroups. Although, they are not antineoplastic drugs, the pharmaceuticals cyproterone (G03HA01) and megestrol acetate (H02AB07), a sex hormone and corticosteroid, respectively, are widely used in cancer treatments.

Like other pharmaceuticals, many cytostatic drugs can be excreted unchanged by urine and feces and be directly discharged into the sewer system from hospitals and from household discharge from outpatients. Many cytostatic compounds have low biodegradability and it can be assumed that biological degradation in surface waters will be negligible (Kosjek and Heath, 2011; Negreira et al., 2014).

The predicted environmental concentrations (PECs) are used to calculate the amounts of drugs expected to be discharged into the

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0013-9351/

environment, and they play an important role in risk assessment. In 2006, the European Medicines Agency (EMEA) developed a model to estimate PECs of pharmaceuticals in water and proposed that a PEC value > 10 ng L^{-1} for an individual drug should be a trigger threshold for further environmental risk assessment (EMEA, 2006). This model or equivalent has been extensively used with or without refinements to estimate PECs of pharmaceuticals (Carballa et al., 2008; Coetsier et al., 2009; Escher et al., 2011; Kugathas et al., 2012; Le Corre et al., 2012; Oosterhuis et al., 2013) or specifically cytostatics in surface or wastewaters (Besse et al., 2012; Booker et al., 2014; Gómez-Canela et al., 2014; Johnson et al., 2008a, 2013; Kümmerer and Al-Ahmad, 2010; Martín et al., 2014; Rowney et al., 2009). As stated by Zhang et al. (2013), PECs only provide a rough insight of the overall contamination at national or regional scale, not accounting for local specificities and contamination hot spots (Zhang et al., 2013), therefore their accuracy depends strongly on the precision of the model used (Carballa et al., 2008). The reliability of the PEC is a key point for the assessment of environmental risks for aquatic organisms and in this context the ratio PEC/MEC (measured environmental concentrations) is a good approach to estimate the relevance of PEC values with an acceptable consistency for the ratio 0.2–4 range (Coetsier et al., 2009).

Catalonia (NE Spain) with a population of 7,570,908 (Idescat, 2012), registered 33,715 cases of cancer in 2007. The aim of this study was to provide data on the occurrence and risk of anticancer drugs in the aquatic environment by calculating PECs based on the consumption of all cytostatic compounds used in Catalonia in the period of 2010–2012. PECs for 132 compounds were estimated using publicly available consumption data, published or calculated excretion values and wastewater elimination rates. It allows predicting the range of concentrations in effluent wastewaters and surface waters, and prioritization of compounds with the highest environmental concern.

2. Material and methods

2.1. PEC calculation

The Catalan Health Service (CatSalut) kindly provided the data consumption for all antineoplastic compounds consumed over the period 2010–2012 in pharmacies and hospitals. A total of 132 cytostatic drugs were consumed along these years. The hospital data was given as the number of pills, capsules or any formulation of an active molecule dispensed (called activities). Knowing the concentration of each activity, the total consumption in g yr^{-1} was calculated. Table SI1 (Supporting information) shows the amounts of cytostatic drugs (in grams) consumed in the period 2010–2012. About 80 different cytostatic drugs were consumed in hospitals. On the other hand, pharmaceutical data consumption was directly provided in mg yr^{-1} by the "Pharmaceutical and Medication Management" of CatSalut. Around 70 different cytostatic drugs were dispensed each year (Table SI1).

To estimate the amounts of cytostatic drugs present in wastewater effluents and surface waters in Catalonia, PECs were calculated in ng L^{-1} as described by Besse et al. (2012) using the following equation:

$$\mathrm{PECs(ng\ L^{-1})} = \frac{\text{consumption} \times \text{Fexc} \times (1 - \text{Fwwtp})}{\text{WW inhab} \times \text{inhab} \times \text{dilution}} 10^9 \quad (1)$$

Consumption (g d^{-1}) is the quantity of an active pharmaceutical ingredient (API) consumed in a defined zone; *Fexc* is the excreted fraction of the unchanged API; *Fwwtp* is the removal fraction in WWTP. Then, *1-Fwwtp*, is the fraction of pharmaceutical's emission from WWTPs to surface waters; *WWinhab* (L $inhab^{-1}$ d^{-1}) is the water consumption per person per day (about 130.9 L $inhab^{-1}$ d^{-1} in Catalonia); *Inhab* is the number of inhabitants of the studied area (Catalonia) and *dilution* is the factor used from WWTP effluents to surface waters. The values of the parameters in the equation were selected using the following criteria:

- Utilization of median dilution factor for Spain. The dilution factor has usually a default value of 10. However, in this study a value of 25.92 was used, based on values from Keller et al. who estimated dilution factors around the world using geographically reference data sets at $0.5^\circ \times 0.5^\circ$ resolution. The value used in the equation is the median predicted for Spain (Keller et al., 2014).
- Selection of highest excretion value of a drug. The primary route of excretion of parent pharmaceuticals is via urine/feces and ranges from negligible to $>90\%$, depending on the compound. Excretion also depends on several factors, like patient age, health and co-medication (Johnson et al., 2013) so, it is difficult to consider an unique excretion value. In cases where a range of values were found for the same compound, the maximum percentage of elimination was considered to calculate PECs to simulate worst case scenario. On the contrary, there are 35% of compounds that have not been studied and no date are available. For these cases a default value (0.5) was used.
- Selection of the lowest WWTP removal value. Removal rates are influenced by the different treatment types of WWTPs. In the cases that different removal ratios were found, the lowest value was used as the worst case scenario. Similar to excretion behavior, there is a lack of experimental data for most of the studied compounds. In these cases, a theoretical model was used (EPISuite 4.1) (U.S. EPA, 2013). This model attempts to predict the environmental fate of organic compounds, considering elimination processes like biodegradation, evaporation or sorption to sludge, among others. It considers a conventional WWTP that uses activated sludge as secondary treatment which is also the most common type of treatment (Ortiz de García et al., 2013). If no data was described or could not be calculated, 0% elimination was considered (30% of compounds).

2.2. Estimation of risks

The risk quotient (RQ), also called hazard quotient (HQ), is used to express the risk posed by a particular chemical to the environment or a particular organism. It is calculated combining the results of the exposure assessment (PEC) with the results of the effects assessment (predicted no effect concentration or PNECs) (van Leeuwen and Hermens, 1995). RQ was calculated according to:

$$\mathrm{RQ} = \frac{\mathrm{PEC}}{\mathrm{PNEC}} = \frac{\mathrm{PEC}}{\mathrm{EC_{50}}/f} \quad (2)$$

where, PEC is the predicted environmental concentration and PNEC is the concentration below which an unacceptable effect will most likely not occur. PNEC can also be estimated as the quotient of the toxicological relevant concentration (EC_{50} or LC_{50}) and a security factor ($f = 1000$). The use of a factor 1000 on short-term toxicity data is a conservative/protective factor and is designed to ensure that substances with the potential to cause adverse effects are identified in the effects assessment. For data interpretation, the maximum probable risk for ecological effects from contaminated water was followed (Marcus et al., 2010):

RQ < 1.0 indicates no significant risk;

$1.0 \leq$ RQ < 10 indicates a small potential for adverse effects;

$10 \leq RQ < 100$ indicates significant potential for adverse effects;

$RQ \geq 100$ indicates that potential adverse effects should be expected.

3. Results and discussion

3.1. Global consumption of cytostatic drugs along ATC codes in Catalonia

According to the data provided by CatSalut, the total consumption of anticancer drugs ranged from 4.7 t to 4.9 t during the period 2010–2012. Table SI1 shows the consumptions of cytostatics in pharmacies and hospitals classified by ATC codes.

Out of 132 drugs consumed, 77 cytostatics belong to the L01 group, 17 to L02, 14 to L03, 22 to L04, 1 to H02 and 1 to G03. Their consumption followed the order L04 > L01 > H02 > G03 > L02 > L03. Consumption is discussed following these codes numerically:

L01 group. It is the second group of cytostatics most consumed. Specifically, the antimetabolite capecitabine (L01BC06) and the antineoplastic agent hydroxycarbamide (L01XX05) represent the 17% and 13% respectively of the total amount of anticancer drugs consumed. Capecitabine, a 5-fluorouracil prodrug, is used against breast and colorectal cancer, which have the highest incidence in women from Catalonia, whereas hydroxycarbamide is a DNA replication inhibitor used against leukemia, but also against multiple diseases such as psoriasis, AIDS or myeloproliferative disease. During 2010–2012 period, a decrease in pharmacies and an increase in hospitals of L01 drugs were observed due to the marked shift of prescriptions from pharmacies to hospitals of capecitabine, imatinib (L01XE01), other L01XE compounds and bexarotene (L01XX25) (see Table SI2). In 2012, similar amounts were prescribed (0.8 t) in both premises (Fig. 1). On the contrary, celecoxib (L01XX33) which was prescribed just in pharmacies (7 kg in 2010), was discontinued in 2012 due to the poor efficiency in cancer treatments (Table SI2).

L02 group. Endocrine therapy compounds are also used against cancer, with the progesterone derivative megestrol acetate (L02AB01), and two non-steroidal antiandrogens bicalutamide (L02BB03) and flutamide (L02BB01) as the most prescribed. L02 drugs were not dispensed in Catalonian hospitals until 2011, but their consumption has risen from 1 g to 19 kg yr^{-1} in the last two years (Fig. 1) due to the high prescription of abiraterone (L02BX03) which was not registered until 2011 but raised to 19 kg in 2012 (Table SI2). Similar prescriptions were obtained for all other L02 drugs along these three years.

L03 group. Immunostimulants are the substances used in fewer amounts. In 2012, only 0.3 g were dispensed in pharmacies and 5 kg in hospitals (Fig. 1). Glatiramer acetate (L03AX13), which is currently used to treat multiple sclerosis, was the most consumed (4 kg yr^{-1}).

L04 group. Immunosuppressants are the main drugs used in Catalonia with consumptions from 2.2 t to 2.4 t in the last three years (Fig. 1). L04 are rather dispensed in pharmacies than in hospitals. Mycophenolic acid (L04AA06) used to prevent rejection in organ transplantation, represents 40% of the global cytostatics administered (1.9 t yr^{-1}). Ciclosporin (L04AD01) and azathioprine (L04AX01) were the other two compounds more prescribed (0.12 t yr^{-1} and 0.20 t yr^{-1}, respectively).

G03 group. Cyproterone (G03HA01), which was exclusively dispensed in pharmacies, was the only compound prescribed and is used as an antiandrogen and progestin. Its consumption has remained fairly stable with a slight decrease from 26 to 22 kg yr^{-1} in the 2010–2012 period (Table SI2).

H02 group. From the corticosteroids, prednisone was the only one administered (H02AB07). It was also exclusively dispensed in pharmacies with an increased consumption (from 222 to 229 kg yr^{-1}, Fig. 1). Prednisone is used to treat hormone-sensitive tumors, such as leukemia, and also for many other indications including asthma, multiple sclerosis or tuberculosis.

3.2. Comparison with consumption rates of cytostatic drugs in Europe

Table 1 displays the different per capita consumption rates of relevant cytostatics in Europe in μg $inhab^{-1}$ d^{-1} (Besse et al., 2012; Booker et al., 2014; Coetsier et al., 2009; Johnson et al., 2008a; Kümmerer and Al-Ahmad, 2010; Martín et al., 2014; Ortiz de García et al., 2013; Rowney et al., 2009). The most consumed cytostatics were mycophenolic acid, capecitabine and hydroxycarbamide, with consumptions ranging from 221 to 704 μg $inhab^{-1}$ d^{-1}. Mycophenolic acid has not been previously reported in Europe but the other two drugs are in the range of other European countries (Johnson et al., 2013; Besse et al., 2012). Other widely consumed compounds are prednisone, azathioprine and megestrol acetate (consumption ranges from 77.7 to 82.0 in μg $inhab^{-1}$ d^{-1}), the latter agrees with the consumption obtained from Spain (79.3 μg $inhab^{-1}$ d^{-1}) (Ortiz de García et al., 2013).

Following, and at much lower doses, gemcitabine,

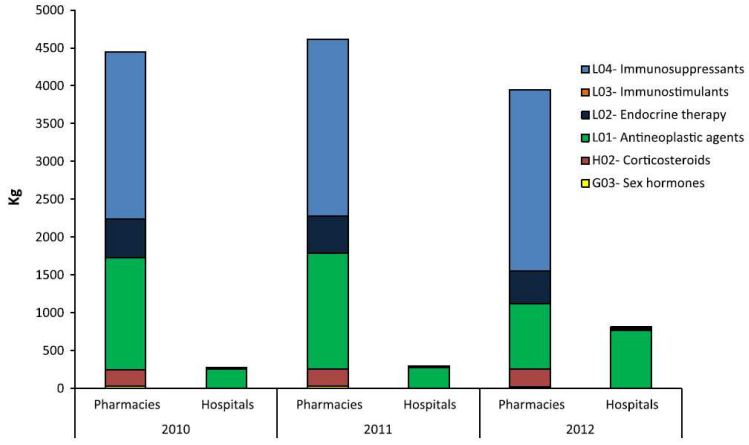

Fig. 1. Annual consumption (kg) of cytostatic drugs, classified according to the ATC code, in pharmacies and hospitals from Catalonia.

Table 1
Comparison of consumption (C, μg inhab^{-1} d^{-1}), PEC_{river} (ng L^{-1}) and PEC river corrected value (PEC_c) for some European countries and regions. Complete table at SI3.

"tbchmode-val"Max ATC group	Name	France[a]			UK									Germany			Spain						Catalonia	
		Besse et al., 2012 (E)			Rowney et al., 2009 (E+R)			Booker et al., 2014 (E+R)			Johnson et al., 2008a, 2008b (E)			Kümmerer and Al-Ahmad, 2010 (E+R)			Ortiz de García et al., 2013 (E+R)			Martín et al., 2014 (R)			This study (mean) (E+R)	
		C	PEC_{river}	PEC_c	C	PEC_{eff}	PEC_c	C	PEC_{river}	PEC_c	C	PEC_{eff}	PEC_c	C	PEC_{river}	PEC_c	C	PEC_{river}	PEC_c	C	PEC_{river}	PEC_c	C	PEC_{river}
L01AA01	Cyclophosphamide	12.7	> 1.74	0.23	20.6	70.2	1.89	40	4.1	1.10				8.90	0.60	0.19	74.5	4.35	1.68	4.70	4.56[b]	1.76	1.54	0.11
L01AA06	Ifosfamide	4.28	1.18	0.16				1	0.1	0.03				8.90	0.60	0.19	20.5	3.80	1.47	3.56	1.15	0.44	2.28	0.34
L01BA01	Methotrexate	3.10	1.54	0.20				1	0.20	0.05										1.53	0[b]	0	3.19	0.04
L01BC02	5-fluorouracil	71.9	7.91	1.04				12	0.90	0.24	46.0	23	0.71							45.4	44.1[b]	17.0	0.70	0.01
L01BC05	Gemcitabine	15.7	0.87	0.11	14.2	3.56	0.10	7	0.20	0.05							51.2	1.83	0.71	6.65	6.46[b]	2.49	12.0	0.21
L01BC06	Capecitabine	213	3.52	0.46	312[c]	13.7	0.37	183	2.3	0.62	79.0	39	1.21										280	7.76
L01XA02	Carboplatin	3.47	1.91	0.25	8.01	21.9	0.59	3	0.20	0.05													0.04	0.01
L01XE01	Imatinib	36.3	4.99	0.66				10	0.50	0.13													33.6	2.34
L01XE07	Lapatinib	4.82	1.86	0.25				2	0	0													8.59	0.26
L01XX05	Hydroxycarbamide	284	78.07	10.3				33	0.5	0.13													221	32.1
L01XX23	Mitotane	9.70	3.20	0.42				2	0	0													3.38	0.05
L02AB01	Megestrol																79.3	15.96	6.16				77.7	0.72
L02BA01	Tamoxifen	15.6	8.61[d]	1.14													15.0	0.209	0.08				17.7	0.05
L02BB01	Flutamide	21.6	< 1.19	0.16													116	0.646	0.25				27.2	0.72
L02BB03	Bicalutamide	35.8	10.84	1.43																			38.1	6.03
L04AA06	Mycophenolic acid																						704	77.4
L04AA13	Leflunomide																						5.99	0.86
L04AX01	Azathioprine																						78.6	0.46
G03HA01	Cyproterone																						8.88	0.73
H02AB07	Prednisone																						82.0	1.58

C: consumption (μg inhab^{-1} d^{-1});
E: excretion rate considered (see the reference for further information);
R: removal rate considered (see the reference for further information);
PEC_{river}: PEC in river (ng L^{-1}) reported by the authors;
PEC_{eff}: PEC in WWTP effluent (ng L^{-1}) reported by the authors;
PEC_c: PEC in river (ng L^{-1}), recalculated according to dilution factors proposed by Keller et al. (2014). France: 75.73; Spain: 25.92; UK: 37.16; Germany: 32.30.

[a] Coetsier et al. (2009) ($C/PEC_{river}/PEC_c$): Ifosfamide: 5.3/8/0.32 ; Tamoxifen: 15.1/7/0.29.
[b] Recalculated in the present table.
[c] Capecitabine with 5-fluorouracil.
[d] Conservative PEC (0% removal, 0% excretion).

Table 2
List of cytostatic drugs consumed in Catalonia, ATC codes, excretion (F_{exc}) and removal fraction (F_{wwtp}), annual consumption (C) and PEC in WWTP effluent and river, sorted by decreasing PEC (≥ 0.1 ng L^{-1}). Complete table: see Table SI3.

Therapeutic group	ATC code	Name	F_{exc}	Ref	F_{wwtp}	Ref	C-2010	C-2011	C-2012	PECeff (mean)	±	SD	PECriver (mean)	±	SD
G03-Sex hormones and modulators of the genital system	G03HA01	Cyproterone	0.33	g	0.15	l	72.3	70.1	60.0	19.0	±	1.9	0.73	±	0.07
H02-Corticosteroids for systemic use	H02AB07	Prednisone	0.5	a	0.87	m	608	626	635	40.9	±	0.9	1.58	±	0.04
L01-Antineoplastic agents	L01XX05	Hydroxycarbamide	0.5	c	0.02	b	1598	1742	1697	832	±	36	32.1	±	1.4
	L01BC06	Capecitabine	0.11	h	0.15	c	2259	2273	1863	201	±	22	7.76	±	0.85
	L01XE01	Imatinib	0.25	c	0.06	l	245	259	262	60.6	±	2.2	2.34	±	0.09
	L01XC02	Rituximab	0.5	a	0[a]	a	17.1	17.2	17.7	8.76	±	0.16	0.34	±	0.01
	L01AA06	Ifosfamide	0.5	c	0	c	12.9	22.3	16.8	8.75	±	2.39	0.34	±	0.09
	L01BB02	Mercaptopurine	0.4	j	0.02	b	19.8	21.1	23.3	8.49	±	0.70	0.33	±	0.03
	L01XE08	Nilotinib	0.69	j	0.78	l	41.6	53.4	64.7	8.14	±	1.77	0.32	±	0.07
	L01XC06	Cetuximab	0.5	a	0[a]	a	15.8	15.5	14.6	7.74	±	0.31	0.30	±	0.01
	L01BA04	Pemetrexed	0.9	j	0.02	b	8.16	8.43	8.78	7.54	±	0.28	0.29	±	0.01
	L01XC03	Trastuzumab	0.5	a	0[a]	a	12.9	14.1	15.3	7.12	±	0.61	0.27	±	0.03
	L01XE07	Lapatinib	0.67	k	0.85	l	68.0	66.1	61.7	6.62	±	0.33	0.26	±	0.02
	L01XC07	Bevacizumab	0.5	a	0[a]	a	14.2	10.9	8.46	5.64	±	1.46	0.22	±	0.06
	L01BC05	Gemcitabine	0.1	j	0.40	c	97.1	89.0	87.0	5.51	±	0.32	0.21	±	0.02
	L01XE05	Sorafenib	0.51	k	0.85	l	73.1	73.1	62.6	5.31	±	0.47	0.21	±	0.02
	L01XA03	Oxaliplatin	0.5	j	0[a]	a	8.71	13.3	9.08	5.24	±	1.30	0.20	±	0.05
	L01XX19	Irinotecan	0.5	c	0.03	l	9.59	9.21	10.3	4.77	±	0.28	0.18	±	0.02
	L01AA01	Cyclophosphamide	0.25	j	0	c	12.4	11.5	11.1	2.94	±	0.17	0.11	±	0.01
	L01BC03	Tegafur	0.05	k	0.02	b	55.3	48.2	50.1	2.54	±	0.18	0.10	±	0.01
	L01XE11	Pazopanib	0.7	j	0.08	l	0	0.44	9.39	2.14	±	3.45	0.08	±	0.14
	L01XA01	Cisplatin	0.6	h	0.02	l	3.66	3.56	4.09	2.23	±	0.16	0.08	±	0.01
	L01CD01	Paclitaxel	0.18	j	0.09	l	11.0	12.8	14.3	2.09	±	0.28	0.08	±	0.01
	L01AX03	Temozolomide	0.21	j	0.02	b	10.5	10.6	10.2	2.17	±	0.04	0.08	±	0.00
	L01BC07	Azacitidine	0.85	j	0.02	b	0	3.29	3.36	1.87	±	1.62	0.07	±	0.06
	L01XX23	Mitotane	0.6	c	0.92	b	17.1	21.4	38.5	1.32	±	0.58	0.05	±	0.03
	L01XE06	Dasatinib	0.19	j	0.02	b	5.97	6.33	8.12	1.28	±	0.22	0.05	±	0.01
	L01BA01	Methotrexate	0.9	c	0.95	c	22.6	24.9	25.3	1.10	±	0.06	0.04	±	0.00
	L01CD02	Docetaxel	0.14	j	0.04	l	6.10	9.02	6.37	0.97	±	0.22	0.04	±	0.01
	L01XC08	Panitumumab	0.5	a	0[a]	a	1.45	1.30	1.75	0.76	±	0.11	0.03	±	0.00
	L01BC53	Tegafur combinations	0.5	a	0	a	2.7	1.58	0.56	0.81	±	0.54	0.03	±	0.02
	L01XE04	Sunitinib	0.16	k	0.04	b	3.23	4.31	3.87	0.59	±	0.09	0.02	±	0.01
	L01XE03	Erlotinib	0.02	c	0.04	b	33.5	28.7	22.9	0.55	±	0.11	0.02	±	0.01
	L01CB01	Etoposide	0.93	c	0.02	l	0.61	0.60	0.52	0.53	±	0.04	0.02	±	0.00
	L01XE02	Gefitinib	0.05	c	0.19	b	1.98	11.9	18.0	0.44	±	0.33	0.02	±	0.01
	L01XD03	Methyl aminolevulinate	0.5	a	0.02	b	0.88	0.85	0.74	0.41	±	0.04	0.02	±	0.01
	L01DB01	Doxorubicin	0.5	c	0.02	l	0.52	0.60	0.34	0.24	±	0.07	0.01	±	0.00
	L01BB05	Fludarabine	0.6	h	0.02	b	0.28	0.32	0.28	0.18	±	0.01	0.01	±	0.00
	L01BC01	Cytarabine	0.1	c	0.50	c	2.20	3.46	3.82	0.16	±	0.04	0.01	±	0.003
	L01AA09	Bendamustine	0.5	a	0.16	l	0	0	0.44	0.06	±	0.11	2E-3	±	0.01
L02-Endocrine Therapy	L02BB03	Bicalutamide	0.55	c	0.03	b	302	302	263	156	±	12	6.03	±	0.47
	L02BB01	Flutamide	0.1	c	0.10	b	259	208	153	18.8	±	4.8	0.73	±	0.19
	L02AB01	Megestrol	0.78	j	0.96	n	619	614	538	18.6	±	1.4	0.72	±	0.06
	L02AB02	Medroxyprogesterone	0.5	a	0.13	b	9.48	10.4	10.1	4.38	±	0.20	0.17	±	0.01
	L02BA01	Tamoxifen	0.13	j	0.93	b	133	136	135	1.22	±	0.01	0.05	±	0.00
	L02BX03	Abiraterone	0.55	j	0.87	b	0	0	51.8	1.27	±	2.21	0.05	±	0.09
	L02BG04	Letrozole	0.06	f	0.03	b	16.1	17.7	18.7	1.03	±	0.08	0.04	±	0.00
	L02BG03	Anastrozole	0.1	f	0.03	b	4.03	3.54	2.92	0.35	±	0.06	0.01	±	0.01
	L02AE04	Triptorelin	0.42	k	0[a]	a	0.78	0.80	0.72	0.33	±	0.02	0.01	±	0.00
	L02AE03	Goserelin	0.9	g	0[a]	a	0.20	0.18	0.14	0.16	±	0.03	0.01	±	0.00
L03-Immunostimulants	L03AX13	Glatiramer acetate	0.5	a	0[a]	a	8.64	9.94	10.9	4.96	±	0.57	0.19	±	0.02
	L03AX03	BCG vaccine	0.5	a	0[a]	a	2.56	2.69	2.29	1.27	±	0.11	0.05	±	0.01
L04-Immunosupressants	L04AA06	Mycophenolic acid	0.63	i	0.41	b	5092	5408	5554	2008	±	88	77.4	±	3.4
	L04AA13	Leflunomide	0.5	a	0.03	b	44.1	46.2	46.2	22.3	±	0.6	0.86	±	0.02
	L04AX01	Azathioprine	0.02	f	0.02	l	553	606	633	11.8	±	0.8	0.45	±	0.03
	L04AB01	Etanercept	0.5	a	0[a]	a	15.3	14.4	14.4	7.43	±	0.26	0.29	±	0.01
	L04AB02	Infliximab	0.5	a	0[a]	a	12.2	12.3	12.8	6.26	±	0.16	0.24	±	0.01
	L04AB04	Adalimumab	0.5	a	0[a]	a	7.69	8.16	8.53	4.10	±	0.21	0.16	±	0.01
	L04AC07	Tocilizumab	0.5	a	0[a]	a	2.03	3.94	5.33	1.90	±	0.84	0.07	±	0.03
	L04AA23	Natalizumab	0.5	a	0[a]	a	3.03	3.40	3.50	1.67	±	0.12	0.07	±	0.01
	L04AC03	Anakinra	0.5	a	0[a]	a	2.31	2.73	4.06	1.53	±	0.46	0.06	±	0.02
	L04AA24	Abatacept	0.5	a	0[a]	a	2.62	2.69	3.08	1.41	±	0.12	0.05	±	0.01
	L04AX04	Lenalidomide	0.82	k	0.02	l	1.57	1.29	1.44	1.17	±	0.12	0.05	±	0.01
	L04AB05	Certolizumab pegol	0.5	a	0[a]	a	0.02	0.56	1.41	0.33	±	0.35	0.01	±	0.02
	L04AA25	Eculizumab	0.5	a	0[a]	a	0.52	0.57	0.76	0.31	±	0.06	0.01	±	0.00
	L04AD01	Ciclosporin	0.001	f	0[a]	a	330	321	305	0.32	±	0.01	0.01	±	0.00

C – consumption (g d^{-1}); Fexc – excreted fraction (worst case value); Fwwtp – removal fraction in WWTPs (worst case value); SD – Standard Deviation;
a – no data; b – Chem Spider (Royal Society of Chemistry, 2014); c – Besse et al. (2012); d – Booker et al. (2014); e – Johnson et al. (2013); f – HSDB (U.S. National Library of Medicine);
g – (Gómez-Canela et al., 2013); h – Rowney et al. (2009); i – www.drugs.com (Wolters Kluwer Health, 2000); j – BCCA (Provincial Health Services Authority, 2013); k – EMC (DataPharm, 2014);
l – EPISuite (U.S. EPA, 2013); m – (Fan et al., 2011); n – (Chang et al., 2011).

[a] The removal fraction could not be modeled by EPISuite.

bicalutamide, imatinib, flutamide, tamoxifen, methotrexate and ifosfamide values matched with those reported in France and UK (Besse et al., 2012; Rowney et al., 2009) and other EU countries (Booker et al., 2014; Kümmerer and Al-Ahmad, 2010; Martín et al., 2014).

A major difference with values observed in the literature arises from widely employed cytostatics such as cyclophosphamide, 5-fluorouracil and carboplatin which are by far much less consumed in Catalonia than in other European countries. Cyclophosphamide consumptions are very low when compared to the European mean value of 10.4 $\mu g\,inhab^{-1}\,d^{-1}$ but still in the 15-fold range in consumption for this compound in 12 EU countries (Johnson et al., 2013). For carboplatin, with a European mean value of 3 $\mu g\,inhab^{-1}\,d^{-1}$ (Johnson et al., 2013) and a range of consumptions from 2.1 to 8.01 $\mu g\,inhab^{-1}\,d^{-1}$ in UK and France (Besse et al., 2012; Booker et al., 2014; Johnson et al., 2013; Rowney et al., 2009) the consumption in Catalonia is only 0.04 $\mu g\,inhab^{-1}\,d^{-1}$ which can be explained by the major use of oxaliplatin (1.37 $\mu g\,inhab^{-1}\,d^{-1}$) and in a lesser extent of cisplatin, among the platinum derivatives (see Table S2).

Among the 132 compounds studied,Table SI2 includes the consumption of 38 L03, L04, G03 and H02 compounds, which, to the best of our knowledge, have not been reported until now in any accessible publication.

3.3. Predicted environmental concentrations (PECs)

Two PEC values were calculated for each compound. PEC_{eff} represents the concentration of cytostatic compounds in WWTP effluents (after WWTP treatment) and PEC_{river} represents the drug fraction estimated to be found in surface water, once discharged and diluted. Dilution factor considered was that proposed for Spain (25.92) by Keller et al. (2014). All PEC values are sorted by decreasing value for each group and summarized in Table 2. It also displays the excretion ratio selected and the WWTP removal employed, as well as the consumption in $g\,d^{-1}$.

PEC values were also compared with those results found in the literature. However, each author selected different criteria in the parameters of Eq. (1) (excretion, removal …) and thus the results might not be effectively compared. For example, Besse et al. studied cytostatic drug consumptions in France and they did not take into account the removal rates, due to scarcity of data (Besse et al., 2012). Excretion and WWTP removal rates have a contribution in the final PEC value, and different interpretations could modify the levels by up to 20-fold. But, apart from consumption, the most important factor is dilution rates because it can change the results by up to 1000-fold (Johnson et al., 2013). In almost all the literature, the dilution value used to calculate concentrations in rivers was 10. Coetsier et al. calculated the PEC values of several drugs in the effluent of a WWTP in Alès (France) considering both excretion and removal rates (Coetsier et al., 2009). The dilution factor of STP effluent in surface water varied between 2.3 and 4.7, depending on the period of time studied. However, the factor proposed by Keller et al. is in that region 16-fold higher (Keller et al., 2014). Other dilution factors predicted in that work were 75.73 (France), 37.16 (UK) and 32.30 (Germany), significantly higher than the default value. Therefore, in the present study all the PEC values were recalculated taking into account the dilution factors predicted by Keller et al. (2014). Table 1 displays the PEC_{river} values of relevant cytostatics ($> 1\ ng\,L^{-1}$) in Catalonia and in different European countries with the original PEC_{river} or PEC_{eff} obtained from the bibliography and the corrections applied considering the different national dilution factors, PEC_c. In the present study, the mean PEC of the three years was calculated in order to compare our results with those of other studies.

L01 group. The antineoplastic agent hydroxycarbamide (L01XX05) was the most consumed L01 drug and also the compound expected to be found at higher levels. Hydroxycarbamide is stable for months at room temperature (Heeney et al., 2004). However, there is no experimental data for excretion and WWTP removal, and EPISuite model predicts an insignificant elimination (2%), resulting in a high PEC_{eff} (mean value 832 $ng\,L^{-1}$). Significant differences are reported for hydroxycarbamide such as the PEC_{eff} values of 781 $ng\,L^{-1}$ and 5 $ng\,L^{-1}$ for France (Besse et al., 2012) and NW England (Booker et al., 2014), respectively. Taking into consideration the dilution factor from WWTPs to river, the PEC_{river} of hydroxycarbamide raises up to 32.1 $ng\,L^{-1}$ higher than the 10.3 and 0.13 $ng\,L^{-1}$ calculated in France and NW England (Besse et al., 2012; Booker et al., 2014).

The second highest PEC_{eff} corresponds to capecitabine (L01BC06) with a mean value of 201 $ng\,L^{-1}$ for these three years and considering an 11% excretion (Rowney et al., 2009) and a 15% removal in the WWTP (Besse et al., 2012). This value is much higher than the maximum PEC level of 87 $ng\,L^{-1}$ reported by Johnson et al. (2013) for the Czech Republic. However, normalized data employing the same excretion values and removals used in our study, give a similar PEC (92 $ng\,L^{-1}$) which is roughly 45% of the PEC_{eff} of capecitabine in Catalonia. European PEC_{river} values for capecitabine corrected by the dilution factor proposed by Keller et al. (2014) are consistently lower (0.37–1.21 $ng\,L^{-1}$) (Besse et al., 2012; Booker et al., 2014; Johnson et al., 2008a; Rowney et al., 2009).

On the contrary, 5-fluorouracil (L01BC02) with a mean PEC_{eff} value of 0.21 $ng\,L^{-1}$ and a mean PEC_{river} $< 0.01\ ng\,L^{-1}$ is far from the 0.24–17.0 $ng\,L^{-1}$ corrected range reported for different countries (Besse et al., 2012; Booker et al., 2014; Johnson et al., 2008a; Martín et al., 2014) (Table 1). As capecitabine is the prodrug of 5-fluorouracil, it is metabolized to 5-fluorouracil in the body and both data can be combined (Johnson et al., 2008a; Keller et al., 2014) giving a PEC_{eff} and PEC_{river} mean values of 201 $ng\,L^{-1}$ and 7.77 $ng\,L^{-1}$, respectively.

Following, imatinib (L01XE01) has a PEC_{eff} mean value of 60.6 $ng\,L^{-1}$ calculated according to an excretion of 25% (Besse et al., 2012) and a WWTP removal of 6% predicted by EPISuite (U.S. EPA, 2013). The other 35 compounds of the L01 group haTable 1 d PEC_{eff} and PEC_{river} mean values $< 10\ ng\,L^{-1}$ and $< 0.5\ ng\,L^{-1}$, respectively. Among them, the widely studied cyclophosphamide and ifosfamide with mean PEC_{eff} values of 2.94 and 8.75 $ng\,L^{-1}$, respectively, were obtained considering excretion rates of the unchanged product of 25 and 50% (Besse et al., 2012; Provincial Health Services Authority, 2013). In Spain these PEC_{eff} values were 43.5 and 38.0 $ng\,L^{-1}$ (Ortiz de García et al., 2013) and specifically 45.6 and 11.5 $ng\,L^{-1}$ in Seville (Martín et al., 2014). Our values are in general closer to those obtained by Besse et al. (2012) in France who reported PEC_{river} values of 0.23 and 0.16 $ng\,L^{-1}$ for these two compounds or Germany with 0.19 $ng\,L^{-1}$ for both compounds (Kümmerer and Al-Ahmad, 2010). Other European data are given in Table 1 with values around 0.03–1.89 $ng\,L^{-1}$ range (Booker et al., 2014; Coetsier et al., 2009; Rowney et al., 2009).

L02 group. The endocrine therapy drug megestrol acetate (L02AB01) was the most consumed having a PEC_{eff} mean value of 18.6 $ng\,L^{-1}$ calculated assuming a 78% excretion rate of the parent compound (Provincial Health Services Authority, 2013) and a removal of 96% in WWTPs (Chang et al., 2011). Its PEC_{river} mean value is 0.72 $ng\,L^{-1}$ which is lower than the corrected value (6.16 $ng\,L^{-1}$) given by Ortiz de García et al. (2013) for Spain who consider 92% excretion and 30% removal estimated with STPWIN.

The antiandrogen bicalutamide (L02BB03) had the highest PEC_{eff} (mean value 156 $ng\,L^{-1}$) after considering an excretion rate of 55% (Besse et al., 2012) and a 3% of WWTP removal (Royal Society of Chemistry, 2014). The PEC_{river} was 6.03 $ng\,L^{-1}$ which is higher than the level of 1.43 $ng\,L^{-1}$ reported in France (Besse et al.,

2012). On the other hand, flutamide (L02BB01), tamoxifen (L02BA01) and exemestane (L02BG06), the other three cytostatic drugs with high consumptions (see Table 2), presented excretion values of 1–13% (Besse et al., 2012; Provincial Health Services Authority, 2013; Royal Society of Chemistry, 2014; U.S. National Library of Medicine, 2005) which leads to low PEC_{eff} values of 18.8; 1.22 and 0.06 ng L^{-1}, respectively. As tamoxifen and exemestane are efficiently removed during WWTP (93% and 90%, respectively (Besse et al., 2012; Royal Society of Chemistry, 2014), their PEC_{river} drops to 0.72, 0.05 and 0.002 ng L^{-1}, respectively. Tamoxifen has also been reported in Spain, France and Alès (France) at PEC_{river} levels of 0.08 ng L^{-1}; 1.14 ng L^{-1} and 0.29 ng L^{-1} respectively (Besse et al., 2012; Coetsier et al., 2009; Ortiz de García et al., 2013). A worst case scenario (0% excretion and 0% removal) was considered for tamoxifen in France (Besse et al., 2012) which can explain the much higher value compared to others reported. Flutamide, due to its poor removal (10%) in the WWTP (Royal Society of Chemistry, 2014) presents a PEC_{river} of 0.73 ng L^{-1} which is slightly superior to the 0.25 ng L^{-1} value reported in Spain (Ortiz de García et al., 2013) and 0.16 ng L^{-1} in France (Besse et al., 2012).

L03 group. Immunostimulants, with a global consumption of 1.66 μg $inhab^{-1}$ d^{-1}, had very low PECs. Glatiramer acetate (L03AX13) was the only drug with relative significant PECs in effluent and river (4.96 and 0.19 ng L^{-1}, respectively). Very little is known about the excretion and the WWTP removal of immunostimulant compounds. For most of them, no data was found and could neither be calculated.

L04 group. Immunosuppressants were the most dispensed compounds in the period 2010–2012. Mycophenolic acid was the most consumed drug in this group. It has been reported that 60%

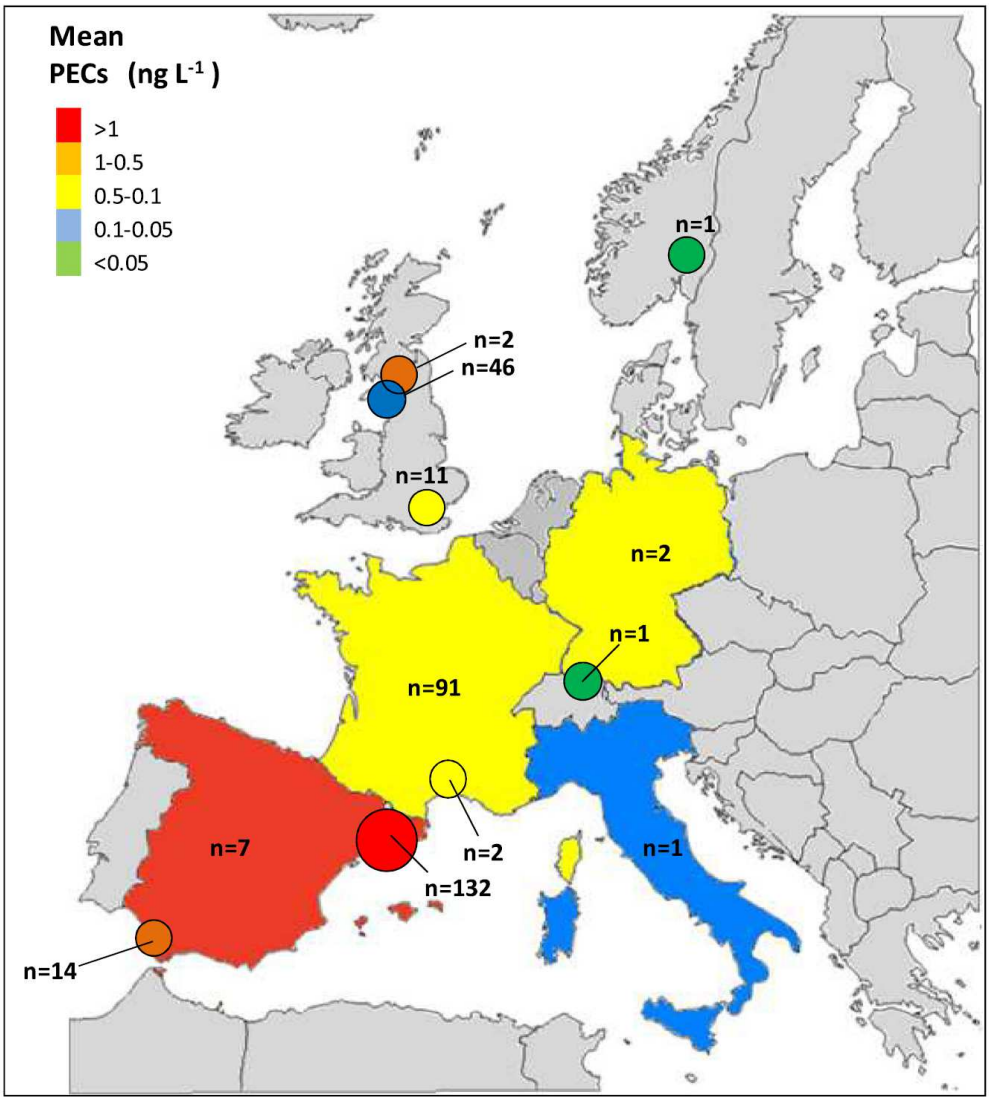

Fig. 2. Mean PECs (ng L^{-1}) values in Europe and number of compounds included in each study region (n). (Besse et al., 2012; Booker et al., 2014; Coetsier et al., 2009; Johnson et al., 2008a, 2013; Kümmerer and Al-Ahmad, 2010; Martín et al., 2014; Ortiz de García et al., 2013; Rowney et al., 2009). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of the administered drug is eliminated in the urine as mycophenolic acid glucuronide and 3% unchanged (Wolters Kluwer Health, 2000). Several studies have proved that deconjugation occurs during WWTP process (Lin et al., 2013) and therefore a value of 63% has been taken into account together with a WWTP removal of 41% (Royal Society of Chemistry, 2014) to calculate the mean PEC_{eff} and PEC_{river} values, which were of 2008 ng L^{-1} and 77.4 ng L^{-1}, respectively. On the other hand, leflunomide (L04AA13), azathioprine (L04AX01) and ciclosporin (L04AD01) had mean PEC_{eff} values of 22.3, 11.8 and 0.32 ng L^{-1}, respectively, and PEC_{river} levels of 0.86, 0.45 and 0.01 ng L^{-1} (see Table 2).

G03 group. For cyproterone (G03HA01), mean values of PEC_{eff} and PEC_{river} were 19.0 and 0.73 ng L^{-1}, respectively, assuming 33% excretion (Gómez-Canela et al., 2013) and 15% removal in WWTP as calculated by EPISuite (U.S. EPA, 2013).

H02 group. Prednisone (H02AB07) was the seventh compound with the highest PECs, with a mean value of 40.9 ng L^{-1} in effluent and 1.58 ng L^{-1} in river. No experimental data was found for the excretion of prednisone, although it is extensively metabolized to prednisolone and 50% excretion was used. Besides, 87% removal was considered as obtained in Chinese WWTPs (Fan et al., 2011) instead of the 2% removal predicted by ChemSpider (Royal Society of Chemistry, 2014).

Fig. 2 represents all the predicted concentration of cytostatic compounds reported in Europe. It displays the mean PEC_{river} values and the number of cytostatic drugs studied. In green are represented the studies with a mean PEC < 0.05 ng L^{-1}, as Oslo and Zurich (Johnson et al., 2013). In blue, the mean PEC < 0.1 ng L^{-1} such in Italy and NW-UK reports (Johnson et al., 2013; Booker et al., 2014). In yellow, the values < 0.5 ng L^{-1}, as in Germany, France and UK (Kümmerer and Al Ahmad, 2010; Besse et al., 2012; Rowney et al., 2009). In orange, the values < 1 ng L^{-1}, as Seville and North UK (Martin et al., 2014; Johnson et al., 2008a). And finally, in red, values > 1 ng L^{-1}, found in Spain (Ortiz de Garcia et al., 2013) and Catalonia (the present study).

Considering all the compounds studied, only the PEC_{river} value of mycophenolic acid and hydroxycarbamide (77.4 and 32.1 ng L^{-1}), were higher than 10 ng L^{-1} which is the EMEA threshold value for the environmental risk assessment of pharmaceuticals (EMEA, 2006). Regarding the other drugs, four cytostatic compounds, capecitabine, bicalutamide, imatinib and prednisone had $PEC_{river} > 1$ ng L^{-1} with average values of 7.76, 6.03, 2.34 and 1.58 ng L^{-1}, respectively in 2010–2012 (see Table 2). On the other hand, 24 cytostatic compounds had PEC_{river} values ranging from 0.1 to 0.99 ng L^{-1} and 102 cytostatic drugs had values lower than 0.1 ng L^{-1} (Table 2).

3.4. PEC_{eff} vs. MECs

PEC estimations were compared with MECs in wastewaters and river waters. Several criteria has been used to evaluate whether the PECs tend to overestimate or underestimate MECs. Among them, Coetsier et al. proposed a scheme ranking in which values $0.2 < PEC/MEC < 1$, PEC are acceptable and slightly underestimated; for those with $1 < PEC/MEC < 4$, PEC results are acceptable but slightly overestimated whereas for $4 < PEC/MEC < 8$, overestimated results are given (Coetsier et al., 2009). Other authors (Ort et al., 2009; Verlicchi et al., 2014) proposed $0.5 < PEC/MEC < 2$ as acceptable. The reliability of PECs compared to MECs has been reported for several pharmaceuticals and very few cytostatics.

The reliability of several PEC_{eff} of the present study has been compared from recent MECs for cytostatics reported in wastewaters of Catalonia (see Table 3). Three compounds, cyclophosphamide, ifosfamide and methotrexate presented a good agreement with their PEC/MEC ratios, in line with those previously reported by other authors (Coetsier et al., 2009; Kümmerer and Al-Ahmad, 2010; Martín et al., 2014; Ortiz de García et al., 2013). PEC/MEC ratios for cyclophosphamide were in the range 0.2–0.73 (one value gave 3.4), for ifosfamide was 0.2– > 1.3 with one value > 6.7 and for methotrexate, the range was > 0.3–1.1 (Table 3). For these compounds PEC_{eff} is similar or higher than their LODs.

Not much information can be obtained from the PEC/MEC ratios of gemcitabine, temozolomide, paclitaxel, imatinib, irinotecan, azathioprine and cyproterone because of the few data reported and concentration levels usually < LOD. The PEC/MEC ratios of chlorambucil, melphalan, fludarabine, vinblastine, vincristine, etoposide, doxorubicin, epirubicin, erlotinib, docetaxel, leuprolide and goserelin could not be estimated either due to their low PEC_{eff} and/or high LODs of the reported analytical method for these compounds (Ferrando-Climent et al., 2014; Gómez-Canela et al., 2014; Negreira et al., 2014, 2013), which precludes their determination. The reported PEC values in other European countries (France, UK) for vincristine (3e−04 ng L^{-1}) (Besse et al., 2012); etoposide (1.3 and 8.7 ng L^{-1}) (Besse et al., 2012; Booker et al., 2014); doxorubicin (0.34 and 1.9 ng L^{-1}) (Besse et al., 2012; Rowney et al., 2009); epirubicin (0.09 ng L^{-1}) (Rowney et al., 2009); erlotinib, docetaxel and goserelin (< 0.7; < 0.5 and 0.3 ng L^{-1}, respectively) (Besse et al., 2012) are consistently similar to those found in this study.

Finally, capecitabine, megestrol and prednisone gave overestimated PEC/MEC ratios. Environmental data in Catalonia from the three latter compounds are scarce and precludes any explanation. However, Liu et al. (2012) have also reported concentrations of glucocorticoids and progestogens in two Chinese WWTPs much lower than the estimated concentrations attributing them to incomplete deconjugation, sorption, degradation or their transformation in the sewer line. The uncertainty of selected estimation data from the literature can be attributed to the scattered removal rates from 11–90% (Fan et al., 2011; Macikova et al., 2014; Ortiz de García et al., 2013; Royal Society of Chemistry, 2014). Measured values of capecitabine are systematically higher than its PEC_{eff} (Table 2) with PEC/MEC ratios in the range of 8.8– > 26.1.

In this section MECs are assumed to be correct, which may not be as they are punctual measurements (Johnson et al., 2008b). And taking into account that several parameters included in the calculation are highly variable (i.e. excretion, elimination rate in WWTPs) or not considered such as environmental degradation or sorption, the ratio PEC/MEC provides only a first attempt to validate the prediction for a reliable evaluation on the presence of cytostatic compounds in the aquatic environment.

3.5. Risk assessment

The risk assessment of cytostatics in Catalonia was performed based on the mean PEC values predicted from 2010–2012 period and the EC_{50} and LC_{50} values obtained from the bibliography. Only the compounds with PECs higher than 0.1 ng L^{-1} were considered to calculate RQ. Ecotoxicity data of these drugs is scarce. Short-term toxicity in aquatic organisms, usually *Daphnia magna*, was mostly found in safety data sheets (MSDS) from pharmaceutical companies (Table 4). Drugs selected were ordered by decreasing PEC for each group.

The toxic concentrations found for these drugs are highly variable. The results obtained by EC_{50} and LC_{50} tests ranges from NOEC (no observed effect concentration) to 1 mg L^{-1}. All the RQ values for these drugs turned out to be much lower than 1.0 showing no risk for the environment. Bicalutamide was the drug with the highest RQ (0.006), calculated considering the *D. magna* 24 h acute toxicity test (1 mg L^{-1}). Secondly, cyproterone had an EC_{50} of 2.4 mg L^{-1} and the RQ was of 0.003 followed by

Table 3
Concentration of cytostatics (ng L^{-1}) in Catalonia published in the literature. PECs vs. MECs.

		Negreira et al. (2013)		Gomez-Canela et al. (2014)		Ferrando-Climent et al. (2014)				Negreira et al. (2014)			
ATC Code	**Cytostatic**	**$WWTP_{inf}$**	**PEC/MEC**	**WWTP-A $_{inf}$**	**PEC/MEC**	**$WWTP_{eff}$[a]**	**PEC/MEC**	**Surface[b]**	**PEC/MEC**	**$WWTP_{eff}$[c]**	**PEC/MEC**	**$WWTP_{eff}$[d]**	**PEC/MEC**
L01AA01	Cyclophosphamide			< 4–10	0.73–3.4	15.7	0.2	< 0.9	> 0.12	8.8	0.33	6.3	0.47
L01AA02	Chlorambucil												
L01AA03	Melphalan			< 11	**↓PEC**								
L01AA06	Ifosfamide	43[e]	0.2	< 6	> 1.3	< 1.3	> 6.7	< 1.1	> 0.31			8.9	0.98
L01AX03	Temozolomide											< 4.2	> 0.52
L01BA01	Metothrexate	20[f]	1.1			< 4.1	> 0.3	1.9	0.02			< 1.8	> 0.61
L01BB05	Fludarabine			< 164	**↑LOD**								
L01BC05	Gemcitabine			< 262	> 1.3 but **↑LOD**							< 9.3	> 0.59
L01BC06	Capecitabine	27[g]	8.8	< 15	> 13							7.7	26.1
L01CA01	Vinblastine			< 4.9	**↓PEC**								
L01CA02	Vincristine			< 5.2	**↓PEC**	< 23.5	**↓PEC**	< 21.3	**↓PEC**				
L01CB01	Etoposide					< 75.7	**↓PEC**	< 72.5	**↓PEC**			< 40	**↑LOD**
L01CD01	Paclitaxel					< 8.7	> 0.24	< 2.9	> 0.03			< 4.0	> 0.52
L01CD02	Docetaxel			< 356	**↑LOD**	< 12.7	**↓PEC**	< 12.7	**↓PEC**				
L01DB01	Doxorubicin			< 54	**↑LOD**							< 2.4	> 10 **↑LOD**
L01DB03	Epirubicin			< 45	**↓PEC**								
L01XE01	Imatinib											< 120	> 0.5
L01XE03	Erlotinib			< 1.8	> 0.3							< 3.4	> 0.16
L01XX19	Irinotecan			< 4	> 1.2					< 1.2	> 3.97		
L02AE02	Leuprolide			< 14	**↓PEC**								
L02AE03	Goserelin			< 16	**↓PEC**								
L02BA01	Tamoxifen	17[h]	1.0	< 0.7	> 1.7	28.7	0.04	32.3	0.002	113.5	4.4 e-4	< 3.0	> 0.41
L02BG01	Aminoglutethimide			< 16	NO PEC								
L04AX01	Azathioprine					< 6.1	> 1.9	< 3.9	> 0.12				
G03AC05	Megestrol			< 3–20 (eff.)	> 1.7–4								
G03HA01	Cyproterone			< 4.1	> 5.4								
H02AB07	Prednisone			< 12	> 4.5								

↑LOD: high limit of detection;
↓PEC: low PEC;
Median PEC_{eff} (in ng/L): Cyclophosphamide (2.94); chlorambucil (0.01); melphalan (0.05); ifosfamide (8.75); temozolomide (2.17); methothrexate (1.10); fludarabine (0.18); gemcitabine (5.51); capecitabine (201.1); vinblastine (1e-05); vincristine (3e-05); etoposide (0.53); paclitaxel (2.09); docetaxel (0.97); doxorubicin (0.24); epirubicin (0.01); imatinib (60.6); erlotinib (0.55); irinotecan (4.77); leuprolide (0.09); goserelin (0.16); tamoxifen (1.22); azathioprine (11.83); megestrol (11.9); cyproterone (19.0) and prednisone (53.4).
Median PEC_{river} (in ng/L): Cyclophosphamide (0.11); ifosfamide (0.34); methothrexate (0.04); tamoxifen (0.05); capecitabine (7.76); paclitaxel (0.08); megestrol (0.44); azathioprine (0.45).
Removals used to calculate WWTP eff.

[a] Average values of the three campaigns.
[b] Average values of the three campaigns.
[c] Large catalan WWTP.
[d] Median of 12 Spanish WWTPs effluents.
[e] Ifosfamide (0%).
[f] Metothrexate (95%).
[g] Capecitabine (15%).
[h] Tamoxifen (93%).

flutamide, with an RQ of 0.0009. The drugs with the highest PECs, such as mycophenolic acid (77.45 ng L^{-1}), hydroxycarbamide (32.08 ng L^{-1}), capecitabine (7.76 ng L^{-1}), imatinib (2.34 ng L^{-1}) and prednisone (1.58 ng L^{-1}) had high EC_{50}, that turned in very low RQ.

In addition, PECs were calculated using a worse dilution factor using the 5th percentile for Spain predicted by Keller et al. (2014) 1.78, instead of the median value 25.92. In this case, PEC_{river} values were 14-fold higher. However, in this scenario all the RQ were still lower than 1.

To determine accumulative effects the ∑RQ values of all compounds showed no risk (0.014). Martin et al. (2014) calculated the RQ in effluent wastewaters and in the receiving waters for seven cytostatics. Two drugs had medium risk ($0.1 < RQ < 1$), for 4 drugs it was low ($0.01 < RQ < 0.1$) and overall, a low risk was observed. However, vinorelbine overcame this limit, showing a RQ value of 5.5 in the WWTP effluents but no ecotoxicological risk was expected after dilution in the receiving waters (RQ=0.004). Due to the complex mixture of these drugs in the environment, the low concentrations and their mechanism of action, Besse et al. (2012) concluded that more accurate test should be performed in order to better simulate the environmental reality.

In terms of bioaccumulation, the partition coefficient (logP) of the most toxic drugs was compiled. The predicted logP for bicalutamide, cyproterone and flutamide was 4.94, 2.52 and 3.72 respectively (Royal Society of Chemistry, 2014). If the logP is higher than 4, bioaccumulation can be expected. For these molecules, bicalutamide is the only one that can potentially be bioaccumulated.

4. Conclusion

The list of antineoplastic drugs used is constantly revised; every year new drugs are included and used in chemotherapy

Table 4
Ecotoxicity data (EC_{50} and LC_{50}) for the selected drugs (PEC > 0.1 ng L^{-1}) and risk quotient (RQ).

ATC group	Name	Organism	Test	Reference	Toxicity (mg L^{-1})	PEC_{river} ng L^{-1} (mean)	±	SD	RQ
G03HA01	Cyproterone	*D. magna*	48 h, EC_{50}	(Publichem, 2013)	2.4	0.73	±	0.07	3.06E−03
H02AB07	Prednisone	*D. magna*	EC_{50}	(Kar and Roy, 2010)	3822	1.58	±	0.04	4.13E−07
L01XX05	Hydroxycarbamide	*D. magna*	Acute toxicity, 48 h, EC_{50}	(Bristol-Myers Squibb Company, 2010)	> 100	32.1	±	1.4	3.21E−04
L01BC06	Capecitabine	*D. magna*	LC_{50}, 48 h	(Parrella et al., 2014)	224	7.76	±	0.85	3.46E−05
		D. magna	Reproduction 48 h, EC_{50}	(Besse et al., 2012)	> 850				9.13E−06
L01XE01	Imatinib	*D. magna*	48 h, LC_{50}	(Parrella et al., 2014)	11.97	2.34	±	0.09	1.95E−04
L01XC02	Rituximab	*nd*				0.34	±	0.01	
L01AA06	Ifosfamide	*D. magna*	48 h, EC_{50}	(Martín et al., 2014)	1795	0.34	±	0.09	1.88E−07
L01BB02	Mercaptopurine	*D. magna*	48 h, EC_{50}	(Duchefa Biochemie, 2011)	55	0.33	±	0.03	5.96E−06
L01XE08	Nilotinib	*nd*				0.32	±	0.07	
L01XC06	Cetuximab	*nd*				0.30	±	0.01	
L01BA04	Pemetrexed	*D. magna*	48 h, EC_{50}	(Publichem, 2013)	462	0.29	±	0.01	6.30E−07
		Fish (unknown)	96 h, LC_{50}	(Publichem, 2013)	1099.6		±		2.65E−07
L01XC03	Trastuzumab	*D. magna*	48 h, EC_{50}	(Roche, 2014)	369	0.27	±	0.03	7.44E−07
		B. sunfish	95 h, LC_{50}	(Roche, 2014)	10				2.75E−05
L01XE07	Lapatinib	*D. magna*	Static test, 48 h, EC_{50}	(GlaxoSmithKline, 2014)	(NOEC) > 0.17	0.26	±	0.02	
		O. mykiss	Static test, 96 h, EC_{50}	(GlaxoSmithKline, 2014)	(NOEC) 43.7				
L01XC07	Bevacizumab	*D. magna*	48 h, EC_{50}	(Roche, 2014)	(NOEC) > 100	0.22	±	0.06	
L01BC05	Gemcitabine	*D. magna*	Acute test 48 h, EC_{50}	(Zounkova et al., 2010)	110	0.21	±	0.02	1.93E−06
		O. mykiss	Survival 96 h, LC_{50}	(Besse et al., 2012)	> 1000				2.13E−07
L01XE05	Sorafenib	*nd*				0.21	±	0.02	
L01XA03	Oxaliplatin	*nd*				0.20	±	0.05	
L01XX19	Irinotecan	*nd*				0.18	±	0.02	
L01AA01	Cyclophosphamide	*D. magna*	Immobilization, EC_{50}	(Zounková et al., 2007)	> 1000	0.11	±	0.01	1.14E−07
		B. rerio	Mortality, EC_{50}	(Zounková et al., 2007)	70				1.62E−06
L01BC03	Tegafur	*nd*				0.10	±	0.01	
L02BB03	Bicalutamide	*D. magna*	24 h (static), EC_{50}	(AstraZeneca, 2006)	> 1	6.03	±	0.47	6.03E−03
		B. Sunfish	96 h (static), LC_{50}	(AstraZeneca, 2006)	> 4.4				1.37E−03
L02AB01	Megestrol	*D. magna*	48 h, LC_{50}	(FDA, 1996)	5	0.72	±	0.06	1.43E−04
L02BB01	Flutamide	*D. magna*	Mortality, 24 h, EC_{50}	(MistraPharma, 2010)	7.8	0.72	±	0.19	9.29E−04
L02AB02	Medroxyprogesterone	*D. magna*	48 h, EC_{50}	(Publichem, 2013)	100	0.17	±	0.01	1.84E−04
L03AX13	Glatiramer acetate	*nd*				0.19	±	0.02	
L04AA06	Mycophenolic acid	*D. magna*	48 h, EC_{50}	(Roche, 2014)	> 100	77.4	±	3.4	7.75E−04
L04AA13	Leflunomide	*D. magna*	48 h, EC_{50}	(USP, 2007)	17	0.86	±	0.02	5.06E−05
		D. rerio	48 h, LC_{50}	(USP, 2007)	3.74				2.30E−04
L04AX01	Azathioprine	*D. magna*	48 h, EC_{50}	(Publichem, 2013)	> 100	0.45	±	0.03	4.56E−06
L04AB01	Etanercept	*nd*				0.29	±	0.01	
L04AB02	Infliximab	*nd*				0.24	±	0.01	
L04AB04	Adalimumab	*nd*				0.16	±	0.01	

EC_{50} Effective concentration, which affects 50% of the organisms.
LC_{50} Lethal concentration to 50% of the organisms.
NOEC No Observed Effect Concentration.
SD Standard Deviation.

treatments whereas some others are not used anymore because of the lower efficiency. An extensive data compilation on cytostatics drugs consumption in Catalonia was performed. Antineoplastic agents (L01) were extensively used, but the immunosuppressant mycophenolic acid (L04) was the most consumed drug. In general, the consumption per capita is similar to the data reported in Europe, with the exception of some specific drugs (e.g. 5-fluorouracil). PECs were calculated considering the excreted fraction and the WWTP removal. Out of 132 different drugs, only 2 had PECs higher than 10 ng L^{-1} (mycophenolic acid and hydroxycarbamide). However, the available data of excretion and removal of these drugs is scarce and can change significantly depending on the value selected. In the present study, the worst case scenario was represented.

The PEC/MEC ratio showed that PEC estimation is a good method to estimate concentrations on the aquatic environment. When this ratio could be calculated, it was in an acceptable range for almost all cytostatics. However, for a high number of compounds, the high LODs of the experimental methodology or the low PECs obtained precluded its calculation.

A risk assessment was performed for those drugs with ecotoxicological data available, showing no risk for the aquatic environment of Catalonia. Nevertheless, ecotoxicological data is also scarce and only toxicity for a few individual drugs are reported in the literature. Mixture drugs toxicity should be evaluated in order to perform a more accurate risk assessment.

In the light of the results, a better knowledge concerning the excretion and removal of some of these drugs should be acquired, in order to calculate more accurate PECs. In addition, the analysis of cytostatic drugs in river water should include the study of mycophenolic acid and hydroxycarbamide.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2015.02.015.

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Supplementary information

Table SI1. Annual consumption (g) in pharmacies and hospitals from Catalonia.

		G03	H02	L01	L02	L03	L04
2010	Pharmacies	26400	221940	1481040	513310	0	2203540
	Hospitals	0	0	256290	0	4210	17420
2011	Pharmacies	25590	228690	1532220	494170	0	2336170
	Hospitals	0	0	273150	1	4710	18380
2012	Pharmacies	21890	231870	868990	430230	0.29	2393550
	Hospitals	0	0	768820	18920	4920	20740

Table SI2. Consumption (g year^{-1}) in pharmacies and hospitals from Catalonia.

			2010 (g year^{-1})			2011 (g year^{-1})			2012 (g year^{-1})		
Therapeuticgroup	**ATC code**	**Name**	**Pharmacies**	**Hospitals**	**Total**	**Pharmacies**	**Hospitals**	**Total**	**Pharmacies**	**Hospitals**	**Total**
G03-Sex hormones and modulators of the genital system	G03HA01	Cyproterone	26399		26399	25589		25589	21888		21888
H02-Corticosteroids for systemic use	H02AB07	Prednisone	221940		221940	228687		228687	231869		231869
L01-Antineoplastic agents	L01AA01	Cyclophosphamide	4525		4525	4186		4186	4066		4066
	L01AA02	Chlorambucil	195		195	199		199	157		157
	L01AA03	Melphalan	101	5	106	110	7	117	113	6	118
	L01AA06	Ifosfamide		4714	4714		8152	8152		6123	6123
	L01AA09	Bendamustine		0	0		0	0		160	160
	L01AB01	Busulfan	7		7	8	2	10	6	7	14
	L01AD02	Lomustine		0	0		0	0		0	0
	L01AD04	Streptozocin		291	291		407	407		364	364
	L01AD05	Fotemustine		27	27		50	50		23	23
	L01AX03	Temozolomide	105	3720	3824	73	3783	3856	14	3723	3737
	L01AX04	Dacarbazine	31		31	15		15	41		41
	L01BA01	Methotrexate	7828	413	8240	8581	515	9096	8679	573	9252
	L01BA03	Raltitrexed		3	3		3	3		2	2
	L01BA04	Pemetrexed		2978	2978		3079	3079		3204	3204

			2010 (g year^{-1})			2011 (g year^{-1})			2012 (g year^{-1})		
Therapeuticgroup	**ATC code**	**Name**	**Pharmacies**	**Hospitals**	**Total**	**Pharmacies**	**Hospitals**	**Total**	**Pharmacies**	**Hospitals**	**Total**
	L01BB02	Mercaptopurine	7246		7246	7703		7703	8523		8523
	L01BB03	Tioguanine	53		53	32		32	34		34
	L01BB04	Cladribine		2	2	0	2	2		2	2
	L01BB05	Fludarabine	47	55	103	52	63	116	37	65	102
	L01BB06	Clofarabine		0	0.0		0.4	0.4		0.7	0.7
	L01BB07	Nelarabine		0	0.0		0.5	0.5		4.0	4.0
	L01BC01	Cytarabine		803	803		1265	1265		1395	1395
	L01BC02	Fluorouracil	2789		2789	2493		2493	535		535
	L01BC03	Tegafur	20184		20184	17608		17608	18288		18288
	L01BC05	Gemcitabine		35440	35440		32476	32476		31772	31772
	L01BC06	Capecitabine	824466	0.0	824466	829548	0	829548	186054	493940	679994
	L01BC07	Azacitidine		922	922		1200	1200		1226	1226
	L01BC53	Tegafur, combinations	984		984	576		576	204		204
	L01CA01	Vinblastine	0.5		0.5	0.5		0.5	0.6		0.6
	L01CA02	Vincristine	0.1		0.1	0.1		0.1	0.1		0.1
	L01CA04	Vinorelbine		555	555		577	577		575	575
	L01CA05	Vinflunine		2	2		121	121		15	15
	L01CB01	Etoposide	224		224	219		219	191		191
	L01CD01	Paclitaxel		4018	4018		4662	4662		5239	5239
	L01CD02	Docetaxel		2226	2226		3291	3291		2326	2326
	L01CD04	Cabazitaxel		0.00	0		0	0		4	4
	L01CX01	Trabectedin		0.08	0		0	0		0	0
	L01DB01	Doxorubicin	22	166	188	18	201	219	12	112	124
	L01DB03	Epirubicin	52		52	29		29	19		19
	L01DB06	Idarubicin	0.1		0.1	0.5		0.5	0.4		0.4
	L01DB07	Mitoxantrone	1		1	1		1			0
	L01DC01	Bleomycin	0.0		0.0	0.0		0.0	0.0		0.0

			2010 (g year^{-1})			2011 (g year^{-1})			2012 (g year^{-1})		
Therapeuticgroup	ATC code	Name	Pharmacies	Hospitals	Total	Pharmacies	Hospitals	Total	Pharmacies	Hospitals	Total
	L01DC03	Mitomycin	46		46	40		40	35		35
	L01XA01	Cisplatin		1337	1337		1299	1299		1491	1491
	L01XA02	Carboplatin	167		167	117		117	70		70
	L01XA03	Oxaliplatin		3181	3181		4871	4871		3313	3313
	L01XC02	Rituximab		6261	6261		6295	6295		6467	6467
	L01XC03	Trastuzumab		4714	4714		5144	5144		5591	5591
	L01XC04	Alemtuzumab		13	13		10	10		9	9
	L01XC06	Cetuximab		5773	5773		5675	5675		5349	5349
	L01XC07	Bevacizumab		5196	5196		3964	3964		3088	3088
	L01XC08	Panitumumab		528	528		475	475		637	637
	L01XD03	Methylaminolevulinate	321		321	312		312	271		271
	L01XD04	Aminolevulinicacid			0						
	L01XE01	Imatinib	1116	88178	89294	678	93817	94495	150	95387	95537
	L01XE02	Gefitinib	0	724	724	60	4280	4340	0	6578	6578
	L01XE03	Erlotinib	514	11731	12245	273	10213	10486	90	8268	8358
	L01XE04	Sunitinib	3	1176	1179		1574	1574		1412	1412
	L01XE05	Sorafenib	672	26020	26692	112	26588	26700	22	22822	22845
	L01XE06	Dasatinib		2179	2179	4	2306	2310		2964	2964
	L01XE07	Lapatinib	595	24228	24823	455	23677	24132		22515	22515
	L01XE08	Nilotinib	22	15154	15177		19486	19486		23616	23616
	L01XE09	Temsirolimus		17	17		29	29		26	26
	L01XE10	Everolimus		0	0		35	35		13	13
	L01XE11	Pazopanib		0	0		162	162		3428	3428
	L01XX05	Hydroxycarbamide	583400		583400	635790		635790	619590		619590
	L01XX08	Pentostatin		0.5	0.5		0.1	0.1		0.3	0.3
	L01XX09	Miltefosine	0.6		0.6	2		2	0.6		0.6

			2010 (g year^{-1})			2011 (g year^{-1})			2012 (g year^{-1})		
Therapeuticgroup	**ATC code**	**Name**	**Pharmacies**	**Hospitals**	**Total**	**Pharmacies**	**Hospitals**	**Total**	**Pharmacies**	**Hospitals**	**Total**
	L01XX11	Estramustine	9744		9744	8442		8442	6454		6454
	L01XX14	Tretinoin	211		211	233		233	190		190
	L01XX17	Topotecan		12	12		4	4		12	12
	L01XX19	Irinotecan		3500	3500		3362	3362		3768	3768
	L01XX23	Mitotane	6250		6250	7800		7800	14050		14050
	L01XX25	Bexarotene	2130	0	2130	2648	0	2648	923	1179	2101
	L01XX27	Arsenictrioxide		4	4		2	2		0.5	0.5
	L01XX32	Bortezomib		22	22		20	20		21	21
	L01XX33	Celecoxib	6816		6816	3624		3624			0
	L01XX35	Anagrelide	174		174	175		175	168		168
	TOTAL L01		1481043	256289	1737332	1532215	273146	1805362	868987	768815	1637803
L02-Endocrine Therapy	L02AA01	Dietilestilbestrol		0	0		1.0	1.0		0.6	0.6
	L02AA04	Fosfestrol	2		2			0			0
	L02AB01	Megestrol	225998		225998	224275		224275	196474		196474
	L02AB02	Medroxyprogesterone	3462		3462	3794		3794	3680		3680
	L02AE01	Buserelin	7		7	6		6	3		3
	L02AE02	Leuprorelin	744		744	691		691	540		540
	L02AE03	Goserelin	74		74	65		65	53		53
	L02AE04	Triptorelin	284		284	290		290	263		263
	L02BA01	Tamoxifen	48640		48640	49731		49731	49372		49372
	L02BA02	Toremifene	197		197	10		10	6		6
	L02BA03	Fulvestrant	181		181	254		254	284		284
	L02BB01	Flutamide	94651		94651	76184		76184	55803		55803
	L02BB03	Bicalutamide	110436		110436	110279		110279	96075		96075
	L02BG03	Anastrozole	1470		1470	1291		1291	1066		1066
	L02BG04	Letrozole	5864		5864	6470		6470	6815		6815

			2010 (g year^{-1})			2011 (g year^{-1})			2012 (g year^{-1})		
Therapeuticgroup	**ATC code**	**Name**	**Pharmacies**	**Hospitals**	**Total**	**Pharmacies**	**Hospitals**	**Total**	**Pharmacies**	**Hospitals**	**Total**
	L02BG06	Exemestane	21296		21296	20831		20831	19796		19796
	L02BX03	Abiraterone		0	0	0	0	0		18915	18915
	TOTAL L02		513308	0	513308	494169	1	494170	430229	18916	449145
L03-Immunostimulants	L03AA02	Filgrastim		23	23		26	26		30	30
	L03AA10	Lenograstim		0.7	0.7		0.5	0.5		0.5	0.5
	L03AA13	Pegfilgrastim		42	42		24	24		22	22
	L03AB03	Interferon gamma	0.1		0	0.1		0	0.1		0.1
	L03AB04	Interferon alfa-2a	0.04		0.04	0.02		0.02	0.01		0.01
	L03AB05	Interferon alfa-2b	0.4		0	0.3		0	0.2		0.2
	L03AB07	Interferon beta-1a		5	5		4	4		3	3
	L03AB08	Interferon beta-1b		42	42		40	40		41	41
	L03AB10	Peginterferon alfa-2b		2	2		1	1		1	1
	L03AB11	Peginterferon alfa-2a		6	6		6	6		4	4
	L03AC01	Aldesleukin		0.1	0.1		0.1	0.1		0.1	0.1
	L03AX03	BCG vaccine		933	933		982	982		835	835
	L03AX13	Glatirameracetate		3155	3155		3629	3629		3981	3981
	L03AX16	Plerixafor		0	0		1	1		0.4	0.4
	TOTAL L03		0.6	4209	4210	0.4	4714	4715	0.3	4918	4918
L04-Immunosupressants	L04AA04	Antithymocyteimmunoglobulin (rabbit)		0.5	0.5		1.5	1.5		0.5	0.5
	L04AA06	Mycophenolicacid	1858604	205	1858809	1973844	75	1973919	2026832	365	2027197
	L04AA10	Sirolimus	318		318	313		313	307		307
	L04AA13	Leflunomide	16103		16103	16877		16877	16853		16853
	L04AA18	Everolimus	239		239	313		313	362		362

			2010 (g year^{-1})			2011 (g year^{-1})			2012 (g year^{-1})		
Therapeuticgroup	**ATC code**	**Name**	**Pharmacies**	**Hospitals**	**Total**	**Pharmacies**	**Hospitals**	**Total**	**Pharmacies**	**Hospitals**	**Total**
	L04AA23	Natalizumab		1105	1105		1240	1240		1277	1277
	L04AA24	Abatacept		958	958		982	982		1123	1123
	L04AA25	Eculizumab		191	191		207	207		276	276
	L04AA27	Fingolimod		0	0		0	0		10	10
	L04AB01	Etanercept	90	5504	5594	45	5214	5259	8	5266	5274
	L04AB02	Infliximab		4445	4445		4484	4484		4669	4669
	L04AB04	Adalimumab		2806	2806		2977	2977		3113	3113
	L04AB05	Certolizumabpegol		8	8		205	205		514	514
	L04AB06	Golimumab		2	2		33	33		82	82
	L04AC03	Anakinra		842	842		996	996		1480	1480
	L04AC05	Ustekinumab	39	28	68	34	50	83	9	85	94
	L04AC07	Tocilizumab		740	740		1438	1438		1946	1946
	L04AC08	Canakinumab		0	0		2	2		3	3
	L04AD01	Ciclosporin	120429	5	120434	117332	0	117332	111538	6	111544
	L04AD02	Tacrolimus	5825	0	5825	6222	0	6222	6688	0	6688
	L04AX01	Azathioprine	201890	8	201898	221190	0	221190	230955	1	230956
	L04AX04	Lenalidomide		574	574		473	473		525	525
	TOTAL L04		2203538	17421	2220958	2336170	18377	2354546	2393550	20742	2414292

Table SI3. Consumption (mg inhab^{-1}day^{-1}) of the cytostatics studied.

		France[+]			UK[++]						Germany			Spain						Catalonia	
		Besse et al. 2012 (E)			Rowney et al. 2009 (E+R)			Booker et al. 2014 (E+R)			Kummerer et al. 2010 (E+R)			Ortiz de Garcia et al. 2013 (E+R)			Martín et al. 2013 (R)			This study (mean) (E+R)	
ATC	Name	C	PEC_{river}	PECc	C	PEC_{eff}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}
G03HA01	Cyproterone																			8.88	0.73
H02AB07	Prednisone																			82.0	1.58
L01AA01	Cyclophosphamide	12.7	>1.74	0.23	20.6	70.2	1.89	40	4.1	1.10	8.90	0.6	0.19	74.5	4.35	1.68	4.70	4.56†	1.76	1.54	0.11
L01AA02	Chlorambucil	0.34	*0.19	0.03																0.07	2E-4
L01AA03	Melphalan	0.20	*0.11	0.01				0	0	0										0.04	2E-3
L01AA06	Ifosfamide	4.28	1.18	0.16				1	0.1	0.03	8.90	0.6	0.19	20.5	3.80	1.47	3.56	1.15	0.44	2.28	0.34
L01AA09	Bendamustine							0	0	0										0.02	2E-3
L01AB01	Busulfan	0.00	*0.001	0.00																4E-3	2E-05
L01AD02	Lomustine	0.13	*0.07	0.01																0.00	0
L01AD04	Streptozocin	0.35	0.02	0.00																0.13	4E-03
L01AD05	Fotemustine	0.05	*0.03	0.00																0.01	2E-03
L01AX03	Temozolomide	2.22	0.12	0.02	0.84	0.418	0.011	1	0	0										1.37	0.08
L01AX04	Dacarbazine	1.22	0.34	0.04				0	0	0										0.01	0.00
L01BA01	Methotrexate	3.10	1.54	0.20				1	0.2	0.05							1.53	0†	0	3.19	0.04
L01BA03	Raltitrexed	0.00	*0.001	0.00																9E-4	1E-4
L01BA04	Pemetrexed	1.55	0.77	0.10				1	0	0										1.11	0.29
L01BB02	Mercaptopurine	3.94	0.15	0.02																2.82	0.33
L01BB03	Tioguanine	0.09	*0.05	0.01																0.01	2E-3
L01BB04	Cladribine	0.00	*0.001	0.00																6E-4	8E-5
L01BB05	Fludarabine	0.23	*0.13	0.02	0.21	0.336	0.009	0	0	0										0.04	0.01
L01BB06	Clofarabine	0.01	*0.003	0.00																2E-4	2E-5
L01BB07	Nelarabine																			5E-4	1E-5
L01BC01	Cytarabine	5.55	<0.31	0.04				1	0	0							2.23	0.93	0.36	0.42	0.01
L01BC02	Fluorouracil	71.9	7.91	1.04				12	0.9	0.24							45.4	44.1†	17.0	0.70	0.01
L01BC03	Tegafur	1.55	*0.85	0.11				0	0	0										6.74	0.10
L01BC05	Gemcitabine	15.7	0.87	0.11	14.2	3.56	0.10	7	0.2	0.05				51.2	1.83	0.71	6.65	6.46†	2.49	12.0	0.21
L01BC06	Capecitabine	213	3.52	0.46	**312	13.7	0.37	183	2.3	0.62										280	7.76
L01BC07	Azacitidine							0	0	0										0.29	0.07
L01BC53	Tegafur, combinations																			0.21	0.03
L01CA01	Vinblastine	0.03	*0.02	0.00																3E-4	5E-7

		France[+]			UK[++]						Germany			Spain						Catalonia	
		Besse et al. 2012 (E)			Rowney et al. 2009 (E+R)			Booker et al. 2014 (E+R)			Kummerer et al. 2010 (E+R)			Ortiz de Garcia et al. 2013 (E+R)			Martín et al. 2013 (R)			This study (mean) (E+R)	
ATC	Name	C	PEC_{river}	PECc	C	PEC_{eff}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}
L01CA02	Vincristine	0.01	*0.003	0.00																4E-5	1E-6
L01CA04	Vinorelbine	0.54	0.003	0.00				0	0	0							0.12	0.12	0.05	0.21	4E-3
L01CA05	Vinflunine																			0.02	2E-4
L01CB01	Etoposide	1.71	0.87	0.11				1	0.1	0.03							0.40	0†	0	0.08	0.02
L01CD01	Paclitaxel	1.61	0.16	0.02				0	0	0							0.80	0.78	0.30	1.67	0.08
L01CD02	Docetaxel	1.14	<0.05	0.01				0	0	0							0.29	0.28	0.11	0.94	0.04
L01CD04	Cabazitaxel																			5E-4	3E-6
L01CX01	Trabectedin	0.00	*0.0003	0.00																9E-5	3E-7
L01DB01	Doxorubicin	0.70	0.19	0.03	1.52	0.343	0.009	0	0	0							0.16	0.16	0.06	0.06	0.01
L01DB03	Epirubicin	0.73	*0.40	0.05	0.90	0.09	0.002	0	0	0							0.25	0.25	0.10	0.01	3E-4
L01DB06	Idarubicin	0.01	*0.004	0.00																9E-5	2E-6
L01DB07	Mitoxantrone	0.01	*0.006	0.00				0	0	0										2E-4	9E-6
L01DC01	Bleomycin	0.04	*0.02	0.00				0	0	0										8E-7	2E-7
L01DC03	Mitomycin	0.12	>0.007	0.00				0	0	0				0.78	0.028	0.011	0.09	0.09	0.03	0.01	4E-4
L01XA01	Cisplatin	0.94	*0.52	0.07	1.09	0.601	0.02	0	0	0										0.50	0.09
L01XA02	Carboplatin	3.47	1.91	0.25	8.01	21.9	0.59	3	0.2	0.05										0.04	0.01
L01XA03	Oxaliplatin	1.39	*0.76	0.10	0.43	0.499	0.013	1	0.1	0.03										1.37	0.20
L01XC02	Rituximab	3.02	*1.65	0.22																2.29	0.34
L01XC03	Trastuzumab	2.32	*1.28	0.17																1.86	0.27
L01XC04	Alemtuzumab	0.03	*0.02	0.00																4E-3	6E-4
L01XC06	Cetuximab	2.28	*1.26	0.17																2.02	0.30
L01XC07	Bevacizumab	3.62	*1.99	0.26				0	0	0										1.47	0.22
L01XC08	Panitumumab	0.05	*0.03	0.00																0.20	0.03
L01XD03	Methylaminolevulinate	0.10	*0.05	0.01																0.11	0.02
L01XD04	Aminolevulinicacid																			0	0
L01XE01	Imatinib	36.3	4.99	0.66				10	0.5	0.13										33.6	2.34
L01XE02	Gefitinib																			1.40	0.02
L01XE03	Erlotinib	6.18	<0.07	0.01				2	0.1	0.03										3.74	0.02
L01XE04	Sunitinib	0.83	*0.46	0.06				0	0	0										0.50	0.02
L01XE05	Sorafenib							5	0	0										9.16	0.20
L01XE06	Dasatinib																			0.90	0.05
L01XE07	Lapatinib	4.82	1.86	0.25				2	0	0										8.59	0.26

		France[+]			UK[++]			Germany			Spain						Catalonia	
		Besse et al. 2012 (E)			Rowney et al. 2009 (E+R)			Booker et al. 2014 (E+R)			Kummerer et al. 2010 (E+R)			Ortiz de Garcia et al. 2013 (E+R)			Martín et al. 2013 (R)			This study (mean) (E+R)	
ATC	Name	C	PEC_{river}	PECc	C	PEC_{eff}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}
L01XE08	Nilotinib	2.44	0.8	0.11				0	0	0										7.00	0.31
L01XE09	Temsirolimus	0.05	*0.03	0.00																0.01	1E-4
L01XE10	Everolimus																			0.01	8E-5
L01XE11	Pazopanib							0	0	0										0.43	0.08
L01XX05	Hydroxycarbamide	284	78.07	10.3				33	0.5	0.13										221	32.1
L01XX08	Pentostatin	0.00	*0.001	0.00																9E-5	3E-5
L01XX09	Miltefosine	0.01	*0.005	0.00																4E-4	5E-5
L01XX11	Estramustine	11.9	0.33	0.04																2.96	0.01
L01XX14	Tretinoin	0.14	*0.07	0.01				0	0	0										0.08	1E-3
L01XX17	Topotecan	0.01	*0.005	0.00				0	0	0										3E-3	6E-4
L01XX19	Irinotecan	1.93	>0.53	0.07				0	0	0							0.34	0.33	0.13	1.28	0.18
L01XX23	Mitotane	9.70	3.20	0.42				2	0	0										3.38	0.05
L01XX25	Bexarotene	0.98	*0.54	0.07																0.83	1E-4
L01XX27	Arsenictrioxide	0.00	*0.001	0.00																7E-4	3E-5
L01XX32	Bortezomib	0.01	*0.005	0.00				0	0	0										0.01	1E-3
L01XX33	Celecoxib																			1.25	3E-3
L01XX35	Anagrelide	0.05	*0.03	0.00																0.06	2E-4
L02AA01	Dietilestilbestrol																			2E-4	6E-6
L02AA04	Fosfestrol																			3E-4	1E-5
L02AB01	Megestrol													79.3	15.96	6.16				77.7	0.72
L02AB02	Medroxyprogesterone																			1.31	0.17
L02AE01	Buserelin	0.00	*0.001	0.00																2E-3	1E-4
L02AE02	Leuprorelin	0.13	*0.07	0.01																0.24	3E-3
L02AE03	Goserelin	0.05	*0.03	0.00																0.02	6E-3
L02AE04	Triptorelin	0.08	*0.05	0.01																0.10	0.001
L02BA01	Tamoxifen	15.6	*8.61	1.14										15.0	0.209	0.08				17.7	0.005
L02BA02	Toremifene	0.04	*0.02	0.00																0.03	2E-4
L02BA03	Fulvestrant	0.28	*0.15	0.02																0.09	2E-5
L02BB01	Flutamide	21.6	<1.19	0.16										116	0.646	0.25				27.2	0.72
L02BB03	Bicalutamide	35.8	10.84	1.43																38.1	6.03
L02BG03	Anastrozole	1.32	*0.72	0.10																0.46	0.01
L02BG04	Letrozole	1.42	*0.78	0.10																2.30	0.04
L02BG06	Exemestane	7.56	*4.16	0.55																7.44	2E-3

		France[+]			UK[++]			Germany			Spain						Catalonia	
		Besse et al. 2012 (E)			Rowney et al. 2009 (E+R)			Booker et al. 2014 (E+R)			Kummerer et al. 2010 (E+R)			Ortiz de Garcia et al. 2013 (E+R)			Martín et al. 2013 (R)			This study (mean) (E+R)	
ATC	Name	C	PEC_{river}	PECc	C	PEC_{eff}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}
L02BX03	Abiraterone																			2.27	0.05
L03AA02	Filgrastim																			9E-3	1E-03
L03AA10	Lenograstim																			3E-4	6E-07
L03AA13	Pegfilgrastim																			0.01	2E-03
L03AB03	Interferon gamma																			8E-6	5E-06
L03AB04	Interferon alfa-2a																			1E-4	1E-06
L03AB05	Interferon alfa-2b																			9E-5	2E-05
L03AB07	Interferon beta-1a																			2E-3	2E-04
L03AB08	Interferon beta-1b																			0.01	2E-03
L03AB10	Peginterferon alfa-2b																			1E-3	8E-05
L03AB11	Peginterferon alfa-2a																			2E-3	3E-04
L03AC01	Aldesleukin																			4E-5	5E-06
L03AX03	BCG vaccine																			0.33	0.05
L03AX13	Glatirameracetate																			1.29	0.19
L03AX16	Plerixafor																			2E-4	3E-5
L04AA04	Antithymocyteimmuno globulin (rabbit)																			2E-4	4E-5
L04AA06	Mycophenolicacid																			704	77.4
L04AA10	Sirolimus																			0.11	2E-4
L04AA13	Leflunomide																			5.99	0.86
L04AA18	Everolimus																			0.11	0.01
L04AA23	Natalizumab																			0.44	0.06
L04AA24	Abatacept																			0.37	0.05
L04AA25	Eculizumab																			0.08	0.01
L04AA27	Fingolimod																			1E-3	2E-6
L04AB01	Etanercept																			1.94	0.29
L04AB02	Infliximab																			1.63	0.24
L04AB04	Adalimumab																			1.07	0.16
L04AB05	Certolizumabpegol																			0.09	0.01
L04AB06	Golimumab																			0.01	2E-3

		France[+]			UK[++]			Germany			Spain						Catalonia	
		Besse et al. 2012 (E)			Rowney et al. 2009 (E+R)			Booker et al. 2014 (E+R)			Kummerer et al. 2010 (E+R)			Ortiz de Garcia et al. 2013 (E+R)			Martín et al. 2013 (R)			This study (mean) (E+R)	
ATC	Name	C	PEC_{river}	PECc	C	PEC_{eff}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}	PECc	C	PEC_{river}
L04AC03	Anakinra																			0.40	0.06
L04AC05	Ustekinumab																			0.03	4E-3
L04AC07	Tocilizumab																			0.50	0.07
L04AC08	Canakinumab																			6E-4	1E-4
L04AD01	Ciclosporin																			42.0	0.01
L04AD02	Tacrolimus																			2.25	3E-3
L04AX01	Azathioprine																			78.6	0.46
L04AX04	Lenalidomide																			0.19	0.04

+ Coetsier et al. 2009 (E+R) ($C/PEC_{river}/PEC_c$): Ifosfamide: 5.3/8/0.32 ;Tamoxifen: 15.1/7/0.29

++ Johnson et al. 2008 (E) ($C/PEC_{river}/PEC_c$): Fluorouracil: 46.0/23/0.71;Capecitabine 79.0/39/1.21

C: consumption (µg $inhab^{-1}$ day^{-1})

PECriver: PEC in river (ng L^{-1})

PECeff: PEC in WWTP effluent (ng L^{-1})

PECriver-dil: PEC in river (ng L^{-1}), according to dilution factors proposed by Keller et al. (Keller et al., 2014). France: 75.73; Spain: 25.92; UK: 37.16; German: 32.30

**capecitabine with 5-fluorouracil

† Recalculated in the present table.

*Conservative PEC (0% removal, 0% excretion).

E: excretion rate considered.

R: removal rate considered

Taula SI4.PEC values of L, G and H ATC drugs studied (sorted by decreasing value for each therapeutic group)

							2010			2011			2012		
Therapeuticgroup	**ATC code**	**Name**	**F_{exc}**	**Ref**	**F_{wwtp}**	**Ref**	**C g day^{-1}**	**PEC_{eff} ng L^{-1}**	**PEC_{river} ng L^{-1}**	**C g day^{-1}**	**PEC_{eff} ng L^{-1}**	**PEC_{river}ng L^{-1}**	**C g day^{-1}**	**PEC_{eff} ng L^{-1}**	**PEC_{river} ng L^{-1}**
G03-Sex hormones and modulators of the genital system	G03HA01	Cyproterone	0.33	g	0.1525	l	72.3	20.4	0.79	70.1	19.8	0.76	60.0	16.9	0.65
H02-Corticosteroids for systemic use	H02AB07	Prednisone	0.5	a	0.87	m	608	39.9	1.54	626	41.1	1.59	635	41.7	1.61
L01-Antineoplastic agents	L01XX05	Hydroxycarbamide	0.5	c	0.0185	b	1598	791	30.5	1742	863	33.3	1697	841	32.4
	L01BC06	Capecitabine	0.11	h	0.15	c	2259	213	8.22	2273	214	8.27	1863	176	6.78
	L01XE01	Imatinib	0.25	c	0.0578	l	245	58.1	2.24	259	61.5	2.37	262	62.2	2.40
	L01XE08	Nilotinib	0.69	j	0.7802	l	41.6	6.36	0.25	53.4	8.17	0.32	64.7	9.90	0.38
	L01BB02	Mercaptopurine	0.4	j	0.0186	b	19.8	7.86	0.30	21.1	8.36	0.32	23.3	9.25	0.36
	L01XC02	Rituximab	0.5	a	0	a	17.1	8.65	0.33	17.2	8.70	0.34	17.7	8.94	0.34
	L01AA06	Ifosfamida	0.5	c	0	c	12.9	6.52	0.25	22.3	11.3	0.43	16.8	8.46	0.33
	L01BA04	Pemetrexed	0.9	j	0.0185	b	8.16	7.27	0.28	8.43	7.52	0.29	8.78	7.83	0.30
	L01XC03	Trastuzumab	0.5	a	0	a	12.9	6.52	0.25	14.1	7.11	0.27	15.3	7.73	0.30
	L01XC06	Cetuximab	0.5	a	0	a	15.8	7.98	0.31	15.5	7.84	0.30	14.6	7.39	0.29
	L01XE07	Lapatinib	0.67	k	0.8499	l	68.0	6.90	0.27	66.1	6.71	0.26	61.7	6.26	0.24
	L01XE11	Pazopanib	0.7	j	0.078	l	0.00	0.00	0.00	0.44	0.29	0.01	9.39	6.12	0.24
	L01BC05	Gemcitabine	0.1	j	0.4	c	97.1	5.88	0.23	89.0	5.39	0.21	87.0	5.27	0.20
	L01XX19	Irinotecan	0.5	c	0.0269	l	9.59	4.71	0.18	9.21	4.52	0.17	10.3	5.07	0.20
	L01XE05	Sorafenib	0.51	k	0.8518	l	73.1	5.58	0.22	73.1	5.58	0.22	62.6	4.77	0.18
	L01XA03	Oxaliplatin	0.5	j	0	a	8.71	4.40	0.17	13.3	6.73	0.26	9.08	4.58	0.18
	L01XC07	Bevacizumab	0.5	a	0	a	14.2	7.18	0.28	10.9	5.48	0.21	8.46	4.27	0.16
	L01BC07	Azacitidine	0.85	j	0.0185	b	0.00	0.00	0.00	3.29	2.77	0.11	3.36	2.83	0.11
	L01AA01	Cyclophosphamide	0.25	j	0	c	12.4	3.13	0.12	11.5	2.89	0.11	11.1	2.81	0.11
	L01BC03	Tegafur	0.05	k	0.0185	b	55.3	2.74	0.11	48.2	2.39	0.09	50.1	2.48	0.10
	L01XA01	Cisplatin	0.6	h	0.0204	l	3.66	2.17	0.08	3.56	2.11	0.08	4.09	2.42	0.09
	L01CD01	Paclitaxel	0.18	j	0.0938	l	11.0	1.81	0.07	12.8	2.10	0.08	14.3	2.36	0.09
	L01AX03	Temozolomide	0.21	j	0.0185	b	10.5	2.18	0.08	10.6	2.20	0.08	10.2	2.13	0.08
	L01XX23	Mitotane	0.6	c	0.9151	b	17.1	0.88	0.03	21.4	1.10	0.04	38.5	1.98	0.08
	L01XE06	Dasatinib	0.19	j	0.019	b	5.97	1.12	0.04	6.33	1.19	0.05	8.12	1.53	0.06
	L01BA01	Methotrexate	0.9	c	0.95	c	22.6	1.03	0.04	24.9	1.13	0.04	25.3	1.15	0.04
	L01XC08	Panitumumab	0.5	a	0	a	1.45	0.73	0.03	1.30	0.66	0.03	1.75	0.88	0.03

								2010			2011			2012		
Therapeuticgroup	ATC code	Name	F_{exc}	Ref	F_{wwtp}	Ref	C g day^{-1}	PEC_{eff} ng L^{-1}	PEC_{river} ng L^{-1}	C g day^{-1}	PEC_{eff} ng L^{-1}	PEC_{river}ng L^{-1}	C g day^{-1}	PEC_{eff} ng L^{-1}	PEC_{river} ng L^{-1}
	L01CD02	Docetaxel	0.14	j	0.0448	l	6.10	0.82	0.03	9.02	1.22	0.05	6.37	0.86	0.03
	L01XE02	Gefitinib	0.05	c	0.1908	b	1.98	0.08	3E-03	11.9	0.49	0.02	18.0	0.74	0.03
	L01XE04	Sunitinib	0.16	k	0.0352	b	3.23	0.50	0.02	4.31	0.67	0.03	3.87	0.60	0.02
	L01CB01	Etoposide	0.93	c	0.0186	l	0.61	0.56	0.02	0.60	0.55	0.02	0.52	0.48	0.02
	L01XE03	Erlotinib	0.02	c	0.0425	b	33.5	0.65	0.03	28.7	0.56	0.02	22.9	0.44	0.02
	L01XD03	Methylaminolevulinate	0.5	a	0.0185	b	0.88	0.44	0.02	0.85	0.42	0.02	0.74	0.37	0.01
	L01BC53	Tegafur, combinations	0.5	a	0	a	2.70	1.36	0.05	1.58	0.80	0.03	0.56	0.28	0.01
	L01BC01	Cytarabine	0.1	c	0.5	c	2.20	0.11	4E-03	3.46	0.17	0.01	3.82	0.19	0.01
	L01AA09	Bendamustine	0.5	a	0.1579	l	0	0	0	0	0	0	0.44	0.19	0.01
	L01DB01	Doxorubicin	0.5	c	0.0192	l	0.52	0.25	0.01	0.60	0.30	0.01	0.34	0.17	0.01
	L01BB05	Fludarabine	0.6	h	0.0185	b	0.28	0.17	0.01	0.32	0.19	0.01	0.28	0.17	0.01
	L01XX11	Estramustine	0.05	c	0.8828	b	26.7	0.16	0.01	23.1	0.14	0.01	17.7	0.10	4E-03
	L01AD04	Streptozocin	0.1	j	0.0185	b	0.80	0.08	3E-03	1.12	0.11	0.00	1.00	0.10	4E-03
	L01CA04	Vinorelbine	0.2	k	0.7037	l	1.52	0.09	4E-03	1.58	0.09	0.00	1.58	0.09	4E-03
	L01XA02	Carboplatin	1	c	0.59	e	0.46	0.19	0.01	0.32	0.13	0.01	0.19	0.08	3E-03
	L01BC02	Fluorouracil	0.39	e	0.9	c	7.64	0.30	0.01	6.83	0.27	0.01	1.47	0.06	2E-03
	L01AX04	Dacarbazine	0.5	c	0.0185	b	0.08	0.04	2E-03	0.04	0.02	8E-04	0.11	0.06	2E-03
	L01AA03	Melphalan	0.16	j	0.018	b	0.29	0.05	2E-03	0.32	0.05	2E-03	0.32	0.05	2E-03
	L01BB03	Tioguanine	0.54	j	0.0185	b	0.15	0.08	3E-03	0.09	0.05	2E-03	0.09	0.05	2E-03
	L01AD05	Fotemustine	0.5	a	0.0187	b	0.07	0.04	1E-03	0.14	0.07	3E-03	0.06	0.03	1E-03
	L01XX32	Bortezomib	0.5	a	0.0225	b	0.06	0.03	1E-03	0.06	0.03	1E-03	0.06	0.03	1E-03
	L01XX14	Tretinoin	0.63	j	0.9309	l	0.58	0.03	1E-03	0.64	0.03	1E-03	0.52	0.02	9E-04
	L01XX17	Topotecan	0.66	k	0.0185	l	0.03	0.02	8E-04	0.01	0.01	3E-04	0.03	0.02	8E-04
	L01XC04	Alemtuzumab	0.5	a	0	a	0.04	0.02	7E-04	0.03	0.01	5E-04	0.03	0.01	5E-04
	L01DC03	Mitomycin	0.1	c	0.018	l	0.13	0.01	5E-04	0.11	0.01	4E-04	0.10	0.01	4E-04
	L01DB03	Epirubicin	0.1	j	0.0192	l	0.14	0.01	5E-04	0.08	0.01	3E-04	0.05	0.01	2E-04
	L01XX35	Anagrelide	0.01	k	0.0185	b	0.48	0.00	2E-04	0.48	0.00	2E-04	0.46	5E-03	2E-04
	L01AA02	Chlorambucil	0.01	k	0.02	l	0.54	0.01	2E-04	0.54	0.01	2E-04	0.43	4E-03	2E-04
	L01XE09	Temsirolimus	0.05	j	0	a	0.05	0.00	9E-05	0.08	4E-03	2E-04	0.07	4E-03	1E-04
	L01XX25	Bexarotene	0.01	j	0.9403	b	5.84	0.00	1E-04	7.25	0.00	2E-04	5.76	3E-03	1E-04
	L01BA03	Raltitrexed	0.5	j	0.0191	b	0.01	0.00	1E-04	0.01	0.00	1E-04	0.01	3E-03	1E-04
	L01CA05	Vinflunine	0.33	k	0.8688	l	0.01	0.00	1E-05	0.33	0.01	6E-04	0.04	2E-03	7E-05
	L01BB04	Cladribine	0.44	j	0.0185	b	0.01	0.00	1E-04	0.00	2E-03	7E-05	0.00	2E-03	7E-05
	L01XE10	Everolimus	0.05	j	0	a	0	0	0	0.10	5E-03	2E-04	0.03	2E-03	7E-05

							2010			2011			2012		
Therapeuticgroup	ATC code	Name	F_{exc}	Ref	F_{wwtp}	Ref	C g day^{-1}	PEC_{eff} ng L^{-1}	PEC_{river} ng L^{-1}	C g day^{-1}	PEC_{eff} ng L^{-1}	PEC_{river}ng L^{-1}	C g day^{-1}	PEC_{eff} ng L^{-1}	PEC_{river} ng L^{-1}
	L01BB06	Clofarabine	0.6	k	0.0185	b	0	0	0	0.00	7E-04	3E-05	0.00	1E-03	4.E-05
	L01XX08	Pentostatin	0.9	k	0.0185	l	1E-03	0.00	4E-05	0.00	2E-04	7E-06	0.00	8E-04	3.E-05
	L01AB01	Busulfan	0.02	j	0.0185	b	0.02	0.00	2E-05	0.03	5E-04	2E-05	0.04	7E-04	3.E-05
	L01XX09	Miltefosine	0.5	a	0.1174	b	0.00	0.00	3E-05	5E-03	2E-03	8E-05	2.E-03	7E-04	3.E-05
	L01BB07	Nelarabine	0.066	i	0.0185	b	0	0	0	1E-03	9E-05	3E-06	0.01	7E-04	3.E-05
	L01XX27	Arsenictrioxide	0.15	k	0.0185	b	0.01	0.00	6E-05	5E-03	7E-04	3E-05	1.E-03	2E-04	8.E-06
	L01CD04	Cabazitaxel	0.023	k	0.2257	l	0	0	0	0	0	0	0.01	2E-04	8.E-06
	L01DB06	Idarubicin	0.07	j	0.0235	b	3E-04	2E-05	8E-07	1E-03	1E-04	4E-06	1.E-03	7E-05	3.E-06
	L01CA02	Vincristine	0.1	j	0.0674	l	4E-04	4E-05	1E-06	3E-04	3E-05	1E-06	2.E-04	1E-05	6.E-07
	L01CA01	Vinblastine	0.01	j	0.1844	l	1E-03	1E-05	5E-07	1E-03	1E-05	4E-07	2.E-03	1E-05	5.E-07
	L01CX01	Trabectedin	0.01	k	0.0212	l	2E-04	2E-06	9E-08	1E-03	1E-05	5E-07	7.E-04	7E-06	3.E-07
	L01DC01	Bleomycin	0.7	j	0	a	5E-06	3E-06	1E-07	7E-06	5E-06	2E-07	6.E-06	4E-06	2.E-07
	L01AD02	Lomustine	0	k	0.0448	b	0	0	0	0	0	0	1.E-04	0	0
	L01DB07	Mitoxantrone	0.23	j	0.026	b	2E-03	4E-04	1E-05	0.00	0.00	1E-05	0	0	0
	L01XD04	Aminolevulinicacid	0.5	a	0.0185	b	0	0	0	0	0	0	0	0	0
	L01XX33	Celecoxib	0.01	k	0.1237	b	18.7	0.17	0.01	9.93	0.09	3E-03	0	0	0
L02-Endocrine Therapy	L02AB01	Megestrol	0.78	j	0.96	n	619	19.5	0.75	614	19.3	0.75	538	16.9	0.65
	L02BB03	Bicalutamide	0.55	c	0.0264	b	303	163	6.31	302	163	6.30	263	142.2	5.49
	L02BB01	Flutamide	0.1	c	0.1004	b	259	23.5	0.91	209	18.9	0.73	153	13.9	0.54
	L02AB02	Medroxyprogesterone	0.5	a	0.1303	b	9.48	4.16	0.16	10.4	4.56	0.18	10.1	4.42	0.17
	L02BX03	Abiraterone	0.55	j	0.8673	b	0	0	0	0	0	0	51.8	3.82	0.15
	L02BA01	Tamoxifen	0.13	j	0.9309	b	133	1.21	0.05	136	1.23	0.05	135	1.23	0.05
	L02BG04	Letrozole	0.06	f	0.0251	b	16.1	0.95	0.04	17.7	1.05	0.04	18.7	1.10	0.04
	L02AE04	Triptorelin	0.42	k	0	a	0.78	0.33	0.01	0.80	0.34	0.01	0.72	0.31	0.01
	L02BG03	Anastrozole	0.1	f	0.0251	b	4.03	0.40	0.02	3.54	0.35	0.01	2.92	0.29	0.01
	L02AE03	Goserelin	0.9	g	0	a	0.20	0.18	0.01	0.18	0.16	0.01	0.14	0.13	0.01
	L02AE02	Leuprorelin	0.05	j	0.019	l	2.04	0.10	4E-03	1.89	0.09	4E-03	1.48	0.07	3E-03
	L02BG06	Exemestane	0.01	f	0.9	c	58.3	0.06	2E-03	57.1	0.06	2E-03	54.2	0.05	2E-03
	L02AE01	Buserelin	0.2	j	0.0186	l	0.02	0.00	1E-04	0.02	3E-03	1E-04	0.01	2E-03	7E-05
	L02BA02	Toremifene	0.5	a	0.9351	b	0.54	0.02	7E-04	0.03	9E-04	3E-05	0.02	5E-04	2E-05
	L02BA03	Fulvestrant	0.01	j	0.9403	l	0.50	0.00	1E-05	0.70	4E-04	2E-05	0.78	5E-04	2E-05
	L02AA01	Dietilestilbestrol	0.5	a	0.798	b	0	0	0	3E-03	3E-04	1E-05	2E-03	2E-04	7E-06
	L02AA04	Fosfestrol	0.5	a	0.669	b	0.01	0.00	0.00	0	0	0	0	0	0

							2010			2011			2012		
Therapeuticgroup	**ATC code**	**Name**	**F_{exc}**	**Ref**	**F_{wwtp}**	**Ref**	**C g day^{-1}**	**PEC_{eff} ng L^{-1}**	**PEC_{river} ng L^{-1}**	**C g day^{-1}**	**PEC_{eff} ng L^{-1}**	**PEC $_{river}$ng L^{-1}**	**C g day^{-1}**	**PEC_{eff} ng L^{-1}**	**PEC_{river} ng L^{-1}**
L03-Immunostimulants	L03AX13	Glatiramer, acetate	0.5	a	0	a	8.64	4.36	0.17	9.94	5.02	0.19	10.9	5.50	0.21
	L03AX03	BCG vaccine	0.5	a	0	a	2.56	1.29	0.05	2.69	1.36	0.05	2.29	1.15	0.04
	L03AB08	Interferon beta-1b	0.5	a	0	a	0.11	0.06	2E-03	0.11	0.05	2E-03	0.11	0.06	2E-03
	L03AA02	Filgrastim	0.5	a	0	a	0.06	0.03	1E-03	0.07	0.04	1E-03	0.08	0.04	2E-03
	L03AA13	Pegfilgrastim	0.5	a	0	a	0.12	0.06	2E-03	0.07	0.03	1E-03	0.06	0.03	1E-03
	L03AB11	Peginterferon alfa-2a	0.5	a	0	a	0.02	0.01	3E-04	0.02	0.01	3E-04	0.01	0.01	2E-04
	L03AB07	Interferon beta-1a	0.5	a	0	a	0.01	0.01	3E-04	0.01	0.01	2E-04	0.01	4E-03	2E-04
	L03AB10	Peginterferon alfa-2b	0.5	a	0	a	0.01	3E-03	1E-04	4E-03	2E-03	7E-05	3E-03	1E-03	5E-05
	L03AX16	Plerixafor	0.7	k	0.0185	l	0	0	0	3E-03	2E-03	7E-05	1E-03	7E-04	3E-05
	L03AB05	Interferon alfa-2b	0.5	a	0	a	1E-03	6E-04	2E-05	9E-04	4E-04	2E-05	5E-04	3E-04	1E-05
	L03AB03	Interferon gamma	0.5	a	0	a	3E-04	2E-04	6E-06	2E-04	1E-04	4E-06	2E-04	1E-04	5E-06
	L03AC01	Aldesleukin	0.5	a	0	a	4E-04	2E-04	7E-06	2E-04	1E-04	5E-06	2E-04	1E-04	4E-06
	L03AA10	Lenograstim	0.01	k	0	a	2E-03	2E-05	7E-07	1E-03	1E-05	6E-07	1E-03	1E-05	5E-07
	L03AB04	Interferon alfa-2a	0.5	a	0	a	1E-04	5E-05	2E-06	6E-05	3E-05	1E-06	3E-05	1E-05	5E-07
L04-Immunosupressants	L04AA06	Mycophenolicacid	0.63	i	0.41	b	5,093	1,910	73.7	5,408	2,029	78.3	5,554	2,083	80.4
	L04AA13	Leflunomide	0.5	a	0.0291	b	44.1	21.6	0.83	46.2	22.6	0.87	46.2	22.6	0.87
	L04AX01	Azathioprine	0.02	f	0.0185	l	553	11.0	0.42	606	12.0	0.46	633	12.5	0.48
	L04AB01	Etanercept	0.5	a	0	a	15.3	7.73	0.30	14.4	7.27	0.28	14.4	7.29	0.28
	L04AB02	Infliximab	0.5	a	0	a	12.2	6.14	0.24	12.3	6.20	0.24	12.8	6.45	0.25
	L04AB04	Adalimumab	0.5	a	0	a	7.69	3.88	0.15	8.16	4.11	0.16	8.53	4.30	0.17
	L04AC07	Tocilizumab	0.5	a	0	a	2.03	1.02	0.04	3.94	1.99	0.08	5.33	2.69	0.10
	L04AC03	Anakinra	0.5	a	0	a	2.31	1.16	0.04	2.73	1.38	0.05	4.06	2.05	0.08
	L04AA23	Natalizumab	0.5	a	0	a	3.03	1.53	0.06	3.40	1.71	0.07	3.50	1.77	0.07
	L04AA24	Abatacept	0.5	a	0	a	2.62	1.32	0.05	2.69	1.36	0.05	3.08	1.55	0.06
	L04AX04	Lenalidomide	0.82	k	0.0182	l	1.57	1.28	0.05	1.29	1.05	0.04	1.44	1.17	0.05
	L04AB05	Certolizumabpegol	0.5	a	0	a	0.02	0.01	4E-04	0.56	0.28	0.01	1.41	0.71	0.03
	L04AA25	Eculizumab	0.5	a	0	a	0.52	0.26	0.01	0.57	0.29	0.01	0.76	0.38	0.01
	L04AD01	Ciclosporin	0.001	f	0	a	330	0.33	0.01	321	0.32	0.01	306	0.31	0.01
	L04AA18	Everolimus	0.5	a	0.576	l	0.65	0.14	0.01	0.86	0.18	0.01	0.99	0.21	8E-03
	L04AC05	Ustekinumab	0.5	a	0	a	0.19	0.09	4E-03	0.23	0.12	4E-03	0.26	0.13	5E-03
	L04AB06	Golimumab	0.5	a	0	a	0.00	0.00	1E-04	0.09	0.05	2E-03	0.23	0.11	4E-03
	L04AD02	Tacrolimus	0.01	i	0.6017	l	16.0	0.06	2E-03	17.0	0.07	3E-03	18.3	0.07	3E-03
	L04AA10	Sirolimus	0.022	i	0.7078	l	0.87	0.01	2E-04	0.86	0.01	2E-04	0.84	0.01	2E-04
	L04AC08	Canakinumab	0.5	a	0	a	0	0	0	0.01	3E-03	1E-04	0.01	4E-03	2E-04

							2010			2011			2012		
Therapeuticgroup	ATC code	Name	F_{exc}	Ref	F_{wwtp}	Ref	C g day^{-1}	PEC_{eff} ng L^{-1}	PEC_{river} ng L^{-1}	C g day^{-1}	PEC_{eff} ng L^{-1}	PEC_{river}ng L^{-1}	C g day^{-1}	PEC_{eff} ng L^{-1}	PEC_{river} ng L^{-1}
	L04AA04	Antithymocyteimmuno globulin (rabbit)	0.5	a	0	a	1E-03	7E-04	3E-05	4E-03	2E-03	8E-05	1.E-03	6E-04	2E-05
	L04AA27	Fingolimod	0.025	k	0.8036	l	0	0	0	0	0	0	0.03	1E-04	5E-06

a-no data;
b- Chem Spider (Royal Society of Chemistry, 2014)
c- (Besse et al., 2012)
d- Booker et al., 2014
e- Johnson et al., 2013
f- HSDB (U.S. National Library of Medicine)
g- Gómez-Canela, 2013
h- Rowney et al., 2009
i- www.drugs.com;(Wolters Kluwer Health, 2000)
j- BCCA; (Provincial Health Services Authority, 2013)
k- EMC; (DataPharm, 2014)
l- EPISuite(U.S. EPA, 2013)
m- Fan et al., 2011
n- Chang et al., 2011

2.1.2. Article científic II:

*Anticancer drugs: consumption trends in Spain, prediction of environmental concentrations and potential risks.*Helena Franquet-Griell, Cristian Gómez-Canela, Francesc Ventura, Sílvia Lacorte.*Environmental Pollution* (2017)(acceptat)

Anticancer drugs: consumption trends in Spain, prediction of environmental concentrations and potential risks

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ABSTRACT

This study presents the occurrence and impact of 78 anticancer drugs compounds in Spanish river basins based on consumption data during the period 2010-2015 and calculation of the predicted environmental concentrations (PEC). The total consumption of anticancer drugs in Spanish pharmacies was of 23.4 tons in 2015, being mycophenolic acid and hydroxycarbamide the drugs with the highest prescription. Their PECs in river at national scale wereup to 80 ng/L.However, the use of different dilution factors revealed major differences between hydrographic basins, and PEC_{river}rose up to 68,014 ng/L in highly populated rivers with low flows. Concerning acute toxicity, there was no expected risk for the aquatic environment. However, chronic toxicity tests revealed possible long-term mutagenic effects for aquatic organisms. This study provides the tools for the estimation of PEC at river basin scale using time trend consumption data compilation. This information is very useful for prioritization of compounds of concern and permit to focus resources in environmental monitoring and risk evaluation.

Keywords: anticancer drugs; Predicted Environmental Concentrations; pharmacies; risk assessment; river.

Capsule: Predicted Environmental Concentrations calculated from consumption data permits to determine the occurrence and risk of anticancer drugs in waste and river waters.

Abbreviations: DF- dilution factor;EMA- European Medicines Agency; NOEC- no-effect concentration; PEC-of predicted environmental concentrations; RQ- risk quotient; WWTP-wastewater treatment plants;

1. Introduction

Over the last years, concern on the presence of anticancer drugs, or cytostatic, in the environment has remarkably increased, associated with the high cancer incidence (estimated 14.1 million new cases in 2012 worldwide (Cancer Research UK)).These compounds are used in chemotherapy and administered in the order of tons per year(Besse et al., 2012; Franquet-Griell et al., 2015).After use, these pharmaceuticals are excreted through urine or feces as metabolites or as the parent compound and can reach the sewer system. For some of these drugs, it is known that their elimination in wastewater treatment plants (WWTP) is low (Negreira et al., 2014; USEPA, 2006; Zhang et al., 2013)and can reach surface waters(Kosjek and Heath, 2011).In fact, nowadays anticancer drugs have been reported as emerging contaminants in European river watersat concentrations up to hundreds of ng/L (Buerge et al., 2006; Coetsier et al., 2009; Ferrando-Climent et al., 2014; Franquet-Griell et al., 2017; Giebułtowicz and Nałęcz-Jawecki, 2016; López-Serna et al., 2012; Martín et al., 2011).Studies considering short-term toxicity showed high lethal concentrations (Bristol-Myers, 2010; Parrella et al., 2014; Roche, 2014)and additionally interaction with DNA can represent long-term effects for the aquatic organisms (Besse et al., 2012).The environmental risk of these compounds can be high.

Anticancer drugs are classified by the World Health Organization (WHO) under the class L according to the organ or system in which they act. This group is divided in four subgroups: L01, which accounts for the antineoplastic agents; L02 classifies estrogens and progestogens used specifically in the treatment of neoplastic diseases; L03 forimmunostimulants; and L04 for immunosuppressants(www.whocc.no/atcddd). G03 are sex hormones and modulators of the genital system and H02 are corticosteroids for systemic use. The number of drugs listed in class L is up to 300 but this list is periodically revised and 18 new drugs are expected to be included in 2017(WHOCC, 2016).

Due to the large number of drugs currently used in themedical treatments, methods have been developed to prioritize those compounds expected to be detected in the environment. The European Medicines Agency (EMA) proposed the calculation of predicted environmental concentrations (PEC)(EMA, 2006) and suggests to evaluate their presence, environmental fate and effects when PEC values in surface water are equal or above 10 ng/L. This model takes into account the consumption of a specific drug in a given area, its excretion and its elimination in WWTP in order to calculate the predicted concentration. This method has been used to estimate the presence of a high number of pharmaceuticals including antibiotics, lipid regulators, antiphlogistics or fibrate regulators, among others (Carballa et al., 2008; Coetsier et al., 2009;Oosterhuis et al., 2013). This method has been useful to prioritize pharmaceuticals in the environment (Guo et al., 2016) and is especially suited when robust and local data on the system

of interest are available and reflect source inputs (Burns et al., 2017). For the specific case of anticancer drugs, this is a suitable model as the consumption can easily be correlated with the sales of these drugs, because all prescribed amount will be taken by the patient. This information can be used to focus and optimize efforts and resources for the control of these pharmaceuticals in different environmental compartments, such as WWTP effluents and surface waters (Besse et al., 2012; Franquet-Griell et al., 2015). PEC foranticancer drugs have been calculated for a few preselected compounds in Germany (Kümmerer and Al-Ahmad, 2010; Kümmerer et al., 2016), France (Besse et al., 2012), severalEuropean countries (Johnson et al., 2013), NW-UK (Booker et al., 2014) and for all L ATC typedrugs in Catalonia (NE Spain) (Franquet-Griell et al., 2015).In these studies, predicted concentrations for some drugs in surface waters were above the threshold of 10 ng/L for environmental risk assessment suggested by EMA, like hydroxycarbamide with 78 ng/L (Besse et al., 2012) or mycophenolic acid with 77 ng/L (Franquet-Griell et al., 2015).The prognostic is that concentrations will increase over the years. According to Kümmerer et al. (2016) consumption will presumably continue growing up due to the share and age of elderly people which increases the probability of suffering cancer, and the use of antineoplastics in palliative medicine.

Spain has 46.7 million people. In 2015, nearly a 200,000 new invasivecancer cases were diagnosed and the five mostcommon cancers were colon–rectum, prostate,lung, breast and urinary bladder(Galceran et al., 2017). The incidence of cancer reverts in a high consumption of chemotherapeutic drugs, which inevitably are discharged to environmental waters. Spain has eight main hydrographic basins (Miño, Duero, Tajo, Guadiana, Guadalquivir, Segura, Júcar and Ebro) which cover 82% of the Spain surface and 57% of the population. River basins in Spain are highly populated areas affected by agricultural run-off, WWTP and industrial discharges. These discharges, together with the dynamics of the rivers(overall water scarcity, flood events, season linked flow variabilities) results in rivers markedly affected by organic contamination.

Given the large amount of anticancer drugs prescribed in Spain,the aim of this study was to prioritize and evaluate theirrisk by calculating PECs according to global consumption in pharmacies from 2010 to 2015. Raw consumption data fromSpainwere used to calculate PECs accordingly to population and dilution factor in the eight main river basins. The predicted concentrations were combined with available toxicological dataincluding acute and chronic effects to identify compounds with potential risk for the aquatic environment.

2. Materials and methods

2.1. Consumption data

The consumption data of anticancer drugs in Spain was kindly provided by the Ministry of Health, Social Services and Equality, and correspondsto the billing of prescriptions from the Spanish National Health Service through pharmacies. During 2010-2015, a total of 78 different drugs belonging to L01-L04, G03 and H02 groups were administered in Spanish pharmacies. This information does not include consumption through mutual insurance companies neither the consumption in hospitals, as this data was not available at national scale. However, pharmacies have proved to be the main path of anticancer drugadministration, as it represented 78-82% of the total administration according to previous studies (Besse et al., 2012; Franquet-Griell et al., 2015; Kümmerer et al., 2016). Therefore, PEC calculationsconsidering only pharmacies administration will be slightly underestimated but will provide reliable data as it considers the most consumed drugs.

Consumption data was provided as the number of pills, capsules,injections or other presentations of a specific drug, as well as its concentration, from 2010 to 2015. Knowing the concentration of each activity, the total consumption in kg per year was calculated.This data was further normalized to µg/inhab/day to compare consumption patterns in Spain with other countries.

2.2. PEC calculations

To calculate the predicted environmental concentrations in WWTP effluents (PEC_{eff}) and surface waters (PEC_{river}), the following equation was used (Besse et al., 2008):

$$PEC\ (ng/L) = \frac{consumption \cdot Fexc\ (1-Fwwtp)}{W \cdot inhab \cdot DF} 10^9 \qquad (1)$$

where,

- *Consumption* (g/day) is the quantity of each cytostaticdelivered in Spanish pharmacies.
- *Fexc* is theexcretedfractionofthe unchanged drug, considering both urine and feces. Compounds with glucuronide metabolites, which are deconjugated in WWTP processes(Domènech et al., 2011), were also considered. When different values were reported in the bibliography, the highest one was used. Selected values ranged from negligible to >90% depending on the compound. For those drugs whose values could not be found, a default value of 0.5 was applied, considering that a pharmaceutical will not be totally excreted as parental compound (Drugbank database).

- *Fwwtp* is the removal fractioninWWTP. Here, when different data were obtained from the bibliography, the lowest value was applied.In the cases that no information was available a default value of 0 was used to consider the worst case scenario.
- *W* (L/inhab per day) is the water consumption per inhabitant per day as mean of Spanish values (in 2013, 130 L/inhab per day were reported)(INE, 2015).
- *Inhab* is the number of inhabitants in Spain(a mean of 46.6 million inhabitantsduring 2010-2015).

More details about the PECs calculation is given in a previous work (Franquet-Griell et al., 2015).

An important parameter in the equation is the dilution factor (DF) applied from WWTP effluents to surface waters, which is used in the PEC_{river} calculation. Changes in this value can vary the results more than 100-fold. Thus, PEC_{river} have been calculated using different values. Firstly, a DF of 25 suggested by Keller et al. (2014) as the median DF for Spain was used to obtain the PEC_{river} at national scale. This data was used to compare PECs with measured environmental concentrations (MEC) according to published data. Secondly, DFs for the main hydrographic basins in Spain were calculated to obtain a better representation of the contamination levels, taking into account the specific characteristics offlow and population. Studied basins were Duero, Ebro, Guadalquivir, Guadiana, Júcar, Miño, Segura and Tajo, which cover most of the peninsula area (basins information in Table 1). These new DFs were calculated adapting the formula from Keller et al. (2014):

$$DF = \frac{Qr \cdot 31536000}{inhab\ basin \cdot Wbasin} \qquad (2)$$

where,

- *Qr(*m^3/s)is the river flow of each river.The flow data were collected from the hydrographic confederations of each basin and considered geographic and seasonal variability along the basin. Maximum, minimum and mean river flows were used to better estimate PEC variability. The flow data reflect the withdrawal of water in each area.
- $Inhab_{basin}$is the population in the basin area (inhabitants).
- W_{basin} is the water use per capita in the basin, including domestic and industrial use(m^3/inhab/year). If data was not available, water consumption at national scale (130 m^3/inhab/year)was used.
- 31536000 are seconds per year used to convert units.

To calculate PEC_{river} for each basin, the DF derived from high, mean and low flows in each river basin were applied to equation 1 and consumption of anticancer drugs was considered to be proportional to the population in the studied area. Finally, the $\sum PEC_{river}$ considering each individual drug represented the global impact of these compounds in each basin.

2.3 Estimation of risk

After calculating PECs in surface water (PEC_{river}), risk assessment was performed to determine if these predicted concentrations might pose the aquatic environmentin danger. The EMA guidelines recommends performing a risk assessment when PEC are higher than 10 ng/L (EMA, 2006), but in the present study it has been calculated for all drugsconsumed in 2015to better evaluate the overall impact. The risk quotient (RQ) was calculated using the following equation, depending on the available data(Cristale et al., 2013):

$$RQ = \frac{PEC}{PNEC} \approx \frac{PEC}{NOEC/f_1} \approx \frac{PEC}{E(L)C_{50}/f_2} \quad (3)$$

where, PEC is the predicted concentration for a specific basin only in 2015 (described in the previous section) and PNEC is the predicted no-effect concentration.In this case, the mean dilution factor for the Tajo river basin was used, as it exemplifies the mean flow throughout the year. Among river basins, Tajo river is the largest river in Iberian Peninsula and is representative of the situation in Spain. PNEC can be estimated using the NOEC (no-observed effect concentration) and a security factor f_1 of 10. If NOEC was not available, PNEC could also be estimated using $E(L)C_{50}$, which is the toxicological relevant concentration (EC_{50} or LC_{50} for acute toxicity) and a security factor f_2 of 1000. Toxic information was collected for several species, as it is important to evaluate the adverse effects at different trophic levels (Česen et al., 2016).

Thus, to determine the risk posed by a specific drug, Wentsel et al. (1996) established a hazard scale based on the maximum probable risk for ecological effect from contaminated water as follows: if RQ<1.0 no significant risk is expected; if 1.0<RQ<10 small potential for adverse effects is expected; if 10<RQ<100 significant potential for adverse effects can be expected andif RQ>100, potential adverse effects should be expected.

On the other hand, chronic toxicity was also evaluated, considering the effects of anticancer drugs on DNA, RNA or their expression. This information was useful to estimate long-term risks.

Table 1. Main hydrographic basins in Spain, territory information with population and its percentage inhabiting in each basin, area and percentage, calculated dilution factors (DF) using mean, maximum and minimum flows. .

Basin	Population	Area	Length	Water use	Riverflow(m^3/s)		DF	
	Inhab (%)	km^2 (%)	km	L/inhab/day	Mean	Min-max*	Mean	Min-max*
Duero	2,210,541 (8)	78,859 (15.6)	897	295	800	50-942	106	6.6-125
Ebro	3,226,921 (11.8)	85,569 (16.9)	930	319	600	123-992	50.4	10.3-83.3
Guadalquivir	4,107,598 (15.2)	57,527 (11.4)	657	232	164	11-563	14.9	1.0-51
Guadiana	1,472,800 (5.6)	55,528 (11.0)	742	255	26	10-800	6.0	2.3-184
Júcar	5,162,163 (19.2)	42,832 (8.5)	498	130	49	12-80	6.3	1.5-10.3
Miño	858,000 (3.2)	17,619 (3.5)	350	130	340	5-520	263	3.9-403
Segura	1,964,636 (7.4)	19,025 (3.8)	325	130	26	0.1-45	8.9	0.03-15.2
Tajo	7,879,123 (29.2)	55,781 (11.0)	1038	130	43	9-335	3.7	0.8-28.3

* Minimum and maximum flow registered during 2014-2015 near the mouth of the river.

**These drugs correspond to those shown in Table 4 for acute toxicity risk assessment.

3. Results and discussion

3.1. Consumption of cytostatic drugs delivered in Spanish pharmacies

In the case of anticancer drugs, where all drugs prescribed are consumed, usage data is very useful to determine the potential environmental impact of chemicals. Considering all reported compounds, the mean annual consumption was of 23.1±1.3 tons(Figure 1). ATC groupsL01 and L04 showed the highest variation. Figure 2shows the consumption trendsfor relevant drugs according to each ATC group ordered by decreasing consumption, and this criterion will be used in the following section.Further, to compare consumption trends in Spain with other countries, data has been normalized to µg/inhab/day. However, only few publications provide an exhaustive view on the consumption of all cytostatic drugs consumed in each country without a preselection, and most are basically focused in some L01 drugs, which make comparison difficult. Therefore, consumption in pharmacies for a high number of drugs from Spain has been compared to data from France (pharmacy data only) (Besse et al., 2012) and Germany (pharmacy data recalculated from total consumption) (Kümmerer et al., 2016)and Catalonia (NE Spain, only considering pharmacy administration)(Franquet-Griell et al., 2015)(Table SI1 of the supplementary material).

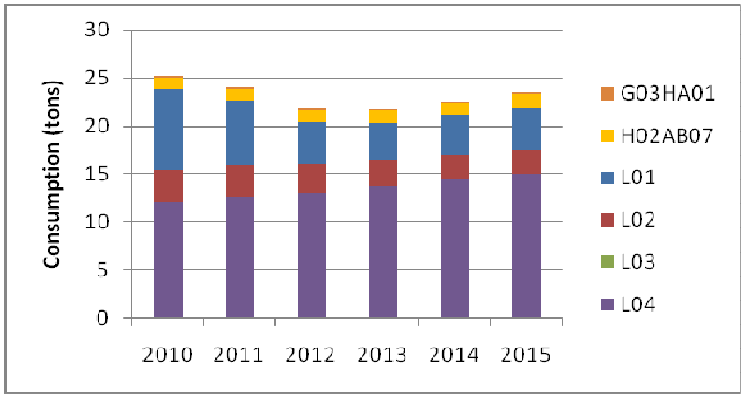

Figure1. Consumption (tn) of cytostatic drugs in Spain during 2010-2015

L01- Antineoplastic agents: 47 drugs belonging to this group were used in 2010with a total consumption of 8.4 tons. In 2015 only 27drugs were registered, with 4.3 tons (Figure 1).Hydroxycarbamide and capecitabine were the most prescribed with levels up to 4.0tonsin 2015and 2010, respectively (Figure 2). However, for capecitabine and imatinib, their consumption in pharmacies significantly decreased in 2013 because these became hospital administered drugs and its consumption data was not further recorded by the Ministry of Health.Other 7 anticancer drugs from this group followed the same decreasing concentration over the period 2010-2015 (tegafur, lapatinib, estramustine, sorafenib, erlotinib, nilotinib and

celecoxib), and mercaptopurine decreased from 2013.Conversely, methotrexate, mitotane increased consumptions andcyclophosphamide stayed constant. For these compounds, consumption was higher than 20 kg per year in 2010. Changes in the posology of anticancer drugs over this period, data from 2015 revealed hydroxycarbamide, tegafur, estramustine, methotrexate, mitotane, and cyclophosphamide as main drugs consumed (>10 kg/year). These, together with capecitabine and imatinib which were highly consumed in 2010, are represented in Figure 2. The rest of the compounds had global consumptions in Spain from 0.01 to 7 kg.

Consumption data was normalized to µg/day/inhab to compare trends in Spain with other countries. For hydroxycarbamide, similar values were found in Spain, Catalonia, France and Germany (265, 277 and 212 µg/day/inhab, respectively)(Table SI1). This trend was also observed for capecitabine, although somewhat lower consumption was observed in Germany. Major differences were observed for imatinib and methotrexate, with consumptions varying more than 100 fold in comparison to other studies. These differences are attributed to different medical protocols and has been suggested that the amounts of cytostatic drugs administered per year and per capita should differ between countries (Kümmerer et al., 2016).

*L02- Endocrine therapy:*A total of 16 drugs which belong to this group were dispensed during 2010-2015, with a decreasing consumption from 3.3 to 2.6 tons(Figure 1).Megestrol acetate was administered up to 1380 kg during this period (Figure 2). It was followed by bicalutamide, tamoxifen, flutamide and exemestane, which had consumptions from 607 to 116 kg respectively in 2015,whereasletrozole, medroxyprogesterone and fulvestrant had consumptions from 41 to 15 kg. The remaining eight drugs were below 10 kg in 2015. Overall, similar amounts were prescribed for endocrine therapy drugs in Spain, Catalonia and France (Table SI1). Because the lack of data from other countries or regions, little conclusions can be extrapolated although it is clear that the ATC L02 group, especially megestrol, deserves attention as it is consumed in high amounts.

*L03- Immunostimulants:*In this group, only 3 drugs were administeredin pharmacies, namely interferon-α2a, interferon-α2b and interferon-γ. Their total consumption decreased from 3 to only 1 gram per yearwhich correspond to $1{\cdot}10^{-5}$ to $3{\cdot}10^{-5}$µg/day/inhab for 2015. (Table 2). It is unlikely that L03 immunostimulats are detected in the environment given those low consumptions. This group has not been reported in any other study.

*L04- Immunosuppressants:*Ten different drugs were consumed from this group, administered with increasing amounts from12 to 15 tons (Figure 1). Of relevance is mycophenolic acid, that represents the 52% of the total anticancer drugs administered and had a consumption of 12.3tons in 2015(Figure 2). This corresponds to 720 and 897 µg/day/inhab in

Spain and Catalonia, 2015, but despite being widely consumed, it has not been considered in other studies. Azathioprine, cyclosporine, leflunomideand tacrolimus had consumptions up to 1906-44 kg, respectively, in 2015. The other compounds, everolimus and sirolimus had consumptions below 3 kg and were not represented in Figure 2. Etanercept, lenalidomide and ustekinumab were consumed in 2010 (0.22 to 1.44 kg) but thereafter were not further dispensed.

*G03 and H02:*Among hormones, cyproterone (G03HA01) had a slightly decreasing consumption in 2010-15 whereasprednisone (H02AB079)increased(Figure 2). Data of these drugs were only available for Catalonia and Spain, which have consumptions of the same order, as expected.

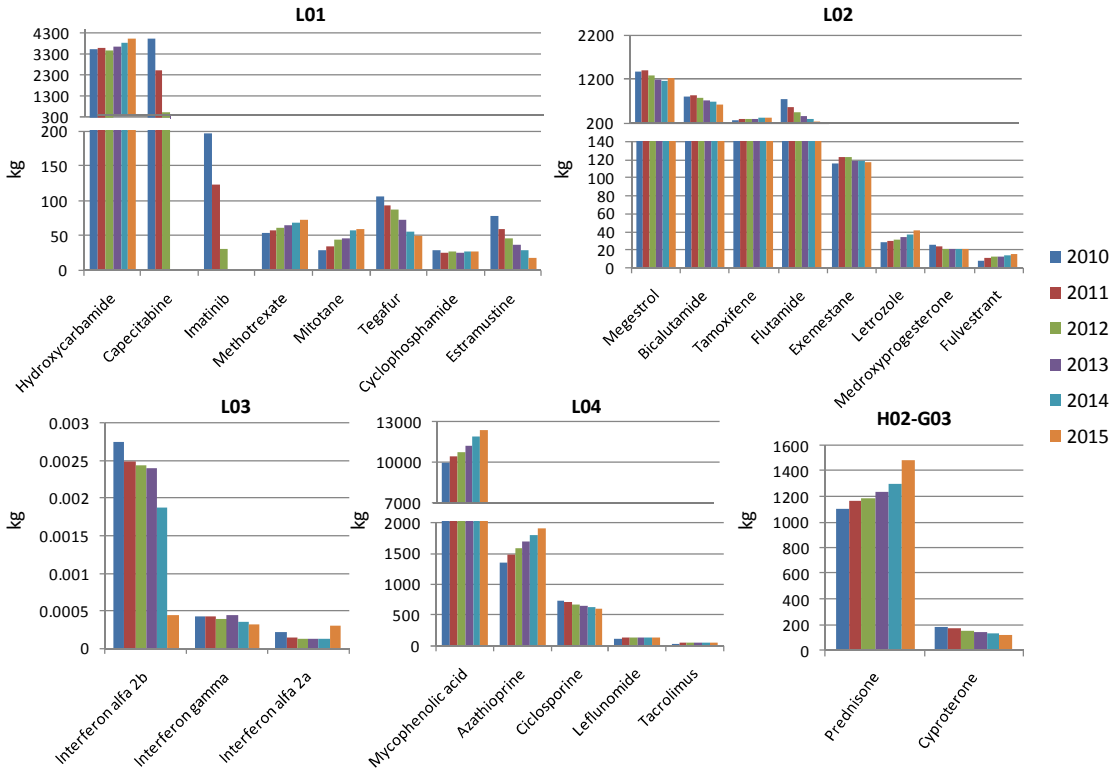

Figure 2. Consumption trends according to each family of cytostatic drugs. Lapatinib and marcaptopurine are not represented as they were not consumed in Spanish pharmacies in 2015, although they were highly consumed in 2010.

Overall, similar pharmacy administrationwas reported for most of the drugs between Spain andother European countries. However, these consumptions are slightly underestimated as no data from hospitals was considered. This underestimation is not significant in the case of some drugs like mycophenolic acid, that had an hospital administration below 0.02% according to a previous study (Franquet-Griell et al., 2015). However, this underestimation is more important in the case of imatinib, which was highly consumed in Catalonia considering hospital

administration (36 µg/day/inhab)according to the same study.For a better global evaluation, more comprehensive data collection is encouraged in order to obtain completeconsumption data especially from L04 group, as drugs like mycophenolic acid proved to be highly administered and were afterwards recurrently detected in the environment (Franquet-Griell et al., 2016; Giebułtowicz and Nałęcz-Jawecki, 2016).

3.2.Predicted environmental concentrations in Spain

Predicted concentrations were calculated for all cytostatic drugs consumed in Spain during the period 2010-2015. Two different PECs (in ng/L) were calculated: PEC_{eff}, which represents the amount expected in WWTP effluents, and PEC_{river},which takes into account the dilution factor from WWTP to surface water and represents the concentration expected in the rivers.In a first step, the DF of 25 proposed by Keller et al. (2014) was taken into consideration. Table 2 shows those drugs with the highest PECs obtained (both PEC_{eff} and PEC_{river}), including their consumption during the six years (g/day), excretion rate and WWTP removal. In fact, the value of PEC calculation relies in having precise information on the study area regarding demography, geographical data and water management issues. One relevant parameter in the PEC calculation is the fraction excreted and the fraction of drugs eliminated in WWTP. For the former, excretion depends on many factors such as age, health and condition of the patient and several values can be considered. This can produce an uncertainty in the calculation. In this study, excretion data used corresponds to published data or data estimated with the EPIsuite software (Episuite, EPA), and we have considered that drugs will never be excreted in 100% (which would be worst case scenario) as drugs have an activity in the body and at least 50% are excreted in a few hours (Drugbank). Regarding the F_{wwtp}, the situation can vary in different countries and thus it is useful to have information on the wastewater management in the study area. In Spain, more than 90% of the urban wastewater is treated, with 37% having secondary treatment and 51% with additional tertiary treatment (http://www.eea.europa.eu/themes/water/water-pollution/uwwtd/interactive-maps/urban-waste-water-treatment-maps-1), which represents the maximum level of elimination of organic matter and contaminants. For such reason, F_{WWTP} has been chosen considering that in the secondary treatment, drugs will be biodegraded. Another factor to take into consideration is the potential sorption of anticancer drugs in sludge. Among the 78 drugs considered in this study, only 15 (indicated in Figure SI5) will have chances to be sorbed to sludge according to their logP value, but these were not among the highest consumed in Spain.

Table SI2 shows all calculated PECs for the 78 cytostatic drugs studied.Out of 78 drugs, only mycophenolic acid and hydroxicarbamide had PEC_{river}> 10 ng/L, which is the EMA threshold value for risk assessment. Four had PEC_{river}>1ng/L in the 2010-2015 period (it includes capecitabine, although in 2013 became a hospital administered drug)(Figure 3). The rest 72 compounds had PEC_{river}< 1 ng/L, as a result of either low consumption, poor excretion or high degradability in the WWTPs. We have selected the concentration of PEC_{river}> 1 ng/L as threshold to evaluate potential effects. Values of predicted concentrations for each drug are explained below, described from higher to lower PEC and according to temporal trends for each compound.

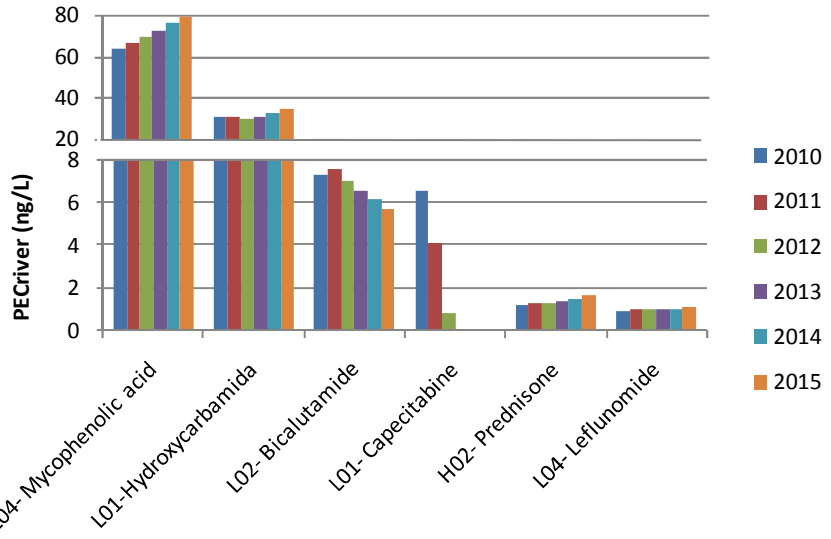

Figure 3. Predicted concentrations in river for cytostatic drugs delivered in pharmacies in Spain (PEC_{river}>1ng/L) in 2010-2015. DF of 25 according to Keller et al. (2014).

Mycophenolic acid was the pharmaceutical with the highest consumption as well as the drug with the highest PEC value, among all L drugs administered in Spain. Its PEC_{eff}increased from 1665-2076 ng/L inthe 2010-2015 period and the PEC_{river} from 64 to 80 ng/L, following consumption patterns. Mycophenolic acid is excreted in a 63% and has only a 41% removal in WWTP (Drugs Information Database, 2014; Royal Society of Chemistry, 2014). Once in river water, it will be quite stable given its low bio and photodegradability (Franquet-Griell et al., 2016). Thus, this compound has high chances to be detected in the environment. However, it has only been studied in surface waters of Llobregat and Besosriver in Catalonia at concentrations from 57 to 656 ng/L of (Franquet-Griell et al., 2016; Franquet-Griell et al., 2017). It has also been detected in WWTP effluents from the Barcelona region at 136 ± 28 ng/L(Franquet-Griell et al., 2017b), confirming the PEC values in effluents. A similar situation was found inVistula and Utrata rivers in Poland where mycophenolic acid was detected at levels up

to 180 ng/L(Giebułtowicz and Nałęcz-Jawecki, 2016). Thus, this compound emerges of environmental importance and to beincluded in monitoring studies dealing with pharmaceuticals.

Hydroxycarbamide was the drug with the highest consumption in the ATC L01 group and accounts for the second higher PEC (PEC_{eff} of 793-901 ng/L and PEC_{river} of 31-35 ng/L, increasing from 2010 to 2015). Its excretion rate was 50% (Besse et al., 2012) and only 2% predicted WWTP removal was reported (Royal Society of Chemistry, 2014).However, it has only been detected onceat 788 ng/L in river water from Bangkok (Usawanuwat et al., 2014).Previous studies have prioritized this compound (Besse et al, 2012; Kümmerer et al., 2016) according to PEC estimations, and a main challenge now is to develop an analytical procedure to detect this very polar compound.

Bicalutamide, of the L02 ATC group, had a decreasing PEC_{eff} of 191-148 ng/L and PEC_{river} of 7.4-5.7 ng/L during the studied period.It has a high excretion rate of 55% (Besse et al., 2012) and WWTP removal of only 3% (Royal Society of Chemistry, 2014). Its presence in the wastewaters or rivers has not been studied.

Capecitabine had a decreasing PEC_{eff} from 171 to 23 ng/L and PEC_{river} from 6.6 to 0.88 ng/L in 2010-2012, because of the reduction in the administration of pharmacies, as mentioned before. For this drug, an 11% of excretion and 15% WWTP removal were reported (Besse et al., 2012; Rowney et al., 2009).Capecitabine was not detected in WWTP effluents (Gómez-Canela et al., 2014) neither in surface waters of Catalonia (Franquet-Griell et al., 2017), but contrarily it wasdetected in WWTP influents from Slovenia at concentrations up to 158 ng/L (Isidori et al., 2016). Its non-detection in wastewaters or rivers from Spain is in line with the non-consumption during the period 2013-2015. However, detection in Slovenian waters suggests different consumption patterns in each country and thus, environmental presence.

Prednisone increased its PEC_{eff} from 33 to 44 ng/L and PEC_{river} from 1.3 to 1.7 ng/L. Prednisone is readily excreted (50% excretion (default value) anda 87% WWTP removal has been published(Fan et al., 2011). A high biodegradation has been reported in controlled laboratory conditions (Franquet-Griell, 2017), which is in line with the high elimination in the WWTP. Thus, it has low probabilities to be discharged to surface waters from WWTP, which is in agreement with its non-detection in WWTP effluents Gomez-Canela et al. (2014). However, it has an intermediate solar photodegradation (Franquet-Griell, 2017), meaning that it might be detectedif discharged to rivers from other sources. Also from ATC code L04, but way down from mycophenolic, leflunomide had PEC_{eff} of 25-29 ng/L and PEC_{river} of 0.96-1.1 ng/L. For this drug, a

50% removal was considered (default value) and 3% excretion (Besse et al., 2012). This compound has not been reported in the environment.

Table 2. Consumption rates (g/day), PEC_{eff} and PEC_{river} values(in ng/L) calculated for the main anticancer drugs in Spain during the period 2010-2015, including its consumption (g/day) excretion rates and removal in WWTP. Compounds are ordered by PECriver in 2010 and for each compound the fraction excreted (Fexc) and the fraction eliminated in the WWTP (Fwwtp) used to calculate the PECs are indicated, with the corresponding reference.

							2010			2011			2012			2013			2014			2015		
	ATC	Name	F_{exc}	Ref.	F_{wwtp}	Ref.	g/day	PEC_{eff}	PEC_{river}	g/day	PEC_{eff}	PEC_{river}	g/day	PEC_{eff}	PEC_{river}	g/day	PEC_{eff}	PEC_{river}	g/day	PEC_{eff}	PEC_{river}	g/day	PEC_{eff}	PEC_{river}
L01	L01XX05	Hydroxycarbamide	0.5	d	0.02	j	9,782	793	31	9,888	798	31	9,644	778	30	10,033	813	31	10,650	865	33	11,070	901	35
	L01BC06	Capecitabine	0.11	c	0.15	a	11,061	171	6.6	6,922	106	4.1	1,481	23	0.88	-	-	-	-	-	-	-	-	-
	L01XE01	Imatinib	0.25	d	0.06	i	535	21	0.80	335	13	0.50	85	3.3	0.13	-	-	-	-	-	-	-	-	-
	L01BB02	Mercaptopurine	0.4	j	0.019	b	139	9.0	0.35	158	10	0.39	177	11.4	0.44	145	9.4	0.36	84	5.4	0.21	0.37	0.024	9.2E-04
	L01XE07	Lapatinib	0.67	k	0.85	l	256	4.25	0.16	140	2.3	0.089	25	0.42	0.016	-	-	-	-	-	-	-	-	-
	L01AA01	Cyclophosphamide	0.25	a	0	d	75	3.1	0.12	69	2.8	0.11	70	2.9	0.11	70	2.9	0.11	70	2.9	0.11	70	2.9	0.11
	L01BC03	Tegafur	0.05	k	0.02	b	291	2.4	0.091	255	2.1	0.079	240	1.9	0.075	196	1.6	0.061	149	1.2	0.047	133	1.1	0.042
	L01BA01	Methotrexate	0.9	c	0.95	c	144	1.1	0.041	157	1.2	0.045	165	1.2	0.047	176	1.3	0.050	185	1.4	0.053	196	1.5	0.056
	L01XX23	Mitotane	0.6	c	0.92	b	79	0.67	0.026	95	0.80	0.031	118	0.99	0.038	125	1.05	0.041	154	1.30	0.050	159	1.34	0.052
	L01XX11	Estramustine	0.05	c	0.88	b	211	0.20	0.0079	162	0.16	0.006	124	0.12	0.0046	101	0.10	0.0038	76	0.07	0.003	48	0.05	0.0018
L02	L02BB03	Bicalutamide	0.55	d	0.03	j	2,163	191	7.4	2,242	197	7.6	2,081	183	7.1	1,938	171	6.6	1,802	160	6.2	1,663	148	5.69
	L02BB01	Flutamide	0.1	d	0.10	j	1,980	29	1.1	1,549	23	0.88	1,174	17	0.67	929	14	0.53	798	12	0.46	642	10	0.37

	L02AB01	Megestrol	0.78	a	0.96	k	3,728	19	0.74	3,783	19	0.75	3,482	18	0.69	3,264	17	0.65	3,168	16	0.63	3,354	17	0.67
	L02AB02	Medroxyprogesterone	0.5	x	0.13	j	67	4.8	0.19	64	4.5	0.18	56	4.0	0.15	54	3.9	0.15	55	4.0	0.15	56	4.0	0.16
L04	L04AA06	Mycophenolicacid	0.63	e	0.41	j	27,108	1,665	64	28,341	1,733	67	29,288	1,791	69	30,664	1,882	73	32,328	1,990	77	33.6	2,076	80
	L04AA13	Leflunomide	0.5	x	0.03	j	311	25	0.96	321	26	0.99	322	26	0.99	330	26	1.0	342	27	1.1	356	29	1.1
	L04AX01	Azathioprine	0.02	f	0.02	i	3,702	12	0.46	4,029	13	0.50	4,307	14	0.54	4,622	15	0.58	4,933	16	0.62	5.2	17	0.66
G03	G03HA01	Cyproterone	0.33	h	0.15	i	517	24	0.92	468	22	0.83	414	19	0.73	382	18	0.68	353	16	0.63	327	15	0.59
H02	H02AB07	Prednisone	0.5	x	0.87	l	3,049	33	1.3	3,202	34	1.3	3,249	35	1.3	3,392	36	1.4	3,565	38	1.5	4,069	44	1.7

[a]Provincial Health Services Authority (2013),[b]Johnson et al. (2013)[c]Rowney et al. (2009)[d]Besse et al. (2012)[e]Drugs Information Database (2014)[f]U.S. National Library of Medicine (2013)[h]Gómez-Canela et al. (2014)[i]U.S. EPA (2013)[j]Royal Society of Chemistry (2014)[k]Chang et al. (2011)[l]Fan et al. (2011)[x] no data.

Finally, there are a series of highly studied compounds according the open bibliography and is worthwhile discussing their potential presence in the environment by comparing the PEC value with monitoring data from WWTP effluents and rivers. These compounds are discussed according to the ATC code to make it more comprehensive.

*L01- Antineoplastic agents:*Imatinib had a decreasing PEC_{eff} of 21-3.3 ng/L and PEC_{river} of 0.80-0.13 ng/L and, like capecitabine, it became of hospital administration. Also considering its low removal of only 6% (U.S. EPA, 2013), its real presence in the environment could be higher than the predicted levels. The rest of drugs in L01 group had PEC_{eff} below 12ng/L and PEC_{river} below 1ng/L, including some compounds usually studied in environmental samples like cyclophosphamide (PEC_{eff}~3 ng/L and PEC_{river}~0.11ng/L) or fluorouracil (with a decreasing PEC_{eff} from 0.06 to 0.012 ng/L and PEC_{river} from 0.0023 to 4.5E-4 ng/L). The presence of cyclophosphamide was confirmed in surface waters from Switzerland at 0.06-0.17 ng/L(Buerge et al., 2006)but was below detection limit in surface waters from Spain(Martín et al., 2011; Valcárcel et al., 2011). Otherwise, 5-fluorouracil studied in surface waters was also below detection limits(Kosjek et al., 2013; Martín et al., 2011), which confirms that these compounds are not expected to be detected in surface waters or at very low concentrations.

*L02- Endocrine therapy:*Megestrol (PEC_{eff} was 19-17 ng/L and PEC_{river}0.74-0.67 ng/L),flutamide (PEC_{eff} 29-10 ng/L and PEC_{river}1.1-0.37 ng/L) and medroxyprogesterone (PEC_{eff} 4.8-4.0 ng/L and PEC_{river}0.19-0.16 ng/L) had low and decreasing PECfrom 2010 to 2015, following the consumption data trends. Only megestrol has been detectedin WWTP effluents at levels up to 20 ng/L detected (Gómez-Canela et al., 2014). The other 12 drugs administered from L02 group had PEC_{eff} below 2 ng/L and PEC_{river}below 0.1ng/L (see Table SI2).

L03- Immunostimulants: The three interferons had very low PEC_{eff} and PEC_{river} due to thesmall dose administered. $PEC_{eff}$was around4-9·10^{-5}ng/L and their PEC_{river} was one order of magnitude lower. Excretion rate and removal for these drugs was not available in the literature, and neither could be calculated with the specific software. Thus, default values of a 50% excretion and 0% removal were considered for the three drugs. These compounds have not been studied in the environment but are not expected to be detected.

*L04- Immunosuppressants:*All the remaining 8 compounds fromthis group hadPEC_{river}< 1 ng/L and theirpresence in environmental waters isexpected to below. For instance, azathioprine had PEC_{eff} of 12-17 ng/L and PEC_{river} of 0.46-0.66 ng/L, considering 2% excretion (U.S. National Library of Medicine, 2013) and 2% WWTP removal (U.S. EPA, 2013)and has been detected in WWTP effluents from Spain at 18-19 ng/L, similar to the predicted concentration (Ferrando-Climent et al., 2013).

*G03 and H02:*Cyproteronehad a decreasing PEC_{eff} of 24-15 ng/L and PEC_{river} of 0.92-0.59 ng/L, considering 33% excretion (Gómez-Canela et al., 2013) and 15% removal (U.S. EPA, 2013).

Overall, out of the 78 cytostatic drugs consumed in Spanish pharmacies, only mycophenolic acid and hydroxycarbamide stand out as the drugs with highest PEC_{river}, with concentrations above the 10 ng/L recommended for the EMA for environmental risk assessment(EMA, 2006). Six had PEC_{river}> 1 ng/L and the rest, even being highly consumed, would not be expected to be found in the environment due to low excretion or high elimination in the WWTPs.

3.4. PECs according to hydrographic basins and comparison with monitoring data

The important role of DF in the calculation of PECs has been outlined previously(Franquet-Griell et al., 2017). Spanish rivers are characterized by high fluctuations in the river flows as a result of seasonal variations linked with precipitations, which can be up to 100-fold. Thus, it is importanttodetermine the PECs in each river basin considering flow variability to obtain more accurate information. DFs were recalculated for the most important hydrographic basins in Spain according to equation 2 and selecting mean flow values from the middle course of the river, and maximum and minimum flows registeredfor each river during 2014-2015 to exemplify the high flow variation (Table 1). To determine the global impact of cytostatic drugs in the different basins, total PEC_{river} ($\sum PEC_{river}$) were calculated considering all compounds using the mean DF of each river.Among the eight Spanish river basins, Miño and Duero were those with the lowest $\sum PEC_{river}$ (13 and 14 ng/L respectively) as these two basins are the ones with the highest DF. Following, Ebro basin had $\sum PEC_{river}$of 27 ng/L with a DF of 50, which is higher than the DF used for Spain(Keller et al., 2014). On the other hand, Guadalquivir, Guadiana and Segura basins had higher $\sum PEC_{river}$, with 128, 290 and 380 ng/L due to the low DF (between 6 and 15). Finally, Júcar and Tajo obtained the highest $\sum PEC_{river}$(535 and 927 ng/L respectively). These two basins are the ones with the highest population and low DF (6.3 and 3.7 respectively). Figure 4 shows the hydrographic basins studied colored according to their $\sum PEC_{river}$.

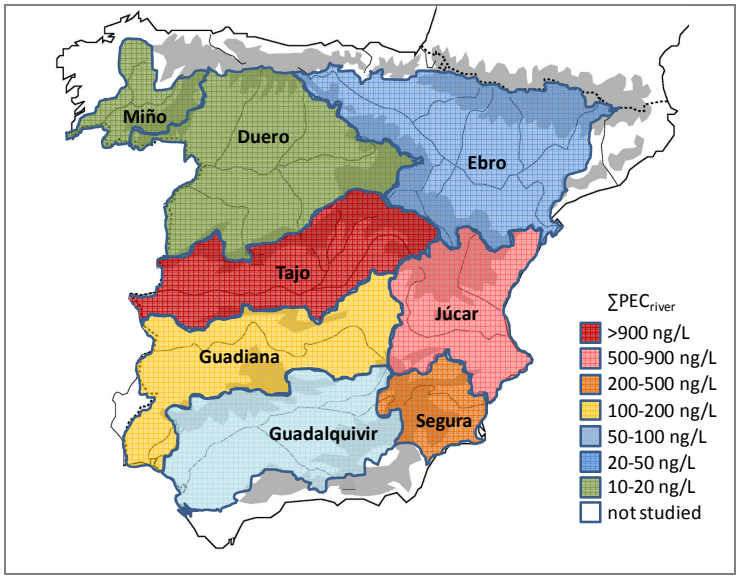

Figure 4.$\sum PEC_{river}$ of cytostatic drugs in the main hydrographic basins of Spain.

Despite the high values, the main contribution in $\sum PEC_{river}$ came from few drugs. Only mycophenolic acid, hydroxycarbamide, bicalutamide, capecitabine and prednisone had PEC_{river} higher than 10 ng/L in, at least, one of the basins. For instance, in Júcar and Tajo, the most impacted river basins, mycophenolic acid had a $PEC_{river}$of 322 and 559 ng/L respectively. Following, hydroxycarbamidehad 140 and 242 ng/L, capecitabine had 27 and 46 ng/L, and bicalutamide had 23 and 40 ng/Lrespectively for the two basins (Table 3). On the other hand, Miño and Duero which accounted for the lowest impacted basins, PEC_{river} for mycophenolic acid was of 7.8 and 8.5 ng/L respectively, much lower than values predicted globally for Spain using DF of 25(Keller et al., 2014). For Tajo, Júcar, Seguraand Guadiana, PEC_{river}values were higher than those calculated at national scale.

Considering the flow variability, Table 3 illustrates the PEC_{river} ranges in each river basin for main compounds detected in each basin. It can be seen that in low DF conditions, the $PEC_{river}$are higher than the 10 ng/L proposed by EMA in Guadalquivir, Júcar, Segura and Tajo. Specifically, concentrations from 939 (leflunomide) to 68,014 ng/L (mycophenolic acid) can be expected in the Segura river given a very low flow in dry periods. Mycophenolic acid, hydroxycarbamide and bicalutamida would always be detected in low river flow conditions. Contrarily, in high DF situation, Guadalquivir, Júcar, Segura and Tajo would still be impacted by high concentrations of anticancer drugs whereas in Duero, Guadiana, Miño and Ebro thePEC_{river} would be 10 ng/L or lower.

Thus, because studied rivers have high variable flow, the use of specific DFs which consider the seasonal variability of water flows can provide a better characterization of the presence of cytostatic drugs in a specific river basin and thus, a more accurate risk assessment.

Table 3.PECriver (ng/L) ranges in each river basin for the 6 cytostatic drugs with highest PECs. Mean represents PEC values calculated with mean DF, and min and max represent PEC values calculated with high DF and low DF, respectively (values given in Table 1).

Hydrographic basin	Mycophenolic acid	Hydroxycarbamide	Bicalutamide	Capecitabine	Prednisone	Leflunomide
	Mean (min-max)	Mean (min-max)	Mean (min-max)	Mean (min-max)	Mean (min-max)	Mean (min-max)
Duero	8.47 (7.2-136)	3.7 (3.1-59)	0.6 (0.5-9.7)	0.7 (1.6-11.2)	0.2 (0.2-2.9)	0.1 (0.1-1.9)
Ebro	16.5 (10-81)	7.2 (4.4-35)	1.2 (0.7-5.7)	1.4 (0.8-6.7)	0.4 (0.2-1.7)	0.2 (0.14-1.1)
Guadalquivir	76.9 (22-1146)	33.3 (9.7-497)	5.5 (1.6-81)	6.4 (1.9-94)	1.6 (0.5-24)	1.1 (0.3-16)
Guadiana	175 (5.7-454)	75.8 (2.5-197)	12.4 (0.4-32)	14.4 (0.5-37)	3.7 (0.1-9.6)	2.4 (0.08-6.3)
Júcar	322 (198-1361)	140 (86-590)	23 (14-97)	26.6 (16-112)	6.8 (4.2-29)	4.5 (2.7-19)
Miño	7.78 (5.08-525)	3.4 (2.2-227)	0.5 (0.36-37)	0.6 (0.4-43)	0.2 (0.1-11)	0.1 (0.07-7.2)
Segura	229 (134-68014)	99.5 (58-29500)	16.3 (9.5-4835)	19 (11-5616)	4.8 (2.8-1436)	3.2 (1.8-939)
Tajo	559 (72-2552)	242 (31-1107)	40 (5.1-181)	46 (5.9-211)	12 (1.5-54)	7.7 (1.0-35)

3.5. Estimation of riskin Spain and its hydrographic basins

Cytostatic drugs aim to kill cancer cells and have different toxicological endpointsdepending on the species tested. Toxic concentrations for cytostatic drugs reported for different trophic levels are summarized in Table 4, ordered by decreasing river basin PEC_{river}according to 2015 values (complete table included in Table SI4). RQ was calculated using the PEC_{river} according to mean DF of the Tajo basin (the most representative basin in Spain and the most impacted), and the lowest aquatic toxic value for each organism. For capecitabine, PEC_{river}calculated with consumption in 2010 was used because, due to the shift of prescriptions, PEC with 2015 datacannot be calculated.

In general, toxic concentrations vary in a wide range, with NOEC values of 1000 mg/L for cyclophosphamide in *D. magna*(Zounková et al., 2007)to an EC_{50} of 0.003 mg/L for tioguanine in green algae (*S. subspicatus*)(GSK, 2014). RQ were calculated for each individual drug using NOEC or EC_{50}/LC_{50}, when NOEC was not reported.

For drugs with PEC_{river}>10 ng/L(Table 4) all obtained RQ were below 1, showing no expected risk for the aquatic environment. In particular, mycophenolic acid was the one with the highest RQ (0.56), considering the algae *P. subcapitata* acute toxicity test (NOEC of 0.01 mg/L)(Santa Cruz Biotechnology, 2010).For the other drugs, with PEC_{river}<10ng/L, the highest RQ value was up to$2.6\cdot10^{-2}$ (*X. laevis* 96h growth inhibition test for methotrexate), meaning no expected risk (Table SI4). It has to be considered here that in low flow conditions, the expected concentrations might have an effect.

Concerningchronic toxicity, effect concentrations were lower than values reported for acute tests. Table 4 summarizes NOEC and EC_{50}values for drugs with basin PEC_{river}>10 ng/L (in 2015) (complete information for all drugs in Table SI5). However, information was available for a few drugs. In this case RQ were not calculated because the security factor *f* applied in the equation is not well defined, and there are different opinions on which value has to be used. For chronic toxicity,values of EC_{50}compiled from bibliography were as low as $1\cdot10^{-5}$mg/L (for tamoxifen, growth test for *P. promelas*)(Besse et al., 2012).However, mycophenolic acid, hydroxycarbamide, capecitabine and prednisone are known to be mutagenic (Vademecum, 2016)(Table 4), which can have negative effects on aquatic organisms or their offspring.

Drugs with PEC_{river}> 10 ng/L EMA threshold showed predicted half-lives of 15-180 days, their Kow ranged from -1.8 to 4.22 and their Henry constant from $2.92\cdot10^{-19}$ to $1.23\cdot10^{-10}$(Royal Society of Chemistry, 2014) (Table 4). All these properties suggest that selected drugs will remain in the waters without adsorption in the sediments or evaporation. Bioconcentration

Table 4. Acute and chronic toxicity and risk assessment for cytostatic drugs with Tajo basin PEC_{river}>10ng/L (2015. calculated with the mean flow) indicated in Table 2.

Name			Specie	Endpoint	Ref.	Basin PEC_{river} 2015 (ng/L)	NOEC (mg/L)	EC_{50}/LC_{50} (mg/L)	RQ_{NOEC}	RQ_{EC50}	$t_{1/2}$* (day)	BCF*	log Kow*	H* atm-m^3/mole	Possible mutagenic**
Mycophenolic acid	Acute	algae	*P. subcapitata*	ErC50 72h	a	7.8-559	0.01		5.6E-01		15	3.2	4.22	3.82E-12	Yes
		crustacean	*D. magna*	EC50 48h	b		27.7		2.0E-04						
		fish	*B. sunfish*	96h	b		1.7		3.3E-03						
	Chronic	algae	*S. subspicatus*	14d	b			(LOEC) 1.6							
Hydroxycarbamide	Acute	crustacean	*D. magna*	48h	c	3.4-243	> 100				15	3.2	-1.8	5.42E-11	Yes
	Chronic	nd	--	--	--										
Capecitabine (2010)	Acute	algae	*P. subcapitata*	growth 72h	d	0.64-46	0.14		3.3E-03		15	3.2	0.56	2.92E-19	Yes
		rotifer	*B. calyciflorus*	24h	e		up to 500								
		crustacean	*D. magna*	48h	e			224		2.1E-04					
		amphibian	*T. platyurus*	24h	e			197.7		2.3E-04					
	Chronic	crustacean	*D. magna*	Reproduction 48h	d			>850							
		crustacean	*D. magna*	21d	e		1.9	20.6							
		crustacean	*C. dubia*	7d	e		0.6	2.4							
		rotifer	*B. calyciflorus*	48h	e		3.12	15.4							
Bicalutamide	Acute	algae	*algae*	EC50	f	0.55-40		>1		4.0E-02	180	12	2.3	2.82E-15	No
		crustacean	*D. magna*	48h	g			>5.3		7.5E-03					
		fish	*B. sunfish*	96h (static)	h		4.4		9.0E-05						
	Chronic	crustacean	*D. magna*	21 d	h	0.56	3.2								
Prednisone	Acute	algae	*P. subcapitata*	IC50. growth 3d	i	0.16-12		31		3.8E-04	60	2.6	1.46	2.83E-10	Yes
		rotifer	*B.calyciflorus*	mortality 24h	j			54.48		2.2E-04					
		crustacean	*D. magna*	immobilisation 24h	j		99.9		1.2E-06						
	Chronic	nd	--	--	--										

[b]Roche (2014)[c]Bristol-Myers (2010)[d]Besse et al. (2012)[e]Parrella et al. (2014)[f]Whitacre (2010)[g]MEDAC (2008) [h]AstraZeneca (2006) [i]Cunningham et al. (2006)[j]DellaGreca et al. (2003). nd : no data; *Royal Society of Chemistry (2014) **Vademecum (2016)

factors (BCF) of 3.2-12(Royal Society of Chemistry, 2014)also suggest that anticancer drugs will not be bioaccumulated.

4. Conclusions

This study reveals the importance of consumption data and temporal patterns for estimating the occurrence and risk of cytostatic compounds in surface waters. The use of exhaustive data compilation covering several years and the inclusion of non-preselected compounds permits and the estimation of PECs.In Spain, over the period 2010-2015, 78 drugs were consumed through pharmacies, but only 6 had PEC_{river} higher than 1 ng/L, according to consumption, excretion rates and degradability. It is clear from this study that PEC calculation permits to better prioritize compounds which have high probability to be detected in the environment.In Spain, mycophenolic acid, hydroxycarbamide and bicalutamida stand out as the drugs with the highest PECsalthough they have hardly been studied in waste or surface waters. In addition, recalculation of PECs according to specific river dilution factors permits to refine the levels likely to be detected at river basin scale.PEC calculations together with acute and chronic toxicological data provide risk assessment of these compounds. Because anticancer drugsare constantly released to surface water, long-term exposure may affect aquatic organisms or their development.

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Supplementary Information

SI1. Consumption of cytostatic drugs inpharmacies(Europe).

		Spain 2015	Catalonia[a]	France[b]	Germany[c]
		Presentstudy	2015	2008	2012
ATC code	**Name**	µg/day/hab	µg/day/hab	µg/day/hab	µg/day/hab
G03HA01	Cyproterone	7	7	-	-
H02AB07	Prednisone	87	103	-	-
L01AA01	Cyclophosphamide	1.5	1.5	3.5	0
L01AA02	Chlorambucil	0.0003	0.0001	0.17	0
L01AA03	Melphalan	0.0001	0.00004	0.20	0
L01AA06	Ifosfamida	-	-	0.0079	0
L01AA09	Bendamustine	-	-	-	0
L01AB01	Busulfan	0.0004	0.0003	0	0
L01AC01	Tiotepa	-	-	-	0
L01AD02	Lomustine	-	-	0.083	0
L01AD04	Streptozocin	-	-	0.0017	0
L01AD05	Fotemustine	-	-	0	0
L01AX03	Temozolomide	-	-	0	1.8
L01AX04	Dacarbazine	0.001	0.005	1.2	0
L01BA01	Methotrexate	4.2	3.9	0.021	9.8
L01BA03	Raltitrexed	-	-	0.00042	0
L01BA04	Pemetrexed	-	-	1.5	0
L01BB02	Mercaptopurine	0.008	0.004	3.8	1.7
L01BB03	Tioguanine	0.0005	-	0.055	0
L01BB04	Cladribine	-	-	0	*
L01BB05	Fludarabine	0.01	0.01	0	0
L01BB06	Clofarabine	-	-	0	0
L01BB07	Nelarabine	-	-	-	*
L01BC01	Cytarabine	0.00004	-	0.17	0
L01BC02	Fluorouracil	0.04	0.18	0.11	0
L01BC03	Tegafur	2.8	3.6	1.53	*
L01BC05	Gemcitabine	-	-	0	0
L01BC06	Capecitabine	236 (2010)	298 (2010)	210	129
L01BC07	Azacitidine	-	-	-	*
L01BC52	Fluorouracilo/salicilico	0.5	0.2	-	0
L01BC53	Tegafur, combinations	-	-	-	0
L01CA01	Vinblastine	0.00002	0.0001	0	0
L01CA02	Vincristine	0.000002	0.00001	0	0
L01CA03	Vindesina	-	-	-	0
L01CA04	Vinorelbine	-	-	0.0021	0
L01CA05	Vinflunine	-	-	-	*
L01CB01	Etoposide	0.1	0.1	0	0
L01CD01	Paclitaxel	-	-	0.44	0
L01CD02	Docetaxel	-	-	0.0033	0
L01CD04	Cabazitaxel	-	-	-	*

		Spain 2015 Presentstudy	Catalonia[a] 2015	France[b] 2008	Germany[c] 2012
ATC code	**Name**	µg/day/hab	µg/day/hab	µg/day/hab	µg/day/hab
L01CX01	Trabectedin	-	-	0	*
L01DB01	Doxorubicin	0.0005	0.003	0.70	0
L01DB03	Epirubicin	0.0004	0.002	0.00042	0
L01DB06	Idarubicin	0.000001	-	0	0
L01DB07	Mitoxantrone	-	-	0	0
L01DC01	Bleomycin	-	-	0.037	0
L01DC03	Mitomycin	0.002	0.01	0	0
L01XA01	Cisplatin	-	-	0	0
L01XA02	Carboplatin	0.002	0.01	3.42	0
L01XA03	Oxaliplatin	-	-	0	0
L01XB01	Procarbazina	-	-	-	0
L01XC02	Rituximab	-	-	1.4	0
L01XC03	Trastuzumab	-	-	2.1	0.76
L01XC04	Alemtuzumab	-	-	0.0025	*
L01XC06	Cetuximab	-	-	0	0.41
L01XC07	Bevacizumab	-	-	0	0
L01XC08	Panitumumab	-	-	0	*
L01XD03	Methylaminolevulinate	0.2	0.1	0	*
L01XD04	Aminolevulinicacid	0.2	0.01	-	*
L01XE01	Imatinib (2010)	11	0.40	36	46
L01XE02	Gefitinib	-	-	-	*
L01XE03	Erlotinib	-	-	5.9	0
L01XE04	Sunitinib	-	-	0	0
L01XE05	Sorafenib	-	-	-	0
L01XE06	Dasatinib	-	-	-	0
L01XE07	Lapatinib	-	-	1.2	*
L01XE08	Nilotinib	-	-	2.4	*
L01XE09	Temsirolimus	-	-	0	*
L01XE10	Everolimus	-	-	-	*
L01XE11	Pazopanib	-	-	-	*
L01XX05	Hydroxycarbamide	237	265	277	212
L01XX08	Pentostatin	-	-	0.00062	0
L01XX09	Miltefosine	-	-	0	0
L01XX11	Estramustine	1.0	0.73	12	0
L01XX14	Tretinoin	0.08	0.1	0.099	0
L01XX17	Topotecan	-	-	0	0
L01XX19	Irinotecan	-	-	0	0
L01XX23	Mitotane	3.4	5.3	0	*
L01XX25	Bexarotene	-	-	0	*
L01XX27	Arsenictrioxide	-	-	0	0
L01XX32	Bortezomib	-	-	0.00042	0
L01XX33	Celecoxib	-	-	-	
L01XX35	Anagrelide	0.04	0.06	0.00083	

		Spain 2015 Presentstudy	Catalonia[a] 2015	France[b] 2008	Germany[c] 2012
ATC code	**Name**	µg/day/hab	µg/day/hab	µg/day/hab	µg/day/hab
L02AA01	Dietilestilbestrol	-	-		
L02AA04	Fosfestrol	-	-		
L02AB01	Megestrol	72	52		
L02AB02	Medroxyprogesterone	1.2	1.4		
L02AE01	Buserelin	0.001	0.001	0.002	
L02AE02	Leuprorelin	0.2	0.2	0.1	
L02AE03	Goserelin	0.02	0.01	0.05	
L02AE04	Triptorelin	0.08	0.10	0.08	
L02AE05	Histrelina	0.0003	0.001		
L02BA01	Tamoxifen	19	20	16	
L02BA02	Toremifene	-	-	0.04	
L02BA03	Fulvestrant	0.87	0.15	0.28	
L02BB01	Flutamide	14	10	22	
L02BB03	Bicalutamide	36	27	36	
L02BG03	Anastrozole	0.4	0.3	1.3	
L02BG04	Letrozole	2.4	3.2	1.4	
L02BG06	Exemestane	6.8	6.6	7.6	
L02BX03	Abiraterone	-	-		
L03AA02	Filgrastim	-	-		
L03AA10	Lenograstim	-	-		
L03AA13	Pegfilgrastim	-	-		
L03AB03	Interferon gamma	0.00002	0.00002		
L03AB04	Interferon alfa-2a	0.00002	0.000004		
L03AB05	Interferon alfa-2b	0.00003	0.00001		
L03AB07	Interferon beta-1a	-	-		
L03AB08	Interferon beta-1b	-	-		
L03AB10	Peginterferon alfa-2b	-	-		
L03AB11	Peginterferon alfa-2a	-	-		
L03AC01	Aldesleukin	-	-		
L03AX03	BCG vaccine	-	-		
L03AX13	Glatiramer acetate	-	-		
L03AX16	Plerixafor	-	-		
L04AA04	Antithymocyteimmunoglobulin (rabbit)	-	-		
L04AA06	Mycophenolicacid	720	897		
L04AA10	Sirolimus	0.07	0.1		
L04AA13	Leflunomide	7.6	6.9		
L04AA18	Everolimus	0.1	0.2		
L04AA23	Natalizumab	-	-		
L04AA24	Abatacept	-	-		
L04AA25	Eculizumab	-	-		
L04AA27	Fingolimod	-	-		
L04AB01	Etanercept	-	-		

		Spain 2015 Presentstudy	Catalonia[a] 2015	France[b] 2008	Germany[c] 2012
ATC code	**Name**	µg/day/hab	µg/day/hab	µg/day/hab	µg/day/hab
L04AB02	Infliximab	-	-		
L04AB04	Adalimumab	-	-		
L04AB05	Certolizumabpegol	-	-		
L04AB06	Golimumab	-	-		
L04AC03	Anakinra	-	-		
L04AC05	Ustekinumab	-	-		
L04AC07	Tocilizumab	-	-		
L04AC08	Canakinumab	-	-		
L04AD01	Ciclosporin	35	37		
L04AD02	Tacrolimus	2.6	3.3		
L04AX01	Azathioprine	112	99		
L04AX04	Lenalidomide	-	-		

[a]Data provided by CatSalut (not published).

[b] Besse, J.P., Latour, J.F., Garric, J., 2012. Anticancer drugs in surface waters. What can we say about the occurrence and environmental significance of cytotoxic, cytostatic and endocrine therapy drugs? Environ. Int. 39, 73-86.

[c] Kümmerer, K., Haiß, A., Schuster, A., Hein, A., Ebert, I., 2016. Antineoplastic compounds in the environment—substances of special concern. Environ. Sci. Pollut. Res. 23, 14791-14804.

* % administered in pharmacies is not known

SI2. PEC_{eff} and PEC_{river} for cytostatic drugs administered in Spanish pharmacies

							2010			2011			2012			2013			2014			2015		
Group	ATC code	Name	F_{exc}	Ref	F_{wwtp}	Ref	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L
G03	G03HA01	Cyproterone	0.33	g	0.15	l	517	24	0.92	468	22	0.83	414	19	0.73	382	18	0.68	352.71	16	0.63	327.21	15	0.59
H02	H02AB07	Prednisone	0.5	a	0.87	m	3,049	33	1.3	3,202	34	1.3	3,249	35	1.3	3,392	36	1.4	3,565	38	1.5	4,069	44	1.7
L01	L01AA01	Cyclophosphamide	0.25	j	0.02	c	75	3	0.12	69	2.8	0.11	70	2.9	0.11	70	2.9	0.11	70	2.9	0.11	70	2.9	0.11
	L01AA02	Chlorambucil	0.01	k	0.02	l	3.0	0.0048	1.9E-04	2.6	0.0042	1.6E-04	2.0	0.0032	1.2E-04	2.00	0.0032	1.2E-04	1.1	0.0018	7.1E-05	0.016	2.5E-05	9.8E-07
	L01AA03	Melphalan	0.16	j	0.02	b	1.81	0.047	0.0018	1.72	0.044	0.0017	1.5	0.039	0.0015	1.4	0.037	0.0014	0.73	0.019	7.4E-04	0.0041	1.1E-04	4.1E-06
	L01AB01	Busulfan	0.02	j	0.02	b	0.20	6.4E-04	2.5E-05	0.23	7.5E-04	2.9E-05	0.20	6.5E-04	2.5E-05	0.20	6.4E-04	2.5E-05	0.13	4.2E-04	1.6E-05	0.020	6.4E-05	2.5E-06
	L01AC01	Tiotepa	0.02	i	0.02	b	2.7E-04	8.9E-07	3.4E-08	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0
	L01AX03	Temozolomide	0.21	j	0.019	b	22	0.75	0.029	13	0.44	0.017	2.7	0.091	0.0035	0.0022	7.5E-05	2.9E-06	0.0011	3.7E-05	1.4E-06	-	0	0
	L01AX04	Dacarbazine	0.5	c	0.02	b	0.09	0.0076	2.9E-04	0.052	0.0042	1.6E-04	0.11	0.0091	3.5E-04	0.14	0.011	4.4E-04	0.060	0.0049	1.9E-04	0.041	0.0033	1.3E-04
	L01BA01	Methotrexate	0.9	c	0.95	c	144	1.1	0.041	157	1.2	0.045	165	1.2	0.047	176	1.3	0.050	185	1.4	0.053	196	1.5	0.056
	L01BB02	Mercaptopurine	0.4	j	0.019	b	139	9.0	0.35	158	10	0.39	177	11.4	0.44	145	9.4	0.36	84	5.4	0.21	0.37	0.024	9.2E-04
	L01BB03	Tioguanine	0.54	j	0.019	b	1.3	0.11	0.0044	1.3	0.12	0.0045	0.99	0.086	0.0033	0.90	0.079	0.0031	0.61	0.053	0.0021	0.022	0.0019	7.4E-05
	L01BB05	Fludarabine	0.6	h	0.019	b	1.4	0.14	0.0053	0.98	0.10	0.0037	0.80	0.077	0.0030	0.78	0.076	0.0029	0.64	0.063	0.0024	0.48	0.047	0.0018
	L01BC01	Cytarabine	0.1	c	0.50	c	0.008	0.0	2.7E-06	0.0041	3.4E-05	1.3E-06	0.0014	1.1E-05	4.3E-07	0.0038	3.2E-05	1.2E-06	-	0	0	0.0019	1.6E-05	6.1E-07
	L01BC02	Fluorouracil	0.39	e	0.90	c	9.1	0.058	0.0023	7.8	0.050	0.0019	2.3	0.015	5.6E-04	3.4	0.022	8.5E-04	3.3	0.021	8.2E-04	1.8	0.012	4.5E-04
	L01BC03	Tegafur	0.05	k	0.02	b	291	2.4	0.091	255	2.1	0.079	240	1.9	0.075	196	1.6	0.061	149	1.2	0.047	133	1.1	0.042
	L01BC06	Capecitabine	0.11	h	0.15	c	11,061	171	6.6	6,922	106	4.1	1,481	23	0.88	-	0	0	-	0	0	-	0	0
	L01BC52	Fluorouracilo/salicilico	0.39	e	0.90	c	-	0	0	-	0.0	0.00	-	0	0	-	0	0	14	0	0.0035	21	0	0.0053
	L01BC5	Tegafur,	0.5	a	0	a	23.1	1.9	0.074	15	1.3	0.049	9.07	0.75	0.029	0.59	0.049	0.0019	-	0	0	-	0	0

							2010			2011			2012			2013			2014			2015		
Group	ATC code	Name	F_{exc}	Ref	F_{wwtp}	Ref	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L
	3	combinations																						
	L01CA01	Vinblastine	0.01	j	0.18	l	0.0015	2.0E-06	7.7E-08	1.5E-03	1.9E-06	7.5E-08	1.7E-03	2.3E-06	8.9E-08	1.4E-04	1.8E-07	7.1E-09	-	0	0	8.8E-04	1.2E-06	4.6E-08
	L01CA02	Vincristine	0.1	j	0.07	l	0.00038	5.9E-06	2.3E-07	3.2E-04	5.0E-06	1.9E-07	1.8E-04	2.8E-06	1.1E-07	7.1E-05	1.1E-06	4.2E-08	3.0E-04	4.6E-06	1.8E-07	7.1E-05	1.1E-06	4.2E-08
	L01CA03	Vindesina	0.5	a	0.35	l	1.4E-05	7.4E-07	2.9E-08	-	0.0	0.00	-	0	0	-	0	0	-	0	0	-	0	0
	L01CB01	Etoposide	0.93	c	0.019	l	3.6	0.55	0.021	2.8	0.42	0.016	2.8	0.42	0.016	2.8	0.42	0.016	2.5	0.37	0.014	2.6	0.40	0.015
	L01DB01	Doxorubicin	0.5	c	0.019	l	0.061	0.0050	1.9E-04	0.049	0.0040	1.5E-04	0.032	0.0026	9.9E-05	0.028	0.0023	8.9E-05	0.032	0.0026	1.0E-04	0.022	0.0018	7.0E-05
	L01DB03	Epirubicin	0.1	j	0.019	l	0.14	0.0023	8.9E-05	0.081	0.0013	5.0E-05	0.056	9.0E-04	3.5E-05	0.067	0.0011	4.2E-05	0.046	7.6E-04	2.9E-05	0.020	3.2E-04	1.2E-05
	L01DB06	Idarubicin	0.07	j	0.024	b	0.0021	2.4E-05	9.2E-07	0.0023	2.5E-05	9.8E-07	0.0010	1.1E-05	4.4E-07	0.0019	2.1E-05	8.1E-07	-	0	0	2.7E-05	3.1E-07	1.2E-08
	L01DB07	Mitoxantrone	0.23	j	0.026	b	0.0025	9.1E-05	3.5E-06	0.0016	6.1E-05	2.3E-06	-	0	0	2.7E-04	0	3.9E-07	-	0	0	-	0	0
	L01DC01	Bleomycin	0.7	j	0	a	0.023	0.0026	0.00010	0.024	0.0028	1.1E-04	0.020	0.0023	8.9E-05	0.0065	0.0008	2.9E-05	-	0	0	-	0	0
	L01DC03	Mitomycin	0.1	c	0.02	l	0.20	0.0032	0.00012	0.17	0.0027	1.1E-04	0.12	0.0019	7.5E-05	0.10	0.0016	6.2E-05	0.089	0.0014	5.6E-05	0.088	0.0014	5.5E-05
	L01XA02	Carboplatin	1	c	0.59	e	0.48	0.033	0.0013	0.32	0.022	8.4E-04	0.19	0.013	5.0E-04	0.19	0.013	5.1E-04	0.15	0.010	4.0E-04	0.10	0.0071	2.7E-04
	L01XB01	Procarbazina	0.05	f	0.02	b	0.0068	5.6E-05	2.1E-06	0.0068	5.5E-05	2.1E-06	-	0	0	-	0	0	-	0	0	-	0	0
	L01XD03	Methylaminolevulinate	0.5	a	0.02	b	7.7	0.62	0.024	7.5	0.61	0.023	6.4	0.51	0.020	6.6	0.53	0.021	6.4	0.52	0.020	8.0	0.65	0.025
	L01XD04	Aminolevulinicacid	0.5	a	0.02	b	7.7	0.62	0.024	7.5	0.61	0.023	6.4	0.51	0.020	6.6	0.53	0.021	6.4	0.52	0.020	8.0	0.65	0.025
	L01XE01	Imatinib	0.25	c	0.06	l	535	21	0.80	335	13	0.50	85	3.3	0.13	-	0	0	-	0	0	-	0	0
	L01XE02	Gefitinib	0.05	c	0.19	b	19	0.12	0.0048	30	0.20	0.0078	8.7	0.06	0.0022	-	0	0	-	0	0	-	0	0
	L01XE03	Erlotinib	0.02	c	0.04	b	87	0.28	0.011	52	0.16	0.0064	11	0.036	0.0014	-	0	0	-	0	0	-	0	0
	L01XE04	Sunitinib	0.16	k	0.04	b	9.2	0.23	0.0091	4.4	0.11	0.0043	0.041	0.0010	4.0E-05	0.0041	0.0001	4.0E-06	-	0	0	-	0	0
	L01XE05	Sorafenib	0.51	k	0.85	l	164	2.1	0.079	106	1.3	0.051	21	0.26	0.010	-	0	0	-	0	0	-	0	0
	L01XE06	Dasatinib	0.19	j	0.02	b	11.2	0.35	0.013	7.0	0.22	0.0083	1.3	0.041	0.0016	-	0	0	-	0	0	-	0	0
	L01XE07	Lapatinib	0.67	k	0.85	l	256	4.25	0.16	140	2.3	0.089	25	0.42	0.016	-	0	0	-	0	0	-	0	0
	L01XE08	Nilotinib	0.69	j	0.78	l	74	1.9	0.072	69	1.7	0.066	22	0.56	0.022	-	0	0	-	0	0	-	0	0

							2010			2011			2012			2013			2014			2015		
Group	ATC code	Name	F_{exc}	Ref	F_{wwtp}	Ref	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L
	L01XE10	Everolimus	0.05	j	0	a		0	0		0.0	0.00		0	0		0	0		0	0		0	0
	L01XX05	Hydroxycarbamide	0.5	c	0.02	b	9,782	793	31	9,888	798	31	9,644	778	30	10,033	813	31	10,650	865	33	11,070	901	35
	L01XX09	Miltefosine	0.5	a	0.12	b	0.25	0.018	7.1E-04	0.18	0.013	5.0E-04	0.089	0.0064	2.5E-04	0.11	0.0080	3.1E-04	-	0	0	-	0	0
	L01XX11	Estramustine	0.05	c	0.88	b	211	0.20	0.0079	162	0.16	0.0060	124	0.12	0.0046	101	0.10	0.0038	76	0.07	0.0029	48	0.05	0.0018
	L01XX14	Tretinoin	0.63	j	0.93	l	3.6	0.026	0.0010	3.3	0.024	9.2E-04	3.1	0.022	8.4E-04	3.3	0.024	9.3E-04	3.2	0.023	8.8E-04	3.6	0.026	0.0010
	L01XX23	Mitotane	0.6	c	0.92	b	79	0.67	0.026	95	0.80	0.031	118	0.99	0.038	125	1.05	0.041	154	1.30	0.050	159	1.34	0.052
	L01XX25	Bexarotene	0.01	j	0.94	b	31	0.0031	1.2E-04	25	0.0025	9.6E-05	7.3	7.1E-04	2.8E-05	-	0	0	-	0	0	-	0	0
	L01XX33	Celecoxib	0.01	k	0.12	b	59	0.085	0.0033	29	0.042	0.0016	0.066	9.5E-05	3.7E-06	-	0	0	-	0	0	-	0	0
	L01XX35	Anagrelide	0.01	k	0.02	b	2.6	0.0042	1.6E-04	2.0	0.0032	1.2E-04	1.9	0.0030	1.2E-04	1.8	0.0028	0.00011	1.8	0.0029	1.1E-04	1.9	0.0032	1.2E-04
L02	L02AA04	Fosfestrol	0.5	a	0.67	b	0.005	1.5E-04	5.8E-06	-	0	0.00	-	0	0	-	0	0	-	0	0	-	0	0
	L02AB01	Megestrol	0.78	j	0.96	n	3,728	19	0.74	3,783	19	0.75	3,482	18	0.69	3,264	17	0.65	3,168	16	0.63	3,354	17	0.67
	L02AB02	Medroxyprogesterone	0.5	a	0.13	b	67	4.8	0.19	64	4.5	0.18	56	4.0	0.15	54	3.9	0.15	55	4.0	0.15	56	4.0	0.16
	L02AE01	Buserelin	0.2	j	0.02	l	0.14	0.0047	1.8E-04	0.11	0.0035	1.4E-04	0.074	0.0024	9.2E-05	0.052	0.0017	6.5E-05	0.038	0.0012	4.8E-05	0.027	8.9E-04	3.4E-05
	L02AE02	Leuprorelin	0.05	j	0.02	l	12	0.10	0.0039	12	0.10	0.0037	10	0.082	0.0032	9.6	0.077	0.0030	9.3	0.075	0.0029	8.8	0.072	0.0028
	L02AE03	Goserelin	0.9	g	0	a	2.00	0.30	0.011	1.8	0.26	0.010	1.4	0.21	0.0082	1.1	0.17	0.0066	0.96	0.14	0.0055	0.87	0.13	0.0050
	L02AE04	Triptorelin	0.42	k	0	a	3.1	0.21	0.0082	3.2	0.22	0.0086	3.1	0.21	0.0083	3.1	0.21	0.0083	3.3	0.23	0.0088	3.6	0.25	0.0095
	L02AE05	Histrelina	0.5	a	0	a	0.041	0.0034	1.3E-04	0.078	0.0064	2.5E-04	0.071	0.0058	2.2E-04	0.055	0.0046	1.8E-04	0.039	0.0032	1.2E-04	0.012	0.0010	3.8E-05
	L02BA01	Tamoxifen	0.13	j	0.93	b	700	1.0	0.040	730	1.1	0.042	758	1.1	0.043	799	1.2	0.046	848	1.3	0.049	870	1.3	0.050
	L02BA02	Toremifene	0.5	a	0.94	b	7.2	0.039	0.0015	0.15	8.0E-04	3.1E-05	0.021	1.1E-04	4.4E-06	0.0049	2.6E-05	1.0E-06	-	0	0	-	0	0
	L02BA03	Fulvestrant	0.01	j	0.94	l	20	0.0020	7.7E-05	30	0.0029	1.1E-04	31	0.0031	1.2E-04	34	0.0033	1.3E-04	37	0.0036	1.4E-04	41	0.0040	1.6E-04
	L02BB01	Flutamide	0.1	c	0.10	b	1,980	29	1.1	1,549	23	0.88	1,174	17	0.67	929	14	0.53	798	12	0.46	642	10	0.37
	L02BB03	Bicalutamide	0.55	c	0.03	b	2,163	191	7.4	2,242	197	7.6	2,081	183	7.1	1,938	171	6.6	1,802	160	6.2	1,663	148	5.694

							2010			2011			2012			2013			2014			2015		
Group	ATC code	Name	F_{exc}	Ref	F_{wwtp}	Ref	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L	g/day	PEC_{eff} ng/L	PEC_{river} ng/L
	L02BG03	Anastrozole	0.1	f	0.03	b	27	0.43	0.017	24	0.38	0.015	21	0.34	0.013	20	0.32	0.012	19	0.31	0.012	18	0.30	0.011
	L02BG04	Letrozole	0.06	f	0.03	b	74	0.72	0.028	80	0.77	0.030	84	0.81	0.031	92	0.89	0.034	102	0.99	0.038	112	1.09	0.042
	L02BG06	Exemestane	0.01	f	0.90	c	317	0.052	0.0020	337	0.055	0.0021	335	0.055	0.0021	325	0.054	0.0021	323	0.054	0.0021	319	0.053	0.0020
L03	L03AB03	Interferon gamma	0.5	a	0	a	0.0011	9.4E-05	3.6E-06	0.0012	9.6E-05	3.7E-06	0.0010	8.6E-05	3.3E-06	0.0012	9.9E-05	3.8E-06	0.00094	7.8E-05	3.0E-06	0.00085	7.1E-05	2.7E-06
	L03AB04	Interferon alfa-2a	0.5	a	0	a	6.0E-04	4.9E-05	1.9E-06	4.0E-04	3.3E-05	1.3E-06	3.3E-04	2.7E-05	1.0E-06	3.2E-04	2.7E-05	1.0E-06	0.00034	2.8E-05	1.1E-06	0.00080	6.6E-05	2.5E-06
	L03AB05	Interferon alfa-2b	0.5	a	0	a	0.0075	6.2E-04	2.4E-05	0.0068	5.6E-04	2.2E-05	0.0067	5.5E-04	2.1E-05	0.0066	5.5E-04	2.1E-05	0.0051	4.2E-04	1.6E-05	0.0012	1.0E-04	3.9E-06
L04	L04AA06	Mycophenolicacid	0.63	i	0.41	b	27,108	1,665	64	28,341	1,733	67	29,288	1,791	69	30,664	1,882	73	32,328	1,990	77	33,689	2,076	80
	L04AA10	Sirolimus	0.02	i	0.71	l	3.6	0.0038	1.5E-04	3.3	0.0035	1.3E-04	3.0	0.0032	1.2E-04	3.1	0.0033	1.3E-04	3.2	0.0034	1.3E-04	3.4	0.0036	1.4E-04
	L04AA13	Leflunomide	0.5	a	0.03	b	311	25	0.96	321	26	0.99	322	26	0.99	330	26	1.0	342	27	1.1	356	29	1.1
	L04AA18	Everolimus	0.5	a	0.58	l	3.6	0.13	0.0049	4.2	0.15	0.0056	4.6	0.16	0.0062	4.9	0.17	0.0067	5.6	0.20	0.0076	6.5	0.23	0.0088
	L04AB01	Etanercept	0.5	a	0	a	3.9	0.33	0.013	4.2	0.35	0.013	1.2	0.10	0.0037	-	0	0	-	0	0	-	0	0
	L04AC05	Ustekinumab	0.5	a	0	a	0.61	0.050	0.0019	0.48	0.039	0.0015	0.13	0.011	4.2E-04	-	0	0	-	0	0	-	0	0
	L04AD01	Ciclosporin	0.001	f	0	a	2,004	0.33	0.013	1,929	0.32	0.012	1,811	0.30	0.011	1,755	0.29	0.011	1,718	0.28	0.011	1,623	0.27	0.010
	L04AD02	Tacrolimus	0.01	i	0.60	l	84	0.055	0.0021	89	0.06	0.0023	95	0.062	0.0024	102	0.067	0.0026	109	0.072	0.0028	120	0.079	0.0031
	L04AX01	Azathioprine	0.02	f	0.02	l	3,702	12	0.46	4,029	13	0.50	4,307	14	0.54	4,622	15	0.58	4,933	16	0.62	5,222	17	0.66

SI3. Consumption of cytostatic drugs (g/day) andPEC_{river}(ng/L) according to the DF calculated with the mean flow of each hydrographic basins in Spain (2015).

			Duero		Guadalquivir		Guadiana		Júcar		Miño		Segura		Tajo		Ebro	
Group	ATC code	Name	consumption g/day	PEC_{river} ng/L	Consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	Consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L
G03	G03HA01	Cyproterone	1.5E+01	6.2E-02	2.9E+01	5.6E-01	1.0E+01	1.3E+00	3.6E+01	2.4E+00	6.0E+00	5.7E-02	1.4E+01	1.7E+00	5.5E+01	4.1E+00	2.2E+01	1.2E-01
H02	H02AB07	Prednisone	1.9E+02	1.8E-01	3.5E+02	1.6E+00	1.3E+02	3.7E+00	4.5E+02	6.8E+00	7.4E+01	1.6E-01	1.7E+02	4.8E+00	6.8E+02	1.2E+01	2.8E+02	3.5E-01
L01	L01AA01	Cyclophosphamide	3.3E+00	1.2E-02	6.1E+00	1.1E-01	2.2E+00	2.4E-01	7.7E+00	4.5E-01	1.3E+00	1.1E-02	2.9E+00	3.2E-01	1.2E+01	7.8E-01	4.8E+00	2.3E-02
	L01AA02	Chlorambucil	7.3E-04	1.0E-07	1.4E-03	9.4E-07	4.9E-04	2.1E-06	1.7E-03	3.9E-06	2.8E-04	9.5E-08	6.5E-04	2.8E-06	2.6E-03	6.8E-06	1.1E-03	2.0E-07
	L01AA03	Melphalan	1.9E-04	4.4E-07	3.6E-04	4.0E-06	1.3E-04	9.0E-06	4.5E-04	1.7E-05	7.5E-05	4.0E-07	1.7E-04	1.2E-05	6.9E-04	2.9E-05	2.8E-04	8.5E-07
	L01AB01	Busulfan	9.2E-04	2.6E-07	1.7E-03	2.4E-06	6.2E-04	5.4E-06	2.2E-03	1.0E-05	3.6E-04	2.4E-07	8.2E-04	7.1E-06	3.3E-03	1.7E-05	1.3E-03	5.1E-07
	L01AX04	Dacarbazine	1.9E-03	1.4E-05	3.6E-03	1.2E-04	1.3E-03	2.8E-04	4.5E-03	5.2E-04	7.5E-04	1.3E-05	1.7E-03	3.7E-04	6.9E-03	9.0E-04	2.8E-03	2.7E-05
	L01BA01	Methotrexate	9.2E+00	6.0E-03	1.7E+01	5.4E-02	6.2E+00	1.2E-01	2.1E+01	2.3E-01	3.6E+00	5.5E-03	8.2E+00	1.6E-01	3.3E+01	3.9E-01	1.3E+01	1.2E-02
	L01BB02	Mercaptopurine	1.7E-02	9.8E-05	3.2E-02	8.9E-04	1.2E-02	2.0E-03	4.0E-02	3.7E-03	6.7E-03	9.0E-05	1.5E-02	2.6E-03	6.1E-02	6.5E-03	2.5E-02	1.9E-04
	L01BB03	Tioguanine	1.0E-03	7.9E-06	1.9E-03	7.1E-05	6.9E-04	1.6E-04	2.4E-03	3.0E-04	4.0E-04	7.2E-06	9.1E-04	2.1E-04	3.7E-03	5.2E-04	1.5E-03	1.5E-05
	L01BB05	Fludarabine	2.2E-02	1.9E-04	4.2E-02	1.7E-03	1.5E-02	3.9E-03	5.2E-02	7.2E-03	8.7E-03	1.7E-04	2.0E-02	5.2E-03	8.0E-02	1.3E-02	3.3E-02	3.7E-04
	L01BC01	Cytarabine	9.0E-05	6.5E-08	1.7E-04	5.9E-07	6.0E-05	1.3E-06	2.1E-04	2.5E-06	3.5E-05	6.0E-08	8.0E-05	1.8E-06	3.2E-04	4.3E-06	1.3E-04	1.3E-07
	L01BC02	Fluorouracil	8.5E-02	4.8E-05	1.6E-01	4.3E-04	5.7E-02	9.8E-04	2.0E-01	1.8E-03	3.3E-02	4.4E-05	7.5E-02	1.3E-03	3.0E-01	3.1E-03	1.2E-01	9.3E-05
	L01BC03	Tegafur	6.2E+00	4.4E-03	1.2E+01	4.0E-02	4.2E+00	9.1E-02	1.5E+01	1.7E-01	2.4E+00	4.0E-03	5.5E+00	1.2E-01	2.2E+01	2.9E-01	9.1E+00	8.6E-03
	L01BC06	Capecitabine (2010)	5.2E+02	7.0E-01	9.6E+02	6.4E+00	3.5E+02	1.4E+01	1.2E+03	2.7E+01	2.0E+02	6.4E-01	4.6E+02	1.9E+01	1.8E+03	4.6E+01	7.6E+02	1.4E+00
	L01BC52	Fluorouracilo/ salicilico	1.0E+00	5.6E-04	1.9E+00	5.1E-03	6.7E-01	1.2E-02	2.3E+00	2.1E-02	3.9E-01	5.2E-04	8.9E-01	1.5E-02	3.6E+00	3.7E-02	1.5E+00	1.1E-03
	L01CA01	Vinblastine	4.1E-05	4.8E-09	7.6E-05	4.4E-08	2.7E-05	1.0E-07	9.6E-05	1.8E-07	1.6E-05	4.4E-09	3.6E-05	1.3E-07	1.5E-04	3.2E-07	6.0E-05	9.4E-09
	L01CA02	Vincristine	3.3E-06	4.5E-09	6.2E-06	4.1E-08	2.2E-06	9.3E-08	7.8E-06	1.7E-07	1.3E-06	4.1E-09	3.0E-06	1.2E-07	1.2E-05	3.0E-07	4.9E-06	8.8E-09
	L01CB01	Etoposide	1.2E-01	1.6E-03	2.3E-01	1.5E-02	8.3E-02	3.4E-02	2.9E-01	6.2E-02	4.8E-02	1.5E-03	1.1E-01	4.4E-02	4.4E-01	1.1E-01	1.8E-01	3.2E-03

			Duero		Guadalquivir		Guadiana		Júcar		Miño		Segura		Tajo		Ebro	
Group	ATC code	Name	consumption g/day	PEC_{river} ng/L	Consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	Consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L
	L01DB01	Doxorubicin	1.0E-03	7.3E-06	1.9E-03	6.7E-05	6.9E-04	1.5E-04	2.4E-03	2.8E-04	4.0E-04	6.7E-06	9.2E-04	2.0E-04	3.7E-03	4.9E-04	1.5E-03	1.4E-05
	L01DB03	Epirubicin	9.1E-04	1.3E-06	1.7E-03	1.2E-05	6.1E-04	2.7E-05	2.1E-03	4.9E-05	3.6E-04	1.2E-06	8.1E-04	3.5E-05	3.3E-03	8.6E-05	1.3E-03	2.5E-06
	L01DB06	Idarubicin	1.3E-06	1.3E-09	2.4E-06	1.2E-08	8.6E-07	2.6E-08	3.0E-06	4.8E-08	5.0E-07	1.2E-09	1.1E-06	3.4E-08	4.6E-06	8.4E-08	1.9E-06	2.5E-09
	L01DC03	Mitomycin	4.1E-03	5.8E-06	7.7E-03	5.3E-05	2.8E-03	1.2E-04	9.6E-03	2.2E-04	1.6E-03	5.4E-06	3.7E-03	1.6E-04	1.5E-02	3.9E-04	6.0E-03	1.1E-05
	L01XA02	Carboplatin	4.9E-03	2.9E-05	9.1E-03	2.6E-04	3.3E-03	6.0E-04	1.1E-02	1.1E-03	1.9E-03	2.7E-05	4.3E-03	7.8E-04	1.7E-02	1.9E-03	7.1E-03	5.6E-05
	L01XD03	Methyl aminolevulinate	3.7E-01	2.6E-03	6.9E-01	2.4E-02	2.5E-01	5.4E-02	8.7E-01	1.0E-01	1.5E-01	2.4E-03	3.3E-01	7.1E-02	1.3E+00	1.7E-01	5.4E-01	5.2E-03
	L01XD04	Aminolevulinicacid	3.7E-01	2.6E-03	6.9E-01	2.4E-02	2.5E-01	5.4E-02	8.7E-01	1.0E-01	1.5E-01	2.4E-03	3.3E-01	7.1E-02	1.3E+00	1.7E-01	5.4E-01	5.2E-03
	L01XX05	Hydroxycarbamide	5.2E+02	3.7E+00	9.7E+02	3.3E+01	3.5E+02	7.6E+01	1.2E+03	1.4E+02	2.0E+02	3.4E+00	4.6E+02	9.9E+01	1.8E+03	2.4E+02	7.6E+02	7.2E+00
	L01XX11	Estramustine	2.2E+00	1.9E-04	4.2E+00	1.7E-03	1.5E+00	3.9E-03	5.3E+00	7.2E-03	8.8E-01	1.7E-04	2.0E+00	5.2E-03	8.0E+00	1.3E-02	3.3E+00	3.7E-04
	L01XX14	Tretinoin	1.7E-01	1.1E-04	3.2E-01	9.7E-04	1.1E-01	2.2E-03	4.0E-01	4.1E-03	6.6E-02	9.8E-05	1.5E-01	2.9E-03	6.1E-01	7.1E-03	2.5E-01	2.1E-04
	L01XX23	Mitotane	7.4E+00	5.5E-03	1.4E+01	5.0E-02	5.0E+00	1.1E-01	1.7E+01	2.1E-01	2.9E+00	5.0E-03	6.6E+00	1.5E-01	2.7E+01	3.6E-01	1.1E+01	1.1E-02
	L01XX35	Anagrelide	9.1E-02	1.3E-05	1.7E-01	1.2E-04	6.1E-02	2.7E-04	2.1E-01	4.9E-04	3.5E-02	1.2E-05	8.1E-02	3.5E-04	3.2E-01	8.5E-04	1.3E-01	2.5E-05
L02	L02AB01	Megestrol	1.6E+02	7.1E-02	2.9E+02	6.4E-01	1.1E+02	1.5E+00	3.7E+02	2.7E+00	6.1E+01	6.5E-02	1.4E+02	1.9E+00	5.6E+02	4.7E+00	2.3E+02	1.4E-01
	L02AB02	Medroxyprogesterone	2.6E+00	1.6E-02	4.9E+00	1.5E-01	1.8E+00	3.4E-01	6.1E+00	6.3E-01	1.0E+00	1.5E-02	2.3E+00	4.5E-01	9.4E+00	1.1E+00	3.8E+00	3.2E-02
	L02AE01	Buserelin	1.3E-03	3.6E-06	2.4E-03	3.3E-05	8.6E-04	7.5E-05	3.0E-03	1.4E-04	5.0E-04	3.3E-06	1.1E-03	9.9E-05	4.6E-03	2.4E-04	1.9E-03	7.1E-06
	L02AE02	Leuprorelin	4.1E-01	2.9E-04	7.7E-01	2.7E-03	2.8E-01	6.0E-03	9.7E-01	1.1E-02	1.6E-01	2.7E-04	3.7E-01	7.9E-03	1.5E+00	1.9E-02	6.0E-01	5.7E-04
	L02AE03	Goserelin	4.1E-02	5.3E-04	7.6E-02	4.8E-03	2.7E-02	1.1E-02	9.5E-02	2.0E-02	1.6E-02	4.9E-04	3.6E-02	1.4E-02	1.5E-01	3.5E-02	5.9E-02	1.0E-03
	L02AE04	Triptorelin	1.7E-01	1.0E-03	3.1E-01	9.2E-03	1.1E-01	2.1E-02	3.9E-01	3.8E-02	6.5E-02	9.3E-04	1.5E-01	2.7E-02	5.9E-01	6.7E-02	2.4E-01	2.0E-03
	L02AE05	Histrelina	5.6E-04	4.0E-06	1.0E-03	3.7E-05	3.7E-04	8.3E-05	1.3E-03	1.5E-04	2.2E-04	3.7E-06	5.0E-04	1.1E-04	2.0E-03	2.7E-04	8.2E-04	7.9E-06
	L02BA01	Tamoxifen	4.1E+01	5.3E-03	7.6E+01	4.8E-02	2.7E+01	1.1E-01	9.5E+01	2.0E-01	1.6E+01	4.9E-03	3.6E+01	1.4E-01	1.5E+02	3.5E-01	6.0E+01	1.0E-02
	L02BA03	Fulvestrant	1.9E+00	1.6E-05	3.6E+00	1.5E-04	1.3E+00	3.4E-04	4.5E+00	6.3E-04	7.4E-01	1.5E-05	1.7E+00	4.5E-04	6.8E+00	1.1E-03	2.8E+00	3.2E-05

			Duero		Guadalquivir		Guadiana		Júcar		Miño		Segura		Tajo		Ebro	
Group	ATC code	Name	consumption g/day	PEC_{river} ng/L	Consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	Consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L	consumption g/day	PEC_{river} ng/L
	L02BB01	Flutamide	3.0E+01	3.9E-02	5.6E+01	3.5E-01	2.0E+01	8.1E-01	7.0E+01	1.5E+00	1.2E+01	3.6E-02	2.7E+01	1.1E+00	1.1E+02	2.6E+00	4.4E+01	7.6E-02
	L02BB03	Bicalutamide	7.8E+01	6.0E-01	1.5E+02	5.5E+00	5.2E+01	1.2E+01	1.8E+02	2.3E+01	3.0E+01	5.5E-01	6.9E+01	1.6E+01	2.8E+02	4.0E+01	1.1E+02	1.2E+00
	L02BG03	Anastrozole	8.6E-01	1.2E-03	1.6E+00	1.1E-02	5.8E-01	2.5E-02	2.0E+00	4.6E-02	3.4E-01	1.1E-03	7.7E-01	3.3E-02	3.1E+00	8.0E-02	1.3E+00	2.4E-03
	L02BG04	Letrozole	5.3E+00	4.4E-03	9.8E+00	4.0E-02	3.5E+00	9.2E-02	1.2E+01	1.7E-01	2.1E+00	4.1E-03	4.7E+00	1.2E-01	1.9E+01	2.9E-01	7.7E+00	8.7E-03
	L02BG06	Exemestane	1.5E+01	2.2E-04	2.8E+01	2.0E-03	1.0E+01	4.4E-03	3.5E+01	8.2E-03	5.8E+00	2.0E-04	1.3E+01	5.8E-03	5.3E+01	1.4E-02	2.2E+01	4.2E-04
L03	L03AB03	Interferon gamma	4.0E-05	2.9E-07	7.5E-05	2.6E-06	2.7E-05	6.0E-06	9.4E-05	1.1E-05	1.6E-05	2.7E-07	3.6E-05	7.8E-06	1.4E-04	1.9E-05	5.8E-05	5.6E-07
	L03AB04	Interferon alfa-2a	3.7E-05	2.7E-07	6.9E-05	2.4E-06	2.5E-05	5.6E-06	8.7E-05	1.0E-05	1.5E-05	2.5E-07	3.3E-05	7.3E-06	1.3E-04	1.8E-05	5.4E-05	5.3E-07
	L03AB05	Interferon alfa-2b	5.7E-05	4.1E-07	1.1E-04	3.7E-06	3.8E-05	8.5E-06	1.3E-04	1.6E-05	2.2E-05	3.8E-07	5.1E-05	1.1E-05	2.0E-04	2.7E-05	8.3E-05	8.0E-07
L04	L04AA06	Mycophenolicacid	1.6E+03	8.5E+00	2.9E+03	7.7E+01	1.1E+03	1.7E+02	3.7E+03	3.2E+02	6.1E+02	7.8E+00	1.4E+03	2.3E+02	5.6E+03	5.6E+02	2.3E+03	1.7E+01
	L04AA10	Sirolimus	1.6E-01	1.5E-05	2.9E-01	1.3E-04	1.1E-01	3.0E-04	3.7E-01	5.6E-04	6.1E-02	1.3E-05	1.4E-01	4.0E-04	5.6E-01	9.6E-04	2.3E-01	2.8E-05
	L04AA13	Leflunomide	1.7E+01	1.2E-01	3.1E+01	1.1E+00	1.1E+01	2.4E+00	3.9E+01	4.5E+00	6.5E+00	1.1E-01	1.5E+01	3.2E+00	6.0E+01	7.7E+00	2.4E+01	2.3E-01
	L04AA18	Everolimus	3.0E-01	9.3E-04	5.7E-01	8.5E-03	2.0E-01	1.9E-02	7.1E-01	3.5E-02	1.2E-01	8.6E-04	2.7E-01	2.5E-02	1.1E+00	6.2E-02	4.4E-01	1.8E-03
	L04AD01	Ciclosporin	7.6E+01	1.1E-03	1.4E+02	1.0E-02	5.1E+01	2.3E-02	1.8E+02	4.2E-02	3.0E+01	1.0E-03	6.8E+01	3.0E-02	2.7E+02	7.2E-02	1.1E+02	2.1E-03
	L04AD02	Tacrolimus	5.6E+00	3.2E-04	1.0E+01	2.9E-03	3.8E+00	6.7E-03	1.3E+01	1.2E-02	2.2E+00	3.0E-04	5.0E+00	8.8E-03	2.0E+01	2.1E-02	8.2E+00	6.3E-04
	L04AX01	Azathioprine	2.4E+02	6.9E-02	4.6E+02	6.3E-01	1.6E+02	1.4E+00	5.7E+02	2.6E+00	9.5E+01	6.4E-02	2.2E+02	1.9E+00	8.7E+02	4.6E+00	3.6E+02	1.4E-01

SI4. Acute toxicity and risk assessment for cytostatic drugs according to PEC_{river} in Tajo basin

						TAJO 2015						
			Specie	Endpoint	Referència	PEC riverng/L	NOEC (mg/L)	LOEC (mg/L)	EC50 (mg/L)	LC50 (mg/L)	RQnoec	RQ
G03	Cyproterone	crustacean	*D. magna*	48h, EC50	PubliChem, MSDS	4.1E+00			2.4			1.7E-03
H02	Prednisone	algae	*P. subcapitata*	IC50, growth 3d	Cunningham 2006	1.2E+01			31			3.8E-04
		rotifer	*B.calyciflorus*	mortality 24h	DellaGreca 2003*	1.2E+01				54.5		2.2E-04
		crustacean	*D. magna*	immobilisation 24h	DellaGreca 2003*	1.2E+01	99.9				1.1E-06	
L01	Cyclophosphamide	algae	*P. subcapitata*	grouth-inhibition 96h	Zounkova 2007	7.8E-01	250	500	930		3.1E-08	8.4E-07
		bacteria	*P. putida*	grouth-inhibition 16h	Zounkova 2007	7.8E-01	1000	>1000	>1000		7.8E-09	
		crustacean	*D. magna*	Immobilisation 48h (estimation)	Zounkova 2007	7.8E-01	1000		1795 (QSAR)		7.8E-09	4.4E-07
		fish	*B. rerio*	96-h mortality (estimation)	Zounkova 2007	7.8E-01			70 (QSAR)			1.1E-05
	Chlorambucil	crustacean	*D. magna*	24h	Gomez-canela 2015	6.8E-06				30.6		2.2E-10
	Melphalan	crustacean	*D. magna*	48h	GSK MSDS	2.9E-05			>11			2.6E-09
	Busulfan		Not expected to be harmful to aquatic organisms		GSK MSDS	1.7E-05						
	Dacarbazine		no data			9.0E-04						
	Methotrexate	algae	*S. subspicatus*	Growth 72h	Besse 2012	3.9E-01			260			1.5E-06
		protozoan	*T. pyriformis*	Growth 48h	Besse 2012	3.9E-01			45			8.8E-06
		crustacean	*D. magna*	Immobilization 48h	Besse 2012	3.9E-01			>1000			
		frog	*X. laevis*	Growth 96h	Besse 2012	3.9E-01			0.015			2.6E-02
	Mercaptopurine	algae	*greenalgae*	72h static test	GSK MSDS	6.5E-03	>100					
		crustacean	*D. magna*	48h static test	GSK MSDS	6.5E-03	32		72		2.02E-09	9.0E-08
	Tioguanine	algae	*greenalgae*	72h static test	GSK MSDS	5.2E-04	0.0005		0.0034		1.04E-05	1.5E-04
		crustacean	*D.magna*	48h	GSK MSDS	5.2E-04			16.5			3.1E-08
	Fludarabine		Avoid release into the environment		Caymanchemical	1.3E-02						
	Cytarabine	algae	*D. suspicatus*	growthinhibition 72h	Zounkova 2010	4.3E-06		40	53			8.1E-11

	bacteria	*P. putida*	growthinhibition 16h	Zounkova 2010	4.3E-06		10	17			2.5E-10
	crustacean	*D. magna*	acute test 48h	Zounkova 2010	4.3E-06		100	200			2.1E-11
Fluorouracil	algae	*P. subcapitata*	grouth-inhibition	Zounkova 2007	3.1E-03	0.001	0.01	0.11		3.1E-05	2.9E-05
	bacteria	*P. putida*	grouth-inhibition	Zounkova 2007	3.1E-03	0.003	0.01	0.027		1.1E-05	1.2E-04
	rotifer	*B. calyciflorus*	24h acute	Parrella 2014	3.1E-03	up tp 200					
	crustacean	*D. magna*	Immobilisation	Zounkova 2007	3.1E-03	1	10	36		3.1E-08	8.7E-08
	amphibian??	*T. platyurus*	24h acute	Parrella 2014	3.1E-03			0.28			1.1E-05
Tegafur	fish	*D. rerioembryos*		Weigt 2011	2.9E-01			3.4mM	6064		4.8E-08
Capecitabine	algae	*P. subcapitata*	Growth 72h	Besse 2012	4.6E+01	0.14				3.2E-3	
(2010)	rotifer	*B. calyciflorus*	acute 24h	Parrella 2014	4.6E+01	up to 500					
	crustacean	*D. magna*	acute 48h	Parrella 2014	4.6E+01			224			2.1E-04
	amphibian??	*T. platyurus*	acute 24h	Parrella 2014	4.6E+01			197.7			2.3E-04
Vinblastine		no data in any MSDS			3.2E-07						
Vincristine		no data in any MSDS			3.0E-07						
Etoposide	algae	*P. subcapitata*	grouth-inhibition 96h	Zounkova 2007	1.1E-01	<10	10	250			4.3E-07
	bacteria	*P. putida*	grouth-inhibition 16h	Zounkova 2007	1.1E-01	200	250	630		5.4E-09	1.7E-07
	rotifer	*B. calyciflorus*	acute 24h	Parrella 2014	1.1E-01			74.85			1.4E-06
	crustacean	*D. magna*	Immobilisation 48h	Zounkova 2007	1.1E-01	10	30	30		1.1E-07	3.6E-06
	fish	*O. mykiss*	96h	pfizer MSDS	1.1E-01				12900		8.4E-09
	amphibian??	*T. platyurus*	acute 24h	Parrella 2014	1.1E-01			NE up to 120			
Doxorubicin	algae	*P. subcapitata*	grouth-inhibition 96h	Zounkova 2007	4.9E-04	1	10	13		4.8E-09	3.7E-08
	bacteria	*P. putida*	grouth-inhibition 16h	Zounkova 2007	4.9E-04	1	10	>1000		4.8E-09	4.9E-10
	rotifer	*B. calyciflorus*	acute 24h	Parrella 2014	4.9E-04			12.69			3.8E-08
	crustacean	*D. magna*	Immobilisation	Zounkova 2007	4.9E-04	0.01	0.1	2.0		4.8E-07	2.4E-07
	fishcells	*P.lucida*	NR (PLHC-1)	Caminada 2006	4.9E-04			1.179			4.1E-07
	amphibian??	*T. platyurus*	acute 24h	Parrella 2014	4.9E-04			0.31			1.6E-06
Epirubicin		nd			8.6E-05						
Idarubicin		nd			8.4E-08						

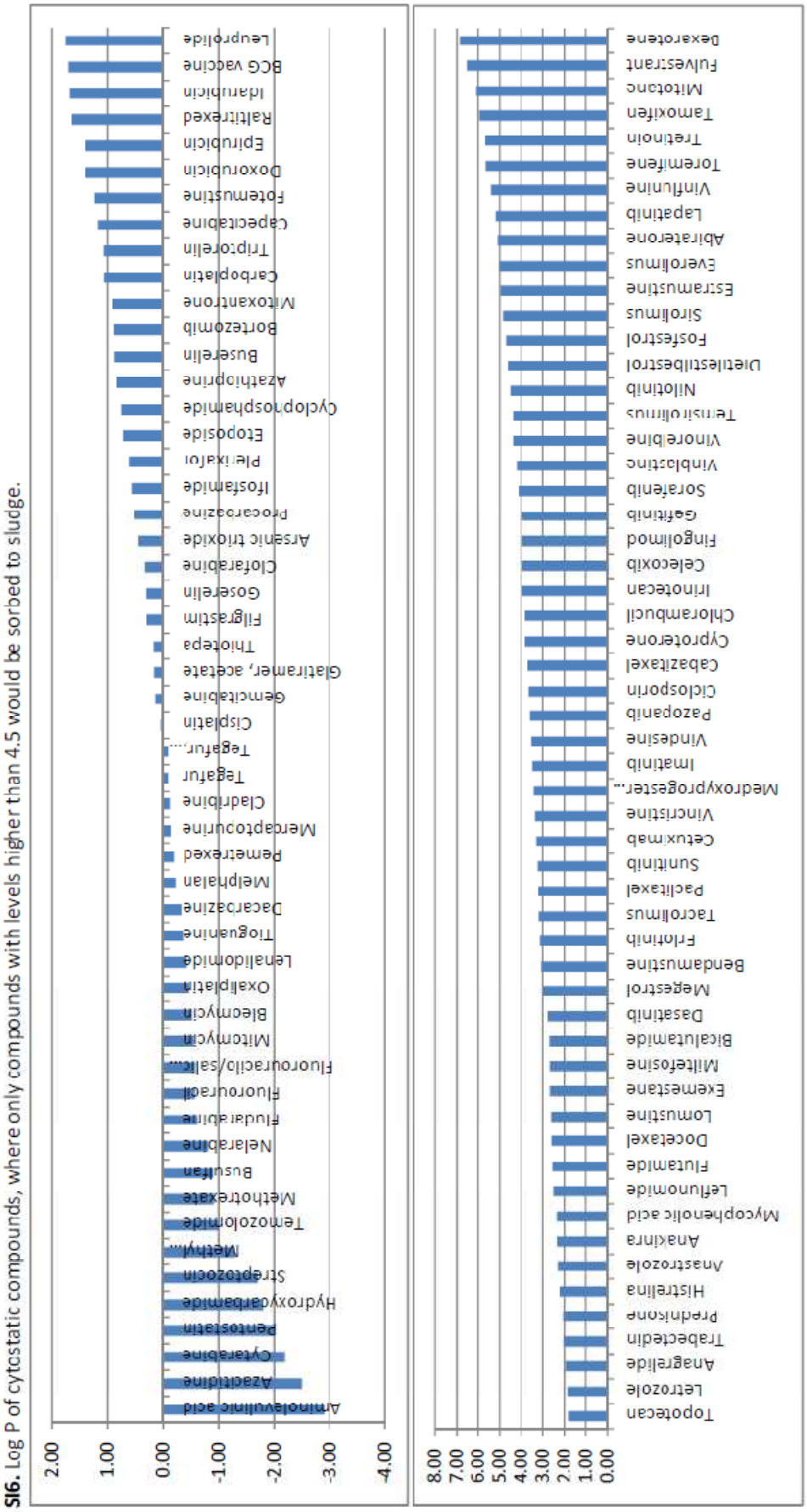
	Mitomycin		nd			3.9E-04			
	Carboplatin		nd			1.9E-03			
	Methylaminolevulinate		nd			1.7E-01			
	Aminolevulinicacid		nd			1.7E-01			
	Hydroxycarbamide	crustacean	*D. magna*	48h	Bristol-Myers_MSDS	2.4E+02	> 100		
	Estramustine		nd			1.3E-02			
	Tretinoin		nd			7.1E-03			
	Mitotane		nd			3.6E-01			
	Anagrelide		nd			8.5E-04			
L02	Megestrol	crustacean	*D. magna*	48h	FDA	4.7E+00		5	9.3E-04
	Medroxyprogesterone		*D. magna*	48h	publichem MSDS	1.1E+00	>100		1.1E-05
	Buserelin		nd			2.4E-04			
	Leuprorelin		nd			1.9E-02			
	Goserelin		nd			3.5E-02			
	Triptorelin		nd			6.7E-02			
	Histrelina		nd			2.7E-04			
	Tamoxifen	fishcells	*P.lucida*	MTT (RTG-2)	Caminada 2006	3.5E-01		7.10	4.9E-05
	Fulvestrant		nd			1.1E-03			
	Flutamide	crustacean	*D. magna*	48h	Kar 2010	2.6E+00		5.301	4.9E-04
	Bicalutamide	algae	*algae*	ec50	Whitacre 2010-	4.0E+01	>1		4.0E-02
		crustacean	*D. magna*	48h	EC safety data sheet	4.0E+01		> 5,3	7.5E-03
		fish?	*B. sunfish*	96h (static)	AstraZeneca MSDS	4.0E+01	4.4		9.0E-05
	Anastrozole		nd			8.0E-02			
	Letrozole		*no acute*			2.9E-01			
	Exemestane		nd			1.4E-02			
	Interferon gamma		nd			1.9E-05			

	Interferon alfa-2a		nd			1.8E-05				
	Interferon alfa-2b		nd			2.7E-05				
L04	Mycophenolicacid	algae	*P. subcapitata*	ErC50 72h	Santa Cruz Biotechnology	5.6E+02	0.01		5.6E-01	
		crustacean	*D. magna*	EC50 48h	Roche 2014	5.6E+02	27.7	>100	2.0E-04	5.6E-03
		fish	*fish*	96h	Roche 2014	5.6E+02	1.7		3.3E-03	
	Sirolimus		nd			9.6E-04				
	Leflunomide	algae	algae	72h	USP Safety data sheet	7.7E+00		22.42		3.4E-04
		crustacean	*D. magna*	48h	USP Safety data sheet	7.7E+00		17		4.5E-04
		fish	Fish	96h	Council of Europe, MSDS	7.7E+00		2.64		2.9E-03
	Everolimus		nd			6.2E-02				
	Ciclosporin	crustacean	*D. magna*	immobilization	Cunningham 2006	7.2E-02		20		3.6E-06
		fish	*O. mykiss*	mortality	Cunningham 2006	7.2E-02		>100		7.2E-07
	Tacrolimus		nd			2.1E-02				
	Azathioprine	crustacean	*D. magna*	EC50 48h	PubliChem, MSDS	4.6E+00	>100			

SI5. Chronic toxicity for cytostatic drugs

Group	Name		Specie	Endpoint	Reference	NOEC mg/L	LOEC mg/L	EC_{50} mg/L	LC_{50}mg/L
L01	Cyclo-phosphamide	crustacean	*D. magna*	reproduction	Evelyne 2011	56			
	Methotrexate	bacteria	*V. fischerii*	Luminescence 30min	Besse 2012			1220	
	Cytarabine	crustacean	*D. magna*	Reproduction 21d	Zounkova2010		3.7	10	
		crustacean	*D.magna*	reproduction 21d	Besse 2012		3.7		
	Fluorouracil	crustacean	*C. dubia*	chronic 7d	Parrella 2014	0.00222	0.0067	0.00335	
		crustacean	*D. magna*	chronic 21d	Parrella 2014	0.00206	0.063	26.4	
		rotifer	*B. calyciflorus*	chronic 48h	Parrella 2014	0.125	0.25	0.322	
		crustacean	*D. magna*	Reproduction 21 days	Zounkova 2010		0.05	0.1	
		bacteria	*V. fischerii*	Luminescence	Besse 2012			0.12	
	Capecitabine	crustacean	*D. magna*	Reproduction 48h	Besse 2012			>850	
		crustacean	*D. magna*	chronic 21d	Parrella 2014	1.9	6.1	20.6	
		crustacean	*C. dubia*	chronic 7d	Parrella 2014	0.6	1.9	2.4	
		rotifer	*B. calyciflorus*	chronic 48h	Parrella 2014	3.12	6.9	15.4	
	Etoposide	crustacean	*D. magna*	chronic 21d	Parrella 2014	0.111	0.33	0.239	
		crustacean	*C. dubia*	chronic 7d	Parrella 2014	0.0976	0.31	0.204	
		rotifer	*B. calyciflorus*	chronic 48h	Parrella 2014	2.5	5	3.7	
	Doxorubicin	crustacean	*D. magna*	chronic 21d	Parrella 2014	nd	nd	nd	
		crustacean	*C. dubia*	chronic 7d	Parrella 2014	nd	nd	nd	
		rotifer	*B. calyciflorus*	chronic 48h	Parrella 2014	5	10	7.7	
L02	Tamoxifen	fish	*P. promelas*	Overall 284d	Besse 2012	0.005			
		fish	*P. promelas*	F1 larvae growth 28d	Besse 2012			0.00008	
		fish	*P. promelas*	F1 growth 112d	Besse 2012			0.00001	

Group	Name		Specie	Endpoint	Reference	NOEC mg/L	LOEC mg/L	EC_{50} mg/L	LC_{50}mg/L
		fish	*P. promelas*	Increase in Vtg, F1 males 112d	Besse 2012			0.00001	
		crustacean	*A. tonsa*	Larval development 5d	Besse 2012			49	
	Flutamide	fish	*P. promelas*	14d LC50	Whitacre 2010			>1000	
		fish	*P.promelas*	mortality 14d	Cunningham 2006				>1000
		rotifer	*B. calicyflorus*	Fertilization of sexual females 96h	Besse 2012		0.001		
		fish	*G. aculeatus*	Spiggin inhibition 21d	Besse 2012		0.5		
		fish	*G. aculeatus*	Male behavior 21d	Besse 2012		0.1		
		fish	*P. promelas*	Testis alterations21d	Besse 2012		0.062		
		fish	*P. promelas*	Increase of estradiol plasma levels 21d	Besse 2012		0.651		
	Bicalutamide	crustacean	*D. magna*	21 d	AstraZeneca MSDS	0.56			3.2
	Letrozole	fish	*O. latipes*	Fecundity 21d	Besse 2012		0.005		
		fish	*O. latipes*	Fertility 21d	Besse 2012		0.005		
		fish	*O. latipes*	Increase in genotypic F1 males 21d	Besse 2012		0.005		
L04	Mycophenolic acid	algae	*S. subspicatus*	14d	Cellcept MSDS		1.6		



SI6. Log P of cytostatic compounds, where only compounds with levels higher than 4.5 would be sorbed to sludge.

2.3. Discussió de resultats

En aquesta tesi s'ha utilitzat el càlcul dels PECs per a la priorització de citostàtics ja que posa de manifest aquells fàrmacs que tenen una major probabilitat de ser detectats en el medi ambient.

Per poder dur a terme aquest càlcul és imprescindible conèixer el consum d'aquests compostos, que en el marc d'aquesta tesi s'han recollit de manera global per primera vegada a nivell de Catalunya i Espanya. A Catalunya, s'han recopilat cadascun dels compostos antineoplàstics administrats en un període de tres anys (2010-2012), dades que s'han recollit a l'article científic I, "*Predicting concentrations of cytostatic drugs in sewage effluents and surface waters of Catalonia (NE Spain)*", i que van ser facilitades per la Secretaria de Gerència de Farmàcia i del Medicament del Servei Català de la Salut (CatSalut). Les dades de l'administració farmacèutica van ser lliurades directament en forma de mg anuals per a cada citostàtic, juntament amb el seu codi ATC. En canvi, per l'administració hospitalària es va recollir el nombre d'activitats dispensades de cada fàrmac, juntament amb la quantitat continguda de principi actiu. Les activitats corresponen al nombre de pastilles, injeccions, càpsules o qualsevol altre presentació que pugui tenir un fàrmac. Així, coneixent les activitats administrades i els mg de principi actiu en cada una es va poder calcular els consum exacte per a cadascun dels 132 citostàtics administrats. El nombre de compostos administrats a Catalunya és molt major al nombre de citostàtics que s'inclouen en els mètodes d'anàlisi i posa de manifest la importància que té poder fer-ne una priorització abans del desenvolupament d'una metodologia analítica.

Malgrat que per alguns citostàtics els valors de consum a Catalunya van ser del mateix ordre que els recollits en altres països europeus, com per la hidroxicarbamida i la capecitabina, per altres compostos el consum a Catalunya van ser menors, com es recull a la Taula 1 de l'Article I. Per aquest motiu en l'article científic II, "*Anticancer drugs: consumption trends in Spain, prediction of environmental concentrations and potential risks*" es va voler recopilar el consum de tots els citostàtics administrats a Espanya, ja que les dades publicades fins al moment només feien referència a uns pocs citostàtics. L'objectiu principal d'aquest treball també ha estat fer-ne la priorització. En aquest segon article, les dades de consum van ser proporcionades per la Secretaria de la Subdirecció General de Qualitat de Medicaments i Productes Sanitaris del Ministeri de Sanitat, Serveis Socials i Igualtat però només es va poder obtenir les dades corresponents a l'administració en farmàcies. A nivell espanyol encara no es disposa d'un sistema validat que permeti agregar la informació de l'administració hospitalària procedent dels diferents centres de l'estat. Així, per l'administració farmacèutica es va facilitar el nombre

d'envasos dispensats de cada medicament, juntament amb el nombre unitats contingudes i la concentració de principi actiu, de manera similar a les dades de Catalunya. Amb aquesta informació es van poder calcular els mg dispensats de cada fàrmac. El consum global de citostàtics a Espanya es recull a la Figura 2.1, juntament amb els valors obtinguts per Catalunya i els publicats per França (Besse et al., 2012) i Alemanya (Kümmerer et al., 2016), que resumeix els valors descrits en els corresponents articles científics. Tots els països esmentats recullen les dades dels fàrmacs corresponents al grup L01, però no la resta de grups terapèutics. Com es pot observar en la figura, el consum anual per càpita d'L01 es troba entre 227 i 263 mg/any/hab, excepte per Espanya que és de 114 mg/any/hab. Aquesta diferència es deu a que aquestes dades corresponen només a l'administració farmacèutica. El que més destaca en la figura, però, és l'alt consum de fàrmacs el grup L04, que no s'havien recollit prèviament. Al no ser un grup de fàrmacs que s'administrin exclusivament contra el càncer, altres autors no n'havien considerat el seu consum. La inclusió del grup terapèutic L04 en el recull de dades d'aquesta tesi ha tingut una importància significativa en els compostos prioritzats, com es comenta a continuació.

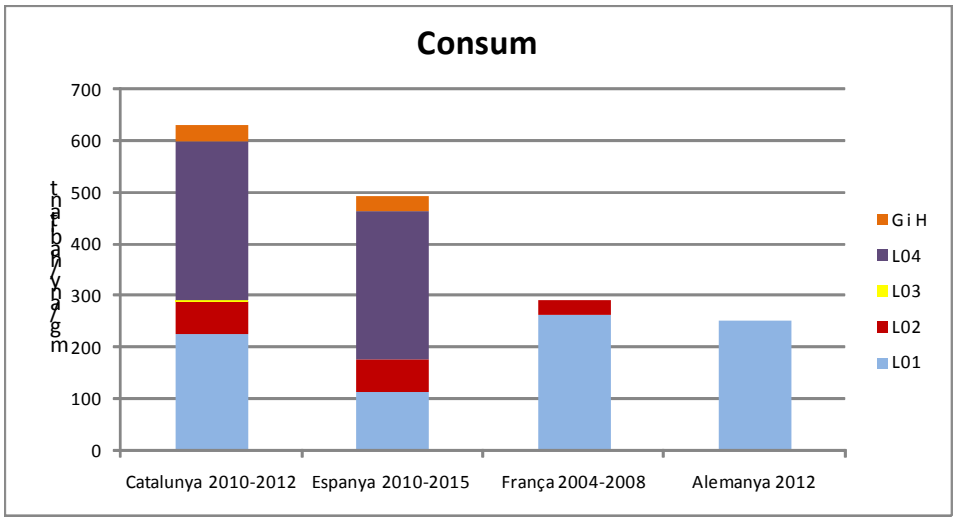


Figura 2.1. Consum de citostàtics en farmàcies i hospitals (només en farmàcies per Espanya), segons les diferents classes terapèutiques.

Amb els consums de cada citostàtic per Catalunya s'ha calculat el seu PEC en rius i s'ha prioritzat l'àcid micofenòlic, la hidroxicarbamida, la capecitabina, la bicalutamida, l'imatinib i la prednisona com els citostàtics amb una concentració prevista més elevada (PEC_{riu}>1 ng/L). Amb les dades d'Espanya, els citostàtics prioritzats van ser l'àcid micofenòlic, la hidroxicarbamida, la bicalutamida, la capecitabina, la prednisona i la leflunomida (PEC_{riu}>1 ng/L). La majoria d'aquests compostos coincideixen amb els prioritzats per Catalunya amb l'excepció de l'imatinib, que és un fàrmac administrat majoritàriament en hospitals i per això no es coneix la totalitat del seu

consum a Espanya. Sense tenir en compte els valors de PECs, els fàrmacs prioritzats a França van ser la hidroxicarbamida, la capecitabina, el 5-fluorouracil, l'imatinib i la bicalutamida (Besse et al., 2012) i a Alemanya van ser la hidroxicarbamida, la capecitabina, el 5-fluorouracil, l'imatinib i la ifosfamida (Kümmerer et al., 2016). Com es pot observar, la majoria de compostos coincideixen en els diferents estudis. No obstant, al no tenir en compte el consum dels L04, cap dels dos autors van prioritzar l'àcid micofenòlic, que a Catalunya i Espanya és el que s'espera trobar a concentracions més elevades al medi. També cal destacar que, així com diversos autors han desenvolupat mètodes per a l'anàlisi del 5-fluorouracil (Mahnik et al., 2004; Martín et al., 2011; Mullot et al., 2009; Usawanuwat et al., 2014; Yu et al., 2006), prioritari tant a França com a Alemanya, i la seva presència ha estat analitzada en efluents d'hospital (Mahnik et al., 2007), EDARs (Kosjek et al., 2013) i rius (Usawanuwat et al., 2014), pels altres citostàtics prioritzats es té poca informació ambiental. Per a la hidroxicarbamida només Usawanuwat et al. (2014) l'han analitzada a Bangkok, l'àcid micofenòlic va ser analitzat per Giebułtowicz i Nałęcz-Jawecki (2016) en un article publicat just després que els articles presentats en aquesta tesi, i no hi ha cap treball enfocat a la presència de la bicalutamida al medi. La diferència entre els compostos prioritzats i els inclosos en metodologies analítiques posa de manifest que sovint, i de forma errònia, es poden analitzar fàrmacs que difícilment poden estar presents en el medi i en canvi, no es determinen aquells fàrmacs que potencialment poden tenir un impacte ambiental, degut al seu elevat consum. Això te greus repercussions tant a nivell ambiental com de recursos (temps i costos) ja que el sistema de vigilància no serà prou adequat.

El càlcul dels PECs té dos objectius, el primer és proposar un llistat de citostàtics prioritaris, però a més es pretén que les concentracions calculades siguin similars a les concentracions reals del medi. Així, també es pot tenir una aproximació de la qualitat de les masses d'aigua abans de fer la monitorització. Un dels problemes que es va plantejar al moment d'obtenir els valors de PECs va ser l'ús del factor de dilució (DF), el paràmetre que té en compte la dilució dels efluents de depuradora un cop alliberats al riu. La majoria d'autors que donen valors de PEC utilitzen un DF de 10, proposat per la EMA. S'ha vist però, que aquest paràmetre pot variar àmpliament. En els càlculs que s'han dut a terme en aquesta tesi s'ha utilitzat en primer lloc el valor de DF de 25, proposat per Keller et al. (2014) com a valor mitjà per Espanya. Aquest valor s'ha aplicat tant per a Catalunya com per Espanya, ja que es va considerar que donaria uns valors de PEC més acurats que el factor de 10. No obstant, en el segon article científic,"*Anticancer drugs: consumption trends in Spain, prediction of environmental concentrations and potential risks.*", es va decidir optimitzar el DF per a les principals conques hidrogràfiques d'Espanya, que es va calcular en base al cabal mitjà i la població de cada zona. Rius que travessen o estan propers a grans

poblacions rebran més abocaments de depuradores i, per tant, de fàrmacs, mentre que rius més cabalosos podran diluir millor aquesta càrrega. Conseqüentment, l'ús d'un DF adequat permet obtenir uns valors de PEC més realistes i identificar les zones o èpoques on pot haver-hi un major risc per al medi ambient. Amb aquesta nova aproximació es va identificar la conca del riu Tajo com la més afectada per la possible presència de citostàtics. Malauradament, no hi ha dades bibliogràfiques sobre la presència de citostàtics en aquest riu que permetin corroborar els resultats obtinguts.

Per determinar el possible risc que aquestes concentracions previstes poden suposar per als organismes aquàtics, la EMA proposa un límit de 10 ng/L per dur-ne a terme una avaluació. Aquest límit d'acció està basat en valors de toxicitat aguda i la mateixa EMA indica que caldrà que sigui revisat quan es disposi de més dades de toxicitat crònica (EMA, 2006). Per aquest motiu, en els articles presentats en aquesta tesi s'han considerat també alguns citostàtics amb un PEC inferior a 10 ng/L, per bé que no s'ha trobat que les concentracions previstes en el medi suposin un risc immediat per als organismes aquàtics. Això no obstant, el consum dels citostàtics ha demostrat tenir una lleugera tendència a l'augment i per aquest motiu és recomanable mantenir un control de la seva presència al medi. A més, encara hi ha poca informació sobre la toxicitat crònica d'aquests compostos i no es poden descartar efectes a llarg termini.

L'estudi del consum dels citostàtics i el càlcul dels PECs ha estat molt important en el desenvolupament d'aquesta tesi ja que ha permès obtenir una visió global de l'abast de l'administració d'aquests compostos i les possibles implicacions per al medi ambient. De manera similar també es podria aplicar aquesta metodologia a altres famílies de fàrmacs com a punt de partida, per tal de conèixer aquells compostos més importants. No obstant, no sempre és possible calcular els PECs principalment degut a la falta d'informació respecte el seu consum. La manera de recollir les dades pot variar en funció de cada país, i pot incloure la recopilació de dades a nivell hospitalari, farmacèutic o d'administració privada, i es poden expressar a nivell local, nacional o concretes per a un hospital determinat. En el cas que no es disposi de dades de consum, se sol optar per dur a terme un mostreig i determinar les concentracions reals del medi (MECs) per alguns fàrmacs pre-seleccionats. Ambdues metodologies tenen els seus avantatges i els seus inconvenients, que tot i haver-se mencionat en diferents punts d'aquesta tesi, s'ha considerat oportú de comentar conjuntament (Taula 2.1).

El principal avantatge dels PECs és la senzillesa del seu càlcul, que permet aplicar-lo a un nombre molt elevat de fàrmacs. En funció de les dades, s'obté el valor de PEC per efluents de depuradora i rius, a partir dels valors de consum de diferents anys o zones geogràfiques. En el nostre estudi s'ha creat un full de càlcul que permet obtenir ràpidament aquests valors per a

tots els citostàtics administrats i elaborar una llista de compostos prioritaris que facilita plantejar una campanya de monitorització de les aigües. A més, el seguiment d'aquests valors al llarg del temps permet veure si el PEC d'algun fàrmac augmenta significativament i així, incloure'l en la selecció de citostàtics prioritaris. Aquesta avaluació prèvia al mostreig ajuda a centrar els esforços en aquest grup de citostàtics i així optimitzar el temps i els costos de desenvolupar una metodologia. Amb aquest càlcul també s'eludeixen alguns dels principals inconvenients que comporta fer el monitoratge de les aigües sense informació prèvia del seu estat. Sovint, se sol començar a analitzar els mateixos compostos que estan descrits a la bibliografia, sense poder saber si aquests s'administren en el país estudiat. A més, el nombre de compostos que es poden analitzar en un sol mètode és limitat i per tant, cal realment posar esforços en analitzar aquells compostos que es trobaran, amb elevada probabilitat, en aigua.

Taula 2.1. Avantatges i inconvenients dels PECs i els MECs

PEC	**MEC**
Avantatges	
• no cal fer una selecció prèvia • estalvi econòmic • permet prioritzar els compostos amb més probabilitat de ser detectats	• valors reals • permet una avaluació de risc més acurada
Inconvenients	
• és un valor aproximat • te una incertesa associada (Fexc, Fwwtp, DF) • dificultat d'aconseguir les dades	• cal seleccionar els compostos prèviament • cost elevat (material, dissolvents, equip) • dificultat de l'extracció de la mostra

No obstant, el càlcul de PECs també té alguns inconvenients. El seu desavantatge principal és que es tracta d'un valor aproximat, amb una gran incertesa associada. A més del consum, s'apliquen a l'equació els valors d'excreció i eliminació que poden oscil·lar en un ampli ventall. Cal afegir que tampoc hi ha dades experimentals de tots ells, fent que els valors teòrics calculats amb models matemàtics puguin diferir dels valors reals. Aquests valors tampoc són sempre calculables, ja que per determinades molècules els programes no ho permeten i cal recórrer a utilitzar un valor arbitrari. Així, el valor de PEC obtingut pot divergir del valor real. En aquest aspecte és on rau el principal avantatgedels MECs. Els valors que s'obtenen corresponen a la concentració real d'aquelles aigües i permeten fer una avaluació de risc més acurada per la zona d'estudi sempre i quan es disposi d'una elevada freqüència de mostreig. Un altre inconvenient

en el càlcul dels PECs pot ser la dificultat d'obtenir les dades. En el cas dels consums, no sempre hi ha un organisme que reculli aquesta informació o pot ser que calguin llargs temps d'espera per poder aconseguir-la. O simplement que no sigui accessible.

Per determinar si els valors de PEC han estat ben calculats se solen comparar amb els MECs, on la ràtio PEC/MEC ha d'estar entre 0,2 i 4 per ser considerat acceptable. Si aquest criteri es compleix, és indicador de que les concentracions que s'han calculat coincideixen amb les que s'han detectat al medi i confirma que el càlcul de PECs és una eina adequada. Contràriament, si el quocient no s'ajusta al rang d'acceptació, tampoc és indicatiu de que els PECs siguin completament erronis. El problema principal es troba en que alguns fàrmacs es troben a concentracions molt baixes i al fer el càlcul dividint dos nombres petits, el resultat pot trobar-se molt per fora del rang d'acceptació. Així mateix, el valor de PEC correspon a una concentració mitjana per a tots els rius de l'àrea estudiada, mentre que els MECs corresponen a la concentració present en el moment del mostreig, que no té perquè ser constant.

En conjunt, tant el càlcul dels PECs com la mesura dels MECs tenen certs avantatges i inconvenients associats. Per aquest motiu, i per tal d'obtenir informació complementària, es recomana l'ús de les dues metodologies. En primer lloc, fer ús dels PECs per preveure quins compostos i nivells es pot esperar detectar al medi per, en segon lloc, desenvolupar una metodologia que permeti detectar aquestes concentracions i fer-ne l'estudi de la seva presència i impacte reals. La comparació dels PECs amb els mesures reals permet la validació del càlcul de les concentracions teòriques i per tan, assegura que la seva aplicació en termes de vigilància sigui adequada.

3. COMPORTAMENT I PRESÈNCIA DE CITOSTÀTICS AL MEDI AMBIENT

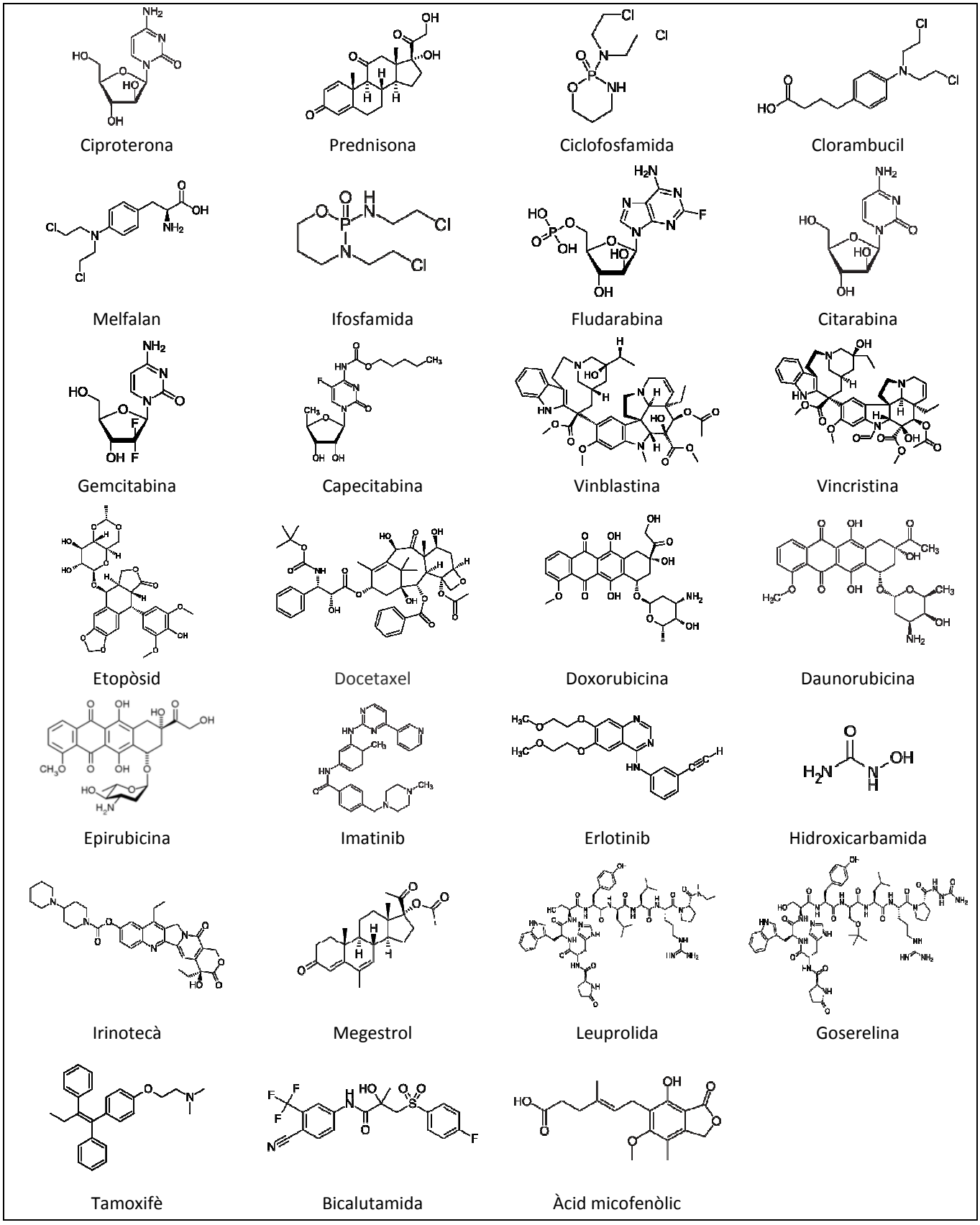
3.1. Introducció

Com s'ha descrit anteriorment, els fàrmacs que s'administren en els tractaments contra el càncer són molt nombrosos. Per aquest motiu cal fer-ne una selecció per monitoritzar la seva presència al medi. Per al desenvolupament d'aquesta tesi s'han escollit els fàrmacs amb un PEC més alt calculat per Catalunya, juntament amb altres fàrmacs que també han estat àmpliament estudiats en la bibliografia. Aquesta selecció es troba recollida a la Figura 3.1, on es mostra la seva estructura. Com es pot observar, es tracta de compostos polars que tenen estructures molt diferents entre ells, tot i que algunes parelles són isòmers estructurals, com la ifosfamida i la ciclofosfamida, o estereoisòmers, com la daunorubicina i l'epirubicina.

A la Taula 3.1 es recullen les seves propietats fisicoquímiques més importants, que són molt diverses, juntament amb el nombre CAS (*Chemical Abstracts Service*) i la fórmula molecular. La massa nominal oscil·la entre 243 i 1269, amb l'excepció de la hidroxicarbamida que és de 76. La solubilitat varia entre 1,5 mg/L per la ciproterona i 1E6 mg/L per la hidroxicarbamida, degut a l'alta polaritat que li donen el grup hidroxil i amina. Cal esmentar que no hi ha valors experimentals per a molts d'aquests fàrmacs i que la major part de les propietats mostrades són teòriques i varien en funció de la font consultada. Els valors de log Kow indiquen que aquests compostos no tindran tendència a acumular-se en la matèria orgànica, ja que són inferiors a 4,5. Només el tamoxifè presenta un log Kow de 7 degut a la seva baixa polaritat, fent possible la seva bioacumulació. El pKa varia entre 1,29 per al melfalan i 17,83 per la ciproterona, però majoritàriament es troben per sobre de 9, valor que indica que es trobaran al medi en forma neutra. La pressió de vapor és més baixa per aquells compostos amb un pes molecular més alt, indicant la seva baixa volatilitat. La hidroxicarbamida, amb pressió de vapor de 2,9E-4 mm Hg és els que tindria una major tendència a evaporar-se. De manera similar, la constant d'Henry (H) també està relacionada amb la pressió de vapor i indica la poca tendència que tindran aquests fàrmacs a passar a la fase gasosa un cop solubilitzats. En aquest cas però, és el megestrol qui té una constant més alta (1,1E-8 atm m^3/mol), però no suficient per ser considerat un compost volàtil.

Aquestes propietats fisicoquímiques indiquen que els citostàtics es trobaran al medi aquàtic preferiblement en solució, des d'on no tindran tendència a evaporar-se (amb excepció, potser, de la hidroxicarbamida).

Figura 3.1. Estructura dels citostàtics seleccionats per estudiar la seva presència al medi.



Taula 3.1. Propietats fisicoquímiques.

Grup ATC	Nom	Nº CAS	Formula molecular	Massa nominal	Solubilitat[1] (mg/L)	log Kow[2]	pKa[1]	Pressió de vapor[2] (mm Hg)	H[2] (atm m^3/mol)
G03	Ciproterona	427-51-0	$C_{22}H_{27}ClO_3$	374,5	1,5	3,59	17.83	1,13E-10	3,05E-09
H02	Prednisona	53-03-2	$C_{21}H_{26}O_5$	358,4	111	1,46*	12.58	2,89E-13	2,83E-10
L01	Ciclofosfamida	6055-19-2	$C_7H_{15}Cl_2N_2O_2P$	261,1	10.000*	0,8*	12.78	4,40E-05	1,36E-11
	Clorambucil	305-03-3	$C_{14}H_{19}NO_2Cl_2$	304,2	12.400*	1,7*	4.46	9,31E-07	2,71E-10
	Melfalan	148-82-3	$C_{13}H_{18}Cl_2N_2O_2$	305,2	358	-0,52*	1.29	3,03E-10	4,19E-13
	Ifosfamida	3778-73-2	$C_7H_{15}Cl_2N_2O_2P$	261,1	3.780*	0,86*	13.24	8,15E-05	1,36E-11
	Fludarabina	21679-14-1	$C_{10}H_{12}FN_5O_4$	285,2	3.530*	-2,8*	12.45	3,71E-15	1,30E-22
	Citarabina	69-74-9	$C_9H_{13}N_3O_5$	243,2	43.800	-2,8*	12.55	8,74E-12	1,57E-19
	Gemcitabina	122111-03-9	$C_9H_{11}F_2N_3O_4$	263,1	22.300	-1,4*	11.52	1,70E-09	1,70E-17
	Capecitabina	154361-50-9	$C_{15}H_{22}FN_3O_6$	359,3	26.000*	0,4*	8.23	1,12E-12	2,92E-19
	Vinblastina	143-67-9	$C_{46}H_{58}N_4O_{19}$	810,9	16,9	3,7*	10.87	1,43E-27	1,03E-27
	Vincristina	2068-78-2	$C_{46}H_{56}N_4O_{10}$	824,9	30	2,82*	10.85	4,78E-29	2,08E-30
	Etopòsid	33419-42-0	$C_{29}H_{32}O_{13}$	588,5	978	0,6*	9.33	1,20E-19	1,57E-30
	Docetaxel	114977-28-5	$C_{43}H_{53}NO_{14}$	807,9	12,7	2,4*	10.96	5,61E-27	8,09E-24
	Doxorubicina	25316-40-9	$C_{27}H_{29}NO_{11}$	543,5	1.180	1,27*	9.53	2,53E-23	2,23E-23
	Daunorubicina	23541-50-6	$C_{27}H_{29}NO_{10}$	527,5	39,2*	1,83*	9.53	9,50E-21	1,45E-25
	Epirubicina	56390-09-01	$C_{27}H_{29}NO_{11}$	543,5	93*	*-0,5	9.53	2,53E-23	2,23E-23
	Imatinib	152959-95-5	$C_{29}H_{31}N_7O$	493,6	14,6	3,01	12,45	3,63E-17	8,07E-24
	Erlotinib	183319-69-9	$C_{22}H_{23}N_3O_4$	393,4	8,91	2,7*	16.14	7,06E-11	1,03E-17
	Hidroxicarbamida	127-07-1	$CH_4N_2O_2$	76,0	1,00E+06*	-1,8*	10.14	2,95E-04	5,42E-11
	Irinotecà	100286-90-6	$C_{33}H_{38}N_4O_6$	586,6	107	3,2*	11.71	2,26E-23	8,82E-23
L02	Megestrol	595-33-5	$C_{22}H_{30}O_3$	342,4	2*	3,2*	17.83	3,48E-10	1,14E-08
	Leuprolida	74381-53-6	$C_{59}H_{84}N_{16}O_{12}$	1209,4	--	1,16	--	--	--
	Goserelina	145781-92-6	$C_{59}H_{84}N_{18}O_{14}$	1269,4	28,3	-2*	9.27	--	--
	Tamoxifè	10540-29-1	$C_{26}H_{29}NO$	371,5	0,167*	7,1*	9.27	3,46E-08	4,49E-10
	Bicalutamida	90357-06-5	$C_{18}H_{14}F_4N_2O_4S$	430,3	5*	2,5*	11.95	7,54E-15	2,82E-15
L04	Àcid micofenòlic	24280-93-1	$C_{17}H_{20}O_6$	320,3	35,5	2,8*	3.57	1,59E-10	3,82E-12

*Valor experimental; [1]Drug Bank Database (2013)[2]Royal Society of Chemistry (2014)

Aquests fàrmacs, un cop excretats, entren en contacte amb les aigües residuals i poden patir diversos processos que contribueixen a la seva degradació al llarg del cicle de l'aigua, (Figura 3.2). La resistència enfront d'aquests mecanismes determinarà que puguin arribar als rius o a les aigües de consum en major o menor mesura. No obstant, el comportament dels citostàtics durant aquest circuit només ha estat estudiat per uns pocs compostos, que es descriuen a continuació.

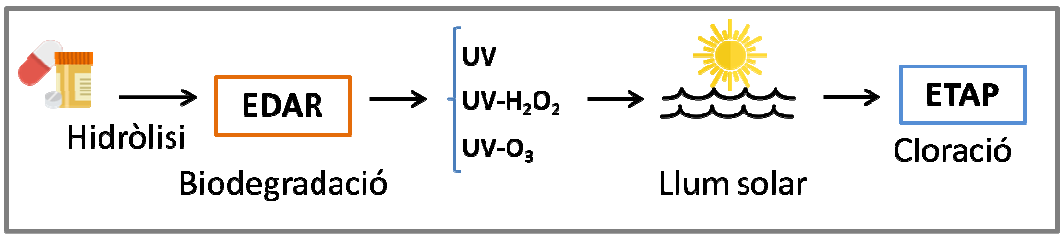


Figura 3.2. Processos que poden contribuir en la degradació dels citostàtics.

La hidròlisi és el procés principal que té lloc des de l'excreció del fàrmac fins arribar a la depuradora. L'estudi d'aquest procés de manera individual permet també identificar els productes de degradació. Per exemple, la hidròlisi de la mitoxantrona i el clorambucil en aigua preparada (*ASTM hard water*) es va estudiar per determinar la toxicitat dels seus productes de degradació (Gómez-Canela et al., 2013a). Els resultats van mostrar que la mitoxantrona es degrada instantàniament i el clorambucil en 24h, i que ambdós fàrmacs tenen productes de degradació actius estables. Per altra banda,Negreira et al. (2014b) van determinar l'estabilitat de 26 citostàtics en aigües residuals. En aquest cas l'objectiu va ser establir les condicions òptimes d'emmagatzematge de les mostres abans de l'extracció. Aquest estudi també senyala la inestabilitat del clorambucil a temperatura ambient, juntament amb altres fàrmacs com la doxorubicina, la vinblastina o la capecitabina, que es degraden en més d'un 50% de la seva concentració inicial en 24h. En canvi, en aigua pura, l'estabilitat de tots els citostàtics estudiats va ser en general més gran, atribuïble a la manca d'activitat biològica (Negreira et al., 2013b). Aquesta informació també permet interpretar que els fàrmacs amb baixa estabilitat tindran menys probabilitats de trobar-se presents en aigua.

Aquells citostàtics que no s'hidrolitzin arribaran a les depuradores, on el tractament secundari basat en la biodegradació és el principal mecanisme d'eliminació de contaminants. Els fangs actius estan formats per un cultiu de diferents microorganismes que s'alimenten de la matèria orgànica que porta l'aigua residual i la transformen idealment en diòxid de carboni i aigua. Però com s'ha vist anteriorment, els processos de tractament primari i secundari de les EDARs no són prou efectius per eliminar tota la càrrega de citostàtics dels influents ja que alguns

d'ells encara es detecten a la sortida. A més, no només són citostàtics els fàrmacs que arriben a les depuradores, sinó que les EDAR reben contínuament altres fàrmacs i compostos que podrien afectar els microorganismes del fang. Concretament, s'ha vist que el tractament continu d'aigües residuals d'hospitals amb fangs activats a escala de laboratori va provocar un augment de la fracció orgànica juntament amb un canvi en la proporció dels diferents tipus de bacteris presents en la biomassa (Stalder et al., 2013). En aquest estudi la qualitat de l'aigua tractada no es va veure afectada, tot i que si es va observar un canvi genètic en els microorganismes. Per tal de millorar l'eficàcia d'eliminació de citostàtics en aigües residuals s'ha proposat augmentar el temps de retenció hidràulic (temps que les aigües estan en contacte amb els fangs actius) o bé utilitzar un reactor biològic de membrana (MBR) (Zhang et al., 2013). Aquests reactors inclouen una membrana d'ultrafiltració i permeten treballar amb concentracions més altes de fangs, que pot implicar millors eliminacions. Per exemple, utilitzant fangs convencionals es va obtenir menys d'uns 60 % d'eliminació pel 5-fluorouracil en un període d'incubació de 50 dies (Yu et al., 2006). En canvi, amb un MBR pilot l'eliminació va ser gairebé completa en 24 h (Mahnik et al., 2007). Altres citostàtics han estat més resistents a aquest procés, com la ciclofosfamida, el carboplatí i el cisplatí, pels quals el tractament amb MBR va eliminar-ne entre el 20 i el 60 % (Zhang et al., 2013). En aquest cas, serien necessaris processos addicionals, com aplicar tractaments terciaris, per eliminar-los completament i així evitar la seva arribada el medi ambient.Això permet obtenir aigües de millor qualitat i fins i tot propicia la seva reutilització.

Els tractaments terciaris que poden ser una alternativa per a la completa eliminació dels citostàtics més persistents i evitar la seva presència als efluents són la fotòlisi directa (UV), la fotòlisi en combinació amb peròxid d'hidrogen (UV-H_2O_2) i l'ozó (O_3). Aquestes processos s'utilitzen si les aigües tractades no compleixen els requisits per poder ser abocades o si es destinen a usos que requereixen una bona qualitat de l'aigua. La degradació mitjançant fotòlisi directa consisteix en irradiar una solució amb els analits amb llum ultraviolada (UV). L'eficiència de degradació dependrà principalment de l'estructura molecular dels analits i de la dosi de radiació utilitzada que en els processos de desinfecció habituals no és suficient per a molts contaminants emergents (Yang et al., 2014). Les làmpades utilitzades poden generar una radiació monocromàtica a 254 nm (làmpades LP), o radiació policromàtica (làmpades MP), que també influenciarà en l'eficàcia de degradació dels analits. Chen et al. (2008) van estudiar la degradabilitat de la ciclofosfamida, l'irinotecà i el tamoxifè amb diferents tractaments. La degradació amb UV va resultar no ser efectiva per als tres citostàtics, que van tenir un percentatge de degradació d'entre el 20 i el 40% després de 30 min d'irradiació amb una làmpada monocromàtica. De forma similar, Llewellyn et al. (2011) van observar una degradació

negligible de la ciclofosfamida, ja que en van detectar les mateixes concentracions abans i després del tractament amb UV d'una EDAR, i Lester et al. (2011) també van obtenir una baixa degradació de la ciclofosfamida utilitzant una làmpada MP. Els estudis de degradació de Lutterbeck et al. (2016) per a la ciclofosfamida i el 5-fluorouracil amb radiació UV corroboren els resultats obtinguts en els anteriors treballs per la ciclofosfamida. Per al 5-fluorouracil l'eliminació va ser completa després de 32 min de radiació. Finalment, la irradiació de citarabina va resultar en només un 10% d'eliminaciódesprés de dues hores, amb una làmpada MP (Ocampo-Pérez et al., 2010).

Degut a la poca eficàcia d'eliminació per alguns citostàtics, els processos d'oxidació avançada (AOPs) s'han proposat com un mètode complementari als anteriorment esmentats per acabar d'eliminar els fàrmacs més recalcitrants. El peròxid d'hidrogen és un potent oxidant que, irradiat amb UV, produeix radicals hidroxil (·OH). Aquests radicals tenen un alt potencial d'oxidació i per això el tractament amb *UV/H_2O_2*s'ha proposatcom a mètode per a l'eliminació de contaminants (Yang et al., 2014). La dosi d'H_2O_2 utilitzada tindrà una influència directa en la degradació dels analits. S'ha descrit que augmentar-ne la concentració fins a 150 mg/L millora el percentatge de degradació però a partir d'aquesta concentració, l'H_2O_2 reacciona amb els radicals ·OH i també adsorbeix radiació UV, competint amb els compostos a degradar (Lester et al., 2011). Així, s'ha determinat una eliminació del 90% per a l'irinotecà després de 15 min d'irradiació amb una concentració inicial d'H_2O_2 de 119 mg/L, però no va ser suficient per a la ciclofosfamida i el tamoxifè (50% d'eliminació) (Chen et al., 2008). Wols et al. (2013) també han determinat una baixa eliminació per a la ciclofosfamida i la ifosfamida (no n'indiquen el percentatge) utilitzant una làmpada MP. Aquesta baixa degradació de la ciclofosfamida coincideix amb els resultats de Tuerk et al. (2010) que van establir que és necessari un temps d'irradiació molt més alt per tal d'obtenir millors eliminacions. Concretament, van ser necessaris 285 min amb una làmpada LP i 52 min amb una làmpada MP. Per a la citarabina, l'addició d'H_2O_2 va permetre obtenir una eliminació superior al 90%, i es va determinar que la reacció és dependent del pH i de la concentració de peròxid addicionada (Ocampo-Pérez et al., 2010).

Un altre AOP que s'ha estudiat per l'eliminació de contaminants és l'ús d'ozó. L'eliminació de fàrmacs amb O_3 té dos mecanismes principals, que consisteixen en la reacció directa de l'analit amb l'O_3 molecular o en la reacció amb els radicals ·OH, producte de la descomposició d'aquest (Zhang et al., 2013). Aquest procés ha resultat ser efectiu per l'eliminació d'irinotecà i tamoxifè, però no per la ciclofosfamida (en aigua pura i pH neutre) (Chen et al., 2008). En canvi, Tuerk et al. (2010), determinen un temps necessari de 40 min per eliminar un 95% de la concentració de ciclofosfamida en aigua residual. Una avaluació més detallada va determinar que un augment

del pH (entre 9 i 11) millora la seva degradació, però que l'addició de H_2O_2 no té un efecte significatiu (Fernández et al., 2010). En canvi, la combinació d'ozó amb peròxid d'hidrogen i radiació ultraviolada ($UV/H_2O_2/O_3$) permet augmentar la constant de degradació de la ciclofosfamida, sent més efectiva que la combinació d'UV/O_3 o UV sol (Lester et al., 2011). Per altra banda,Somensi et al. (2012) van estudiar la degradació de dos citostàtics, doxorubicin i metotrexat, per ozonòlisi i amb combinació amb ultrasons (sonòlisi/ozonòlisi). Així, amb O_3 es va observar que l'eliminació de la doxorubicina és dependent del pH i es va obtenir una degradació de fins el 40% després de 20 min a pH 9, que es va veure augmentada a un 47% amb l'ajuda de la sonòlisi. Per al metotrexat, es va obtenir una degradació màxima del 75% amb O_3 a pH7 i del 80 % en combinació amb la sonòlisi a pH 11.

L'ús d'AOPs permet, doncs, degradar aquells citostàtics més recalcitrants, com la ciclofosfamida, que resisteixen els tractaments d'aigua convencionals. En general, cal optimitzar les dosis de radiació i reactius aplicats per tal de trobar la proporció i les condicions adequades que permetin una eliminació completa. Cal tenir en compte també que l'ús de tècniques avançades suposa una despesa energètica més gran, i no sempre són aplicables a gran escala. Tuerk et al. (2010) van fer un estudi del costos derivats d'aplicar radiació UV o ozó per l'eliminació de ciclofosfamida en efluents d'hospital, obtenint uns costos totals del mateix ordre per ambdós tractaments. Però tenint en compte que la major part de la càrrega de fàrmacs que arriben a les depuradors prové de les aigües residuals domèstiques, els mateixos tractaments aplicats en una EDAR tindrien un cost molt més elevat. En aquest mateix article s'indica com a exemple que eliminar 1g de l'antibiòtic sulfametoxazol pot tenir un cost de 5 € o de 50-200 € segons si s'aplica el tractament a l'efluent d'hospital o a l'EDAR, respectivament.

Aquells citostàtics que no s'hagin eliminat en els processos anteriors i arribin al riu estaran exposats a la llum solar, que també pot contribuir en la degradació d'aquests fàrmacs ja que té un espectre de radiació més ampli que la llum UV. Tot i que ha estat menys estudiat que la resta de processos, conèixer el seu comportament i degradabilitat és important per tal d'avaluar la seva persistència al medi, els possibles productes de degradació que es puguin generar i llur toxicitat, ja que poden afectar directament els organismes aquàtics. La degradació amb un simulador de llum solar per la prednisona durant 8 hores va generar set productes de degradació, alguns d'ells amb una toxicitat aguda més alta que el compost original i d'altres més baixa(DellaGreca et al., 2003). Per al tamoxifè, la irradiació durant 80h amb un simulador de llum solar va produir un 10% de degradació en aigua pura, i en presència de nitrats i àcids húmics a pH 9 la degradació va ser negligible i del 70% a pH 4 (DellaGreca et al., 2007). En canvi, l'exposició a llum solar natural durant 1 mes, a pH 4, va resultar en un 5 % de degradació. Tant el

tamoxifè com els seus derivats van donar valors de concentracions tòxiques per sobre les concentracions detectades al medi però que podrien tenir efectes a llarg termini. Lutterbeck et al. (2016), van estudiar l'eliminació de la ciclofosfamida i el 5-fluorouracil. La irradiació amb un simulador de llum solar no va degradar la ciclofosfamida, i només va degradar parcialment el 5-fluorouracil, donant lloc a productes de degradació no menys tòxics.

Per als citostàtics més persistents, la seva presència als rius fa que hi hagi la possibilitat que arribin a l'aigua de beguda. Per això, les estacions de tractament d'aigua potable (ETAP) sotmeten les aigües a diferents processos per eliminar la càrrega de contaminants i matèria orgànica que els arriben. La degradació amb clor és una etapa molt important en les ETAP, on s'utilitza per desinfectar i oxidar tant metalls com matèria orgànica. Aquest tractament és també determinant en l'eliminació de fàrmacs antineoplàstics, que ha estat estudiat per tal de determinar-ne l'eliminació del compost parental així com dels productes de degradació. El comportament de l'etopòsid es va estudiar a diferents concentracions de clor lliure (10-200 mg/L) i es va observar que a altes concentracions es degrada en pocs segons, mentre que a baixes dosis de clor, es manté en solució i no s'elimina completament després de 2h (Negreira et al., 2015a). Per al tamoxifè es va estudiar la degradació juntament amb els seus metabòlits principals en aigua pura i residual amb clor lliure. En ambdues matrius es va observar que el tamoxifè es va mantenir estable després de 90 min d'exposició (94% de la concentració inicial) i que els metabòlits es van degradar ràpidament (Negreira et al., 2015c). En canvi, l'erlotinib es va eliminar un 95% de la concentració addicionada inicialment en aigua pura però en aigua residual només es va produir un 40% d'eliminació, diferència que va ser atribuïda a la presència de matèria orgànica (Negreira et al., 2015b). Per la vincristina, la vinblastina i la vinorelbina també es va estudiar la seva degradació en clor. En aigua pura, la presència de vinblastina i vinorelbina després de 30 min va ser menor del 10% de la concentració inicial. En canvi, la presència de la vincristina va ser d'un 70% (Negreira et al., 2016). Roig et al. (2014) van estudiar la degradació del metotrexat amb una solució amb clor, que es va eliminar gairebé completament després de 120 minuts i van calcular un temps de vida mitjana de 21min. Finalment, Huber et al. (2005), van estudiar la degradabilitat de la ciclofosfamida, juntament amb altres fàrmacs. A diferents concentracions de ClO_2 la degradació de la ciclofosfamida va ser mínima, ja que després de 30 min, la seva presència va ser d'un 88-97% de la concentració inicial addicionada.Així doncs, l'eliminació de citostàtics amb clor és un mètode generalment efectiu amb l'excepció de la ciclofosfamida, però pot dependre de les dosis de clor utilitzades i del tipus d'aigua a tractar, fent que sigui menys efectiu si hi ha presència de matèria orgànica o altres compostos que puguin competir.

3.2. Resultats

Tenint en compte el cicle de l'aigua, és important conèixer els processos de degradació que poden tenir lloc en la natura oque es poden aplicar en el tractament de les aigües, però la informació disponible se centra en uns pocs fàrmacs. Per aquest motiu, a l'article científic III, titulat "*Biological and photochemical degradation of cytostatic drugs under laboratory conditions"* publicat al*Journal of Hazardous Materials* 323: 319-328 (2017) s'ha seleccionat un grup de citostàtics en funció dels seus consums, de la freqüència de detecció en aigües i de la seva capacitat per afectar l'ADN. Amb aquest grup de citostàtics s'han estudiat diferents tractaments de degradació, incloent tractaments d'oxidació avançada, per tal de determinar la seva degradabilitat i poder establir les seves constants de degradació. Aquesta informació és útil per determinar els compostos més persistents al medi i pels quals caldrà un estudi més exhaustiu per avaluar el seu impacte ambiental.

Amb aquests resultats, i degut a les altes concentracions de citostàtics que es preveuen detectar als rius de Catalunya d'acord amb els PECs calculats, s'ha dut a terme la monitorització de la conca del riu Besòs, un dels rius històricament més afectats per la contaminació antropogènica. Al llarg de la seva conca hi ha nombroses depuradores que hi aboquen les seves aigües, a més d'activitats industrials, fent que la major part del seu cabal provingui dels efluents d'aquestes. La presència d'altres contaminants, com els retardants de flama organofosforats (Cristale et al., 2013), ful·lerens (Sanchís et al., 2015), pesticides i fàrmacs (Matamoros et al., 2010) ha estat detectada amb anterioritat i per tant, no es descarta que també hi hagi presència d'antineoplàstics, tot i que no s'hagin estudiat en aquest riu.Aquest estudi es recull en l'article científic IV, "*Determination of cytostatic drugs in Besòs River (NE Spain) and comparison with predicted environmental concentrations", Environmental Science and Pollution Research* (2017) 24:6492-6503 on s'ha seleccionat un grup de citostàtics, d'acord amb el seu PEC i incloent-hi altres citostàtics sovint analitzats a la bibliografia, que s'han analitzat al llarg del riu Besòs. Els nivells detectats s'han comparat amb els seus PECs i se n'ha avaluat el risc per als organismes aquàtics.

Un altre riu important de les conques catalanes és el Llobregat. Al seu tram baix es localitza l'ETAP de Sant Joan Despí, que en capta l'aigua, la tracta i la distribueix a Barcelona i part de la seva àrea metropolitana. Tot i que els tractaments de potabilització són eficaços per a la majoria de compostos que arriben a l'entrada de la planta, s'ha detectat la presència d'alguns fàrmacs a la seva sortida però mai s'ha estudiat la presència de citostàtics. Per aquest motiu, i degut que l'àcid micofenòlic és el compost amb un PEC més elevat, en l'article científic V "*Do cytostatic drugs reach drinking water? The case of mycophenolic acid" (Short communication)*

Environmental Pollution (2016) 208 (B): 532–536 s'ha analitzat la seva presència a l'entrada de l'ETAP de Sant Joan Despí i al llarg de tots els processos de potabilització de la planta fins a l'aigua final.

3.2.1. Article científic III:

*Biological and photochemical degradation of cytostatic drugs under laboratory conditions.*Helena Franquet-Griell, Andrés Medina, Carme Sans, Silvia Lacorte. *Journal of Hazardous Materials* (2017) 323: 319-328.

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Biological and photochemical degradation of cytostatic drugs under laboratory conditions

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HIGHLIGHTS

- Cytostatic drugs with reactive chemical groups are rapidly hydrolyzed.
- Biodegradation is not capable to eliminate all cytostatic drugs.
- UV-C and photolysis are not effective for the elimination of cytostatics.
- UV-H_2O_2 eliminates all compounds and no traces are present after 4 min.
- Unless AOP is used, WWTP effluents are a main source of cytostatics to rivers.

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ABSTRACT

Cytostatic drugs, used in chemotherapy, have emerged as new environmental contaminants due to their recurrent presence in surface waters and genotoxic effects. Yet, their degradability and environmental fate is largely unknown. The aim of this study was to determine the degradation kinetics of 16 cytostatic drugs, prioritized according to their usage and occurrence in hospital and wastewater treatment plants (WWTP) effluents, through the following laboratory scale processes: hydrolysis, aerobic biodegradation, UV-C photolysis, UV-C/H_2O_2 and simulated solar radiation. Some drugs were unstable in milli-Q water (vincristine, vinblastine, daunorubicin, doxorubicin and irinotecan); others were photodegraded under UV-C light (melphalan and etoposide) but some others were found to be recalcitrant to biodegradation and/or UV-C, making necessary the use of advanced oxidation processes (AOPs) such as UV-C/H_2O_2 for complete elimination (cytarabine, ifosfamide and cyclophosphamide). Finally, radiation in a solar box was used to simulate the fate of cytostatic drugs in surface waters under natural radiation and complete removal was not observed for any drug. The degradation process was monitored using liquid chromatography coupled to high resolution mass spectrometry and pseudo-first order kinetic degradation constants were calculated. This study provides new data on the degradability of cytostatic compounds in water, thus contributing to the existing knowledge on their fate and risk in the environment.

1. Introduction

In the last years, cancer incidence in the global population is gradually increasing. In 2012, an estimated 14.1 million new cases of cancer occurred worldwide and caused 8.2 million deaths [1]. The incidence of cancer leads to the production and consumption of a large number and quantities of cytostatic drugs. On a country-wide basis, consumption of the most commonly used anticancer drugs (~10–20 drugs) is in the order of tonnes yr^{-1} [2,3] which warrants research on their fate and behaviour in the environment.

Once administered, cytostatic compounds are directly discharged into the sewer system by urinary and/or faecal excretions [4,5] and $\mu g L^{-1}$ concentrations have been detected in hospital effluents [6,7], in wastewater treatment plant (WWTPs) effluents [8] and in river waters [9]. Thus, sewage waters from urban areas and hospitals are the main source of cytostatic compounds towards WWTPs, although hospitals account for 5.5% [10] to 17% of the total discharged [11]. Cytostatics have been reported to have low biodegradability [12]. Indeed, some cytostatic compounds such as cyclophosphamide, ifosfamide and tamoxifen have been fre-

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Table 1
Physicochemical parameters of the primary treatment effluent and the activated sludge used in the biodegradation experiments.

Primary effluent		Bioreactor		
			t0	48h
pH	7.6	pH	7.4 ± 0.1	7.7 ± 0.2
DOC (mg C L^{-1})	51.8	TOC (mg/l) (1:1)	176 ± 34	9.4 ± 0.9
IC (mg C L^{-1})	80.2	TSS (mg/L)	1153 ± 67	1167 ± 72
TN (mg L^{-1})	37.3	TVSS (mg/L)	887 ± 146	990 ± 85
COD (mg O_2 L^{-1})	253			
BOD5 (mg L^{-1})	76.88			
BOD5/COD	0.30			
SS (mg L^{-1})	110			
UV_{254} (m^{-1})	36.2			
Turbidity (NTU)	70			
Alcalinity (mg$CaCO_3$ L^{-1})	369			
Conductivity (μS)	1945			
DOC; disolved organic carbon		TOC; total organic carbon		
IC; inorganic carbon		TSS; total suspended solids		
TN; total nitrogen		TVSS; total volatile suspended solids		
COD; chemical oxigen demand				
BOD5; biochemical oxigen demand				
UV; adsorbance at 254 nm				

quently reported in WWTP effluents indicating partial removal [5] attributed to their high stability [13].

Studies focused on the degradation of pharmaceuticals only include few cytostatic drugs. The elimination using activated sludge and membrane-bio-reactors was studied for cyclophosphamide [14] and ifosfamide [8,15], 5-fluorouracil, doxorubicin, epirubicin and daunorubicin [16]. No degradation was observed in some cases [8] and some compounds affected sludge viability [14]. Advanced Oxidation Process (AOP) techniques with UV-H_2O_2 or ozone have been proven to eliminate cyclophosphamide and ifosfamide at laboratory and pilot plant scale [17–19]. However, degradation constants have only been calculated for a few cytostatic compounds.

This paper aims to study the degradation kinetics of 16 widely used cytostatic drugs under laboratory conditions using hydrolysis, biodegradation, UV-C and simulated solar radiation. Because of the urgent need to eliminate these compounds from hospital and WWTP effluents to reduce their impact in the environment, AOP with UV-C/H_2O_2 was also tested to reach complete removal. The degradability herein studied includes a systematic and comprehensive approach that covers the most relevant natural and chemical degradation processes. Degradability in batch experiments were monitored using liquid chromatography coupled to Orbitrap mass spectrometry (LC-HRMS) due to its high sensitivity and selectivity [20].

2. Materials and methods

2.1. Chemicals and reagents

In this study sixteen cytostatic drugs were studied, prioritized according to major medical prescriptions, those usually detected in hospital and wastewater treatment plants effluents and those affecting DNA [6,8,16,21–23]. Compounds studied, their classification and physico-chemical properties are indicated in Table SI1. Stock solutions were prepared at 2000 mg L^{-1} and working solution at 100 mg L^{-1} in methanol (MeOH from Merck, Germany). Milli-Q water was produced from an Integral Water Purification System from Millipore (Billerica, MA, USA). Metavanadate, sodium hydrogen sulfite (40% w/v) and hydrogen peroxide solution (30% w/w) were supplied by Panreac Quimica Inc. (Spain).

Effluent from primary treatment and activated sludge collected from Calafell WWTP (Catalonia, Spain) were used to study the biodegradation of cytostatic drugs and their characteristics are shown in Table 1.

2.2. Experimental devices and conditions

To determine the simultaneous degradability of 16 cytostatic drugs under various controlled conditions, bench scale experiments were conducted at a concentration of 50 μg L^{-1} each, which is a concentration where cytostatics are often detected in wastewater [5]. Hydrolysis, UV-C, UV-C/H_2O_2 and photolysis experiments were carried out in spiked milli-Q water (pH of 6.3). Hydrolysis was used as control condition to evaluate the water stability of cytostatic drugs in water. For biodegradation experiments, cytostatic drugs were spiked in real wastewater (pH of 7.6) to simulate an activated sludge treatment and to test the effect of cytostatic drugs on bacteria adaptation and degradation potential.

To determine the losses due to adsorption in the reactors and other material used in the laboratory, we compared the spiked concentration and the measured concentration at t = 0 min. For the 5 conditions tested, the recovery of non-hydrolyzed compounds was of between 72 ± 4 and 98 ± 15%, indicating high efficiency of the experimental setup. The specific conditions for each experiment and the time aliquots analysed are detailed below.

2.2.1. Hydrolysis

Hydrolysis has been selected as the first step in the evaluation of the stability of cytostatic compounds in water. Cytostatic drugs have a solubility ranging from 0.0446 and 1.76E5 mg L^{-1}, and have reactive groups, which favour hydrolytic reactions. 1 L of milli-Q water was placed in a flask (covered with aluminium foil) and spiked with the mixture of the drugs and kept at room temperature (22 °C). 1 mL aliquots were taken at 0, 2, 5, 7, 10, 15, 30, 60 min, 2, 4, 24 and 48 h.

2.2.2. Biodegradation

A sequential batch reactor (SBR) to simulate activated sludge treatment was used. Two 1 L aerated reactors magnetically stirred and covered with aluminium foil to avoid light interference were inoculated with activated sludge to obtain an initial Total Suspended Solids (TSS) concentration of 1–1.2 g L^{-1}. Reactors were filled with WWTP primary effluent and one was spiked with cytostatics (B1) and the other was kept as control. A constant saturation level of O_2 was obtained by means of an air bubbling system with ceramic submerged diffusers. Aliquots were taken at 0, 15, 30, 45, 60 min, 2, 4, 8, 24 and 48 h. For this experiment, time zero corresponds to the moment just before starting the aeration of the bioreactor, with the spiked water already in contact with the

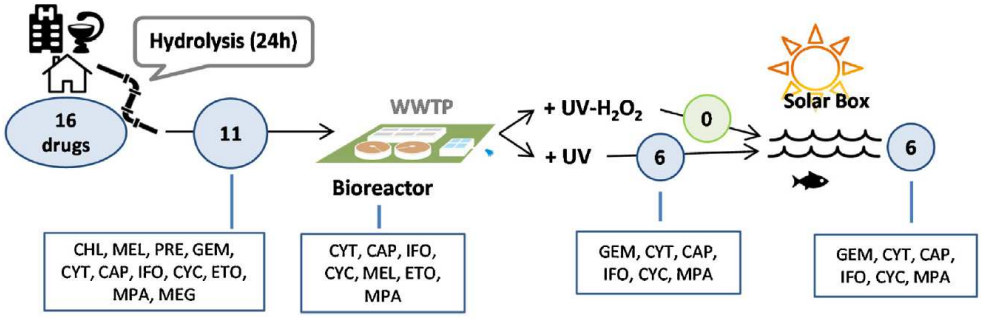

Fig. 1. "Life-cycle" of cytostatic drugs in the environment including the number of drugs studied, the degradation processes studied and the number of compounds remaining after each treatment.

sludge. The hydraulic retention time (HRT) was considered in this experimental design to study the effect of cytostatic drugs on sludge acclimation and bacterial adaptation and thus, on the biodegradation potential. Cyclic experiments were performed where after two days incubation, the sludge was left to settle, the supernatant was removed and new freshly spiked primary effluent was added to the same sludge, which corresponds to a regime of fill-react-settle-decant-refill of a SBR. This process was repeated during five cycles and each cycle had a duration of 48 h, and it is referred as B5. To control the physicochemical parameters of the reactor and the sludge content, the total organic carbon (TOC), pH, TSS and Total Volatile Suspended Solids (TVSS) were measured for each cycle and as there were no differences between cycles, the mean concentration is given in Table 1. In addition to that, we determined background concentration of the target compounds in the WWTP effluent used as matrix for the biodegradation experiment. Only traces of mycophenolic acid were detected and this concentration was negligible regarding possible effects on the biological system.

2.2.3. UV-C light system

UV light is used in many WWTP plants as a disinfection system. The fluence applied for wastewater disinfection generally range between 40 and 100 mJ cm^{-2}. We evaluated the capability of UV-C lamp with a photonic flow of 0.49E-5 Einsteins s^{-1}, to remove cytostatic compounds. Under those conditions, it is expected that compounds having aromatic moieties or double bonds may be degraded in some extent. 2 L of milli-Q water were spiked with the 16 cytostatic drugs and placed in a jacketed reactor. The irradiation system was a low-pressure Hg UV lamp (Philips TUV 8 W, G8T5) immersed in the photo-reactor, emitting at 254 nm (average fluence rate 2.68 mW cm^{-2}), and the temperature was kept at 25 °C. The lamp was initially warmed for at least 15 min to ensure a relatively stable output and then samples were taken at time 0 (before irradiation), 1, 2, 5, 7, 10, 15, 30, 60 and 90 min.

2.2.4. UV-H_2O_2 reactor

To complete the work, we decided to study the removal capacity of an Advanced Oxidation Process over the most recalcitrant cytostatic drugs. Among AOP's, UV/H_2O_2 is been widely studied to deplete pharmaceuticals in wastewater, and it is also implemented in many WWTP. The oxidation capacity is mainly due to the in-situ generation of hydroxyl radicals. On the other hand, ozonation is also a well known advanced process applied for the removal of micropollutants in water. At neutral or slightly basic pH the degradation pathways include both direct ozone reaction but also the participation of hydroxyl radicals. We selected UV/H_2O_2 due to its easy deployment and low cost, especially if compared to ozone treatments. 2 L of milli-Q water were spiked and placed in a jacketed reactor with 15 mg H_2O_2 L^{-1} added in a single dose before starting the radiation. Three low-pressure Hg UV lamps (8 W, Philips TUV, G8T5) emitting 254 nm light (average fluence rate of 8.04 mW cm^{-2} in the reactor) were placed equally separated in the reactor with each protected by a quartz sleeves. The UV lamps were also initially warmed up outside the reactor. The reaction solution temperature was controlled at 25 °C. Throughout the experiment, aliquots were taken at 0, 1, 2, 5, 7, 10, 15, 30, 60 and 90 min. The remaining concentration of H_2O_2 was determined using the metavanadate spectrophotometric procedure at 450 nm [24]. Sodium hydrogen sulphite was added to these samples to avoid further reactions.

2.2.5. Solar simulator

Solar irradiation simulates degradation of cytostatic drugs in surface waters. 1 L of spiked milli-Q water was placed in a SOLAR BOX® (Co. fo.me.gra 220 V, 50 Hz) solar simulator with a 280 nm light filter. The solution was continuously pumped (peristaltic pump Ecoline VC-280) into the Solar Box and recirculated back to the reservoir tank. In the Solar Box, the Duran tubular photoreactor (0.078 L) was placed in the axis of a parabolic mirror and it was irradiated by a Xe-OP lamp (Phillips 1 kW). The photonic flow of the photoreactor in the 290–400 nm range was evaluated by *o*-nitrobenzaldehyde actinometry [25], being 2.68E – 06 Einstein s^{-1}. The temperature of the stirred tank was kept at 25 °C with an ultra-thermostat bath (Haake K10). Aliquots were taken at 0, 1, 2, 4, 7, 10, 15, 30, 60 min, 2, 3 and 4 h.

In all cases, sample aliquots were placed in amber chromatographic vials and kept at −20 °C until analysis (within nine days). Samples from the biodegradation experiments were filtered through 0.2 μm nylon syringe filters to eliminate suspended solids and biomass which could affect the chromatographic performance. By doing so, no matrix effect was observed and mean recoveries at t = 0 were 98 ± 15% except for hydrolysed compounds.

2.3. Analytical determinations

Total organic carbon (TOC) was measured using a Shimadzu TOC-VCSN TOC analyzer. Hydrogen peroxide concentration was evaluated spectrophotometrically with a Hach Lange DR 3900. Cytostatic compounds were analyzed using a LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, UK) with electrospray ionization operated in positive mode (ESI+). 10 μL were injected and gradient and instrumental conditions were adapted from those previously reported [20]. Table SI2 shows the quality parameters obtained and the accurate mass of the ions monitored.

Table 2
Degradation constants (k) in min^{-1} for the different degradation processes studied: hydrolysis (k_{HYD}), biodegradation (k_{BIO}), UV-C radiation (k_{UV}), UV-H_2O_2 (k_{H2O2}) and solar simulator (k_{SUN}).

k (min^{-1})	VINB	VINC	DAU	DOX	IRI	CHL	MEL	MEG	GEM	PRE	ETO	CAP	MPA	CYT	IFO	CYC
Hydrolysis	0.511	0.3262	0.246	0.4586	0.3643	0.0195	0.0046	0.0009	0.0017	0.0004	0.0004	0.0005	0.0002	0.0003	0.0004	0.0004
Bio1	na	na	nd0′	nd1′	na	0.0100	0.0045	nd0′	nd30′	0.0124	0.0082	0.0009	0.0017	0.0031	na	na
Bio5	na	na	nd0′	nd0′	na	0.0045	0.0028	nd0′	0.0974	0.0054	0.0004	0.0005	0.0006	0.0135	0.0015	0.0016
UV	0.3004	nd1′	na	na	0.2127	nd1′	nd1′	0.0165	0.0869	1.622	0.1262	0.0532	0.0284	0.0352	na	0.0001
UV-H_2O_2	nd0′	nd0′	nd2′	nd2′	2.4041	nd1′	nd1′	nd2′	1.7089	nd1′	nd1′	nd2′	nd2′	nd2′	1.141	1.1059
Sun	nd0′	nd0′	0.6697	na	0.1272	0.0266	0.0225	0.0601	0.0398	0.0104	0.0011	0.0024	0.0040	0.0388	0.0007	0.0066

na; not applicable.
nd0′: not detected at time t_0 min.
nd1′: not detected at the first sampling time.
nd2′/…: the compound was detected in <3 sampling points, which precludes calculating the K.

3. Results and discussion

3.1. Summary information

Degradability of cytostatic compounds was evaluated under different chemical and biological processes to simulate the path drugs would follow after excretion and release to sewage water, wastewater treatment and final release to surface waters (Fig. 1). Figs. 2–4 present the degradation kinetics of compounds under study, represented by the dimensionless concentration (C/Co) versus treatment time for each studied process. In these figures, only the first 100–500 min are represented to better observe the degradation trends. The full degradation graphs are included in Figs. S1 and S2 . The pseudo-first order kinetic degradation constants (k) obtained are indicated in Table 2 and the R2 values are in Table S3. According to k values, degradability followed the trend Hydrolysis < Biodegradation ~Solar < UV-C < UV-H_2O_2.

3.2. Hydrolysis

Out of sixteen drugs, seven were completely hydrolysed within 24 h namely vinblastine, vincristine, daunorubicin, doxorubicin, irinotecan, chlorambucil and melphalan. Very fast degradation was observed for five of these compounds (SI1). Daunorubicin was rapidly degraded and only 10% of the initial concentration was detected after 5 min of exposure and had a $k_{HYD} = 0.246\ min^{-1}$. A similar behaviour was observed for doxorubicin and irinotecan where 90% of the initial concentration was removed in 5 min, but approximately 10% remained until the end of the experiment (48 h). The $k_{HYD} = 0.459\ min^{-1}$ for doxorubicin and $k_{HYD} = 0.364\ min^{-1}$ for irinotecan indicate rapid degradation. Vinblastine was degraded in 97% after 15 min and totally degraded after 240 min ($k_{HYD} = 0.511\ min^{-1}$). In a slower rate, vincristine was also hydrolysed and 15% of the initial concentration was detected after 5 min, and at 240 min no traces were observed ($k_{HYD} = 0.326\ min^{-1}$). These compounds are reported to be stable for at least 7 d in pure water and at room temperature [26] and vinblastine sulfate is relatively stable in aqueous solution at or below room temperature (estimated t90 values: 150 d at 25 °C) [27]. However, milli-Q water used in the present experiment had a pH of 6.3, and these compounds are most stable at pH < 4 and pH > 7 [28], which would explain their hydrolysis.

Chlorambucil and melphalan were also degraded to a large extent during the first 240 min although its degradation kinetic were significantly lower ($k_{HYD} = 0.019\ min^{-1}$ and $0.0046\ min^{-1}$, respectively). This pattern is in agreement with the degradation kinetics reported elsewhere [29,30]. These two drugs have also a similar structure, both with acidic groups. Hydrolysis of chlorambucil occurs rapidly in the presence of water due to its high dielectric constant of water [31]. Melphalan has a similar chemical structure and was hydrolysed after 24 h, similar to the 8 h hydrolysis at 37 °C reported by Chang et al. [32]. Therefore, those drugs that undergo rapid hydrolysis (~10% of the initial concentration remains after 60 min) are expected to be quickly eliminated in sewage waters.

On the other hand, 9 out of the 16 compounds tested were stable, with removals below 50% after 48 h (Fig. SI1), namely, prednisone, gemcitabine, cytarabine, capecitabine, ifosfamide, cyclophosphamide, etoposide, megestrol and mycophenolic acid.

In accordance to OECD 312 guidelines, compounds hydrolyzed should not be further assessed in the other degradation processes, although they were included in all experiments to corroborate results obtained and also, in the case of biodegradation, to evaluate the effect of the water matrix in the degradation behavior.

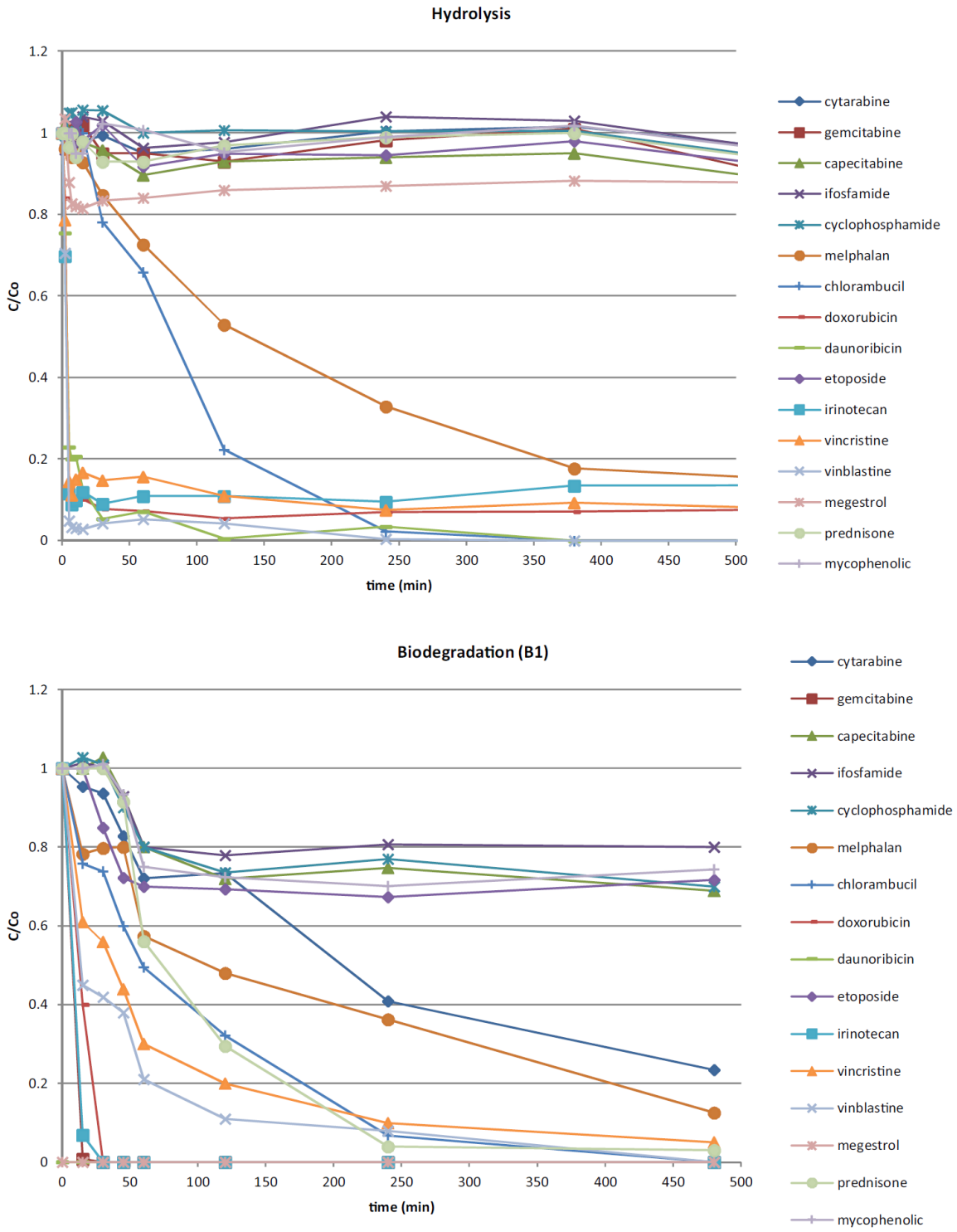

Fig. 2. Degradation curves for hydrolysis and biodegradation experiments for the 16 cytostatic compounds studied.

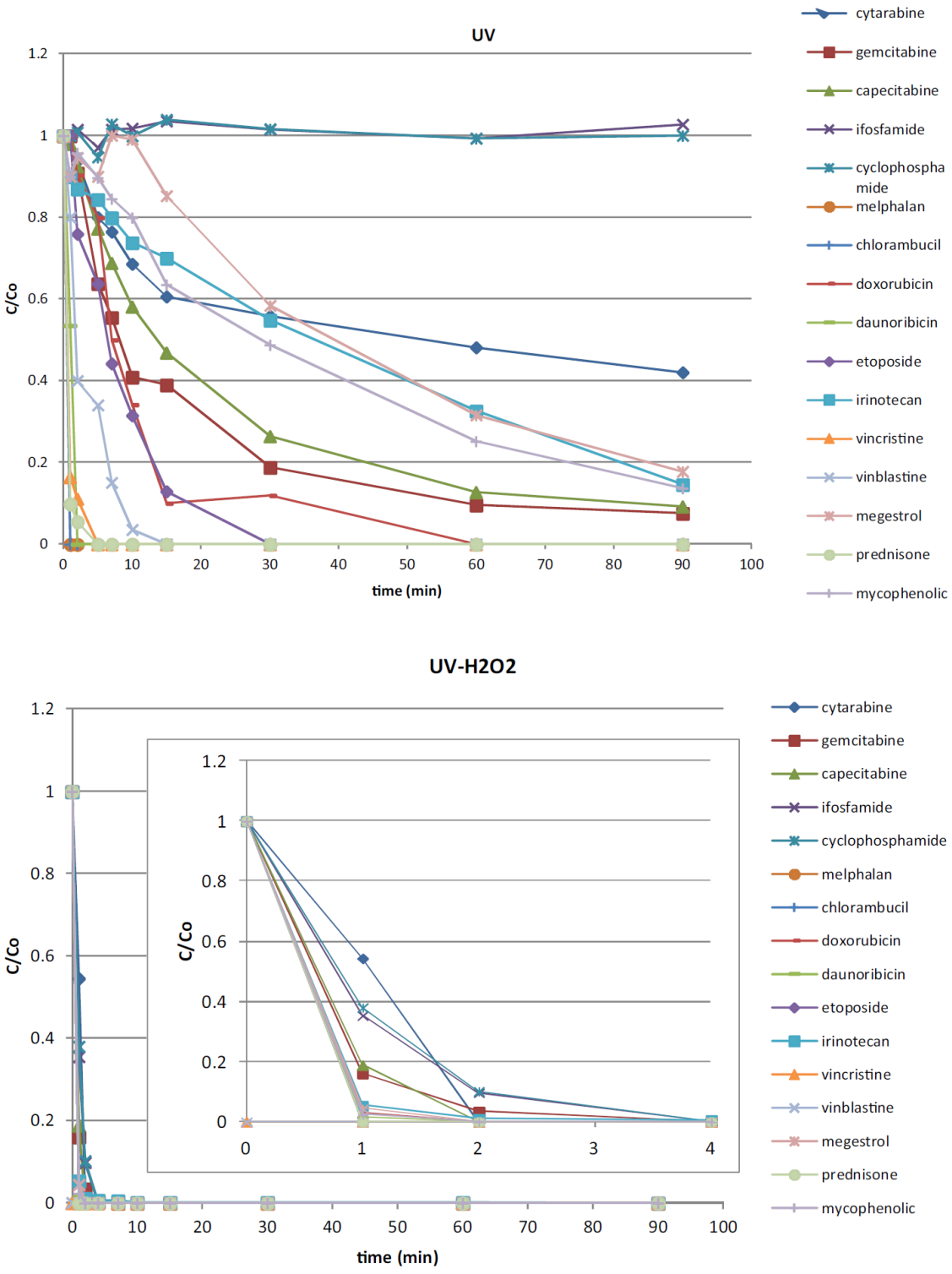

Fig. 3. Degradation curves for UV and UV-H_2O_2 tests.

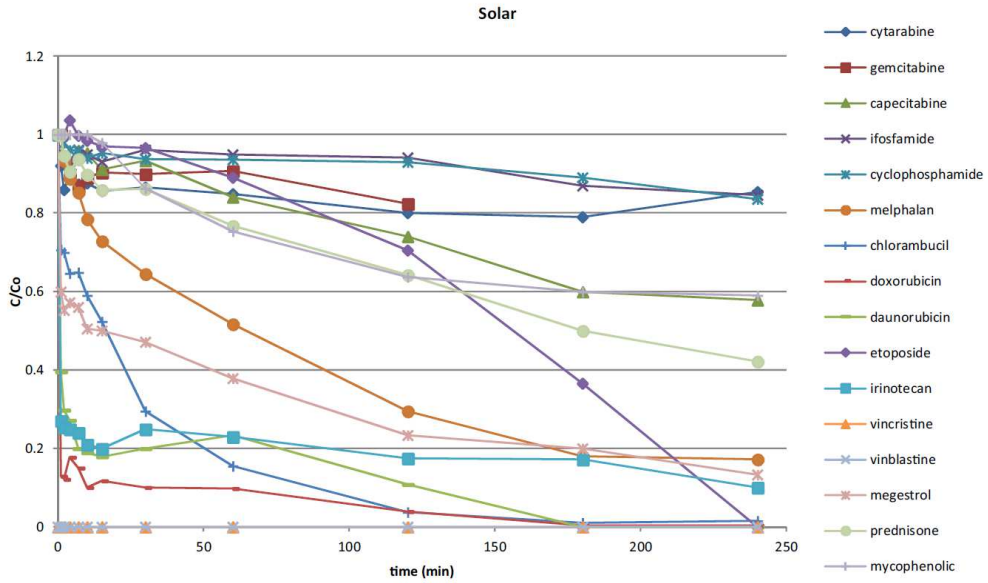

Fig. 4. Degradation curves for the Solar box degradation test.

3.3. Biodegradation

The biodegradability test aims to determine the capability of activated sludge to degrade cytostatic drugs contained in primary effluent. B1 corresponds to the first batch load of spiked wastewater with activated sludge at an initial concentration of 1–1.2 g L^{-1} during 48 h under O_2 saturation and continuous stirring conditions (Fig. 2). Vincristine and vinblastine, classified as non biodegradable [33], were more stable than in the hydrolysis experiments due to the higher pH of wastewater. Daunorubicin was not detected as it hydrolyses and irinotecan and doxorubicin remained at 7 and 40% of the initial concentration, respectively, after 15 min, but they were not detected in the next reaction time (30 min). Kosjek and Heath (2011) indicate that daunorubicin and doxorubicin are not biodegraded but can be adsorbed onto the sludge. However, given the low Koc of these compounds (0.766 and 1.27, respectively, Table SI1), it is likely that these compounds are hydrolysed before being sorbed to sludge. For chlorambucil and melphalan, 6% and 36% were detected after 240 min, and were completely removed within 24 h, following the same trend as the hydrolysis test. On the other hand, megestrol was not detected in wastewater at time 0, differing from the 80% of the initial concentration detected in hydrolysis experiments after 48 h. This compound has a Koc of 4 (Table S1), indicating that it would be rapidly sorbed to particulate matter and sludge.

Finally, gemcitabine, which showed high stability in water, was rapidly biodegraded and only traces were detected after 15 min, as previously described [12]. Prednisone was also eliminated in a faster rate compared with hydrolysis as only 30% was present after 120 min and it was completely eliminated at 240 min. The apparent first order constant obtained k_{BIO} was of 0.0124 min^{-1}. Cytarabine, capecitabine and mycophenolic acid were not detected after 24 h. For etoposide, 5% of the initial concentration was detected after 48 h (60% in hydrolysis). For those compounds, their k_{BIO} is indicated in Table 2 and their values are in all cases higher than k_{HYD}, indicating the degradation capacity of aerobic activated sludge for those contaminants. However, ifosfamide and cyclophosphamide were not biodegraded as 85% of the initial concentration remained after 24 h which indicates the biological recalcitrance of these drugs.

The biodegradation described above was tested at 48 h, however, a conventional activated sludge WWTP has an hydraulic retention time between 2 and 8 h. Considering 2 h of retention time in the bioreactor, 11 out of 16 compounds (Fig. 2) would remain in the effluents and at 8 h of retention time, 7 compounds (Figs. 1 and 2) would remain at concentrations between 10 and 80% of the initial concentration, suggesting that unless AOP are used, WWTP effluents are a direct source of cytostatic compounds to surface waters. The non-completely degraded drugs (Fig. 1) can be expected to enter the aquatic systems due to incomplete removal in WWTPs [7,8,23,34].

We also tested the effect of the mixture of cytostatics on sewage acclimation and bacterial adaptation to evaluate the long-term biodegradation capacity. After 5 operation cycles, k_{BIO5} was lower for all compounds in comparison to k_{BIO1}, except for cytarabine, indicating a sort of decrease of the reactor performance attributed to the lower activity of the bacteria exposed to influent waters containing cytostatics. At the end of the biodegradation process, etoposide, ifosfamide and cyclophosphamide remained in the solution (30–85%). Biodegradation curves for B1 and B5 during 3000 min are included Figs. S1 and S2. Whereas differences were observed in the degradation of the cytostatic drugs after the 5th cycle in comparison to the 1st cycle, no significant differences on the TOC removal and sludge content were observed. TOC removal in the spiked and control bioreactors were of 95% ± 0.7 and 85 ± 13 respectively, which indicates that the degradation process is taking place and is in agreement with standard removal rates of 85% [35]. The pH was maintained during all operation cycles between 7.2 and 8.2. And finally, there was no significant difference in the suspended solids content comparing the initial concentration in B1 (1153 ± 67 mg L^{-1}) and B5 (1167 ± 72 mg L^{-1}), showing the stable sludge production during the process. Therefore, although most of the organic matter content in the influent is eliminated in the conditions reproduced in this study, the biological treatment itself is not able to cope with all cytostatic depletion. It has to be consid-

ered, however, that the presence of particles or other oxidizable compounds may influence the degradation rate of cytostatics under real conditions. In addition, water temperature and the presence of organic matter may result in different biodegradation rates.

3.4. UV-C light photolysis

Three of the drugs underwent rapid photolysis under 254 nm radiation: melphalan and etoposide were completely removed for the first time in this sequence of experiments, whereas prednisone was eliminated at a faster rate than biodegradation. Melphalan was removed after 1 min and its k_{UV} could not be calculated because it was only detected at t_0. For etoposide, only 13% of the initial concentration remained after 15 min of irradiation and it was not detected at 30 min (k_{UV} = 0.1262 min^{-1}). And finally, prednisone, with a k_{UV} = 1.622 min^{-1}, remained in 5% of the initial dose after 2 min and was totally removed after 5 min. All these compounds have aromatic moieties that explain the absorbance of UV light, leading to a rapid degradation.

The remaining compounds were gradually degraded but were not completely removed at final exposure time (90 min, corresponding to a UV fluence of 14,472 mJ cm^{-2}). At this irradiation time, gemcitabine and capecitabine were almost completely eliminated (10% remaining in the solution) and mycophenolic acid, megestrol and cytarabine were still present at 14, 18 and 40% of the initial concentration (k_{UV} indicated in Table 2).

Cyclophosphamide and ifosfamide again proved to be the most refractory drugs, with negligible degradation after 90 min of UV_{254} irradiation. They have a stable structure, with an alkyl chain with 2 chlorine atoms. Minimum removal (up to 3%) was previously described for cyclophosphamide in a pilot-scale hospital wastewater treatment plant using a membrane bioreactor (MBR) followed by UV radiation [15]. Low k_{UV} for cyclophosphamide (0.0049 min^{-1}) using 8 W low pressure lamps and pure water was also reported elsewhere [17].

According to the calculated k values, after 90 min UV_{254} irradiation (equivalent to a fluence of 14,472 mJ cm^{-2}), the removal efficiencies of cytostatic drugs were between 10% and 40%, except for ifosfamide and cyclophosphamide which were not eliminated. This fluence is much higher than that recommended for wastewater disinfection (e.g., 40–186 mJ cm^{-2}) [36]. Considering this maximum fluence of 186 mJ cm^{-2} and that the dose we have used is 2.68 mW cm^2, the time needed to reach the recommended dose is of 1.16 min. At this irradiation time, 10 compounds out of the 16 studied were present at 76–100% of the initial dose. This means that these drugs would not be removed at the irradiation conditions used in WWTP for disinfection and thus, are expected to be discharged to surface waters.

3.5. UV-H_2O_2

In view of the previous results, the conventional treatments performed in WWTP would not be enough to completely remove all the cytostatic drugs, which leads to the crucial need for enhanced technologies that can be used to reduce their release to surface waters. AOPs are clean technologies based on the generation of extremely reactive and non-selective hydroxyl radicals, with very high oxidative power. Because these technologies are implemented in some WWTP where water is reused for irrigation or any other purpose, their outcome for the removal of cytostatic drugs was evaluated.

The degradation of target cytostatic drugs in water is shown in Fig. 3, and the observed pseudo-first order rate constants are included in Table 2. Using UV-H_2O_2 treatment, all cytostatic drugs were removed within 4 min (H_2O_2 consumption = 1.5 mg^{-1} L), including the most recalcitrant cyclophosphamide and ifosfamide. Those compounds not completely removed by UV-C radiation alone (megestrol, mycophenolic acid and irinotecan) were totally removed after 1 min of UV-H_2O_2 exposure. Similarly, gemcitabine and capecitabine, which showed a slow degradation using UV-C light, were removed in 84 and 81% of the initial concentration within 1 min of UV-H_2O_2 exposure and at 2 min no traces were present. These depletions are primarily ascribed to the indirect oxidation of hydroxyl radicals, although direct UV-C photolysis also contributes. Whereas gemcitabine was eliminated by biodegradation, capecitabine was persistent and this confirms the need to apply advanced treatments for its total elimination.

The most recalcitrant compounds to biodegradation and UV-C light were cytarabine, ifosfamide and cyclophosphamide. Cytarabine was eliminated in 50% within 1 min of UV-H_2O_2 and totally removed at 2 min. Ifosfamide and cyclophosphamide, which were resistant to all the previous treatments, were degraded in 65% of the initial concentration in 1 min, and 90% eliminated after 2 min and were completely removed in 4 min (H_2O_2 consumption = 1.5 mg^{-1} L). A previous study reported a 0% removal for ifosfamide and 31–77% for cyclophosphamide in a pilot plant treating hospital effluents (0.25–5.00 m^3 h^{-1}) and H_2O_2 concentrations (0.83–1.11 g H_2O_2 L^{-1}) [19]. Degradation constants were reported for cyclophosphamide, using an 8 W low pressure lamp with 8.2 mg H_2O_2 L^{-1} in pure water, being 0.059 min^{-1} [17], lower than the 1.1 min^{-1} obtained in the present study using more powerful conditions. Although the biodegradability of cyclophosphamide and ifosfamide have been studied [22,37] further knowledge is needed on their removal using AOP [12].

3.6. Solar simulator

The Solar Simulator controls the emitted radiation and temperature in the reactor in order to provide reliable data that degradation occurred exclusively by solar light simulating photolysis in river water.

For chlorambucil and melphalan, that showed medium hydrolysis, solar radiation slightly enhanced their degradation. Only traces of chlorambucil were detected after 120 min (22% present in hydrolysis) and it was not detected after 240 min (traces were detected in hydrolysis). It had a k_{SUN} = 0.0266 min^{-1}. After 240 min, 17% melphalan was present in the solution (32% in hydrolysis) and had a k_{SUN} = 0.0225 min^{-1}.

For other drugs, solar radiation accelerated their degradation. Etoposide had a slow degradation at the beginning of the experiment, with k_{SUN} = 0.0011 min^{-1}, being 37% still present after 180 min. However, it was not detected in the following reaction time (240 min). Megestrol and prednisone were detected at 13% and 42% of the initial concentration after 240 min (90% and 100% in hydrolysis test respectively), and had a k_{SUN} = 0.0601 min^{-1} and 0.0104 min^{-1}.

High amounts of capecitabine and mycophenolic acid were detected (60% of the initial concentration, approximately) in 240 min. Their k_{SUN} were 0.0024 and 0.004 min^{-1} respectively. And finally, >80% of the initial concentration remained for cytarabine, cyclophosphamide and ifosfamide. Their k_{SUN} were 0.0388, 0.0066 and 0.0007 min^{-1} respectively. Thus, it can be concluded that the sun radiation is not capable of removing the drugs present is surface waters, and this might have serious environmental implications.

For stable compounds which might reach river waters through WWTP effluents (Fig. 1), the risk should be considered as they might have an effect on the aquatic ecosystem. Given the *Daphnia magna* EC_{50} values between 5 and 1795 mg L^{-1} [38], the impact they may have in the aquatic system is foreseen to be low. However, sublethal effects concerning DNA damage in aquatic organisms should not be discarded. In addition, these compounds have a Kow between −2.5 and 4.2, showing low bioaccumulation potential. However, during the degradation processes, transformation products, which

eventually can be more toxic than parent compounds, might be generated. Future studies will deal with their characterization of under different photolytic and AOP conditions.

4. Conclusions

This study presents for the first time the degradation kinetics of 16 cytostatics using several approaches that simulate the life cycle of these compounds from excretion to river water, including their passage through WWTP. It has been observed that some compounds are rapidly hydrolysed in water and have a high potential to be eliminated in sewage waters before reaching the WWTP. Other compounds can be efficiently biodegraded in the biological treatment of WWTPs. Recalcitrant compounds to biodegradation and neither eliminated by UV-C light at the typical disinfection dose are potentially released to surface waters unless more effective treatments are applied. UV-C/H_2O_2 efficiently achieved complete removal after 4 min of UV-C radiation and H_2O_2 consumption of 1.5 mg^{-1} L. Finally, we evaluated the solar photolysis of cytostatic compounds to simulate their behaviour in river water and most of the compounds were not removed. Although the aquatic risk is expected to be low according to *Daphnia magna* toxicity, sublethal effects should be studied as most of these compounds, or eventually their degradation products, may affect DNA.

The present study provides new information on the behaviour of cytostatic drugs. On-going studies regarding the degradation pathways and elucidation of transformation by-products are being performed to have an overall vision of the degradation mechanisms.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2016.06.057.

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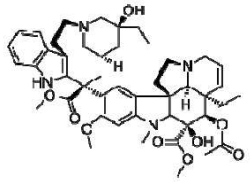
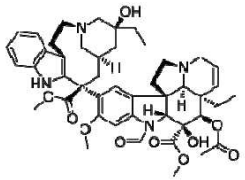
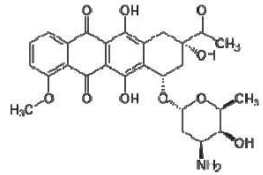
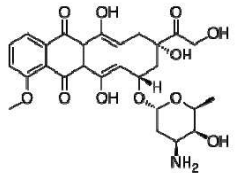
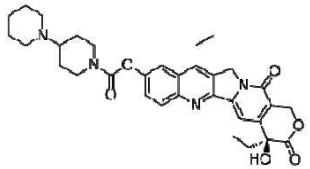
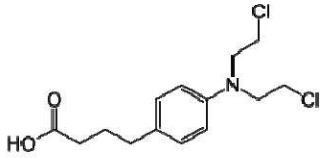
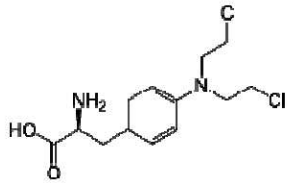
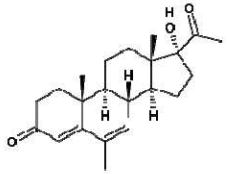
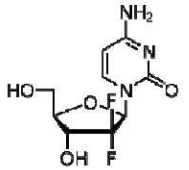
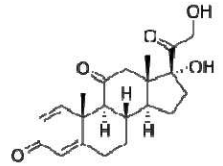
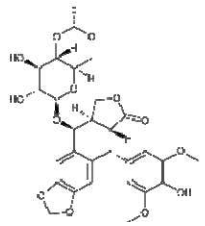
Supplementary Information

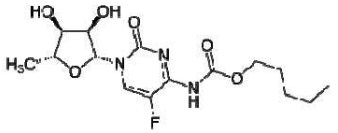
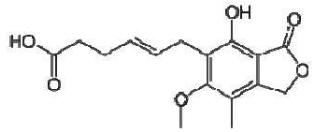
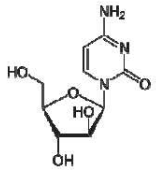
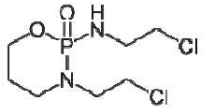
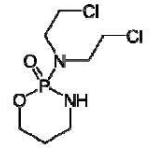
Supplementary information 1.

Cytostatic drugs are classified in five levels according to the organ or system in which they act (L for antineoplastic and immunomodulating agents) and their therapeutic properties (L01 for antineoplastic agents, L02 for endocrine therapy drugs, L03 for immunostimulants and L04 for immunosuppressants). They present a wide range of structures and physico-chemical properties (Table S1) which affect their behaviour and environmental fate.

Table S1. Physicochemical properties of target compounds. Compounds are ordered according to their degradability.

Target Compound	CAS Nº	Molecular formula	MW (g mol^{-1})	Watersolubility (mg L^{-1})	pKa	Log Koc	Structure
Vinblastine	143-67-9	$C_{46}H_{58}N_4O_{19}$	810.9	0.0446	14.41	4.32	
Vincristine	2068-78-2	$C_{46}H_{56}N_4O_{10}$	824.9	0.26	14.41	2.82	
Daunorubicin	23541-50-6	$C_{27}H_{29}NO_{10}$	527.5	39.2	11.02	0.766	
Doxorubicin	25316-40-9	$C_{27}H_{29}NO_{11}$	543.5	10	11.02	1.27	
Irinotecan	100286-90-6	$C_{33}H_{38}N_4O_6$	586.6	107	nd	3.2	

Chlorambucil	305-03-3	$C_{14}H_{19}NO_2Cl_2$	304.2	$1.24 \cdot 10^{+4}$	5.75	1.7	
Melphalan	148-82-3	$C_{13}H_{18}Cl_2N_2O_2$	305.2	45.7	-0.432	-0.52	
Megestrol	595-33-5	$C_{22}H_{30}O_3$	342.4	2	17.61	4	
Gemcitabine	122111-03-9	$C_9H_{11}F_2N_3O_4$	263.1	$5.13 \cdot 10^{+4}$	3.6	-1.4	
Prednisone	53-03-2	$C_{21}H_{26}O_5$	358.4	312	13.90	1.46	
Etoposide	33419-42-0	$C_{29}H_{32}O_{13}$	588.5	200	9.8	1.16	

Capecitabine	154361-50-9	$C_{15}H_{22}FN_3O_6$	359.3	26	1.9	0.56	
Mycophenolic acid	24280-93-1	$C_{17}H_{20}O_6$	320.3	22	3.57	2.8-4.2	
Cytarabine	69-74-9	$C_9H_{13}N_3O_5$	243.2	$1.76 \cdot 10^5$	4.22	-2.51	
Ifosfamide	3778-73-2	$C_7H_{15}Cl_2N_2O_2P$	261.1	3780	4.75	0.86	
Cyclophosphamide	6055-19-2	$C_7H_{15}Cl_2N_2O_2P$	261.1	40	nd	0.76	

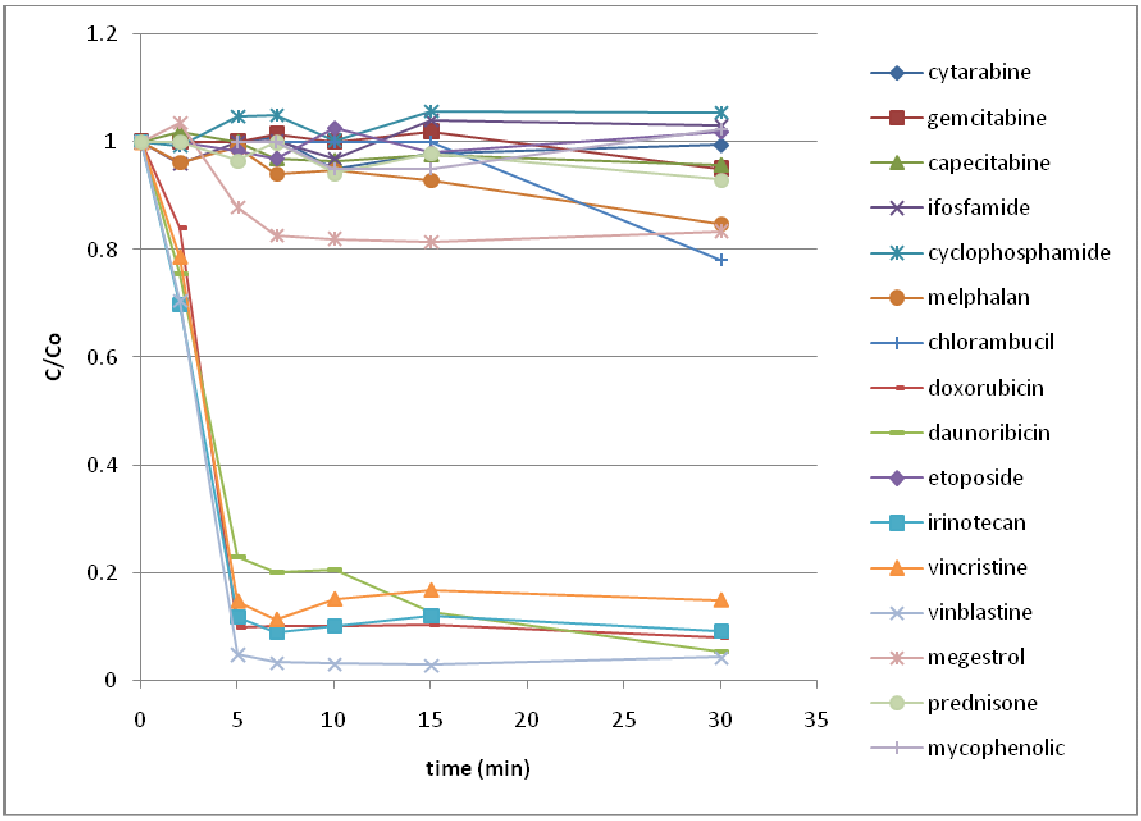
Supplementary information 2: method description

Capillary voltage was 3.5 kV and the temperature was set at 375 ºC. Sheath gas flow was 50 au (arbitrary units) and auxiliary gas flow was 20 au. A Luna C18 column (150 mm x 2.00mm, ID, particle size 5 µm, Phenomenex, Torrance, USA) was used and the mobile phase was 0.1 % HCOOH in HPLC water (A) and 0.1% HCOOH in acetonitrile (both from Merck, Darmstadt, Germany) (B). Gradient started at 5% B and was kept for 1 min, then increased to 70% B in 29 min and reached 100% in minute 31. It was kept for 5 min, then got back to initial conditions in 4 min, and kept for 10 min to stabilize the system. Direct sample introduction of 10 µL permitted to obtain overall good quality parameters for all studied compounds, with linearity range of 0.001 - 0.7 ng μL^{-1} for most of them, $r^2 > 0.99$, IDL ≤ 0.1 ng and reproducibility RSD ≤ 13%.

Table SI2. Retention time, ions monitored and quality parameters obtained by LC-Orbitrap-MS.

Target compounds	Retention time (min)	Precursor ionmonitored $[M+H]^+$	Secondary ion	Linearity (ng µL)	R^2	LOQ (µg/L)	Reproductibility (0.5 ng μL^{-1}) RSD%
Cytarabine	1.82	244.0927	112.0506	0.001-0.7	0.999	1	3.6
Gemcitabine	1.98	264.0790	112.0506	0.001-0.7	0.998	1	4.8
Irinotecan	14.57	587.2871	195.1495	0.001-0.7	0.998	5	7.3
Vincristine	14.97	825.4060	353.1861	0.01-0.7	0.997	10	13.1
Doxorubicin	15.31	544.1807	361.0707	0.01-0.7	0.999	10	6.7
Vinblastine	15.7	811.4264	355.2017	0.01-0.7	0.996	10	2.4
Ifosfamide	16.24	261.0313	78.0105	0.001-0.7	0.998	1	2.7
Melphalan	16.36	305.0810	288.0554	0.005-0.7	0.999	5	6.8
Cyclophosphamide	16.7	261.0313	140.0029	0.001-0.7	0.997	10	2.7
Daunorubicin	17.17	528.1863	363.0868	0.01-0.5	0.996	10	6.7
Capecitabine	18.32	360.1553	71.0855	0.001-0.7	0.999	5	7.1
Prednisone	18.32	359.1841	299.0608	0.005-0.7	0.998	1	8.7
Etoposide	18.36	589.1911	229.0496	0.01-0.7	0.999	10	3.1
Mycophenolic acid	23.97	321.1332	207.0651	0.001-0.7	0.999	1	4.8
Chlorambucil	29.74	304.0864	241.0868	0.001-0.7	0.997	1	6.1
Megestrol	31.56	385.2371	267.1746	0.005-0.7	0.997	5	3.2

Figure SI1. Partial (0-50 min) and complete (3000 min) degradationcurves for hydrolysis



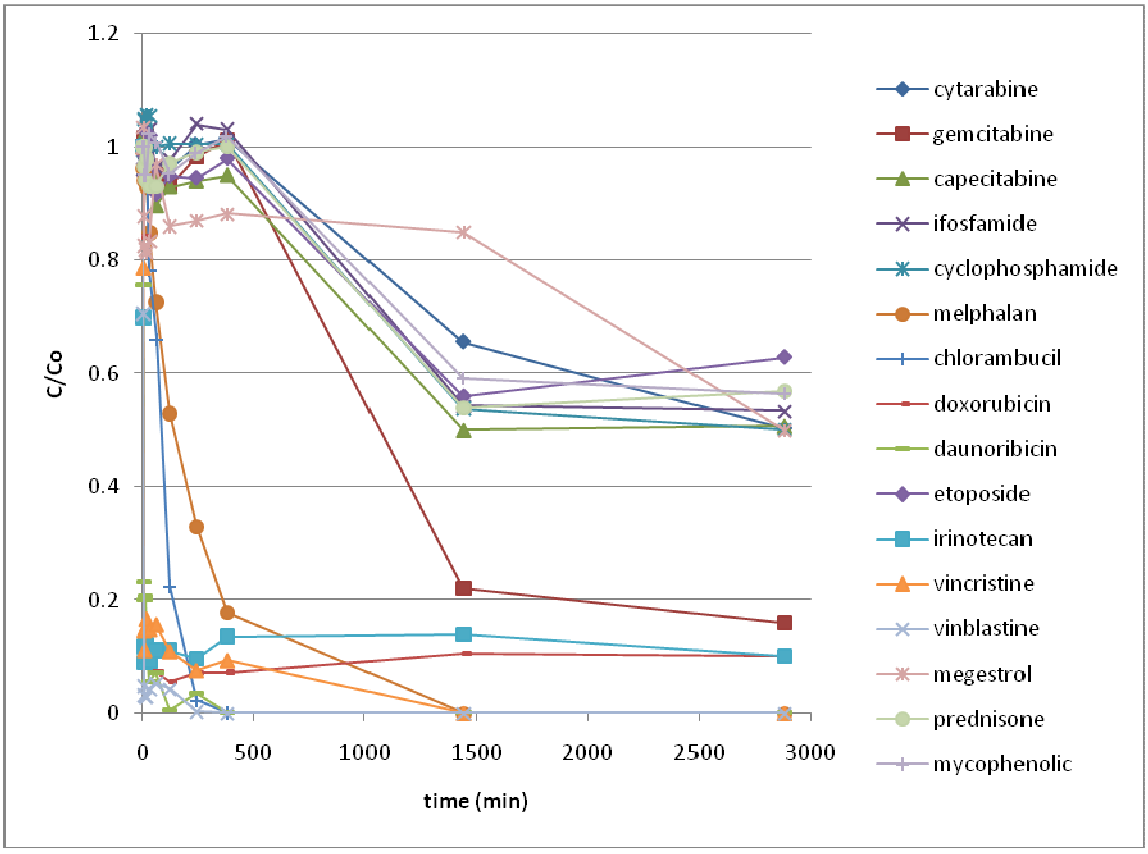
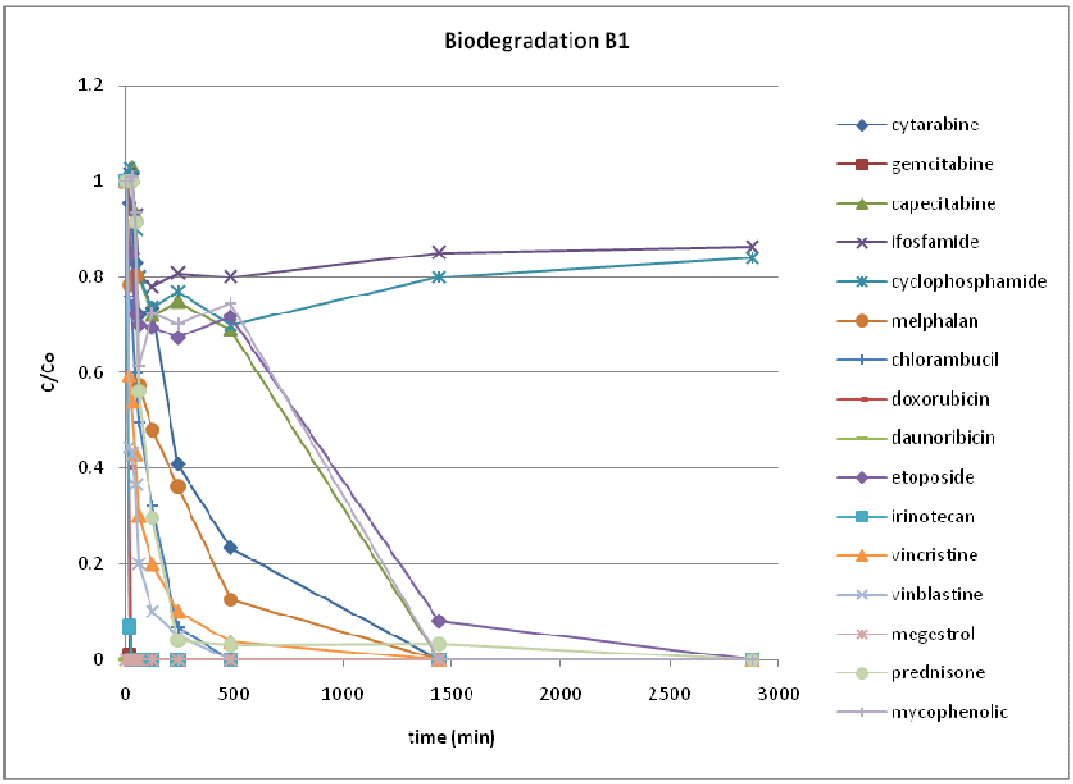


Figure SI2. Degradation curves for the first (B1) and last (B5) cycles of the biodegradation experiments.



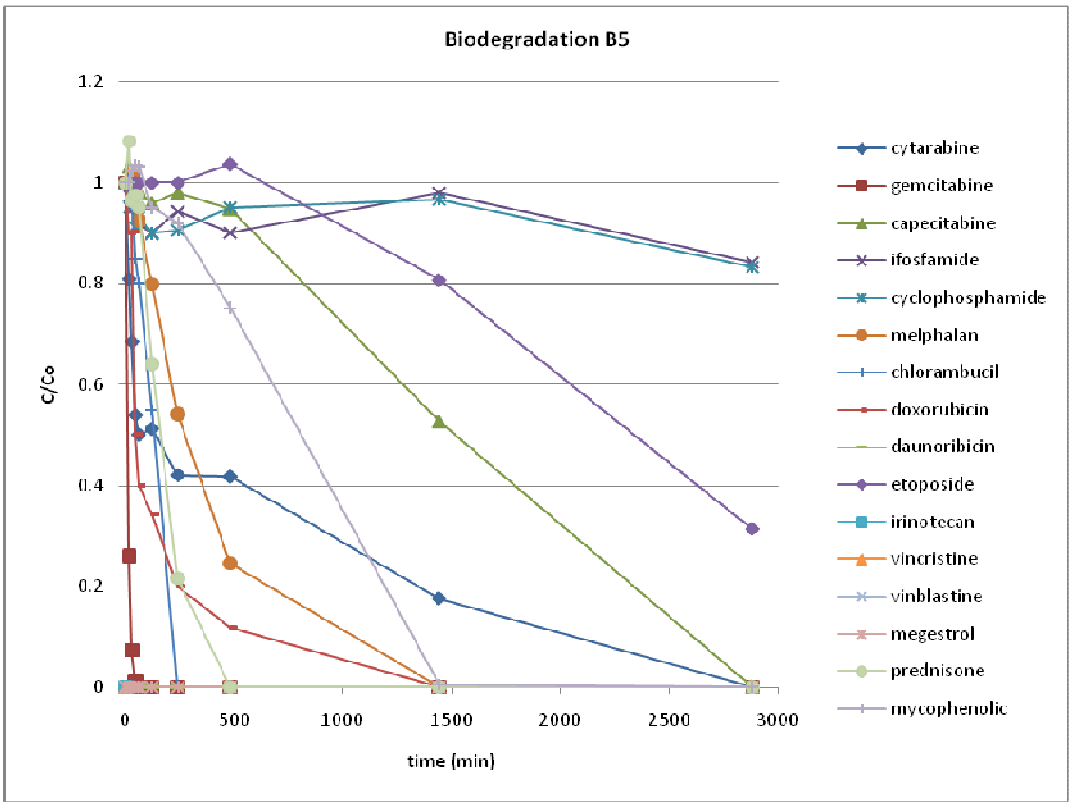


Table SI3. R^2 values from the pseudo-first order kinetic degradation constants calculations

R^2	VINB	VINC	DAU	DOX	IRI	CHL	MEL	MEG	GEM	PRE	ETO	CAP	MPA	CYT	IFO	CYC
Hydrolysis	0.92	0.930	0.942	0.852	0.942	0.952	0.979	0.917	0.937	0.854	0.917	0.88	0.953	0.980	0.98	0.861
Bio1	--	--	--	--	--	0.961	0.953	--	--	0.945	0.961	0.917	0.858	0.866	--	--
Bio5	--	--	--	--	--	0.918	0.979	--	0.987	0.870	0.976	0.804	0.860	0.996	0.91	0.892
UV	0.942	--	--	0.982	0.990	--	--	0.943	0.986	0.870	0.973	0.995	0.976	0.989	--	0.999
UV-H_2O_2	--	--	--	--	0.967	--	--	--	0.997	--	--	--	--	--	0.99	0.990
SolarBox	--	--	0.897	0.88	0.825	0.969	0.971	0.997	0.955	0.908	0.933	0.960	0.988	0.996	0.93	0.871

na; notapplicable

nd0'; not detected at time t_0

nd1'; not detected at the first sampling time

nd2'/...: the compound was detected in <3 sampling points, which precludes calculating the *K*

3.2.2. Article científic IV:

Determination of cytostatic drugs in Besòs River (NE Spain) and comparison with predicted environmental concentrations. Helena Franquet-Griell, Deborah Cornadó, Josep Caixach, Francesc Ventura, Sílvia Lacorte. *Environmental Science and Pollution Research* (2017) 24:6492-6503.

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RESEARCH ARTICLE

Determination of cytostatic drugs in Besòs River (NE Spain) and comparison with predicted environmental concentrations

Helena Franquet-Griell[1] · Deborah Cornadó[1] · Josep Caixach[1] · Francesc Ventura[1] · Silvia Lacorte[1]

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Abstract The number of cytostatic drugs used in cancer treatments is wide and increases every year; therefore, tools have been developed to predict their concentration in the environment to prioritize those for monitoring studies. In the present study, the predicted environmental concentrations (PECs) were calculated according to consumption data in Catalonia (NE Spain) for 2014. According to PECs and to the most widely reported compounds, 19 cytostatics were monitored in two sampling campaigns performed along the Besòs River. A total of seven drugs were detected at levels between 0.5 and 656 ng L^{-1}. PEC and measured environmental concentrations (MECs) were compared in order to validate PECs. The PEC/MEC ratio presented a good agreement between predicted and measured concentrations confirming the PEC estimations. Mycophenolic acid, prioritized as the compound with the highest PEC, was detected at the highest concentrations (8.5–656 ng L^{-1}) but showed no risk for aquatic organisms (risk quotient <1) considering acute toxicity tests performed in *Daphnia magna*.

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Keywords Cytostatic drugs · PEC · MEC · Validation · River water · Risk assessment

Introduction

Cytostatic drugs are a wide group of chemicals with different structures and action modes used in cancer treatments. They are classified by the World Health Organization (WHO) in the Anatomical Therapeutic Chemical (ATC) category, according to the organ or system in which they act and their therapeutic, pharmacological and chemical properties. Cytostatics mainly belong to the class L, called Antineoplastic and Immunomodulating Agents, which includes more than 260 drugs subclassified in four groups (L01 to L04). This list is revised periodically in order to include new compounds. High consumption of these pharmaceuticals has been reported in several countries such as France (13 t in 2004 and 17.5 t in 2008) or NW England (0.5 t) (Besse et al. 2012; Booker et al. 2014). In Catalonia (NE Spain), over 130 different compounds were administered in the period 2010–2012, with a total consumption between 4.7 and 4.9 t (Franquet-Griell et al. 2015).

Cytostatics might reach wastewater treatment plants (WWTPs) after excretion either as metabolites and/or unchanged parent. Removal rates for these drugs range between 0 and 96% depending on the compound, but for many, values <2% were reported due to low biodegradability (Franquet-Griell et al. 2015). For recalcitrant compounds, the usual treatments in WWTP are not capable of removing them completely (Royal Society of Chemistry 2014; U.S. National Library of Medicine 2013). Therefore, considering their increasing consumption, they have emerged as new water contaminants (Buerge et al. 2006) as several anticancer drugs have been detected in wastewaters (Gómez-Canela et al. 2014;

Negreira et al. 2014a), in rivers (Ferrando-Climent et al. 2014; Giebułtowicz and Nałęcz-Jawecki 2016) and in drinking waters (Mendoza et al. 2016).

Predicted environmental concentrations (PECs) have become a useful tool to identify pharmaceuticals likely to be found in the environment, and it has recently been used for cytostatic drugs (Besse et al. 2012; Coetsier et al. 2009; Franquet-Griell et al. 2015; Martín et al. 2014; Ortiz de García et al. 2013). This method is adequate for this kind of compounds because, unlike other pharmaceuticals, all the prescribed drugs will be consumed by the patient.

$$\mathrm{PECs}\left(\mathrm{ng\,L^{-1}}\right) = \frac{\text{consumption} \times \text{Fexc} \times (1-\text{Fwwtp})}{\text{WWinhab} \times \text{inhab} \times \text{dilution}} 10^9$$

This simple equation considers the consumption, excretion (Fexc) and removal fraction (Fwwtp) of the parent compound to calculate the concentrations expected to be found in WWTP effluents and river water considering the water consumption per inhabitant (WWinhab), the inhabitants of a specific area (inhab) and the dilution factor from WWTPs to the river (dilution). PECs represent an effective way to roughly identify target compounds most likely to be detected in water, before any field sampling is undertaken. In a previous study, PECs in river (PEC_{river}) for cytostatic drugs were calculated in the Catalonia region and mycophenolic acid and hydroxycarbamide were expected to be found at the highest concentrations (77.5 and 32.1 ng L^{-1}, respectively), whereas capecitabine, bicalutamide, imatinib and prednisone showed intermediate PEC (1–10 ng L^{-1}) (Franquet-Griell et al. 2015). In the above-mentioned study, it was also found that 126 out of 132 compounds studied should never be detected in river water due to either low consumption, low excretion rate or high degradability (PEC < 1 ng L^{-1}).

The reliability of the PEC approach should be validated by comparing the predicted values with measured environmental concentrations (MECs) in a given area where the river dynamics and impact of WWTP discharges are known. However, in most of the studies, cytostatic drugs with the highest PECs according to consumption are not considered, indicating that a large proportion of the information regarding their occurrence and potential impact is still missing.

Besòs River borders the city of Barcelona and is one of the most polluted fluvial areas in Catalonia due to the low flow (4.33 m^3 s^{-1}) and high population density of the area (from 1500 to 15558 inhab km^{-2} throughout the basin). It is also a highly industrialized river, with pharmaceutical, food and plastic material industries, among others. In addition, several hospitals and WWTPs are located in the basin. Altogether, Besòs River represents a worst case scenario given the large amount of discharges it receives.

The aim of the present study was to calculate the PECs for 2014 to prioritize the cytostatic drugs most likely to be present in rivers and to monitor 19 compounds belonging to L01–L04 ATC codes in the Besòs River and its tributaries. The 19 cytostatic drugs were selected according to (i) 3 drugs with PEC > 10 ng L^{-1}, which were expected to be detected in the river according to their predicted concentration (mycophenolic acid, capecitabine and prednisone), and (ii) 16 drugs with low PEC but frequently monitored in surface water according to the bibliography. To validate the PEC_{river} estimation, MECs were compared with predicted value calculations in order to provide new data on the reliability of PEC estimation to be used in environmental monitoring. Finally, a risk assessment was performed considering the maximum concentrations detected in river waters. The full set of compounds permits to expand the knowledge on their occurrence and to ensure a holistic assessment of risk.

Experimental

Chemicals and materials

Cytostatic compounds studied, their consumption in Catalonia in 2010 and 2014 and their use are indicated in Table 1. All the target compounds, molecular formula and relevant physicochemical properties are shown in the Supplementary Information (SI1). Nineteen pure analytical standards of 98–99% purity were acquired from Sigma-Aldrich (St. Louis, MO, USA) and from Toronto Research Chemicals, TRC (Ontario, Canada). The surrogates cyclophosphamide-d_4 and ifosfamide-d_4 were purchased from Santa Cruz Biotechnology, USA, and were used as internal standard (IS). Stock and working standard solutions were prepared at 1000 and 100 mg L^{-1}, respectively, in methanol (MeOH). MeOH, acetonitrile (ACN), acetone (SupraSolv grade) and HPLC water (LiChrosolv grade) were supplied by Merck (Darmstadt, Germany). Formic acid (HCOOH) and ammonium acetate (NH_4OAc) were supplied by Sigma-Aldrich. Milli-Q water was produced from an Integral Water Purification System from Millipore (Billerica, MA, USA). Oasis HLB cartridges (6 cc, 200 mg) were purchased from Waters (Milford, MA, USA).

Sampling procedure

Sampling was performed in two campaigns during May and July 2014 along the Besòs River and its tributaries, located in Catalonia. The tributaries Congost and Mogent rivers converge and form the Besòs River which flows 40 km south and south-east and empties into the Mediterranean Sea, north of Barcelona's urban centre. The Besòs catchment area is located in a heavily populated and industrialized area, receiving the authorized discharges of 27 WWTP, 219 industries and 12 hospitals (Fig. 1). The

Table 1 Cytostatic drugs analysed ordered according to ATC code, consumption (kg) in Catalonia in years 2010 and 2014, PECs in river calculated from 2014 consumption data and their therapeutic use

ATC code	Name	Consumption (kg)		PEC (ng L^{-1})	Uses	Ref.
		2010	2014	2014		
G03HA01	Cyproterone	26	19	0.57	Prostate	[a]
H02AB07	Prednisone	222	253	1.75	Acute lymphoblastic leukaemia, non-Hodgkin lymphomas, Hodgkin's lymphoma, multiple myeloma and other hormone-sensitive tumours	[a]
L01AA01	Cyclophosphamide	4.5	4.5	0.12	Breast, lung, myeloma, some type of lymphomas and leukaemia, soft tissue sarcoma, bone, some children's cancers	[a]
L01AA02	Chlorambucil	0.20	0.084	0.00008	Chronic lymphocytic leukaemia, low-grade non-Hodgkin lymphoma and Hodgkin lymphoma	[a]
L01AA03	Melphalan	0.11	0.051	0.00086	Multiple myeloma, advanced ovarian and advanced breast cancer	[a]
L01AA06	Ifosfamide	4.7	7.5	0.39	Breast cancer, testicular cancer and lung cancer, as well as some types of lymphoma	[a]
L01BB05	Fludarabine	0.10	0.061	0.0038	Chronic lymphocytic leukaemia	[a]
L01BC05	Gemcitabine	35	34	0.22	Pancreas, lung, breast that has spread, bladder that has spread, ovarian	[a]
L01BC06	Capecitabine	824	587	5.85	Breast, bowel, stomach, pancreatic, oesophageal	[a]
L01DB01	Doxorubicin	0.19	0.094	0.0049	Many types	[a]
L01DB02	Daunorubicin	0	0	0	Acute leukaemias	[a]
L01DB03	Epirubicin	0.052	0.014	0.00014	Breast, ovarian, stomach, lung, bowel, myeloma, some types of lymphoma and leukaemia	[a]
L01XE03	Erlotinib	12.25	6.69	0.013	Pancreatic cancer that has spread and non-small cell lung cancer that has spread	[a]
L01XX19	Irinotecan	3.5	3.2	0.16	Bowel	[a]
L02AB01	Megestrol	226	145	0.48	Breast and womb	[a]
L02AE02	Leuprolide	0.74	0.48	0.0025	Prostate	[a]
L02AE03	Goserelin	0.07	0.04	0.0033	Breast and prostate	[a]
L02BA01	Tamoxifen	49	55	0.052	Breast	[a]
L04AA06	Mycophenolic acid	1859	2356	93.4	Prevent rejection in organ transplantation	[b]

[a] Cancer Research (UK) (2012)

[b] Vademecum (2016)

average Besòs River flows were 2.29 and 2.08 m^3 s^{-1} in May and July 2014. In relation to tributaries, the Congost River flows were 0.11 and 0.05 m^3 s^{-1}, respectively, for Mogent River, 0.24 and 0.12 m^3 s^{-1}, and much lower flows for Ripoll, Caldes and Tenes rivers were recorded (Agència Catalana de l'Aigua 2015).

Water samples were collected along the river and its tributaries, in 19 different locations including the five river sources, before and after WWTP and before and after the tributary flows into the main river (Fig. 1). Water was taken from the centre of the river, 20 cm (~8 in) below the surface and avoiding stagnant water. Samples were collected in precleaned and 450 °C baked amber glass bottles and kept at 4 °C and processed within 24 h to avoid any degradation, as water sample storage is critical for cytostatics (Negreira et al. 2013).

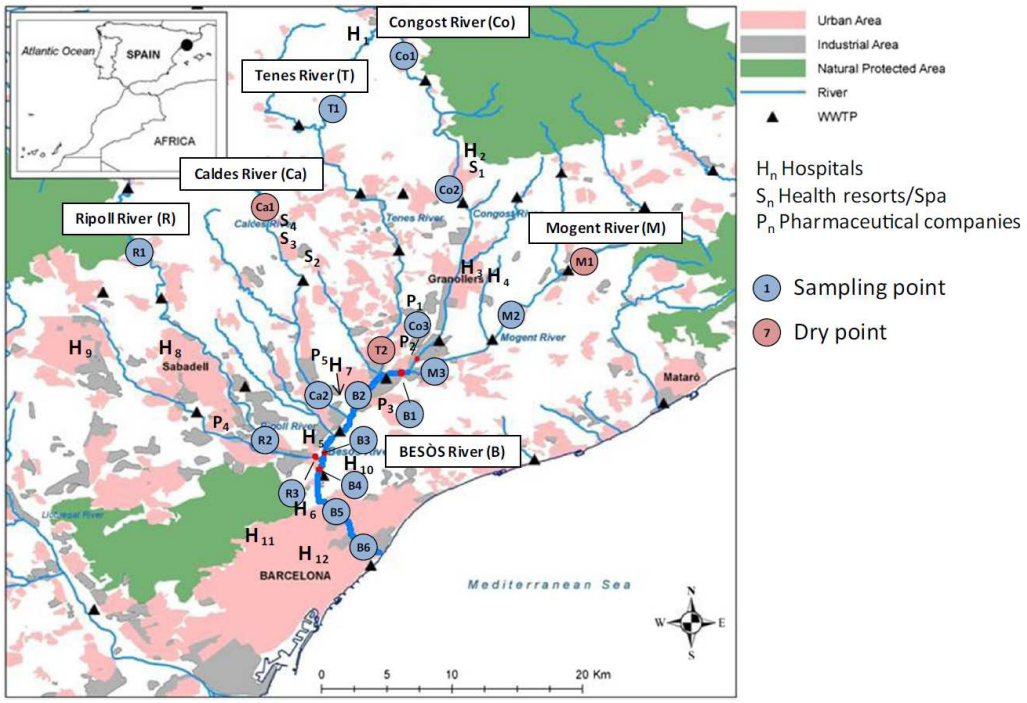

Fig. 1 Sampling points monitored in Besòs River basin. Hospitals (*H*), pharmaceutical companies (*P*), health resorts (*S*) and WWTP (Δ) located along the basin are indicated

Sample extraction and analysis

Samples were analysed adapting the methods developed previously by Gomez-Canela et al. (2013b, 2014). Briefly, 100 mL of filtered river water was acidified at pH 2 with HCl 0.1 N and then spiked with 5 ng IS. Oasis HLB cartridges were conditioned using MeOH (6 mL) and H_2O (6 mL). Samples were loaded at 1 mL min^{-1}. Once preconcentrated, the cartridges were washed with 100 mmol L^{-1} NH_4OAc in Milli-Q water (3 mL), dried over 45–60 min and eluted by gravity with 6 mL MeOH and 6 mL HCOOH/MeOH (5:95). Then, samples were evaporated to almost dryness under a current of N_2 at 25 °C and transferred to a 2-mL chromatographic vial using acetonitrile as washing solvent. Finally, they were evaporated to dryness and reconstituted to 100 μL using 0.1% HCOOH in ACN/water (50:50).

Liquid chromatography coupled to high-resolution mass spectrometry (LC-Orbitrap-MS) was used to analyse the samples. An Orbitrap-Exactive HCD mass spectrometer equipped with heated electrospray ionization (H-ESI+) source (Thermo Fischer Scientific, Bremen, Germany) was used. The system was equipped with an HTC PAL autosampler and a Surveyor MS Plus pump. A Luna C18 column (150 × 2.00 mm ID, particle size 5 μm, Phenomenex, Torrance, USA) was used, and the mobile phase was 0.1% HCOOH in water (A) and 0.1% HCOOH in acetonitrile (B). A summary of gradient and specific mass spectrometric conditions are given in SI2. Full-scan acquisition was performed over a mass range of 50–900 Da. To ensure the identification of target compounds, the following criteria was established: (i) Retention time shift between standards and samples should be lower than 2%; (ii) accurate mass measurements of the molecular and product ions should have an error below 5 ppm, with a high resolving power of 50,000 FWHM, *m/z* 200; and (iii) four decimal numbers should be used to identify precursor and fragment ions.

Extraction efficiency was performed using river water spiked at a concentration of 50 ng L^{-1}, and method detection limits (MDLs) were calculated considering an intensity of the signal of 10^3. Intra and inter-day precision were calculated at 0.5 mg L^{-1}. Quality parameters obtained using this SPE-LC-Orbitrap-MS method are summarized in Table 2.

Estimation of PECs and comparison of PECs vs. MECs

PECs of cytostatics in Catalonia for 2014 were calculated as described previously (Franquet-Griell et al. 2015). PECs is a useful tool to identify the contaminants and concentrations that are likely to be found in the environment, but, as a model, it is necessary to validate the results obtained with empirical data. PEC values represent average concentrations considering all the studied area. Therefore, to compare PECs with MECs, the minimum and maximum concentration of each

Table 2 Quality parameters of the LC-Orbitrap-MS, indicating compounds analysed ordered by retention time, the ion monitored and mass error (ppm), linearity (μg μL^{-1}) and regression coefficient (R^2), instrumental detection limits (ng), intra-day and inter-day precision (%), % recoveries and standard deviation ($n = 3$), matrix effect (%), method detection limits (MDL in ng L^{-1}) and water stability data according to bibliography

Target compounds	Rt (min)	Precursor ion	Mass error (ppm)	Linearity (mg L^{-1})	R^2	IDL (ng)	Intra-day precision (%)	Inter-day precision (%)	$\%R \pm$ SD river water ($n = 3$)	Matrix effect (%)	MDL (ng L^{-1})	Water stability[a,b]
Gemcitabine	2.75	264.0790	2.5	0.001–0.5	0.994	0.02	13	9.3	29 ± 18	−9	8.4	−
Fludarabine	6.58	286.0946	1.1	0.005–0.5	0.995	0.02	7.4	11	68 ± 6	−8	11	na
Goserelin	14.10	635.3264	2.2	0.02–1	0.996	0.1	7.2	23	77 ± 1	49	41	na
Leuprolide	14.11	605.3294	1.0	0.001–1	0.995	0.1	4.1	9.7	79 ± 1	−3	2.4	na
Irinotecan	14.42	587.2864	2.1	0.001–0.7	0.993	0.005	6.6	6.8	33 ± 0	−2	9.9	−
Doxorubicin	15.30	544.1813	3.5	0.001–1	0.995	0.05	6.1	8.6	48 ± 9	6	1.1	−
Cyclophosphamide	15.54	261.0321	0.9	0.001–1	0.999	0.005	16	15	111 ± 7	10	4.1	+
Epirubicin	15.79	544.1813	2.5	0.001–1	0.991	0.005	6.9	5.6	75 ± 8	−28	2.1	na
Ifosfamide	16.26	261.0321	1.6	0.001–1	0.993	0.005	3.9	11	65 ± 9	−5	4.1	+
Melphalan	16.35	305.0818	0.2	0.001–1	0.996	0.005	4.9	5.8	43 ± 4	−20	1.9	−
Daunorubicin	17.14	528.1864	2.3	0.01–1	0.995	0.1	4.1	9.5	62 ± 8	5	26	−
Erlotinib	17.16	394.1761	1.7	0.001–1	0.993	0.005	1.3	2.3	81 ± 3	−8	2.5	+
Capecitabine	17.67	360.1565	1.7	0.001–1	0.995	0.02	9.6	14	96 ± 50	−13	2.9	+
Prednisone	17.84	359.1853	0.9	0.001–1	0.996	0.005	3.3	11	73 ± 6	−16	1.8	+
Mycophenolic acid	23.69	321.1333	1.4	0.001–1	0.997	0.005	6.7	8.8	69 ± 19	−32	1.4	+
Tamoxifen	25.03	372.2322	1.5	0.0005–2	0.997	0.005	4.7	11	44 ± 16	−8	0.5	+
Chlorambucil	29.48	304.0866	1.4	0.001–1	0.994	0.005	3.3	8.0	50 ± 15	−12	1.0	−
Cyproterone	31.04	417.1827	1.5	0.001–1	0.997	0.02	2.5	11	72 ± 22	−13	1.8	na
Megestrol	31.27	385.2373	1.6	0.001–1	0.999	0.005	0.1	9.6	74 ± 15	−7	1.0	+

IDL instrumental detection limit, *MDL* method detection limit, *%R* recoveries spiked at 50 ng L^{-1}, − non-stable in water, + stable in water, *na* not available

[a] Franquet-Griell et al. (2016a)

[b] Negreira et al. (2014b)

compound detected in the river was considered. To evaluate the reliability of PECs, the following criteria has been used (Coetsier et al. 2009): f or 0.2 < PEC/MEC < 1, PECs are acceptable and slightly underestimated; for 1 < PEC/MEC < 4, PECs are acceptable and slightly overestimated, and for 4 < PEC/MEC < 8, PECs are overestimated.

One of the main parameters that influence the calculation of PECs is the receiving river flow and the dilution factor (DF). In the study of Franquet-Griell et al. (2015), the dilution factor used was of 25 specific for Spain, according to Keller et al. (2014) who provided dilution factors for different areas in Europe. However, given that the Besòs River and its tributaries have a very low flow rate, a specific DF was calculated using the formula reported by Keller et al. (2014):

$$DF = \frac{R \times A \times 10^3}{p \times W}$$

where A is the catchment area (1039 km^2), R is the specific run-off for Besòs River (131.4 mm $year^{-1}$) (Keller et al. 2014), p is the population (2,300,000 inhab) and W is the domestic water use per capita (47.8 m^3 cap^{-1} $year^{-1}$). This formula gives a DF of 1.2, which was used for PEC calculations, and therefore provides more accurate results.

Risk assessment

The risk quotient (RQ) was calculated using the measured concentrations to know if cytostatics poses a risk for the aquatic environment, using the following equation:

$$\mathrm{RQ} = \frac{MEC}{\mathrm{PNEC}} = \frac{MEC}{EC_{50}/f}$$

where MEC is the measured concentration in the present study and predicted no effect concentration (PNEC) is the concentration below which an unacceptable effect will most likely not occur. PNEC is estimated as the quotient of the toxicological relevant concentration (EC_{50}) and an assessment factor ($f = 1000$). To interpret the results, the following criteria were established (Marcus et al. 2010): RQ < 1.0 indicates no significant risk; 1.0 ≤ RQ < 10 indicates a small potential for

adverse effects; $10 \leq RQ < 100$ indicates significant potential for adverse effects and $RQ \geq 100$ indicates that potential adverse effects should be expected. EMEA guidelines suggest risk assessment based on long-term toxicity; however, very little information is available for these drugs, and short-term toxicity was considered (EMEA 2006).

Results and discussion

Quality parameters

An SPE-LC-Orbitrap-MS method was used for the determination of 19 cytostatic drugs, and in general, good quality parameters were obtained for most of them. A wide linearity range (0.001–1 mg L^{-1}) and R^2 values >0.99 indicated that internal standard quantification using the two deuterated standards provided a good and linear response. IDL ranged from 0.005 to 0.1 ng, being goserelin, leuprolide and daunorubicin, with high molecular weight (1269.4, 1209.4 and 527.5 Da, respectively), the compounds with lower response due to poor ionization efficiency in the conditions used. Intra-day precision, calculated using a concentration of 0.5 mg L^{-1}, ranged between 0.1 and 13%, and inter-day precision, at the same concentration, ranged from 2.3 to 23%. One of the main difficulties related to the analysis of cytostatic compounds in aqueous solutions is their water stability. Being polar and soluble compounds, many can hydrolyse in water and this affects extraction efficiency. Therefore, water samples need to be analysed upon collection to minimize this effect. For recovery tests, waters were spiked with target compounds at a relatively low concentration (50 ng L^{-1}, Table 2) and analysed immediately. At this low spiking levels, losses due to hydrolysis can occur as loading through the cartridge takes >1 h, and this has already an effect on the recovery values. Five drugs which had recoveries below 60% (gemcitabine, irinotecan, doxorubicin, melphalan and chlorambucil) have been reported to have low stability in water (0–22% after 24-h hydrolysis experiment) (Franquet-Griell et al. 2016a; Gómez-Canela et al. 2013a). For these compounds, degradation during the extraction step contributed to obtain the observed low recoveries. On the other hand, tamoxifen showed good stability (100% after 24 h) (Negreira et al. 2014b), but its recovery was low because it has a logP of 6.3 and would need more apolar solvents to ensure total elution from the Oasis HLB cartridges. However, despite having low recoveries, the inclusion of tamoxifen in this method permits its quantifications at levels higher than its MDL of 0.5 ng L^{-1}. For this compound, a specific method would provide better quality performance parameters. The other 13 drugs analysed presented recoveries ranging from 62 ± 8% (daunorubicin) to 111 ± 7% (cyclophosphamide) and correspond to compounds with high water stability (Franquet-Griell et al. 2016a). Danourubicin has a poor stability, but if extraction is performed immediately after spiking, it is possible to recover it quite satisfactorily. The matrix effect is another parameter that may affect quantification and has to be taken into account when analyzing cytostatic compounds in water, as it can produce enhancement or suppression of the MS signal leading to a subestimation or overestimation of the concentration of target analytes. Matrix effect was calculated by comparing the signal obtained in fortified pure and river water. Except for goserelin, values obtained ranged from −2 (for irinotecan) to −32% (for mycophenolic acid) which indicates that the response of the analytes was not affected by the humic and fulvic acids and salt content present in river waters, thus leading to a precise quantification. Goserelin had a signal enhancement of 49% and is explained by its low analytical response in the LC-HRMS which is then more affected by matrix components. Overall, by preconcentrating 100 mL of water, the MDL ranged between 0.5 and 41 ng L^{-1}, which is adequate for the trace analysis of cytostatic compounds in river waters. Precautions that have to be taken in river water monitoring studies to ensure high sensitivity as the one herein obtained are the following: (i) need to extract water samples within 24 h (or less) after sample collection to minimize degradation of cytostatic compounds and (ii) determination of matrix effects considering the specific composition of the water to be monitored.

PECs

Consumption data for cytostatic drugs increased in Catalonia from 4.7 t in 2010 to 5.1 t in 2014. According to 2014 consumption data, PECs were calculated for the cytostatic drugs used in Catalonia (PECs for the 19 drugs studied are indicated in Table 1). Data obtained is slightly different from the period 2010–2012 (Franquet-Griell et al. 2015), indicating the need to annually update the PECs so that they can be used in monitoring studies. Mycophenolic acid was by far the compound consumed at the highest amount (2365 kg in 2014 in comparison to 1859 in 2010) and had a PEC_{river} of 93.4 ng L^{-1}. This was followed by hydroxycarbamide (36.4 ng L^{-1}), capecitabine (5.85 ng L^{-1}), bicalutamide (4.65 ng L^{-1}), imatinib (2.57 ng L^{-1}) and prednisone (1.75 ng L^{-1}). All the other drugs had PEC < 1 ng L^{-1}. Compared to 2010–2012 (Franquet-Griell et al. 2015), the consumption, and consequently their PEC, increased for most of the drugs (PEC was 73.7 ng L^{-1} for mycophenolic acid in 2010) except for capecitabine and bicalutamide that slightly decreased (8.22 and 6.31 ng L^{-1} in 2010). Accordingly, compounds prioritized for the monitoring study followed the following criteria: (i) high probability of being detected such as mycophenolic acid, capecitabine and prednisone, with PEC > 10 ng L^{-1}. Hydroxycarbamide and bicalutamide could not be included in this multiresidue method as a specific method should be developed for these two drugs; (ii) low probability:

cyproterone, megestrol, gemcitabine, irinotecan, cyclophosphamide and ifosfamide, with PEC between 0.1 and 10 ng L^{-1}; (iii) very low probability: tamoxifen, erlotinib, doxorubicin, fludarabine, goserelin, leuprolide, melphalan, epirubicin, chlorambucil and daunorubicin, with PEC < 0.1 ng L^{-1}. These low probability drugs were included because some of them were detected in hospital and WWTP effluents in Catalonia (Gómez-Canela et al. 2014).

MECs in Besòs River

Water samples from Besòs River and its tributaries collected in two campaigns (May–July 2014) showed different concentration profiles. Out of 19 compounds analysed, 7 cytostatic compounds were detected at increasing concentrations from source to mouth of the river and a decrease in the last point due to the intrusion of the sea water. The minimum and maximum concentrations of cytostatic compounds were of 0.5 (tamoxifen) and 89.5 ng L^{-1} (mycophenolic acid) in May and 1.4 and 656 ng L^{-1} for the same compounds in July, and the relatively higher concentrations in this month are attributed to the low water flow in the dry season. In the first campaign (May 2014), only three drugs were detected: cyclophosphamide from the therapeutic group L01 (antineoplastic drugs), tamoxifen from the endocrine therapy group (L02) and mycophenolic acid from the immunosuppressants group (L04). Mycophenolic acid, mainly found along the lower stream of Besòs River (Fig. 2), was the most prevalent compound, present in 7 out of 19 samples and also the cytostatic measured at the highest concentration with values ranging from 13.1 to 89.5 ng L^{-1}. Cyclophosphamide was detected in four samples, at 7.8–10.0 ng L^{-1} mostly in three tributaries (see Fig. 2). Tamoxifen was determined in two samples at 0.5–0.9 ng L^{-1} in one tributary and in the Besòs River. In July, seven drugs were detected: ifosfamide, erlotinib and chlorambucil from the L01 therapeutic group and megestrol from L02 therapeutic group and those previously identified in the former campaign (mycophenolic acid, cyclophosphamide and tamoxifen). Mycophenolic acid was again the one at the highest concentration (8.5–656 ng L^{-1}), detected in 4 out of 19 samples with increasing concentrations downstream. Chlorambucil was the most prevalent drug, found in seven samples at 1.6–4.8 ng L^{-1} range in both river and tributaries. Tamoxifen and cyclophosphamide were identified in five samples from the tributaries mainly at 1.4–25.1 and 5.0–13.7 ng L^{-1}, respectively. Ifosfamide was detected at 10.1–13.9 ng L^{-1} in three samples, and erlotinib and megestrol were only measured once at 3.9 and 6.3 ng L^{-1}, respectively. Finally, 12 drugs (cyproterone, prednisone, melphalan, fludarabine, gemcitabine, capecitabine, doxorubicin, daunorubicin, epirubicin, irinotecan, leuprolide and goserelin) were not detected in any sample.

The type of compounds and levels detected in the Besos River are in the range of previous studies performed around the world. In Poland, mycophenolic acid was the compound continuously detected in Utrata and Vistula rivers, detecting the highest concentrations (up to 180 ng L^{-1}) in spring (Giebułtowicz and Nałęcz-Jawecki 2016). In the Somes River (Romania), cyclophosphamide was detected at concentrations up to 65 ng L^{-1}, but in a following campaign, it was <LOQ (10 ng L^{-1}) after a WWTP upgrade (Moldovan et al. 2009). In south France, ifosfamide and tamoxifen were

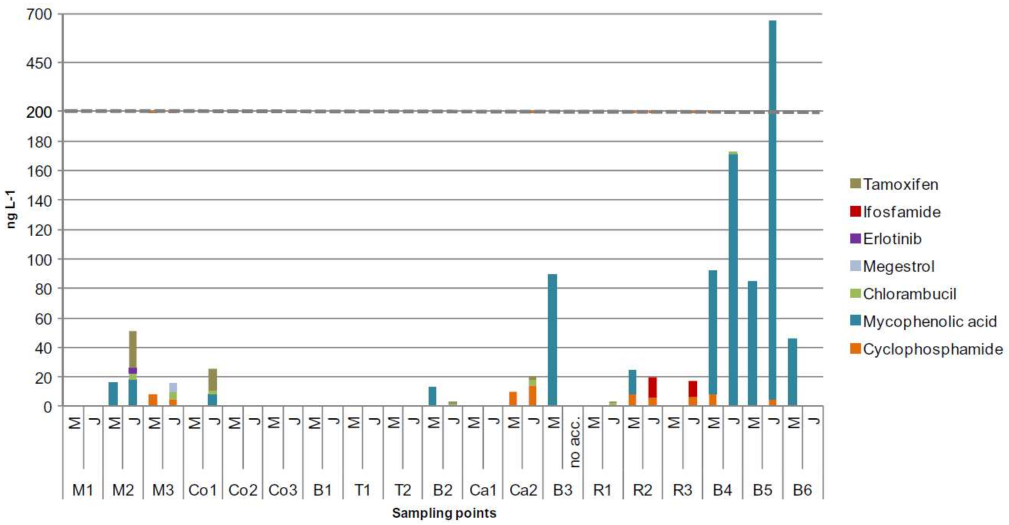

Fig. 2 Concentrations (ng L^{-1}) of the seven cytostatic drugs detected in the Besòs River and tributaries on May (*M*) and July (*J*) 2014 from source to mouth: Mogent (*M*), Congost (*Co*), Tenes (*T*), Caldes (*Ca*), Ripoll (*R*) and Besòs (*B*) (*no acc* sampling location not accessible)

analysed downstream of the WWTP and were detected at <3.8 and <5.8–25 ng L^{-1}, respectively (Coetsier et al. 2009). Cyclophosphamide and ifosfamide were studied in the Limmat River, Zurich, and were detected at concentrations up to 0.17 and 0.14 ng L^{-1}, respectively (Buerge et al. 2006). In Chao Phraya River, Bangkok, 5-fluorouracil was detected at 578 ng L^{-1}, cyclophosphamide at 1907 ng L^{-1} and hydroxycarbamide at 788 ng L^{-1} (Usawanuwat et al. 2014). So overall, in different areas of Europe, similar compounds and concentrations are detected, and this is indicative that cytostatic compounds can reach surface waters through either direct discharge of sewage waters, poor WWTP elimination efficiency or eventually release through pharmaceutical production plants.

PECs vs. MECs

MECs were compared with refined PECs ($PEC_{1.2}$), considering the low dilution factor (1.2) in the Besòs River. In addition, the $PEC_{1.2}$/MEC ratio was calculated considering the maximum MEC. Table 3 indicates compounds with PEC > 1 ng L^{-1} and PECs < 1 ng L^{-1}, the maximum and minimum concentrations detected in the two sampling campaigns and the PEC/MEC ratio.

For those drugs with PEC > 10 ng L^{-1} (mycophenolic acid, capecitabine and prednisone), only mycophenolic acid was detected. Its $PEC_{1.2}$ was 2018 ng L^{-1}, meaning a $PEC_{1.2}/MEC_{max}$ ratio of 3.1 (acceptable but slightly overestimated). This was the compound detected at the highest concentration and the one with the highest PEC, which reinforce the reliability of the PEC estimations. On the other hand, capecitabine (PEC = 5.85 ng L^{-1}) and prednisone (PEC = 1.75 ng L^{-1}) were not detected. Concerning the drugs with PEC between 0.1 and 10 ng L^{-1}, megestrol, ifosfamide and cyclophosphamide were identified. Their $PEC_{1.2}$ was 10.4, 8.48 and 4.1 ng L^{-1}, respectively, and the $PEC_{1.2}/MEC_{max}$ ratio was 1.7, 0.6 and 0.2, which is in the acceptable rank. Cyproterone, gemcitabine and irinotecan were not detected in any sample and correspond to compounds with low probability to be detected. For these compounds, water stability and solar degradation may play an important role in their presence and fate in river waters (Franquet-Griell et al. 2016a; Negreira et al. 2014b).

For the other drugs with PEC < 0.1 ng L^{-1} (tamoxifen, erlotinib and chlorambucil), they were unexpectedly detected at concentrations higher than their PECs. However, the sites where these compounds were detected correspond to the tributaries of Besòs, which have a very low flow rate, and thus, the PECs are underestimated. Finally, doxorubicin, fludarabine, goserelin, leuprolide, melphalan, epirubicin and daunorubicin were not detected in any sample, which is in agreement with their low PEC. In the specific case of daunorubicin, frequently monitored in the literature but not consumed in Catalonia, this non-consumption corresponds to its non-detection in Besòs river water.

For those drugs whose PEC/MEC was overestimated, this could be due to other processes which can occur in the river and are not taken into account in the PEC calculations, as solar degradation or hydrolysis. This is the case of capecitabine and prednisone which were removed in 40% after 3 h of simulated solar radiation, and 50% degradation was observed after 24 h of hydrolysis experiment in laboratory conditions (Franquet-Griell et al. 2016a). For those drugs whose PEC/MEC was underestimated (tamoxifen, erlotinib and chlorambucil), this could be attributed to a combined effect of poor elimination in WWTP and poor dilution in receiving waters as the sites where these compounds were detected had a river flow rate <0.2 m^3 s^{-1} which represents a very low DF. This suggests that point source contamination can influence the PEC/MEC ratio.

Table 3 Compounds detected in the Besòs River at PEC levels higher or lower than 1 ng L^{-1}, the PEC calculated at a DF of 25 and 1.2 from consumption data of 2014, method detection limit (MDL), the minimum and maximum concentrations and the number of detected samples out of 37 analysed, and the $PEC_{1.2}$/MEC ratio considering the maximum concentrations detected in the Besòs River

		PEC_{river} (2012)	PEC_{river} (2014)	$PEC_{1.2}$ (2014)	MDL (ng L^{-1})	MEC (ng L^{-1})			$PEC_{1.2}/MEC_{max}$
						Min	Max	*n*	
PEC >1 ng L^{-1}	Mycophenolic acid	80.4	93.4	2018	1.4	8.6	656	11	3.1
	Capecitabine	6.78	5.85	126	2.9	nd	nd	0	–
	Prednisone	1.61	1.75	37.9	1.8	nd	nd	0	–
PEC <1 ng L^{-1}	Megestrol	0.65	0.48	10.4	1.0	–	6.0	1	1.7
	Ifosfamide	0.33	0.39	8.48	4.1	10.1	13.9	2	0.6
	Cyclophosphamide	0.11	0.12	2.57	4.1	5.0	13.7	9	0.2
	Tamoxifen	0.05	0.052	1.13	0.5	0.5	25.1	7	0.05
	Erlotinib	0.02	0.013	0.29	2.5	–	3.9	1	0.08
	Chlorambucil	0.0002	0.00008	0.002	1.0	1.7	4.8	7	0.0004

PEC_{river}: PEC for Catalonian rivers (DF = 25), *$PEC_{1.2}$*: PEC in Besòs River (DF = 1.2)

When calculating PECs, the DF from WWTP to surface water is an important parameter. In the present case, DF of 1.2 for the particular case of Besòs River and its tributaries was used, as most of their flow comes directly from WWTPs and the DF is minimal. Still, PECs are calculated on a large geographical area (Catalonia), and to validate the PECs with MECs, consumption of cytostatics in a specific area would provide more exact results. However, more accurate consumption or estimated percentage of cytostatic consumption data in the Barcelona area and its surroundings was not available. Even so, PEC calculations are a good approach to prioritize drugs that should be given more attention in monitoring studies, and mycophenolic acid is a great example. It was, by far, the drug with the highest PEC and also the one detected at the highest concentration in river water. On the other side, drugs with low PECs were not detected in the river water, which again proves the validity of the PEC estimation.

In other studies, the PEC/MEC relation was also evaluated showing, in general, a good agreement between predicted and measured concentrations. For six drugs detected in surface waters in Alès (France), that included tamoxifen and ifosfamide, the relation PEC/MEC was acceptable ($0.2 < PEC/MEC < 4$ range). However, for five other widely consumed pharmaceuticals (ibuprofen, fenofibrate, norfloxacin, acebutolol and pravastatin), the $PEC/MEC > 8$ was attributed to an additional elimination in the sewage treatment plant (like sorption in sludge or in the river sediment) (Coetsier et al. 2009). In a WWTP from Barcelona, predicted and measured concentrations were of the same magnitude order for irinotecan, ifosfamide and capecitabine, showing a good PEC/MEC agreement (Gómez-Canela et al. 2014). PECs for pharmaceuticals (including ifosfamide and cyclophosphamide) and personal care products were calculated and compared with MECs taken from other studies, and PECs tended to be overestimated in a 57.4% (Ortiz de García et al. 2013).

The concentrations detected in Besòs River were in the same order than those reported in other areas of Spain, and the PEC/MEC ratio was calculated using a DF of 25 as a way to support the procedure. Ten anticancer drugs were analysed in the Ter River (NE Spain), 500 m upstream and downstream of the WWTP discharge (Ferrando-Climent et al. 2014). In that study, ifosfamide, docetaxel and vincristine were not found in agreement with the low PECs estimated (<0.08 ng L^{-1}), whilst cyclophosphamide and tamoxifen were detected at levels up to 20 and 38 ng L^{-1}, respectively, close to the highest concentrations of 13.7 and 25.1 ng L^{-1} determined in Besòs. However, this represents underestimated PEC/MEC values of 0.006 and 0.001 for these two compounds detected in Ter River. Tamoxifen was not detected in the Llobregat River (NE Spain) (López-Serna et al. 2010), but it was detected at 12.4–20.1 ng L^{-1} in the Ebro (NE Spain) and at 22.4–26.8 ng L^{-1} in its tributaries (López-Serna et al. 2012), which corresponds to a PEC/MEC of 0.004–0.002. Mycophenolic acid was detected in the Llobregat River in the drinking water treatment plant intake at concentrations from 17 to 56 ng L^{-1} in samples collected during seven consecutive days (Franquet-Griell et al. 2016b), and although the levels are lower than those detected in Besòs, a good agreement (PEC/MEC = 0.6) was observed according to its PEC of 93.4 ng L^{-1}. The presence of 14 frequently used cytostatics in Spain was analysed in Guadalquivir River (SW Spain) (Martín et al. 2011). Cytarabine and gemcitabine were found at 13 and 2.4 ng L^{-1}, respectively (not detected in Besòs River), which are levels higher than their PECs (0.007 and 0.22 ng L^{-1}, respectively). On the other hand, cyclophosphamide, ifosfamide, irinotecan, docetaxel, doxorubicin and epirubicin were <MDL, which is in agreement with the low PECs estimated by Franquet-Griell (2015). Cytostatics were detected in the main rivers of the Madrid region at concentrations <3 and <1–41 ng L^{-1} (Valcárcel et al. 2011), and cyclophosphamide had a LOD higher than the PECs and precludes the estimation, whilst the PEC/MEC ratio for ifosfamide was of 0.008.

For most of the cytostatic in the different monitoring studies, PEC/MEC was underestimated, which could be due to the low removal in WWTP or the use of inaccurate DF for each particular river. Among all the parameters used to calculate PECs, key factors influencing the PEC are Fwwtp, DF and water degradability. Fwwtp may vary among areas as removability in WWPTs is highly dependent on the configuration, hydraulic retention time and efficiency of each WWTP, affecting biodegradability of cytostatics. Accordingly, a high variability in the fraction of cytostatics discharged through the effluent is expected. DF is the most critical parameter and the one with the widest range of data in the bibliography (Keller et al. 2014). DF is also affected by seasonal differences in the river flow (periods of drought and rainfall) which have a high impact on PEC estimation, especially in rivers with low flows, such as rivers in the Mediterranean area. Therefore, we highlight the importance to determine the dilution factor for each river (if possibly in different seasons) to have a more precise estimation of PECs. Finally, PEC estimation is dependent on the degradation of cytostatic drugs in the natural environment, as unlike other pharmaceuticals, many of these compounds can undergo hydrolysis or photodegradation (Franquet-Griell et al. 2016a; Negreira et al. 2013) and thus would influence the PEC/MEC ratio. Besides, the potential for bioaccumulation is low according to their Kow (Royal Society of Chemistry 2014), and once discharged to receiving waters, cytostatic will likely remain solubilized in water.

Contrarily, Fexc is not expected to affect significantly the PEC calculation as ratios of human metabolism are anticipated to be similar among geographically different areas, although variable data has been found in the bibliography and this might influence PECs. Overall, more precise data implies

more accurate PECs and an improvement of the model. If available, geographically distributed consumption data permits accuracy in PEC estimation which guarantees its use for prioritizing compounds for environmental monitoring.

Risk assessment

To know if the concentrations detected in Besòs River represented a risk for the aquatic environment, the worst case scenario was considered and the highest MECs were used for RQ calculations. Table 4 summarizes the compounds studied, the highest concentrations detected in river water, the EC_{50} according with the bibliography and the RQ calculated. All the ecotoxicity elucidation was performed using *Daphnia magna*, as a toxicological model for aquatic organisms, because most of the toxicity results available in the bibliography are performed using this organism and it covers a major number of drugs.

For the compounds detected, their toxicity is between >1000 mg L^{-1} (cyclophosphamide) and 0.21 mg L^{-1} (tamoxifen). According to these values, tamoxifen had a RQ of 0.12, indicating no significant risk. Despite the high concentration of mycophenolic acid, the reported EC_{50} (>100 mg L^{-1}) (Roche 2014) indicates a low toxicity (RQ = 0.006). For the other drugs detected, their RQ is ≤0.001. Therefore, according to the above-mentioned criteria, none of the drugs had a significant risk (all RQ < 1.0) considering immobilization in acute toxicity tests.

Other studies report in general low acute toxicity of cytostatic compounds in water. Gemcitabine, cytarabine, docetaxel and epirubicin were previously evaluated considering different organisms (bacteria, algae, yeast and invertebrates among others) as endpoints (Martín et al. 2014). Considering only the acute toxicity in *D. magna*, the calculated RQ in the receiving waters was below 0.001, but in the other species, doxorubicin showed medium risk (0.1 < RQ < 1); cytarabine, gemcitabine, doxorubicin and epirubicin showed low risk (0.01 < RQ < 0.1) according to their criteria (Martín et al. 2014). No risk was either predicted for cyclophosphamide at the output of the WWTP, considering immobilization for *D. magna* (Ferrando-Climent et al. 2014). The ∑RQ was also calculated to simulate the accumulative effect, and it was 0.128, meaning no significant risk. However, it has to be taken into consideration that cytostatic compounds affect DNA and RNA, and thus, subchronic effects might be observed on cell replication or on protein synthesis in the long term. Therefore, there is an urgent need to evaluate the genotoxic effects of cytostatics to better determine their impact in aquatic organisms.

Conclusions

This study reports the concentration level of 19 cytostatic compounds in river water from an area impacted by a large population density and suffering from water pollution and scarcity. Seven compounds were detected at concentrations from 0.5 ng L^{-1} (tamoxifen) to 656 ng L^{-1} (mycophenolic acid), with levels increasing towards the mouth of the river. MECs detected in the Besòs River are of the same order or lower than PECs, which is a reasonable result as PECs provide average concentrations considering overall consumptions in Catalonia and water flows. To validate the method, the PEC/MEC ratio showed reliable results for several drugs (mycophenolic acid, megestrol, ifosfamide, cyclophosphamide), whereas most of the non-detected compounds (doxorubicin, fludarabine, goserelin, leuprolide, melphalan, epirubicin and daunorubicin) corresponds to those with low PEC and confirmed that this could be a useful tool to prioritize contaminants and estimate their concentration in the river. In hot spot areas, such as downstream rivers flowing through populated areas, the concentrations detected were much higher than predicted, and then, sampling monitoring following PEC calculations is recommended. The concentrations of the seven cytostatic drugs detected in the Besòs River did not show any risk for the aquatic environment considering acute toxicity in *D. magna*. This evaluation could be more accurate if long term and mixture drugs' toxicity could be considered, but the data available in the literature is very scarce. Overall, this study confirms the usability of PEC calculation to prioritize

Table 4 Risk assessment: the highest measured concentration (MECs) detected in Besòs River, *D. magna* ecotoxicity data (EC_{50}) and risk quotient (RQ)

	MEC (ng L^{-1})	EC_{50} (mg L^{-1})	Endpoint	Ref.	RQ
Mycophenolic acid	656	>100	48 h, EC_{50}	Roche (2014)	6.6E−03
Tamoxifen	25.1	0.21	Immobilization, 48 h EC_{50}	Orias et al. (2015)	1.2E−01
Ifosfamide	13.9	1795	48 h, EC_{50}	Martín et al. (2014)	7.7E−06
Cyclophosphamide	13.7	>1000	Immobilization, EC_{50}	Zounková et al. (2007)	1.4E−05
Megestrol	6.0	5	48 h, LC_{50}	FDA (1996)	1.2E−03
Chlorambucil	4.8	>10	EC_{50} 48 h	EDQM (2015)	4.8E−04
Erlotinib	3.9	>100	EC_{50} 48 h	Genentech (2015)	3.9E−05

cytostatic compounds to be monitored in a given area, as consumption patterns and the removal dynamics of each compound reflect the environmental concentrations. However, care has to be taken to validate the PEC/MEC ratios as DF has an important role in PEC estimation. Geographical distributed consumption data would improve the PEC estimation within a specific river basin.

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Supplementary Information

Table SI1: Target compounds, molecular formula, and relevant physicochemical properties

Target Compound		*CAS Nº*	*Molecular formula*	*Mm* ($gmol^{-1}$)	*Watersolubility* ($mg\ L^{-1}$)	*pKa*	*logP*	*Pv (mmHg)*	*Ctt. Henry* ($atmm^3mol^{-1}$)
CAP	Capecitabine	154361-50-9	$C_{15}H_{22}FN_3O_6$	359.3	26	1.9	0.56	$1.12\cdot10^{-12}$	$2.9\cdot10^{-19}$
CHL	Chlorambucil	305-03-3	$C_{14}H_{19}NO_2Cl_2$	304.2	$1.24\cdot10^{4}$	5.75	1.7	$9.3\cdot10^{-7}$	2.71E-10
CYC	Cyclophosphamide	6055-19-2	$C_7H_{15}Cl_2N_2O_2P$	261.1	40	-	0.76	$1.15\cdot10^{-4}$	-
CYP	Cyproterone	427-51-0	$C_{22}H_{27}ClO_3$	374.5	6.65	17.61	3.37	$3.18\cdot10^{-9}$	$2.688\cdot10^{-9}$
DAU	Daunorubicin	23541-50-6	$C_{27}H_{29}NO_{10}$	527.5	39.2	11.02	0.766	$6.13\cdot10^{-25}$	$2.1\cdot10^{-13}$
DOX	Doxorubicin	25316-40-9	$C_{27}H_{29}NO_{11}$	543.5	10	11.02	1.27	$9.64\cdot10^{-28}$	$2.23\cdot10^{-23}$
EPI	Epirubicin	56390-09-01	$C_{27}H_{29}NO_{11}$	543.5	1.18	11.02	1.41	$9.64\cdot10^{-28}$	-
ERL	Erlotinib	183319-69-9	$C_{22}H_{23}N_3O_4$	393.4	810	3.37	2.79	$2.69\cdot10^{-12}$	$2.61\cdot10^{-12}$
FLU	Fludarabine	21679-14-1	$C_{10}H_{12}FN_5O_4$	285.2	3530	6.26	-1.18	$3.7\cdot10^{-15}$	$1.3\cdot10^{-22}$
GEM	Gemcitabine	122111-03-9	$C_9H_{11}F_2N_3O_4$	263.1	$5.13\cdot10^{4}$	3.6	-1.4	$2.41\cdot10^{-11}$	$1.7\cdot10^{-17}$
GOS	Goserelin	145781-92-6	$C_{59}H_{84}N_{18}O_{14}$	1269.4	20	6.2	-	-	-
IFO	Ifosfamide	3778-73-2	$C_7H_{15}Cl_2N_2O_2P$	261.1	3780	4.75	0.86	$2.98\cdot10^{-5}$	$1.36\cdot10^{-11}$
IRI	Irinotecan	100286-90-6	$C_{33}H_{38}N_4O_6$	586.6	107		3.2	$1.31\cdot10^{-32}$	-
LEU	Leuprolide	74381-53-6	$C_{59}H_{84}N_{16}O_{12}$	1209.4	-	9.6	-	-	-
MEG	Megestrol	595-33-5	$C_{22}H_{30}O_3$	342.4	2	17.61	4	$4.37\cdot10^{-9}$	$1.23\cdot10^{-9}$
MEL	Melphalan	148-82-3	$C_{13}H_{18}Cl_2N_2O_2$	305.2	45.7	-0.432	-0.52	$3\cdot10^{-10}$	$4.2\cdot10^{-13}$
MPA	Mycophenolic acid	24280-93-1	$C_{17}H_{20}O_6$	320.3	22	3.57	2.8-4.2	$1.59\cdot10^{-10}$	$3.04\cdot10^{-12}$
PRE	Prednisone	53-03-2	$C_{21}H_{26}O_5$	358.4	312	13.9	1.46	$5.09\cdot10^{-13}$	$2.83\cdot10^{-10}$
TAM	Tamoxifen	10540-29-1	$C_{26}H_{29}NO$	371.5	0.167	5.31	6.3	$3.46\cdot10^{-8}$	$4.49\cdot10^{-10}$

SI2. LC-Orbitrap-MS analysis

The gradient started at 95% A and 5% B, increased to 70% B in 30 min, then increased to 100% A in 1 min, and then held for 10 min. Initial conditions were attained in 4 min and the system was stabilized for 5 min. The flow was 200 µL min^{-1} and 5 µL were injected. Cytostatics were measured under positive electrospray ionization (ESI+). Full scan acquisition was performed over a mass range of 50-900 Da at 50,000 full width at half maximum (FWHM), with the spray voltage at 3.5 kV, capillary voltage at 30 V, skimmer voltage at 28 V, and tube lens voltage at 130 V. All these conditions were chosen on the basis of a previous optimization study(Gómez-Canela et al. 2013).

SI3. Concentrations (ng L^{-1}) of the 7 cytostatic drugs detected in the Besòs River and its tributaries on May (M) and July (J) 2014.No acc.= no accessible

		IFO	CYC	ERL	MPA	TAM	CHL	MEG
M1	M							
	J							
M2	M				16.0			
	J	<lod	<lod	3.9	18.1	25.1	4.1	
M3	M		8.1					
	J	<lod	5.0				4.8	6.0
Co1	M				<lod	<lod		
	J	<lod	<lod		8.6	14.3	2.5	<lod
Co2	M							
	J			<lod				
Co3	M					<lod		
	J	<lod	<lod					
B1	M					0.5		
	J							
T1	M					0.9		
	J							
T2	M							
	J							
B2	M				13.1			
	J	<lod	<lod			1.4	1.7	
Ca1	M							
	J							
Ca2	M		10.0					
	J	<lod	13.7			2.3	4.4	
B3	M				89.5			
	no acc.							
R1	M							
	J		<lod			1.4	2.0	
R2	M		8.4		15.9			
	J	13.9	5.5					
R3	M							
	J	10.1	6.9					
B4	M		7.8		84.0			
	J	<lod	<lod		171.3		1.9	
B5	M				84.9			
	J		5.2		656.2		<lod	
B6	M				46.4			
	J	<lod	<lod				<lod	

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Short communication

Do cytostatic drugs reach drinking water? The case of mycophenolic acid

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ABSTRACT

Mycophenolic acid (MPA) has been identified as a new river contaminant according to its wide use and high predicted concentration. The aim of this study was to monitor the impact of MPA in a drinking water treatment plant (DWTP) that collects water downstream Llobregat River (NE Spain) in a highly densified urban area. During a one week survey MPA was recurrently detected in the DWTP intake (17–56.2 ng L^{-1}). The presence of this compound in river water was associated to its widespread consumption (>2 tons in 2012 in Catalonia), high excretion rates and low degradability. The fate of MPA in waters at each treatment step of the DWTP was analyzed and complete removal was observed after pretreatment with chlorine dioxide. So far, MPA has not been described as water contaminant and its presence associated with its consumption in anticancer treatments is of relevance to highlight the importance of monitoring this compound.

1. Introduction

Cytostatic drugs have emerged as new water contaminants due to their wide use in cancer treatments (Buerge et al., 2006). In Catalonia (NE Spain), the total consumption of 132 different anticancer drugs ranged from 4.7 t to 4.9 t during the period 2010–2012 (Franquet-Griell et al., 2015). Among them, mycophenolic acid (MPA) accounted for 40% of total drugs administered, equaling 1.9 t yr^{-1} corresponding to a consumption per capita of 704 μg $inhab^{-1}$ d^{-1}. Consumption data has been used recently to calculate the predicted environmental concentration in rivers (PEC_{river}), which is a value that gives an estimate of the level of exposure for a given scenario and is thus essential for an initial indication of environmental impact (Kelly et al., 2003). According to a previous study (Franquet-Griell et al., 2015), among 132 cytostatic compounds, MPA was expected to be found in Catalonian rivers at the highest concentration (77.4 ng L^{-1}), which exceeds the threshold value for an individual drug for further environmental risk assessment (10 ng L^{-1}), according to the European Medicines Agency (EMEA) (EMEA, 2006). MPA, classified in the Anatomical Therapeutic Chemical system (ATC) as L04AA06, is used basically as renal, cardiac and hepatic allogeneic prophylactic treatment against organ rejection. After administration, 60% of the drug is excreted in the urine as mycophenolic acid glucuronide whereas 3% remains unchanged (Drugs Information Database, 2014). Once it reaches sewage water and enters a wastewater treatment plant (WWTP), the glucuronide metabolite is deconjugated and the parent compound is formed again. Its estimated removal rate is of 41% (Royal Society of Chemistry, 2014), and therefore, this compound has great chances to reach surface waters. MPA is a weak organic acid with a solubility of 22 mg L^{-1} (Royal Society of Chemistry, 2014), a predicted pKa from 3.57 to 4.61 and a partition coefficient octanol–water from 2.8 to 4.2 (depending on the database) (Chemical Book, 2008; Drug Bank Database, 2013; Royal Society of Chemistry, 2014) and this suggests that it will be preferentially detected in water. Once introduced in the water cycle it could reach tap water if the treatment is not efficient. The toxicological effects of MPA on human beings are unknown, but its mode of action is based on the non-competitive, selective and reversible inhibition of the inosinmonophosphate dehydrogenase, so it inhibits *de novo* synthesis of the nucleotide guanosin, without incorporation to DNA. It can have negative effects on the long term like cyclophosphamide, tamoxifen or melphalan, which are known to be carcinogenic (IARC, 2015). Thus, the importance of MPA monitoring in water intended for human consumption.

Whereas ultra high performance liquid chromatography

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coupled to tandem mass spectrometry (UHPLC-MS/MS) methods have been developed to analyze this compound in biological samples for clinical therapeutic drug human biomonitoring (Klepacki et al., 2012), to our knowledge, no one has attempted to determine this compound as a water contaminant. Because of its potential occurrence in river waters, drinking water becomes at risk, especially when river water constitutes the main source for purification. The study site is the drinking water treatment plant (DWTP) that supplies water to the Barcelona city (2,000,000 inhabitants). This plant collects water from the Llobregat River, a river which receives the impact of 139 cities and villages, the discharges of 94 WWTPs and 571 authorized waste discharges from industries and agricultural fields (Agència Catalana de l'Aigua, 2015). The DWTP intake is settled near the mouth of the river and close to the city of Barcelona, downstream of many urban, industrial discharges and run-off and therefore, it represents a worst case scenario. The aim of this study was to determine the occurrence of MPA in a drinking plant considering water collection and all the purification treatments. For such purpose, we developed and validated an UPLC-MS/MS method to accurately quantify MPA in water.

2. Experimental

2.1. Chemicals and reagents

MPA and MPA-d_3 pure analytical standards of ≥98% purity were acquired from Sigma–Aldrich (St. Louis, USA). MPA stock standard solution was prepared at a concentration of 1000 ng μL^{-1} in methanol, and working solutions at 10 ng μL^{-1}. Methanol, acetonitrile, acetone (SupraSolv grade) and HPLC water (LiChrosolv grade) were supplied by Merck (Darmstadt, Germany). Oasis HLB 200 mg solid phase extraction cartridges (SPE) were from Waters (MA, USA).

2.2. Sampling

Water samples (1 L) were collected from a DWTP located close to the city of Barcelona (Catalonia, Spain). This plant collects raw water from the Llobregat River and is treated following the order: dioxychlorination of river raw water, coagulation, flocculation, settling, sand filtration and groundwater addition to improve water quality. The filtered water is then split in two parallel purification lines, one employing conventional treatment with ozonization and GAC (granular activated carbon) filtration (~70% of the total flow), and the other, advanced treatment with ultrafiltration and reverse osmosis (~30%). Both treated waters are then blended, chlorinated and distributed. Samples were collected during 7 consecutive days (25th to 31st January 2014) and since MPA was always detected, its evolution along the treatment lines and in finished water was determined in two further sampling campaigns performed in the following days. Samples were taken throughout the DWTP according its hydraulic retention time. The sampling campaign was performed in winter, which is the season when the highest contaminant concentrations of drugs are detected in this area (Boleda et al., 2009).

2.3. Extraction and analysis

Samples were acidified at pH 2 with HCl 37% and afterward extracted with an automated solid-phase extraction apparatus (Dionex Autotrace 280, Thermo Scientific). One hundred mL of water were spiked with 5 ng of MPA-d_3 as internal standard (IS). Oasis HLB 200 mg SPE cartridges were conditioned with 6 mL MeOH and 6 mL H_2O at 3 mL min^{-1} and then the sample was loaded at a flow rate of 1 mL min^{-1}. Cartridges were rinsed with 3 mL 100 mM NH_4OAc in H_2O, dried during 30–45 min under a current of nitrogen at 5 mL min^{-1} and eluted with 4 mL MeOH and further 4 mL HCOOH:MeOH (5:95). Samples were then evaporated to almost dryness in a TurboVap under a current of nitrogen at 25 °C and transferred to a 2 mL chromatographic vial with 1 mL of ACN as washing solvent. Finally, samples were evaporated to dryness and reconstituted to 100 μL of a 50:50 mixture (0.1% HCOOH in ACN and 0.1% HCOOH in HPLC water).

UHPLC conditions were optimized to obtain good resolution and sample throughput. An Acquity Waters, USA, system connected to a Quattro-micro triple quadrupole detector (UHPLC-MS/MS) was used to determine MPA. An Acquity UPLC BEH C18 column (100 mm × 2.1 mm ID, particle size 1.7 μm) was used at a flow rate of 0.3 mL min^{-1}. The mobile phase composition consisted of binary mixtures with 0.1% HCOOH in water (A) and 0.1% HCOOH in acetonitrile (B). Gradient elution started at 95% A and 5% B, increased to 70% B in 10 min (using a slight convex curve) and increased to 100% B in 5 min. Initial conditions were attained in 1 min and the system was stabilized for 1 min. Ten μL were injected. MPA was measured under positive electrospray ionisation (ESI+). Flow injection analysis (FIA) was performed to determine the optimum cone voltage (between 10 and 100 V) that produced the molecular ion and the optimum collision energies (between 5 and 50 eV) to obtain at least two intense fragments. Finally, acquisition was performed in selected reaction monitoring (SRM) mode using two transitions from $[M+H]^+$ precursor ion to product ions to identify each compound. The transitions used as well as the optimized cone voltages and collision energies are given in Fig. 1. Internal standard quantification was performed. The data were acquired and processed using the MassLinx v.4.1 software package.

2.4. Quality control/Quality assurance

The method was assessed for accuracy, linearity, sensitivity, selectivity, extraction efficiency and matrix effects. Intra and inter-day accuracy were determined by injecting a 0.5 ng μL^{-1} standard during 5 consecutive injections and in 5 different days. Linearity was studied over a concentration range of 0.005–1 ng μL^{-1}, with IS kept at a constant concentration of 0.4 ng μL^{-1}. Sensitivity was determined by calculating the instrumental limits of detection (IDL) and method detection limits (MDL). IDL was calculated as the amount of analyte that gives a signal to noise ratio of 3 (S/N = 3) using the standard at 0.005 ng μL^{-1}. MQL was calculated as the concentration that gave a S/N = 10 and was determined from the spiked river water at 10 ng L^{-1} and gives information on the sensitivity of the method, considering the extraction and analytical procedure. Extraction efficiencies were determined by spiking Llobregat river water and from pristine mountain creek water at a concentration of 50 ng L^{-1} and performing the analysis in triplicates. At the same concentration, the matrix effect (ME) was evaluated using the following equation:

$$ME\ \% = \left(1 - \frac{I_{river} - I_{blank}}{I_{creek}}\right) \cdot 100 \quad (1)$$

where I_{river} was the MPA peak intensity in spiked Llobregat river water, I_{blank} in unspiked Llobregat river water (as MPA was always detected) and I_{creek} in the spiked pristine creek water. Identification criteria included the retention time and two transitions, one used for quantification and the other for confirmation, and the ion ratio, as suggested by the European Union Decision 202/657/EC (August 17, 2002).

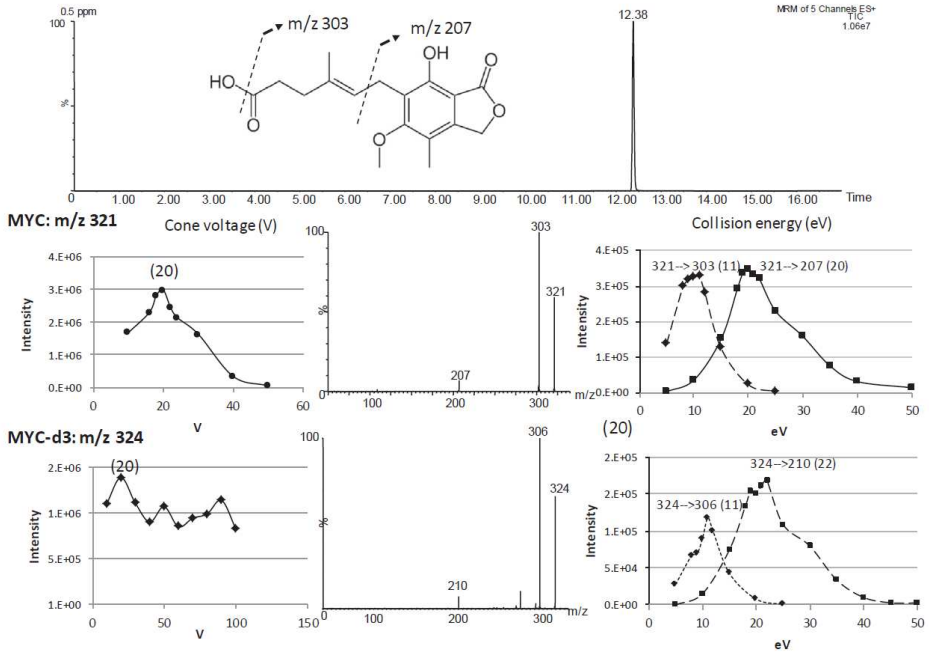

Fig. 1. UHPLC-MS/MS chromatogram of a standard at 0.5 ng μL^{-1}, spectra of MPA and MPA-d_3, and method optimization for the trace identification of MPA.

3. Results and discussion

3.1. Method performance

MPA and MPA-d3 coeluted at 12.5 min. MPA was detected at *m/z* 321$[M+H]^+$ and several product ions were identified. From this precursor ion, the transition *m/z* 321 → 207 was used for quantification, which corresponds to the loss of $C_6O_2H_8$ (see Fig. 1). It was produced at 20 V of cone voltage and 20 eV of collision energy. The transition *m/z* 321 → 303 $[M-H_2O]^+$ was used as confirmation, produced at 11 eV of collision energy. These two transitions were observed by other authors, who also selected the *m/z* 207 (Martins Duarte Byrro et al., 2013; Nguyen Thi et al., 2013) and *m/z* 303 (Delavenne et al., 2011). MPA-d_3 was detected at *m/z* 324 $[M+H]^+$, and the transition *m/z* 324 → 210 was used for quantification (20 V cone voltage and 22 eV collision energy).

Quality parameters include intra and inter-day accuracies, precision, linearity, sensitivity, selectivity, extraction efficiency and matrix effects (Table 1). Good correlation was obtained ($r^2 = 0.998$) over a concentration range of 0.005–1 ng μL^{-1} using isotopic dilution with MPA-d_3. The intra and inter-day accuracies of the method were 3% and 13% respectively, at 0.5 ng μL^{-1}. Recoveries in Llobregat raw water were 59 ± 4% and 71 ± 2% in pristine creek water, respectively (at 50 ng L^{-1} spiking level). The matrix effect was 39% attributed to the initial water quality of the Llobregat River with a TOC of 3.2 mg C L^{-1} and a turbidity of 78.8 NTU (normalized turbidity units). The IDL was 0.0003 ng and the MQL 0.5 ng L^{-1}. These quality parameters ensure the trace monitoring of this compound in water.

Table 1
Quality parameters of the UHPLC-MS/MS method developed for the trace analysis of MPA in water.

Accuracy (0.5 ng μL^{-1})	
Intra-day	3%
Inter-day	13%
Linearity (ng μL^{-1})	0.005–1
Regression equation	y = 2.0937x–0.0446
R^2	0.998
Sensitivity	
IDL (ng)	0.0003
MQL (ng L^{-1})	0.5
Extraction efficiency (±sd)	
River	59 ± 4%
Pristine creek	71 ± 2%
Matrix effect	39%

MPA has only been analyzed in several biological matrices but not in the aquatic environment. Other studies where MPA was analyzed for therapeutic applications also provided low sensitivity and good reproducibility. An automated high-throughput UHPLC–MS/MS assay using liquid-handling robotic extraction was developed and validated for the quantification of MPA and its metabolites in plasma and urine (Klepacki et al., 2012). In another study, MPA was analyzed in human kidney biopsies by LC-MS/MS in MRM using ESI+ and excellent intra and inter-day variability were obtained at concentrations from 0.6 to 10 ng mL^{-1} (Md Dom et al., 2014). MPA was determined in the vitreous humor of rabbits (Martins Duarte Byrro et al., 2013) and human peripheral blood mononuclear cells (Nguyen Thi et al., 2013) using HPLC-ESI + MS/MS, with good linearities observed in both cases over a concentration ranges of 0.003–10 ng μL^{-1} and 0.0001–0.5 ng μL^{-1}, respectively.

3.2. Occurrence of MPA in the DWTP intake

MPA was detected in all river samples at the DWTP intake at concentrations ranging from 17.0 to 56.2 ng L^{-1} (Fig. 2). This compound despite not being identified before as a water contaminant, presented concentrations in the Llobregat River close to

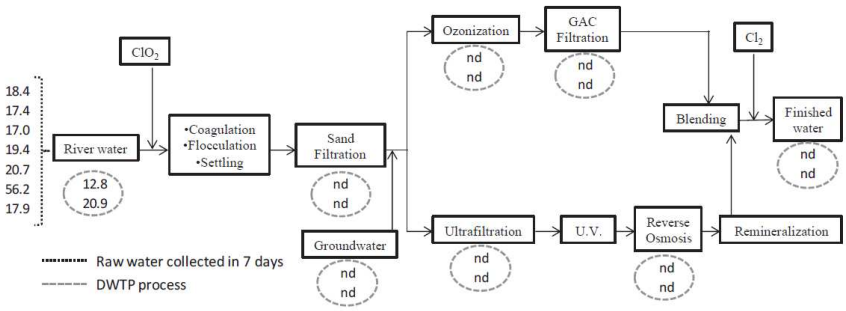

Fig. 2. DWTP processes and MPA concentrations detected (ng L^{-1}) in the different sampling campaigns: raw water in 7 consecutive days and the DWTP process in 2 different days.

other cytostatic compounds detected in Spanish river waters. Among them, tamoxifen and cyclophosphamide were measured at 12–36 ng L^{-1} and nd-20 ng L^{-1} respectively in Ter River (NE Spain) (Ferrando-Climent et al., 2014), cytarabine at 13 ng L^{-1} and gemcitabine at 2.4 ng L^{-1} in Guadalquivir River (SW Spain) (Martín et al., 2011) and ifosfamide was detected at <1–41 ng L^{-1} in rivers near Madrid region (Central Spain) (Valcárcel et al., 2011). The predicted concentrations for these drugs were in the range 0.006–0.3 ng L^{-1}, much lower than PEC_{river} for MPA (77.4 ng L^{-1}) (Franquet-Griell et al., 2015). The identification of MPA, among all the other cytostatics studied, is of relevance as it confirms the validity and usability to calculate the PECs as a preliminary step to determine the presence of drugs in the environment. PECs consider consumption and excretion rates as well as elimination efficiency in WWTPs. Therefore, they provide accurate data of the real situation where excreted drugs are directed to the sewage system and are discharged to surface waters after treatment in the WWTPs. Because cytostatic compounds are polar compounds and some have low degradability in WWTPs, they are released by the effluents to receiving waters and become potential water contaminants (Gómez-Canela et al., 2014). The case of MPA is interesting because it is a compound largely used; a significant amount (60%) of the drug is excreted in the urine (Drugs Information Database, 2014) and only 41% is removed in the WWTPs (Royal Society of Chemistry, 2014). This is the reason that leads to its systematic detection during the monitored week at the DWTP intake indicating its ubiquitous presence in the Llobregat River downstream waters.

3.3. Elimination in the DWTP

In view of the results, samples from both parallel purification treatment lines were analyzed: river water, sand filtration, ozonization, GAC filtration, ultrafiltration, reverse osmosis and finished water. MPA was detected again in raw water at concentrations of 12.8 and 20.9 ng L^{-1}. A complete elimination was observed after dioxychlorination, sedimentation and sand filtration (Fig. 2). The MPA elimination occurs after the ClO_2 addition, which is actually the first step in the purification process used for disinfection. The oxidative removal by chlorine dioxide has been studied for several pharmaceuticals, including antibiotics and hormones among others (Huber et al., 2005; Lee and von Gunten, 2010; Sharma, 2008; Wang et al., 2015). In these cases, the phenolic moiety reacts with ClO_2. For MPA and taking into account that chlorine dioxide reacts very slowly with olefins and is likely unreactive with carboxylic acids, the primary attack should be the phenolic moiety to yield the corresponding quinone and further unidentified transformation products. This degradation pathway will be studied in the future.

Thus, whether MPA reaches drinking water, the answer should be no considering the current purification treatments carried out in this DWTP. However, MPA is a river contaminant based on its reiterative presence and PEC_{river}. The example herewith provided represents a case of a DWTP which treats river water from a highly urbanized and industrialized area, so it may represent one of the worst case scenarios regarding contamination by cytostatic compounds or other pharmaceuticals. In this sense, we provide an accurate UHPLC-MS/MS method to monitor MPA in river waters and indicate the need for its inclusion in environmental surveys. Generally speaking, the incidence of cytostatic compounds can be assessed by first calculating the PEC_{river} considering their consumption in each area and prioritizing the monitored compounds according to these values, as has been performed in the present study.

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3.3. Discussió de resultats

En el capítol 2 d'aquesta tesi s'han destacat un grup de citostàtics amb majors probabilitats de ser detectats a les aigües superficials mitjançant el càlcul dels PECs. No obstant, aquests compostos prioritzats no coincideixen amb aquells que s'analitzen al medi d'acord amb les dades bibliogràfiques. A més, per alguns d'ells es desconeix la seva degradabilitat, ja que no hi ha dades experimentals al respecte. Aquest tema és important per tal de determinar llur comportament en aigües i determinar quins són els compostos més recalcitrants i per tant, amb més probabilitat de detectar-se en aigües superficials.

Per aquest motiu, en l'article científic III, "*Biological and photochemical degradation of cytostatic drugs under laboratory conditions*", s'han estudiat els processos de degradació que poden experimentar aquests fàrmacs des de la seva excreció fins que arriben al medi, passant per les EDARs. Concretament, els processos estudiats van ser la hidròlisi, la biodegradació, la radiació UV i l'UV-H_2O_2 per un conjunt de 16 citostàtics. Els compostos es van seleccionar d'entre aquells amb valors de PEC més elevat calculats per Catalunya, per haver estat prèviament detectats en aigües segons la bibliografiai entre aquells que interaccionen directe o indirectament amb l'ADN de manera que podrien afectar el procés de biodegradació en el tractament secundari de les EDARs. Amb la combinació d'aquests criteris es va seleccionar la ciclofosfamida, la ifosfamida, la citarabina, l'àcid micofenòlic, la capecitabina, l'etopòsid, la prednisona, la gemcitabina, el megestrol, el melfalan, el clorambucil, l'irinotecà, la doxorubicina, la daunorubicina, la vincristina i la vinblastina. Els experiments de degradació es van dur a terme als laboratoris del Departament d'Enginyeria Química de la Universitat de Barcelona.

Inicialment es va estudiar la hidròlisi dels citostàtics, ja que conèixer llur estabilitat és important per tal d'avaluar tots els altres processos. Es va observar que alguns compostos s'eliminaven ràpidament en aigua, tals com la vincristina, la vinblastina, la daunorubicina, la doxorubicina, l'irinotecà i el clorambucil, com es mostra a la Figura 2 de l'Article III. Això indica que aquests compostos s'eliminaran ràpidament un cop excretats i abocats a la xarxa de clavegueram, i que difícilment es detectaran en influents de les EDARs i encara menys, en rius. Un cop es van descartar aquells compostos hidrolitzats, es va avaluar el comportament dels citostàtics més estables al llarg del cicle de l'aigua, amb l'objectiu final de predir quins són els compostos que s'eliminaran durant el tractament de les EDARs i quins seran els que potencialment s'alliberaran al riu a través dels efluents de les plantes de tractament.

Un dels factors més importants a l'hora d'avaluar la presència de citostàtics en el efluents de depuradores, i conseqüentment en rius, és el càlcul de la fracció eliminada o altrament

coneguda com l'eficàcia d'eliminació de les EDARs. En el càlcul dels PECs dels articles científics I i II del capítol "Consum i priorització de fàrmacs citostàtics" es va tenir en compte el percentatge d'eliminació en depuradores d'acord amb les dades experimentals de la bibliografia i quan aquestes no existien, de models teòrics (per a la majoria de compostos). Els models teòrics comporten una incertesa degut que sovint s'estimen en funció dels paràmetres fisicoquímics i poden no ser reals. Per tant, disposar de dades experimentals en condicions controlades però el més similars possibles a les reals (tipus d'aigua i fang de depuradora, concentració d'analits, temps de permanència, etc.) permet obtenir una informació molt més acurada de la capacitat de biodegradació dels citostàtics, que simularia la biodegradació en els tancs aeròbics d'una depuradora. Els estudis de degradació en el reactor biològic han permès comparar els resultats obtinguts amb els Fwwtp aplicats en el càlcul de PECs (Taula 3.2). Concretament, es mostren els % de degradació obtinguts després de 2 i 8h, que és el temps més habitual de permanència de les aigües residuals amb els fangs activats. Les dades s'han ordenat de menor a major degradació en 2h de reacció. A la taula s'observa que els valors a 2h de degradació són més similars als Fwwtp que els valors a 8h i que, com és d'esperar, s'apropen més als valors que provenen de dades experimentals (senyalat amb *). Per la ifosfamida i la ciclofosfamida, ambdós valors indiquen una baixa degradació si es té en compte un temps baix de permanència al reactor. Per a la prednisona i el megestrol també coincideixen en determinar una alta degradació. No obstant, per a la resta de citostàtics les dades no són gaire coincidents. Per a la citarabina i l'àcid micofenòlic es va obtenir una menor degradació que la prevista, mentre que per la capecitabina i l'etopòsid va ser lleugerament major. Per la vincristina, la vinblastina, el melfalan, el clorambucil, la gemcitabina, l'irinotecà, la doxorubicina i la daunorubicina les dades són contradictòries, ja que les degradacions obtingudes experimentalment són molt majors que les esperades segons els models teòrics. Aquestes diferències són degudes a que les condicions experimentals utilitzades tenen en compte molts altres factors. Amb excepció de la gemcitabina, aquests citostàtics van ser hidrolitzats ràpidament i per tant, l'eliminació en el bioreactor es deu a aquest procés. Aquest factor no es comptabilitza en el càlcul teòric. A més, la biodegradació també depèn molt de la càrrega de citostàtics en les aigües residuals, ja que concentracions elevades de citostàtics podrien afectar la biomassa bacteriana dels reactors biològics i per tant, el seu rendiment.

Taula 3.2. Degradació al reactor biològic en 2h i 8h (%) i Fwwtp (%)

	% bioreactor 2h*	% bioreactor 8h*	% Fwwtp	Referència
Ifosfamida	23	20	0*	Besse et al. (2012)
Ciclofosfamida	27	30	0*	Besse et al. (2012)
Citarabina	27	77	50*	Besse et al. (2012)
Àcid micofenòlic	28	26	41	(Royal Society of Chemistry, 2014)
Capecitabina	28	32	15*	Besse et al. (2012)
Etopòsid	31	29	2	U.S. EPA (2013)
Melfalan	52	88	2	(Royal Society of Chemistry, 2014)
Clorambucil	68	100	2	U.S. EPA (2013)
Prednisona	71	97	87*	Fan et al. (2011)
Vincristina	80	94	7	U.S. EPA (2013)
Vinblastina	90	97	18	U.S. EPA (2013)
Gemcitabina	100	100	40*	Besse et al. (2012)
Megestrol	100	100	96*	Chang et al. (2011)
Irinotecà	100	100	3	U.S. EPA (2013)
Doxorubicina	100	100	2	U.S. EPA (2013)
Daunorubicina	100	100	2	U.S. EPA (2013)

* valor experimental

Finalment, el tipus d'aigua residual i nivells de matèria orgànica poden també afectar la biodegradació per temes de competència, factors que tampoc es tenen en compte en els càlculs teòrics. Això posa de manifest la necessitat de tenir dades experimentals per tal de poder avaluar amb precisió l'eliminació d'aquests compostos durant el tractament secundari.

En l'estudi dels processos de degradació es va determinar quins dels citostàtics analitzats són més recalcitrants i per tant s'espera que puguin arribar a les aigües superficials. Per corroborar aquests resultats i per confirmar els citostàtics prioritzats amb els PECs es va dur a terme la monitorització del riu Besòs, corresponent a l'article científic IV "*Determination of cytostatic drugs in Besòs River (NE Spain) and comparison with predicted environmental concentrations*".

A l'hora de fer la monitorització del riu Besòs es va adaptar el mètode desenvolupat per aigües residuals de Gómez-Canela et al. (2014). A aquest mètode es va afegir l'àcid micofenòlic i la hidroxicarbamida, ja que van ser els fàrmacs amb PECs més alts. Aquest mètode també inclou altres citostàtics, a més dels estudiats en el treball de degradacions, ja que es va considerar oportú incloure'ls degut que són compostos àmpliament estudiats segons la bibliografia, la qual cosa permet obtenir la màxima informació possible sobre llur presència al riu.

Les mostres es van analitzar per cromatografia de líquids acoblada a l'espectrometria de masses d'alta resolució (LC-Orbitrap-MS), fent l'escombratge de tots els ions (mode *full-scan*) i utilitzant uns voltatges fixes per a tots els compostos (esprai a 3,5 kV, capil·lar a 30 V, *skimmer* a 28 V i *tube lens voltage* a 130 V). L'àcid micofenòlic es va poder incorporar a l'anàlisi sense problemes, però no la hidroxicarbamida, per la qual les condicions de l'equip per a l'anàlisi de 19 citostàtics no van ser les adequades. En fer la optimització mitjançant injecció directa no es va poder observar el seu ió molecular, ja que adquirint en mode d'escombratge d'ions total en la zona de masses baixes (la hidroxicarbamida té un pes molecular de 76 g/mol) s'observen molts ions que poden interferir.A més, la columna analítica utilitzada en la separació (Luna C18, 150 × 2,00 mm de diàmetre intern i 5 µm de mida de partícula, Phenomenex, Torrance, USA) no és adequada per a compostos molt polars, i la hidroxicarbamida eluiria en el front d'elució. Per aquest motiu es va decidir fer la monitorització amb el mètode establert per a l'àcid micofenòlic i la resta de citostàtics i optimitzar un segon mètode més adequat per a la hidroxicarbamida. La Figura 3.3 mostra la separació cromatogràfica dels 19 citostàtics analitzats per LC-Orbitrap-MS.

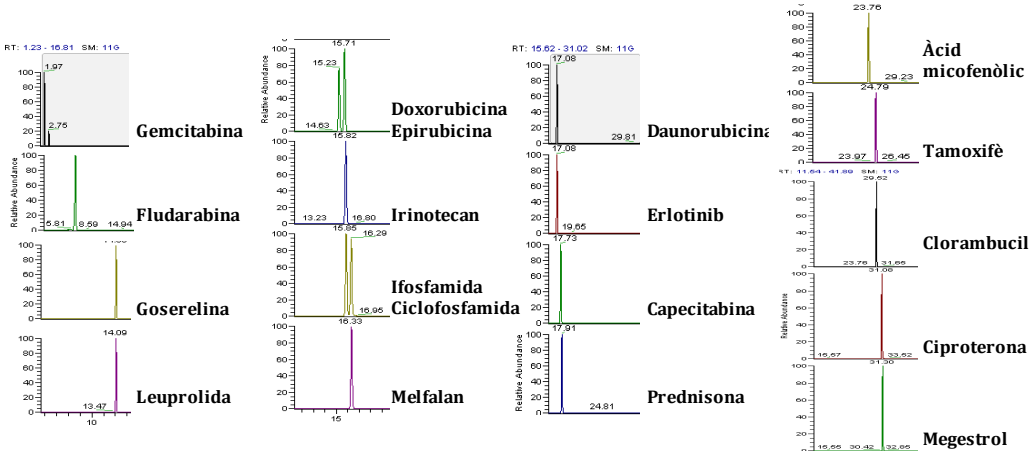

Figura 3.3. Separació cromatogràfica dels 19 citostàtics analitzats al riu Besòs

Els paràmetres de qualitat obtinguts per aquest mètode es troben recollits a la Taula 2 de l'Article IV. En general, es va obtenir una àmplia linealitat (0,001-1 mg/L) i límits de detecció metodològic entre 0,5 i 41 ng/L. Comparat amb l'article de Gómez-Canela et al. (2014) on s'analitzen 26 citostàtics en aigua residual, els MDL són més baixos per a 11 citostàtics (la gemcitabina, la fludarabina, la leuprolida, la doxorubicina, l'epirubicina, el melfalan, la daunorubicina, la capecitabina, la prednisona, la ciproterona i el megestrol), iguals per a 5 citostàtics (la ciclofosfamida, la ifosfamida, l'erlotinib, el tamoxifè i el clorambucil) i més alts per a 2 compostos (la goserelina i l'irinotecà). Aquestes diferències es poden atribuir a que l'aigua de riu és una matriu més neta, i s'obtenen MDLs en general més baixos. Comparat amb altres mètodes per a aigües superficials, s'han publicat límits més baixos per a la ciclofosfamida i la

ifosfamida (0,1-1 ng/L) extraient més volum de mostra (400 mL) (Martínez Bueno et al., 2010) o per l'irinotecà (0,9 ng/L) amb l'extracció de 250 mL (Martín et al., 2011). Però també altres autors han determinat límits més alts per a la ciclofosfamida i la ifosfamida (4,2 i 10 ng/L) amb l'extracció d'1 L d'aigua subterrània (Sacher et al., 2001) o per la doxorubicina i l'epirubicina (5,3 i 3,5 ng/L respectivament) (Martín et al., 2011). En general, els límits de detecció metodològic són del mateix ordre (ng/L) que els determinats en els altres mètodes descrits a la bibliografia.

En aquest estudi es va detectar la presència de 7 citostàtics al llarg de la conca del riu Besòs. Entre ells, destaca la presència de l'àcid micofenòlic, que va ser el citostàtic detectat a una concentració més elevada. A la Taula 3.3 s'han recollit els citostàtics que s'espera trobar al medi i aquells que posteriorment s'han pogut detectar segons els diferents estudis que s'han realitzat en aquesta tesi.

Taula 3.3. Comparació dels citostàtics que s'espera detectar al medi i els compostos posteriorment detectats, segons el treball desenvolupat en aquesta tesi.

	PECs[1]	*Presència al medi*	**Degradacions**[2]	*Detecció*	**Besòs**[3]
Cat.	MPA, HYD, CAP, BIC, IMA, PRE	*si*	MPA, IFO, CYC, CAP, CIT, ETO	*si*	MPA, IFO, CYC, TAM, ERL, MEG, CHL
Esp.	MPA, HYD, BIC, CAP, PRE, LEF	*no*	DAU, IRI, CHL, MEL, MEG, PRE, DOX, VINC, VINB, GEM	*no*	CIP,PRE, MEL, FLU, GEM, CAP, DOX, DAU, EPI, IRI, LEU, GOS

[1]Article científic I i II;[2] Article científic III; [3] Article científic IV;Cat.: Catalunya; Esp.: Espanya.

En primer lloc es recullen els compostos prioritzats per Catalunya i Espanya, segons els PECs. En segon lloc s'inclouen aquells que s'espera que arribin al medi segons els estudis de degradació, tenint en compte el procés d'hidròlisi i la biodegradació (2h), que és el tractament més habitual a les EDAR properes als punts de mostreig del riu Besòs. Finalment s'inclouen els compostos detectats i no detectats en la monitorització del Besòs.

Per a l'àcid micofenòlic, la seva priorització mitjançant els PECs va ser determinant per incloure aquest compost en el mètode d'anàlisi, i això ha permès confirmar per primera vegada la seva presència al medi. La ifosfamida i la ciclofosfamida van mostrar una gran resistència als tractaments d'eliminació, i conseqüentment es va poder detectar la seva presència en aigües de riu. Per contra, el clorambucil i el megestrol, que mostren una ràpida eliminació en contacte amb l'aigua, es van detectar a concentracions de 1,7-6 ng/L . Aquest fet pot ser degut a l'alliberament d'altes concentracions d'aquests compostos que, malgrat tinguin percentatges

d'eliminació elevats, la seva entrada al medi és superior al percentatge d'eliminació. També cal tenir en compte que les mostres d'aigua del riu són puntuals i que hi pot haver diferències en el temps, tal i com s'ha constatat en les dues campanyes realitzades al Besòs.

Un cop determinada la presència de citostàtics en aigües superficials a Catalunya, es va plantejar la possibilitat que aquests compostos poguessin arribar a les aigües de consum. Per aquest motiu es va dur a terme un mostreig preliminar al riu Llobregat, a l'entrada de la planta potabilitzadora d'Aigües de Barcelona a Sant Joan Despí. En aquestes mostres es van analitzar els mateixos citostàtics que al Besòs, però només es va detectar l'àcid micofenòlic, confirmant una vegada més la seva importància. La Figura 3.4 mostra el cromatograma d'una mostra positiva, on es mostren els ions corresponents a aquest compost i la massa exacta del patró i de la mostra, que permet fer-ne la confirmació.

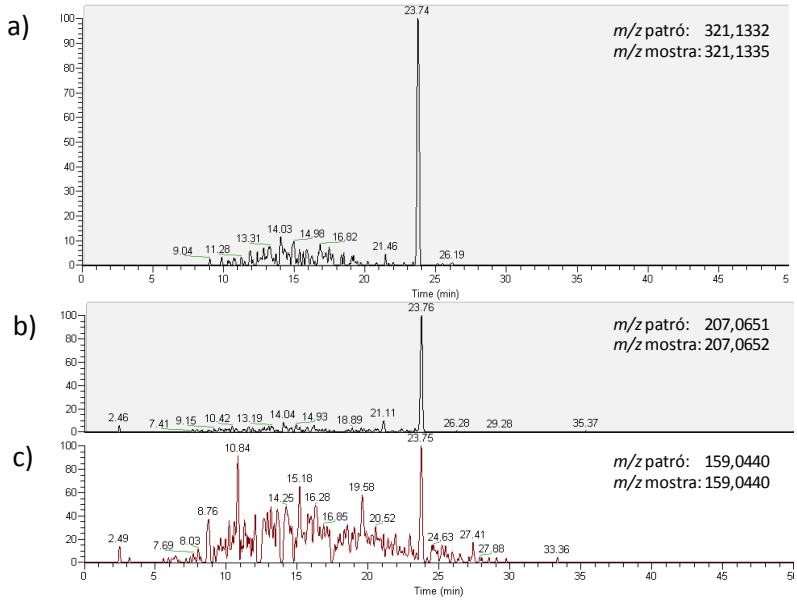

Figura 3.4. Cromatograma d'una mostra positiva per l'àcid micofenòlic;
a) ió molecular 321; b) ió fragment 207; c) ió fragment 159.

Per això, l'estudi en la planta es va centrar en aquest fàrmac, tal i com es descriu a l'article científic V, "*Do cytostatic drugs reach drinking water? The case of mycophenolic acid"*. Els nivells detectats a l'entrada de l'ETAP, entre 18 i 56 ng/L, són més baixos que al riu Besòs, on es van detectar fins a 656 ng/L, degut al major cabal del Llobregat. L'anàlisi de l'aigua després de cada un dels processos de la planta va determinar que l'àcid micofenòlic s'elimina completament després de l'addició de diòxid de clor, a l'inici del tractament.

Per aquest motiu, es va estudiar la seva degradació amb ClO_2, tal com es fa el pretractament de l'aigua crua en aquesta ETAP. Aquesta informació no està inclosa en els articles científics

publicats en aquesta tesi però s'ha considerat oportú incloure breument aquest estudi, ja que permet justificar que l'àcid micofenòlic no s'hagi detectat al llarg de la planta de tractament ni en l'aigua de distribució. En primer lloc es va fer el seguiment de la cinètica de degradació per MRM i es va observar que l'eliminació va ser completa en 30 segons (temps mínim necessari per agafar una alíquota i aturar la reacció). En el seguiment de la reacció de degradació en *full-scan* només es van observar 4 pics molt minoritaris que no es van poder identificar (Figura 3.5). Només es va identificar el pic del temps 12,0 min com a la cloració de l'àcid micofenòlic, corresponent a les masses $[M+35]^+$ i $[M+37]^+$ en proporció 3:1.

Així i tot, es va confirmar la ràpida degradació de l'àcid micofenòlic amb ClO_2. Aquest estudi va permetre descriure el comportament d'aquest compost al llarg del procés de potabilització i garantir la seva absència en l'aigua de distribució de l'ETAP de Sant Joan Despí.

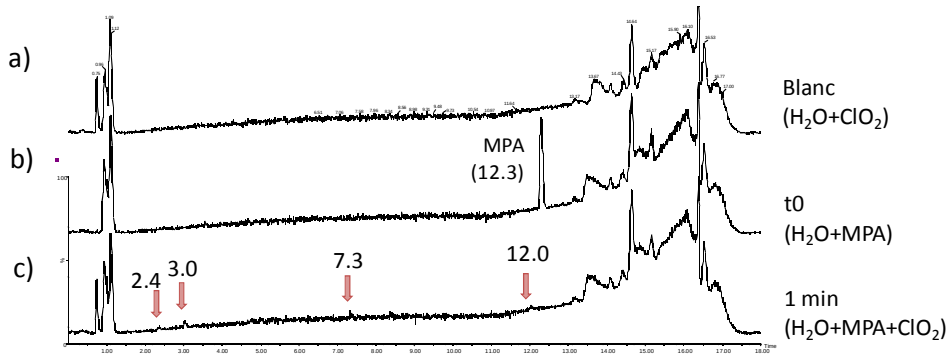

Figura 3.5. Productes de degradació de l'àcid micofenòlic (MPA) amb ClO_2: cromatograma en *full-scan*. a) blanc; b) temps zero; c) després d'un minut de reacció.

El segon fàrmac prioritzat, després de l'àcid micofenòlic, va ser la hidroxicarbamida. Ambdós compostos van tenir un PEC de 77 i 32 ng/L respectivament, un ordre de magnitud per sobre del següent citostàtic prioritzat (la capecitabina amb un PEC de 7,7 ng/L). Però degut a l'alta polaritat de la hidroxicarbamida i al seu baix pes molecular no es va poder afegir al mètode cromatogràfic amb la resta de citostàtics. No obstant, igual que la presència de l'àcid micofenòlic es va poder confirmar, es podria esperar que la hidroxicarbamida també fos present en les aigües superficials.

Per aquest motiu es va voler desenvolupar un mètode en base als resultats dels citostàtics de PEC més elevat (>1 ng/L a Catalunya): l'àcid micofenòlic, la hidroxicarbamida, la capecitabina, la bicalutamida, l'imatinib i la prednisona. Aquest treball no s'ha pogut publicar degut a les nombroses dificultats que s'han trobat, però s'ha considerat oportú afegir aquestes dades, ja que es té molt poca informació ambiental d'aquest fàrmac.

El grup de citostàtics seleccionats té una massa nominal que varia entre 320 i 493, excepte la hidroxicarbamida que és 76, un pKa entre 8,23 i 12,58, amb excepció de l'àcid micofenòlic (pKa=3,57) i tenen unes estructures molt diferents. Aquestes propietats dificulten el seu anàlisi simultani. En la separació cromatogràfica amb una columna Acquity BEH C18 (2,1 x 100 mm, 1,7 µm), la hidroxicarbamida no va quedar retinguda i va eluir a 0,8 min. Al tenir una massa molt petita i unes transicions poc selectives, no és adequat que surti en el front d'elució, ja que en l'anàlisi de mostres ambientals podria coeluir amb àcids húmics i fúlvics presents en la matriu. Amb una columna XBridge Amide (4,6x150mm, 5µm), més adequada per a compostos molt polars, es va poder retenir la hidroxicarbamida, tot i que la resta de compostos surten en un temps de retenció molt similar (Figura 3.6).

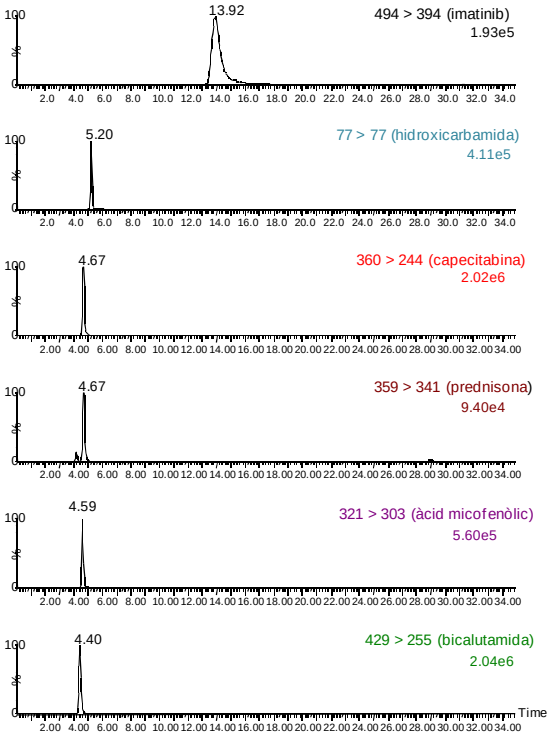

Figura 3.6. Separació cromatogràfica per als sis citostàtics prioritzats; columna XBridge Amide. Solució patró a 0,5 mg/L per UHPLC-MS/MS (Acquity UPLC -TQD, Waters, USA).

Per a l'extracció, es van provar diferents cartutxos d'SPE de diferents modes de retenció. En les condicions que van donar un millor resultat es van utilitzar cartutxos d'intercanvi iònic febleStrata-XL-AW ja que els citostàtics estudiats tenen grups àcids en la seva estructura i poden tenir càrrega negativa (Taula 3.4). Amb aquest mètode es van obtenir recuperacions iguals o superiors al 65% per a tots els citostàtics amb excepció de la hidroxicarbamida, que no es va poder recuperar amb cap dels mètodes provats.

Taula 3.4. Proves d'extracció SPE: condicions i recuperacions (%)

	Cartutx	Càrrega mostra	Elució I	Elució II	Recuperació %		
						Elució I	Elució II
B	Strata-XL-AW	100 mL (pH 2)	6 mL MeOH	6 mL MeOH:NH_4OH (95:5)	HYD	n.d.	n.d.
					MPA	72±4	n.d.
					PRE	101±8	n.d.
					CAP	95±3	n.d.
					IMA	113±16	n.d.
					BIC	65±2	n.d.

HYD: hidroxicarbamida, MPA: àcid micofenòlic, PRE: prednisona, CAP: capecitabina, IMA: imatinib, BIC: bicalutamida.
nd: no detectat

Després de nombroses proves, es va observar que la hidroxicarbamida s'evaporava en l'etapa de concentració dels extractes. Aquest compost només es manté en la solució amb MeOH:HCOOH (95:5), treballant a 20ºC i amb un corrent de nitrogen suau, mentre que en altres condicions s'evapora entre el 43 i el 100%. Malauradament, les condicions que estabilitzen la hidroxicarbamida no són adequades per a la seva extracció, fent que fins al moment no s'hagi pogut desenvolupar un mètode adequat per a l'anàlisi dels citostàtics prioritzatsamb el càlcul dels PECS.Caldrà doncs seguir aquesta línia d'investigació provant altres mètodes alternatius que no requereixin preconcentració de les mostres o evaporació com podria ser l'extracció en línia o l'injecció directa de grans volums de mostra. Malauradament aquests mètodes no han estat disponibles i no s'ha pogut completar l'estudi.

4. DESENVOLUPAMENT I OPTIMITZACIÓ

DEMOSTREJADORS PASSIUS

4.1. Introducció

Per tal de determinar la presència de citostàtics al medi aquàtic calen sistemes de mostreig que assegurin la captació dels analits de manera representativa. En els diferents treballs publicats que descriuen la presència d'aquests fàrmacs en aigües residuals i superficials, les mostres es recullen de manera puntual (Llewellyn et al., 2011) o amb instruments que permeten la captació d'aigua puntualment durant 24 h per analitzar una part d'aquesta mostra composta (Buerge et al., 2006). Els tractaments de quimioteràpia, però, s'administren en cicles d'un o més dies seguit d'un període de descans per permetre que es recuperin les cèl·lules sanes (Booker et al., 2014) i per tant, no es pot saber amb certesa quan és més adequat fer la presa de mostra. Per això alguns treballs on s'analitzen aigües procedents dels efluents d'hospital planifiquen l'hora del mostreig en funció dels tractaments de quimioteràpia programats (Gómez-Canela et al., 2014). No obstant, si es té també en compte el consum domèstic l'alliberament de citostàtics al medi procedent conjuntament dels hospitals i de les aigües residuals domèstiques es preveu que sigui continu. Un mètode alternatiu al mostreig puntual i que permetria obtenir valors més representatius és l'ús de mostrejadors passius. Aquests sistemes s'han utilitzat per al mostreig de diversos compostos orgànics en diferents matrius aquoses (Seethapathy et al., 2008; Vrana et al., 2005).

Els mostrejadors passius es defineixen com un sistema de mostreig que permet la lliure circulació d'analits del medi fins a una fase receptora degut a la diferència de potencial químic (Vrana et al., 2005), tal com es mostra a la Figura 4.1. La major part dels mostrejadors treballen en la zona de règim cinètic, on s'assumeix que la transferència de massa és proporcional a la diferència de concentració entre el medi i la fase a l'interior del mostrejador.

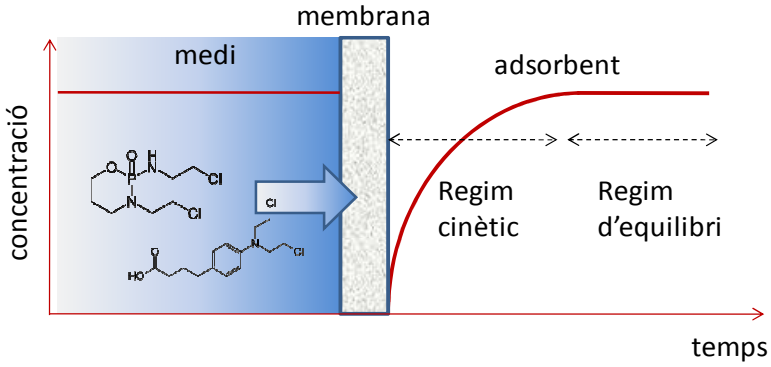

Figura 4.1. Esquema de funcionament d'un mostrejador passiu

L'equació general que descriu la captació dels analits en un mostrejador passiu és:

$$M\ (t) = Cw \cdot Rs \cdot t \quad (4.1)$$

on, M (t) és la massa acumulada de l'analit durant el temps de mostreig; Cw és la concentració al medi; Rs és la velocitat de mostreig (*sampling rate*) i t és el temps de mostreig. La velocitat de mostreig està relacionada amb el coeficient de difusió dels analits a través de la membrana que separa el medi de la fase receptora (De), l'àrea de contacte entre ambdós (A) i el gruix de la membrana (Δg), segons l'equació 4.2:

$$Rs = \frac{De \cdot A}{\Delta g} \quad (4.2)$$

Per poder utilitzar els mostrejadors passius per a la presa de mostra, cal primer fer-ne el calibratge i determinar el De o el Rs, que són característics per a cada analit en un determinat tipus de mostrejador.

Els avantatges que proporcionen els mostrejadors passius respecte el mostreig puntual són diversos. Principalment, permeten mostrejar durant llargs períodes de temps i proporcionen dades integrades que seran més representatives. Una llarga exposició també implicarà mostrejar volums més grans d'aigua i permetrà obtenir límits de detecció més baixos (Jones et al., 2015). L'extracció *in situ* dels analits en aquests dispositius també minimitza els possibles problemes de transport de grans volums de mostra i ajuda a disminuir el risc de degradació durant el transport i l'emmagatzematge (Namieśnik et al., 2005). Existeixen almenys una trentena de tipus de mostrejadors passius, amb diferents configuracions i aplicacions, els més importants dels quals es descriuen a continuació.

SPMD (semi-permeable membrane device): els dispositius de membrana semipermeable estan formats per una membrana de polietilè de baixa densitat amb cavitats d'uns 10 Å que permeten el pas dels compostos dissolts en l'aigua (Figura 4.2a). A l'interior de la membrana hi ha trioleïna, un lípid d'alt pes molecular, que simula la bioconcentració en el greix dels organismes aquàtics i reté els analits no iònics amb K_{ow}>3 (Lu et al., 2002; Vrana et al., 2005). Aquests mostrejadors s'han utilitzat per determinar la presència d'hidrocarburs aromàtics policíclics (PAH), pesticides o retardants de flama en aigües superficials (Chang et al., 2014; Goodbred et al., 2009; Karacik et al., 2013).

POCIS (*polar organic chemical integrative sampler*): aquests mostrejadors tenen una fase receptora sòlida, normalment un adsorbent d'extracció en fase sòlida que pot variar en funció de l'analit, situat entre dues membranes que limiten la difusió de compostos no desitjats (Vrana

et al., 2005) i se subjecta amb dos anells metàl·lics, que s'introdueixen en una estructura amb reixa metàl·lica (Figura 4.2b). Aquest mostrejador va ser desenvolupat per Alvarez et al. (2004) per determinar contaminants hidrofílics amb K_{ow}<4, com diversos pesticides i fàrmacs. Posteriorment s'han utilitzat per monitoritzar aquestes famílies de contaminants en rius (Alvarez et al., 2004; Bayen et al., 2014; Poulier et al., 2014) i aigua de mar (Martínez Bueno et al., 2016).

Chemcatcher: està format per una fase receptora sòlida, normalment discs Empore®, subjecte a una estructura de tefló (Figura 4.2c).A diferència dels POCIS, en el *Chemcatcher* pot no posar-se la membrana que limita la difusió, fet que permet temps de mostreig menors (dies) (Vermeirssen et al., 2013). Aquests mostrejadors també estan dissenyats per mostrejar compostos orgànics polars com pesticides, fàrmacs o metabòlits entre d'altres, en aigües superficials (Vermeirssen et al., 2013) i en efluents de depuradora (Petrie et al., 2016).

Dosímetre ceràmic: aquests mostrejadors estan formats per un cilindre porós ceràmic que actua de capa difusora i està reblert d'una fase receptora sòlida, que pot ser el mateix tipus d'adsorbent utilitzat en l'extracció en fase sòlida (Figura 4.2d) (Vrana et al., 2005). Aquests mostrejadors van ser desenvolupats per Martin et al. (1999) amb l'objectiu de determinar PAH i altres compostos volàtils en aigües subterrànies (Bopp et al., 2005; Martin et al., 2003).

DGT (diffusive gradients in thin films): els DGT estan formats per un filtre i una capa difusora en gel que separen la fase receptora del medi de mostreig, subjectes per una estructura de plàstic inert (Figura 4.2e). El pas dels analits està regit per la difusió a través del gel i per tant, la captació no es veu afectada per l'agitació del medi, al contrari que els POCIS. Els DGT van ser dissenyats per al mostreig de metalls (Davison and Zhang, 1994) però recentment s'ha estudiat la seva aplicació per a l'anàlisi de compostos orgànics, com els antibiòtics (Chen et al., 2012).

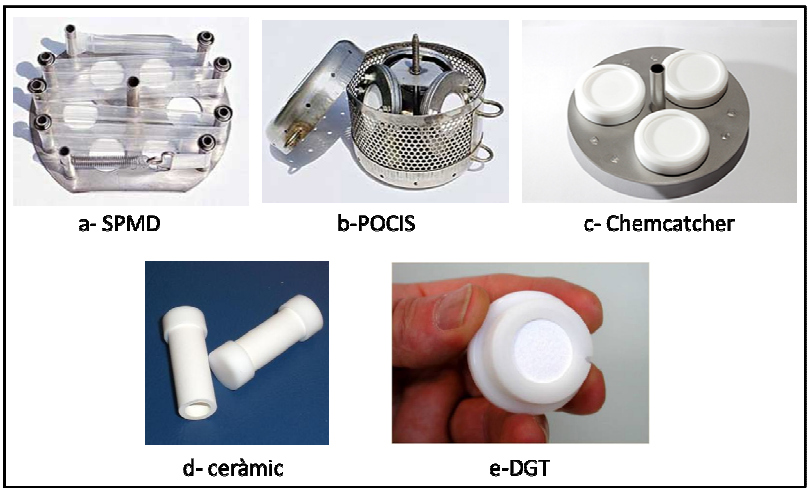

Figura 4.2. Mostrejadors passius utilitzats per a compostos orgànics en aigua

L'ús de mostrejadors passius per a l'estudi de fàrmacs citostàtics és molt limitat. Només Petrie et al. (2016) inclouen 4 citostàtics (azatioprina, metotrexat, ifosfamida i tamoxifè) entre els 88 compostos estudiats amb el mostrejador Chemcatcher. El calibratge d'aquest dispositiu es va fer *in situ*, però la presència de citostàtics en l'aigua analitzada va estar per sota els límits de quantificació del mètode (tant en el mostrejador com a les mostres puntuals). Per tant, no es va poder fer el calibratge per aquests fàrmacs. A part de l'esmentat treball, no s'han trobat altres articles on s'utilitzin els mostrejadors passius en l'anàlisi de citostàtics.

Com totes les tècniques, tant el mostreig actiu com el passiu presenten avantatges i inconvenients que cal tenir en compte i que s'han recollit a la Taula 4.1. El mostreig actiu és el mètode més comú per fer la presa de mostra, ja que és el més fàcil de dur a terme perquè no requereix d'una preparació prèvia. Les concentracions que s'obtenen corresponen al moment que s'ha pres la mostra i poden no ser representatives, com s'ha vist reflectit en les diferents concentracions detectades al riu Besòs. No obstant, si es fan mostrejos periòdics es poden observar les variacions de concentració que tenen lloc en el temps. D'altra banda, els mostrejadors passius requereixen un calibratge previ al laboratori abans de ser utilitzats. Després, es col·loquen en el punt de mostreig durant un període més llarg i les concentracions detectades representen la concentració mitjana durant aquest temps. Amb aquests mostrejadors l'extracció té lloc en el mateix medi i per tant, un cop retirats de l'aigua es minimitza la degradació dels analits i es facilita el seu transport, ja que es tracta de petits dispositius que no fan més d'uns pocs centímetres de llargada o diàmetre. En canvi, per al mostreig actiu cal transportar grans volums de mostra que caldrà conservar en fred al laboratori fins a la seva extracció. Això requereix un gran espai que no sempre està disponible. A més, en el cas dels citostàtics cal organitzar l'extracció amb el mínim temps possible (≤24 h), ja que molts d'ells es degraden ràpidament com s'ha pogut confirmar amb els estudis de degradació de l'article científic III. Un cop al laboratori, les mostres puntuals se solen filtrar abans de ser preconcentrades en cartutxos d'SPE, que és el mètode més habitual per l'anàlisi d'aigües. L'extracció per SPE requereix que la càrrega de la mostra i l'elució amb dissolvents orgànics es dugui a terme a un ritme adequat (1 mL/min) per donar temps a retenir els analits i recuperar-los completament.Entre aquests dos passos cal deixar secar el cartutx durant 30-60 min, fet que allarga el procés d'extracció. En aquest cas, el nombre de mostres que es poden extreure simultàniament està limitat a les posicions del col·lector.En canvi per als mostrejadors passius, se sol fer l'extracció mitjançant ultrasons durant uns 20 min, fet que facilita molt l'extracció de les mostres i permet extreure'n un gran nombre simultàniament.

Taula 4.1. Avantatges i inconvenients del mostreig actiu i el mostreig passiu.

Mostreig actiu	Mostreig passiu
Avantatges	
• mostra les variacions de concentració en el temps • sistema de presa de mostra fàcil i ben consolidat	• concentracions integrades en el temps • fàcil extracció de la mostra • minimitza la degradació dels analits durant el transport i l'emmagatzematge
Inconvenients	
• cal filtrar les mostres per no obturar els cartutxos d'extracció • transport de grans volums d'aigua • mètodes d'extracció més complexes	• requereix un calibratge previ al laboratori • possible obturació • possible efecte de les condicions del medi • robatori/vandalisme/pèrdua

Un dels majors inconvenients que poden presentar els mostrejadors passius és que el seu funcionament es pot veure afectat per les condicions del medi. En primer lloc, si l'aigua a mostrejar conté molta matèria en suspensió o si els mostrejadors es col·loquen durant un llarg període de temps, aquesta matèria o les algues que puguin créixer en la seva superfície poden fer disminuir l'adsorció dels analits. L'agitació de les aigües també pot produir variacions en la captació dels analits, ja que en aigües amb poc corrent l'arribada dels contaminants al mostrejador serà més lenta que en aigües agitades. Altres condicions de l'aigua, com el pH o la força iònica també poden afectar l'eficàcia dels mostrejadors. Per aquest motiu és recomanable fer les proves al laboratori per avaluar la captació dels analits en diferents situacions. En el cas del mostreig actiu, les condicions del medi també poden afectar l'extracció, però és més fàcil de corregir ajustant el pH de les mostres al laboratori.

Quan s'utilitzen els mostrejadors passius per l'anàlisi d'aigües superficials es col·loquen en el punt de mostreig i es deixen durant un llarg període de temps, entre una setmana i mesos (Bopp et al., 2005), sense vigilància. És recomanable triar zones discretes o amagades per evitar que puguin ser retirats sense permís o que pateixin actes de vandalisme. En canvi, si s'utilitzen per al mostreig en EDARs o recintes tancats on només hi te accés el personal autoritzat, la recuperació dels mostrejadors és més segura.

4.2. Resultats

Degut als pocs treballs que s'han dedicat a l'aplicació de mostrejadors passius en l'anàlisi de citostàtics, en aquesta tesi s'ha volgut posar a punt dos tipus de mostrejadors diferents amb l'objectiu de poder captar els compostos citostàtics en aigua residual i superficial.

En l'article científic VI, "Development of a macroporous ceramic passive sampler for the monitoring of cytostatic drugs in water"Chemosphere 2017 (acceptat) s'ha desenvolupat i caracteritzat un mostrejador passiu ceràmic per analitzar citostàtics en aigües. A nivell de laboratori s'ha fet el calibratge i s'ha determinat el coeficient de difusió, que permet relacionar la massa adsorbida en el mostrejador amb la concentració del medi exterior. Finalment, aquests mostrejadors s'han col·locat a l'entrada i a la sortida d'una EDAR i els resultats obtinguts s'han comparat amb les mostres puntuals preses en el mateix període i extretes per SPE.

L'article científic VII, "*Laboratory calibration of o-DGT for analysis of cytostatic drugs"* (en preparació)descriu la optimització del mostrejador passiu DGT per als citostàtics més persistents. En aquest estudi s'han seleccionat els materials més adients per al muntatge d'aquests mostrejadors i s'han determinant els coeficients de difusió. També s'ha estudiat si les condicions del medi (pH i força iònica) poden afectar l'adsorció d'aquests compostos en el mostrejador.

4.2.1. Article científic VI:

*Design and characterization of a new macroporous ceramic passive sampler for the analysis of water contaminants.*Helena Franquet-Griell, Jorge Silva, Víctor Pueyo, Victor M. Orera, Silvia Lacorte. *Chemosphere*182 (2017) 681-690.

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Development of a macroporous ceramic passive sampler for the monitoring of cytostatic drugs in water

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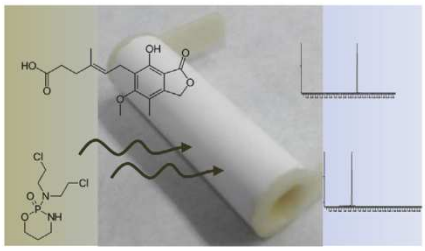
HIGHLIGHTS

- A Macroporous Ceramic Passive Sampler has been designed for monitoring cytostatic compounds in water.
- Water stability affects uptake, and sampling rate was calculated only for 5 out of 16 cytostatics.
- Similar performance was observed between Passive Sampler and grab sampling in WWTP effluents.
- Cytostatics were detected in WWTP effluents wastewaters at low levels.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this study was to develop and calibrate a macroporous ceramic passive sampler (MCPS) for the monitoring of anticancer drugs in wastewater. This system was designed by the Spanish Research Council (CSIC) and consists in a porous ceramic tube to allow a high diffusion of contaminants. The MCPS has been calibrated for 16 cytostatic drugs over time periods up to 9 d in spiked water under controlled laboratory conditions. Optimal uptake was accomplished for 7 compounds, namely ifosfamide, cyclophosphamide, capecitabine, prednisone, megestrol, cyproterone and mycophenolic acid, whereas cytarabine was not adsorbed in the receiving phase and the rest were hydrolyzed over the deployment period. The sampling rate for these 7 compounds was between 0.825 and 3.350 mL day^{-1} and the diffusion coefficients varied from 1.01E-07 to 4.12E-07 cm^2 s^{-1}. To prove the applicability of the MCPSs, samplers (n = 3) were deployed in influent and effluent waters of a WWTP for a period of 6 d and results were compared to grab sampling and extraction with Solid Phase Extraction (SPE). In influent waters, MCPS were clogged due to the high amount of suspended solids in these waters. In effluents, MCPS detected cyclophosphamide and mycophenolic acid at concentrations of 19 ± 3 and 136 ± 28 ng L^{-1} with a good agreement with the levels obtained by grab sampling. The study discusses the use and performance of the MCPS for the monitoring of stable cytostatic compounds in a complex matrix such as wastewater.

1. Introduction

Passive sampling techniques have emerged as an efficient and relatively inexpensive way of sample collection for the characterization of water pollutants. The main advantages of passive

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samplers are high capacity, time integrated response, versatility for the monitoring of different chemical families or types of water, autonomy as no external source of energy is required and easy deployment (Jones et al., 2015; Vrana et al., 2005). Many types of sampling techniques have been developed each providing specific advantages: dialysis membranes (Truitt and Weber, 1981), Polar Organic Chemical Integrative Sampler (POCIS) (Alvarez et al., 2004; Mazzella et al., 2010), Diffusive Gradient in Thin Films (DGT) (Zhang and Davison, 1995) and ceramic dosimeters (Martin et al., 2003). The uptake of the contaminants is usually achieved by means of sorption/binding onto a media which has specific retention properties. Because passive samplers can detect contaminants at ng-μg L^{-1}, they have been deployed for the monitoring of flame retardants in surface waters (Cristale et al., 2013), polycyclic aromatic hydrocarbons (PAHs) in groundwater (Bopp et al., 2005), pharmaceuticals in seawater (Martínez Bueno et al., 2016) and antibiotics in wastewaters (Chen et al., 2013), among other applications.

One of the main constrains in the deployment of passive samplers in environmental waters is the exposure time and the sorption kinetics without clogging the system. The diffusion coefficient (De) and the sampling rate (Rs), are key factors for determining the uptake efficiency and performance of a passive sampler. High De values means high free flow of contaminants to the receiving phase and, eventually, high Rs values if concentration efficiency at the receiving phase is high. The properties that govern the uptake of chemicals by a passive sampler are usually the polarity, water solubility and lipophilicity of target pollutants, and its performance depends on the type of water to be monitored and the sampler receiving phase.

This study is focused on diffusion-controlled ceramic dosimeters, first developed by Martin et al. (2001), which are characterized by an inert and rigid porous cylindrical ceramic membrane which operates as a diffusion barrier and a polymeric sorbent placed in the inner part capable to retain contaminants. Particularly, ceramic dosimeters developed by Martin et al. have a configuration of 50 mm of length, 1.5 mm of wall thickness and 10 mm outer diameter and they have been used to monitor PAHs. A similar structure of 50 mm, 2 mm of wall thickness and outer diameter of 10 mm with a porosity of 30% and lined with 5 μm layer of titanium dioxide (TiO_2) was used to determine dioxin contaminated water (Addeck et al., 2011). Those ceramic dosimeters have a pore diameter of 5 nm which makes diffusion slow and if deployed for wastewaters, clogging may occur.

In the frame of the present study, we have developed a Macroporous Ceramic Passive Sampler (MCPS) (Patent: P201530882, https://www.google.com/patents/WO2016207461A1?cl=es) whose main difference to previous ceramic dosimeters is its high connected porosity values and pore morphology consisting of large pore cavities connected by smaller pores. The idea was to allow for a high water volume inside the ceramic support. In addition, we have used a polymeric sorbent to enrich both polar and nonpolar cytostatic compounds, making the system versatile. As other ceramic dosimeters, the device is flow-independent and permits the sampling of the aqueous environment as diffusion constant through the wall will not be affected by the water flow. Finally, thanks to the high porosity, average weighed concentrations of cytostatics can be obtained in a complex matrix such as wastewater, provided the device is properly calibrated.

This study is focused on cytostatic compounds; class L for antineoplastic and immunomodulating agents according to the ATC classification (Drug Bank Database, 2013). They consist in a broad group of chemotherapy compounds with different chemical structure and modes of action used to treat different types of cancers. The environmental concern is related to the chronic effects that cytostatics may produce towards aquatic organisms as they bind directly to the genetic material or affect cellular protein synthesis affecting the growth and proliferation of both normal and cancer cells (Drug Bank Database, 2013). On a country-wide basis, consumption of the most commonly used anticancer drugs (~10–20 drugs) is in the order of tons/yr (Besse et al., 2012; Booker et al., 2014) and given their genotoxicity, concern has arisen on their emission and discharge to receiving waters (Buerge et al., 2006; Martín et al., 2011). In this sense, Wastewater Treatment Plants (WWTPs) have been identified as one of the main sources of pollution of cytostatic drugs, among other pharmaceuticals, to surface waters (Kosjek and Heath, 2011). Several compounds have been detected in wastewaters such as cyclophosphamide, tamoxifen and megestrol at concentrations in the range of 4.4–220 ng L^{-1} (Ferrando-Climent et al., 2014; Gómez-Canela et al., 2014; Negreira et al., 2014a). In these studies, grab or 24 h composite sampling are generally used and provide information on the compounds and levels detected in a given time. Alternatively, passive sampling provides time averaged concentrations on compounds present in a water body and it is especially suited for the control and time-integrated surveillance of contaminants. For cytostatic compounds, Fabrizi et al. (2012) used a printed glass slide as a passive sampler for surface monitoring. However, to our knowledge, passive sampling techniques have not yet been applied for the monitoring of cytostatic compounds in wastewaters.

The objective of the present study was to develop and assess the analytical capability of a home-made passive sampler (MCPS) for the analysis of cytostatic compounds in wastewater. First, the ceramic membrane was fabricated using alumina and the porogen agent using the slip casting method to obtain the desired high porosity. Then the polymeric sorbent was introduced inside the cylinder and preconcentration efficiency was evaluated. Then, it was calibrated and tested in terms of uptake rate considering the stability of 16 drugs in water. Based on these calibration studies, the Rs and De were determined for each compound to allow for the calculation of water concentrations (Cw) in the sampled medium. Finally, the system was deployed in WWTP influents and effluents, and simultaneously, grab samples from the same sampling points were collected during 4 days and analyzed by Solid Phase Extraction (SPE) to validate the method.

2. Experimental section

2.1. Chemicals and reagents

Sixteen pure analytical standards of cytostatic drugs (98–99%) purity were acquired from Sigma-Aldrich (St. Louis, USA) and from Toronto Research Chemicals (TRC, Ontario, Canada), namely cytarabine (CYT), ifosfamide (IFO), cyclophosphamide (CYC), capecitabine (CAP), prednisone (PRE), melphalan (MEL), doxorubicin (DOX), mycophenolic acid (MPA), tamoxifen (TAM), chlorambucil (CHL), cyproterone (CYP), megestrol acetate (MEG), leuprolide (LEU), irinotecan (IRI), vinblastine (VINB) and vincristine (VINC). Their molecular formula and relevant physicochemical properties are shown in the supplementary information (SI1). Cyclophosphamide-d_4 (CYC-d_4, Santa Cruz Biotechnology, USA) and mycophenolic acid-d_3 (MPA-d_3, Sigma-Aldrich, St. Louis, USA) were used as internal standards (IS). Stock solutions were prepared at 1000 μg mL^{-1} and working solutions at 10 μg mL^{-1} in methanol (MeOH). MeOH, acetonitrile (ACN) (SuperSolv grade) and HPLC water (LiChrosolv grade) were supplied by Merck (Darmstadt, Germany). Formic acid (HCOOH) and ammonium acetate (NH_4OAc) were supplied by Sigma-Aldrich (St. Louis, MO USA). Milli-Q water was produced from an Integral Water Purification System from Millipore (Billerica, MA, USA). Oasis HLB cartridges (200 mg) were purchased from Waters (Mildford, MA, USA) and were used for the solid phase

extraction of wastewaters. Sepra™ ZT (30 μm, 85 Å, Phenomenex, California, USA) bought as bulk material was the sorbent used as retaining phase for the MCPS.

2.2. Development of the MCPS

2.2.1. Fabrication of the MCPS

The MCPS developed [Patent P201530882] consists of a cylindrical ceramic shell of 45 mm length x 13 mm outer diameter and a wall thickness of 1.5 mm. The ceramic membrane of the MCPS holds a water membrane that acts as a diffusion barrier and the sorbent material in the inner part. A hierarchical pore microstructure was developed to maximize the contained water volume in the membrane assuring a high connectivity between pores.

These ceramic membranes were fabricated by slip casting method. Stable and homogeneous ceramic suspensions were prepared using aluminum oxide powders (Ceralox APA-0.5, 8.1 m^2/g, Sasol North America, Inc.) and water following currently developed procedures developed in our laboratory for ceramic pastes and colloids preparation (Laguna-Bercero et al., 2016). In short, ceramic suspensions were stabilized using Duramax D-3005 as dispersant (1% in solid weight) and cornstarch as pore former. Dispersant and water were first mixed by magnetic stirring then alumina powders and pore formation agents were poured into the mix and stirred. The resulting paste was treated with ultrasounds and magnetic stirred again for 30 min. The stable ceramic paste was poured in a plaster mold with the desired cylindrical geometry and remained between 10 and 20 min. The time that the suspension remains in the mold determines the thickness of the raw piece. After casting, the membranes were pre-sintered in air at 950 °C for 4 h and rectified and polished to get homogeneous, defect free, standard dimensions. The membranes were sintered at 1300 °C for 3 h dwelling time and heating and cooling rates of 4 °C min^{-1}. The MCPS is characterized by a high total porosity ($\varepsilon = 0.50$) as determined by gravimetric density measurements. Open and connected porosity and small pore size was measured by means of a Hg porosimeter (Poremaster, Quantachrome; maximum pressure 30,000 psia). The microstructure of the porous ceramic was studied in polished transverse membrane cross-sections using a JEOL 6301F scanning electron microscope (SEM). The SEM specimens were embedded in epoxy resin and polished in cross-sections according to a polishing procedure previously published (Serrano-Zabaleta et al., 2014). Fig. 1a shows the SEM image of the ceramic membrane transverse section. Image 1b shows the cavities with some of the holes which connect the cavities. Image analysis technique (n = 20 SEM images) was used in order to determine the size of cavities. Small pores cannot be seen in SEM images. We have to rely on porosimetry measurements to detect these small pores but except for well-defined cylindrical pores, Hg-porosimetry gives us only the size of the struts or windows connecting pores, not the actual pore size. In order to get a close picture to pore morphology, information provided by both techniques has to be combined. We conclude that the porous ceramic microstructure consists of a homogeneous and continuous area volume of spheroid cavities (10–15 μm) connected by pores of less than 200 nm diameters. Finally, image 1c shows the ceramic cylinder used as passive sampler.

2.2.2. Optimization of sorbent and extraction conditions

We selected Sepra™ ZT as a retaining phase in the MCPS to preconcentrate cytostatic drugs. Sepra™ ZT is a reverse phase pyrrolidone modified styrenedivinylbenzene polymer, and its retention mechanisms are hydrophobic, hydrogen bonding and aromatic and offer high selectivity to extract polar target cytostatics drugs from the aqueous samples. Prior to use, the sorbent has to be conditioned. To do so, 200 mg of Sepra™ ZT were first activated using 3 mL of MeOH and 3 mL of Milli-Q water, which were thereafter decanted. Then, the activated Sepra™ ZT was placed in the inside of the cylinder with a spatula and was spread within the cylindrical cavity so that the diffusion layer and sorbent were in immediate contact with one another to correctly sample the water. Then, both ends of the cylinder were closed with conical thermoplastic rubber caps. Before deployment, empty MCPS were rinsed with MeOH and water and were kept immersed in Milli-Q water until use.

For Sepra™ ZT used in the MCPS, recovery tests were first performed. 25 ng of the mixture of the 16 standards and 25 ng of IS were directly spiked onto the 200 mg activated Sepra™ ZT adsorbent. In a preliminary test, 2 different extraction solvent were tested: i) 6 mL MeOH +6 mL MeOH (REC 1) and ii) 6 mL MeOH + 6 mL MeOH (HCOOH 0.1%) (REC 2). For these tests, the extraction solvent was added in the centrifuge tube containing the sorbent and the solution was vortexed for 1 min and then, ultrasonic extracted during 10 min. This cycle of vortex-ultrasonic extraction was repeated three times. Then, the solvent was removed and 6 mL of fresh solvent was added and the extraction was repeated. This process permitted to desorb target analytes from the sorbent. Afterwards, the solvents were combined (6 + 6 mL), the solution was centrifuged (15 min, 4000 rpm) and the supernatant was filtered through a nylon 0.22 μm syringe filter to eliminate any particle from the sorbent which could obstruct the ion source of the MS. The extracts were dried under N_2, and reconstituted with 500 μL of water:ACN (50:50) both with 0.1% HCOOH. Better results were obtained with MeOH without acidification. However, the process was time consuming and then we tested the extraction with 12 mL of MeOH without changing the solvent (REC 3). This condition was further used.

2.2.3. Calibration of MCPS

MCPS were calibrated to calculate the concentration-time slope (k), the Rs and the effective De for each single compound. For laboratory calibration, a beaker with 4 L of bottled water was prepared and spiked with the mixture of standards at concentrations of 20 μg L^{-1}. This concentration was chosen to emulate real conditions for cytostatic compounds present in hospital or WWTP effluents (Giebułtowicz and Nałęcz-Jawecki, 2016; Gómez-Canela et al., 2012). Calibration was performed in bottled water as its pH and mineral content better simulates natural waters, compared to Milli-Q water. However, it has to be noted that in natural waters both the pH and the amount of organic matter can vary seriously affecting the stability and diffusivity of cytostatic drugs to the receiving phase of the MCPS.

To calibrate the MCPS, ten passive samplers were enmeshed in a support made of tulle mesh, placed in the beaker and were left in an orbital shaker for 9 days. During exposure period, the temperature was kept constant at 22-23 °C. Two MCPS were removed every 2, 4, 7 and 9 days. The adsorbent was removed from the passive sampler and was placed in a centrifuge tube, using a little amount of water to clean the inside of the ceramic cylinder. The tubes were centrifuged (15 min, 4000 rpm) and all the water was removed. Then, the suspension was spiked with 25 ng of CYC-d_4 and MPA-d_3. Then 12 mL MeOH were added to the tubes. Samples were shaken for 1 min in the vortex mixer and ultrasonic extraction was performed as depicted before.

Stability studies were also performed for all target compounds as some of these compounds seems to be easily degraded in aqueous media (Franquet-Griell et al., 2017b). To do so, water was spiked at 20 μg L^{-1} and an aliquot of 500 μL was taken at t = 0, 1, 2, 3, 4, 8 and 9 d, spiked with 25 ng of IS and analyzed by direct sample injection. This was done to control that the concentration in

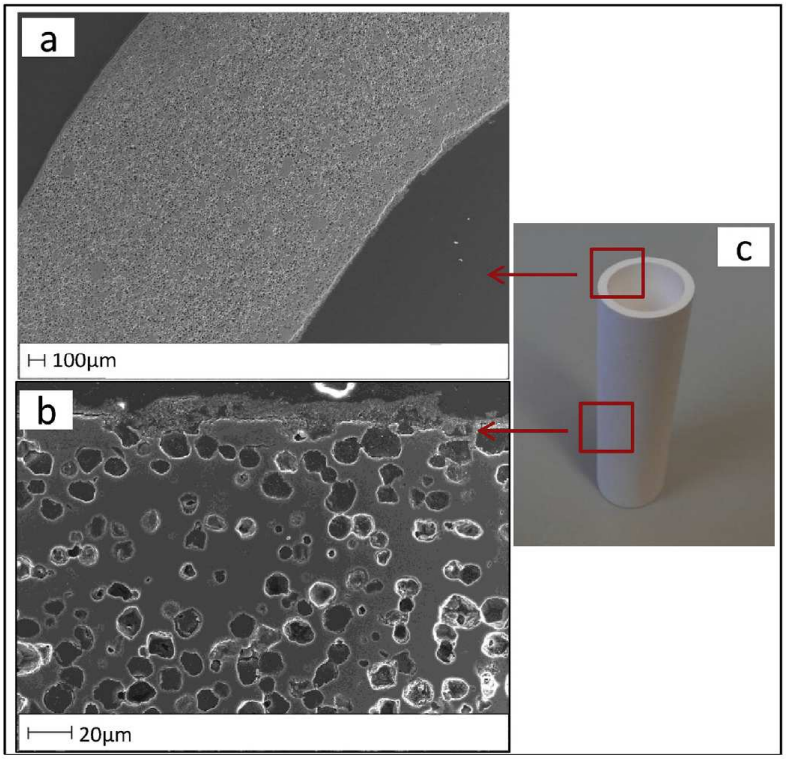

Fig. 1. SEM images showing the microstructure of the ceramic cylinder: a) Transverse tube section at a 100 µm scale; b) Detail of the cavities structure showing the porosities; c) Ceramic cylinder in its final configuration.

the solution was constant during deployment time and to identify the compounds that would eventually degrade in water and thus, would not accumulate in the MCPS receiving phase.

2.2.4. MCPS data modelling

For a steady state, homogeneous and unidimensional system and if the mass flow of contaminants is diffusion limited it can be described by the first Fick's law (Martin et al., 2001).

$$J_s = -D_e\left(\frac{\Delta C_s}{\Delta x}\right) \quad (1)$$

where.

- J_s is the mass flow of solute.
- D_e stands for the effective diffusion coefficient of contaminants in water, and describes the diffusion of analytes through the water filled porous ceramic membrane.
- $\frac{\Delta C_s}{\Delta x}$ is the concentration gradient.

In the normal operating situation a concentrator (sorbent) material is placed inside the sampler. If adsorption of solute by the sorbent is efficient, the concentration of analyte in the solution inside the cylinder is considered to be constant and close to zero. Then, the concentration gradient is constant and the amount of contaminants diffusing across the membrane per unit time is proportional to the membrane area A, and concentration gradient. The accumulated mass M(t) in the concentrator will depends linearly on time (t) and can be given by:

$$M(t) = \frac{D_e C(L)}{L} \cdot A \cdot t = k \cdot t \quad (2)$$

where:

- C(L) is the concentration in the external solution, in this case 20 µg L^{-1}.
- L is the diffusion layer thickness (1.5 mm).
- A is the membrane area (14.1 cm^2).
- t is the deployment time.

The effective diffusion constant, De, was calculated from measured *k* values using eq. (2)

For the MCPS, the sampling rate R_s was calculated as:

$$R_s = \frac{k}{C(L)} = \frac{D_e A}{L} \quad (3)$$

where k is the slope of the MCPS calibration, representing the mass adsorbed vs. time.

2.3. Validation of MCPS to determine cytostatics in wastewater using grab sampling and SPE

The MCPS was deployed in influents and effluents of a WWTP. However, in influents the system was totally clogged with dirt and could not be analyzed. Then, MCPS was only validated in WWTP effluents by comparing the concentration of cytostatic drugs

obtained after 6 d deployment of the MCPS with the mean concentration from grab samplers (n = 4) measured using SPE. The study was done in a WWTP located near Barcelona, which treats a large amount of water (525.000 m^3 per day). It has a capacity of 2,843,750 inhabitants-equivalent and treats 65% of the wastewaters from Barcelona city and surroundings and receives basically urban waters, effluents from 3 large hospitals and industrial waters from the northern part of the city. Influent waters are characterized by waters of 3000–5000 ppm suspended solids, a DQO up to 7000 mg L^{-1} and a mean conductivity around 3000 μS cm^{-1}. This WWTP performs biological treatment without nitrogen and phosphorous removal and treated waters are discharged to the Mediterranean Sea via a marine emissary. Sampling was performed in May 2016. MCPS (n = 3) were enmeshed in the tulle net and were deployed in influent and effluent of the WWTP. For practical reasons, MCPS were placed in small water conduits to avoid that the samplers were lost due to turbulences. According to MCPS calibration, the deployment time was of 6 consecutive days to ensure an efficient and linear uptake. Besides, longer periods would produce fouling and reduce the uptake capacity when used in wastewaters. After deployment time, MCPS were collected and extracted within 24 h as depicted before. Quantification in absolute amount (ng/MCPS) was converted to ng L^{-1}, applying the Rs calculated according to the calibration performed under controlled conditions.

In parallel, 4 grab samples (1 L each, using precleaned amber glass bottles) were collected in 4 different days during the MCPS deployment period from both influent and effluent streams, and were kept at 4 °C. Grab samples were extracted using SPE within 24 h after its collection to avoid degradation. First, water was centrifuged to remove suspended particles. Then, it was filtered initially through 1 μm nylon filters to eliminate gross particulate matter which facilitated filtration through 0.45 μm nylon filters to finally obtain the soluble fraction. Extraction was done using SPE. 100 mL of water were acidified at pH2 and spiked with 25 ng IS. Cartridges were conditioned with 6 mL MeOH and 6 mL H_2O. Samples were loaded through Oasis HLB cartridges and then were washed with 100 mmol L^{-1} NH_4OAc in Milli-Q water (3 mL), dried over 45–60 min and eluted by gravity with 6 mL MeOH and 6 mL MeOH:HCOOH (95:5), following the method developed by Gómez-Canela et al. (2014). Extracts were reconstituted as indicated before. Recovery tests for SPE were calculated at a concentration of 0.25 μg L^{-1} using Milli-Q, influent and effluent wastewater (n = 4). After a preconcentration of 200 times, the levels in the vial were of 50 μg L^{-1}, which is within the calibration range as depicted below. MDL were calculated in effluent wastewaters as the concentration that gave a signal to noise ratio of 3.

2.4. Analytical determination

Analysis was performed by ultra high performance liquid chromatography coupled to a triple quadrupole mass analyzer (Acquity UPLC-TQD, Waters, USA) in positive electrospray (ESI+) following a previous study where chromatographic conditions and the MS transitions were optimized (Franquet-Griell et al., 2016; Gómez-Canela et al., 2013b). An Acquity UPLC BEH C18 column (100 mm x 2.1 ID, 1.7 μm) was used at a flow rate of 0.4 mL min^{-1}. ACN with 0.1% HCOOH (A) and water with 0.1% HCOOH (B) were used as mobile phase. The gradient started with 5% A, and then increased to 30% A in 10 min and to 100% A in 6 min (condition kept for 1 min). Then, initial conditions were attained in 1 min and kept for 2 min to stabilize the system before the next injection. Injection volume was 10 μL in full loop conditions. Acquisition was performed in Multiple Reaction Monitoring (MRM) using 2 transitions per compound. The transitions used and the voltage applied are indicated in Table 1. Internal standard quantification was performed using MPA-d_3 for MPA and CYC-d_4 for the other drugs. Linear range and response factors were calculated for all target compounds. To ensure the identification and quantification of target compounds, retention time shift between standards and samples should be lower than 2%, the two transitions selected for MRM mode should be detected and the S/N should be ≥ 10.

Calibration of the LC-MS/MS system was performed over a concentration range of 1–1000 μg L^{-1}, with standards prepared in ACN/water (50:50) with 0.1% HCOOH. Instrumental limits of detection (IDLs) provide information on the lowest concentration that the MS method used here is able to detect and were determined using a signal to noise ratio (S/N) of 3 calculated from the lowest concentration of the calibration curve for each drug (1 μg L^{-1}). Reproducibility was calculated by injecting a standard at a concentration of 50 μg L^{-1} in 5 different days. Cytostatic concentrations were quantified along the linearity range of the MS calibration curves (Table 1). Concentration in wastewater are given in ng L^{-1} given the low levels detected.

3. Results and discussion

3.1. Method performance and quality control analysis

To obtain high sensitivity and ensure precise quantification with the passive samplers, it is mandatory that the analytical procedure is robust and sensitive. Table 1 indicates the instrumental quality parameters obtained and the recoveries obtained with both SPE and Sepra™ ZT sorbents. Regarding the mass spectrometric calibration, good correlation was obtained ($R^2 \geq 0.99$) over a concentration range of 1–1000 μg L^{-1} for most of the drugs using internal standard calibration, with good reproducibility (0.8–26% RSD). IDL ranged from 0.001 ng (CYT) to 0.45 ng (LEU) by injecting 10 μL of the standard at 1 μg L^{-1}. According to the regression equation, good response factors were obtained using internal standard quantification. The high instrumental sensitivity and good reproducibility allows for the trace determination of cytostatic compounds, which are expected to be present in waters at the μg or ng L^{-1} level.

Table 1 also compares the recoveries of the developed MCPS method with the conventional SPE. Analyte extraction efficiency from the MCPS receiving phase is a critical issue that has to be optimized before calibration of the MCPS to guarantee a complete recovery of the drugs from the adsorbent. Recoveries in 2 steps (6 + 6 mL of MeOH or 6 + 6 mL of MeOH with HCOOH) were not efficient for IRI, and overall the extraction efficiency was similar or lower than 1 step with REC 3 method (Table 1 and SI2). Thus, the optimum recoveries with the lowest dispersion were obtained using 12 mL MeOH and applying vortex and ultrasonication three times without changing the solvent. This procedure provided recovery rates from 69 to 134%, except for CYT, IRI and CHL, with recoveries of 40 ± 3, 38 ± 13 and 51 ± 7% respectively (Table 1). VINB, VINC and LEU were not recovered from the MCPS sorbent with any of the methods. Compounds not recovered was due to poor stability in water that affects extraction efficiency, as shown in Fig. 2. Once the extraction procedure from the MCPS receiving phase was optimized, the system was ready for calibration.

Using SPE which was used to validate the results of the MCPS in wastewaters, recoveries were calculated at a spiking level of 0.25 μg L^{-1} using Milli-Q water and WWTP influents and effluent (Table 1). In Milli-Q water, VINB and VINC were not detected as they are hydrolyzed at the low pH of Milli-Q water, as demonstrated in a previous study (Franquet-Griell et al., 2017b) and LEU was neither detected. In influent waters, recoveries were affected by the high amount of DOM and particulate matter, and 6 compounds were not effectively recovered (n.d. or %R < 14%, Table 1) whereas MPA was detected in influents at concentration higher than the spiking level

Table 1
Compounds studied, ordered by retention time. Collision voltage (C.V., in volts (V), quantification and confirmation transitions, with the collision energy (C.E., in eV) are indicated. Quality parameters to determine method performance include: reproducibility at 50 μg L^{-1} as relative standard deviation (in %), linearity, regression equation and coefficient (R^2) and Instrumental Detection Limits (IDL) calculated at a S/N ratio of 3 in absolute amount corresponding to an injection of 10 μL. Recoveries (Rec) are given for the MCPS in the Sepra™ ZT adsorbent using 12 mL of MeOH and for SPE, recoveries in Milli-Q, influent and effluent wastewater and Method Detection Limits (MDL, ng L^{-1}) in effluents.

	Rt	C.V	Quantification transitions (m/z)	Confirmation transition (m/z)	Instrumental parameters					Recoveries (%), n = 4				MDL
					Reproducibility	Linearity	Regression		IDL	MCPS		SPE		SPE, ng L^{-1}
	(min)	(V)	(C.E., eV)	(C.E., eV)	RSD %	(ng μL^{-1})	equation	R^2	(ng)	Milli-Q	Milli-Q	$WWTP_{inf}$	$WWTP_{eff}$	WWTP eff
CYT	0.6	38	244–112(19)	244–95 (41)	4.0	0.001–1	y = 6.99x − 2.68	0.992	0.001	40 ± 3	46 ± 5	nd	nd	–
IFO	5.4	31	261–233 (20)	261–154 (20)	2.6	0.001–1	y = 1.12x − 0.033	0.999	0.009	107 ± 3	100 ± 3	33 ± 2	52 ± 2	5.0
CYC	5.7	30	261–233 (20)	261–140(20)	2.0	0.001–1	y = 1.44x − 0.037	0.999	0.007	113 ± 7	109 ± 5	57 ± 2	92 ± 2	3.0
IRI	6.4	46	587–167 (44)	587–124 (40)	1.7	0.02–1	y = 0.88x + 0.072	0.997	0.05	38 ± 13	78 ± 26	5 ± 2	18 ± 8	18
LEU	7.2	30	605–249 (34)	605–221 (34)	13	0.1–1	y = 0.045x − 0.047	0.993	0.5	nd	nd	nd	nd	–
MEL	7.1	21	305–288 (20)	305–168 (32)	0.8	0.005 −0.5	y = 0.71x − 0.026	0.997	0.03	86 ± 6	91 ± 10	nd	nd	–
DOX	7.2	16	544–397 (11)	544–130 (8)	1.0	0.01–1	y = 1.28x − 0.39	0.995	0.03	95 ± 23	63 ± 21	14 ± 7	13 ± 7	21
CAP	7.0	20	360–244 (10)	360–130 (25)	1.3	0.001–1	y = 16.4x − 4.35	0.991	0.007	134 ± 8	103 ± 6	64 ± 10	81 ± 5	2.5
PRE	7.4	20	359–341 (10)	359–161 (20)	9.6	0.01–1	y = 0.28x − 0.044	0.992	0.05	103 ± 4	102 ± 4	65 ± 11	71 ± 4	120
VINC	8.3	30	413–383 (20)	413–353 (25)	7.6	0.02–1	y = 8.01x − 0.13	0.997	0.2	nd	nd	84 ± 10	82 ± 11	59
VINB	9.5	30	406–355 (25)	406–346 (20)	26	0.05–1	y = 0.189x − 0.093	0.996	0.08	nd	nd	nd	nd	–
MPA	11.8	20	321–303 (11)	321–207 (20)	2.6	0.001–1	y = 4.07x + 0.21	0.999	0.002	104 ± 14	107 ± 9	[a]	83 ± 3	16
CHL	13.4	30	304–241 (20)	304–192 (25)	2.6	0.001–1	y = 5.13x + 0.47	0.997	0.005	51 ± 7	64 ± 8	73 ± 6	83 ± 5	0.70
TAM	14.7	40	572–129 (25)	572–129 (25)	1.3	0.001–1	y = 1.87x + 0.35	0.998	0.004	69 ± 8	87 ± 22	71 ± 4	89 ± 3	3.2
CYP	13.6	30	417–357 (30)	417–313 (20)	7.2	0.02–1	y = 0.13x + 0.018	0.996	0.09	93 ± 11	106 ± 13	77 ± 10	96 ± 3	10
MEG	13.7	30	385–325 (19)	385–267 (18)	1.9	0.001–1	y = 0.86x + 0.093	0.997	0.008	73 ± 7	97 ± 9	54 ± 6	73 ± 4	3.5

– Not detected at a spiking level of 0.25 μg L^{-1}
[a] Concentration in WWTPinf were much higher than spiked.

and recoveries could not be calculated. In effluent waters, values > 70% were obtained for most compounds. In general, recoveries in wastewater effluents are lower than the ones obtained in river water (Franquet-Griell et al., 2017a) and are attributed to the presence of organic matter in wastewaters, which is typically between 5 and 10 mg L^{-1} in WWTP effluents. IFO was recovered in 52% and CYT, IRI, LEU, MEL, DOX, VINB were poorly recovered. The poor recovery of these compounds is also attributed to low water stability (see below). It has to be mentioned that VINC is recovered in wastewaters but not in Milli-Q given their different pH. The preconcentration factor of 1000 (from 100 mL to 0.1 mL) permitted to obtain MDL between 0.07 and 59 ng L^{-1} in effluent wastewater, except for prednisone (120 ng L^{-1}) which had a low MS signal.

3.2. MCPS calibration and effect of stability

Diffusion and accumulation of cytostatic drugs in the receiving phase of the MCPS depend on their water stability. This effect is especially relevant in the case of cytostatics, as they are known to have high degradability in water (Franquet-Griell et al., 2017b; Negreira et al., 2014b). In addition, the stability of compounds is also important to determine deployment time of the MCPS, as highly degradable compounds should be determined using a deployment time of only a few days.

Fig. 2 shows the stability of cytostatic compounds in water for a period of 9 days. VINB was not detected even at time 0 (neither it was detected by SPE in any type of waters). LEU, CHL and MEL were completely hydrolyzed within 24 h, and thus would not be detected in a sample unless extraction was performed in less than 24 h. In particular, fast hydrolysis has been reported for CHL, which was completely degraded within 4 h (Gómez-Canela et al., 2013a) and MEL was completely hydrolyzed within 25 h (Franquet-Griell et al., 2017b). After 96 h (4 days) 7% DOX, 30% VINC and 35% TAM remained in the solution, and these compounds were completely degraded after 192 h (8 days). These compounds are the ones exhibiting low recoveries (Table 1). After 216 h (9 days), IRI had 17 of the initial concentration. The remaining drugs (MEG, CYP, CYC, CAP, IFO, CYT, PRE, and MPA) were stable with >47% of the initial concentration after 9 days. Thus, only stable cytostatic compounds will be retained in the MCPS receiving phase.

This information was taken into account in the MCPS calibration under the assumption that external concentration is constant during deployment time. For drugs with low or intermediate stability (Fig. 2), adsorbed mass did not follow a linear uptake. The maximum adsorbed mass was detected after only 2 days of deployment, and then decreased almost linearly. In particular VINB (32 ng accumulated), VINC (1.8 ng), CHL (0.8 ng), MEL (6 ng), DOX (35 ng), LEU (111 ng), TAM (5 ng) and IRI (30 ng) were the drugs following this behavior (SI3). In these cases, the MCPS would only be useful to determine their presence/absence in short deployment periods (e.g. 2 d). For these compounds, De and Rs could not be calculated.

On the other hand CYT was stable but was accumulated in very low amounts, which is consistent with the low recoveries obtained with both the MCPS receiving phase and SPE, Rs and De could not be accurately estimated.

Calibration in the MCPS was only successful for 7 compounds (MEG, CYP, CYC, CAP, IFO, PRE and MPA), which showed relatively high uptake dynamics. In all the cases an adsorption linear with exposure time was observed until about 168 h (7 days). This deployment time ensured an accurate and reproducible calibration of the system (Fig. 3). However, it was observed that after 7 d, the concentration of those cytostatics in the MCPS slightly decreased. This decrease is associated to the degradation of compounds in water. Such effect was not observed the ceramic dosimeter of Martin et al. (2003) which was deployed over a period of 80 d and linear absorption was observed for PAHs, which are highly stable in water. At present and given the stability of cytostatic compounds, we limit the sampling time to periods in which we know that the dosimeter kinetics is linear.

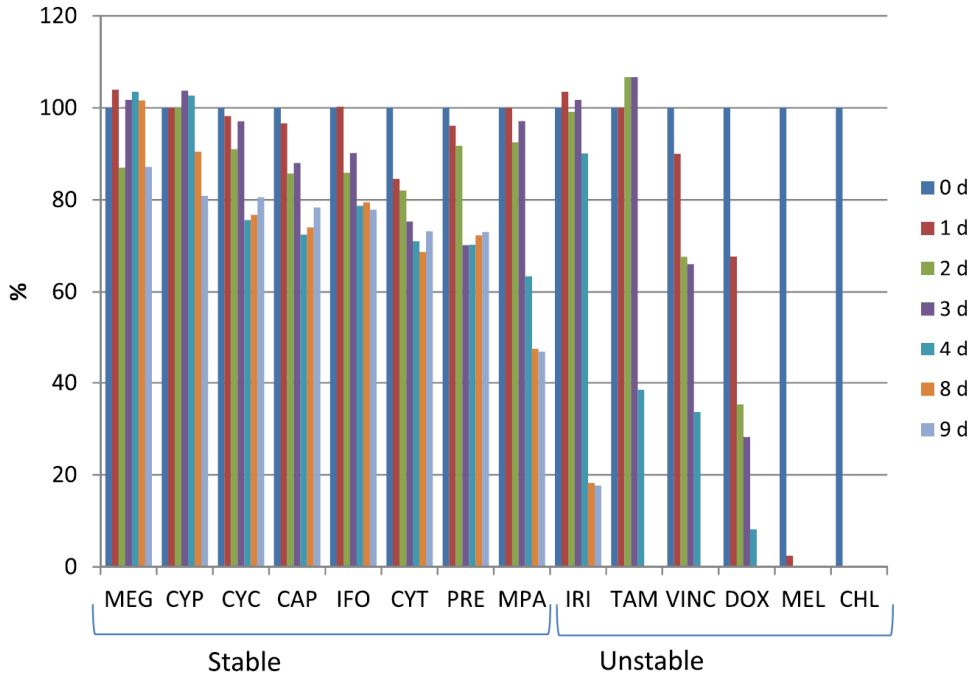

Fig. 2. Stability of cytostatic drugs in water spiked at 20 μg L^{-1} and analyzed by LC-MS/MS with direct sample introduction (no preconcentration). The concentration of each analyte throughout an exposure time of 9 d was normalized to time = 0 and is given as percentage (%). LEU and VINB not detected at t = 0 and are not represented.

3.3. Calculation of sampling rate and diffusion coefficients

Rs and De were only calculated for the 7 stable compounds which were linearly accumulated in the MCPS receiving phase during a period of 7d under controlled conditions. The results obtained for the calibrations, are shown in Fig. 3 and SI3 for CYT. For these compounds, good reproducibility of the MCPS was obtained, which were between 3 and 30%, depending on the compound analyzed. Higher variations were observed at longer exposure times probably due to the effect of degradation of cytostatics which is of 20% or more at 9d.

The final step was to determine the De and Rs of cytostatic drugs, which were calculated according to equations (2) and (3). A slope (k) between 8.25 and 30.5 ng day^{-1} was obtained considering the linear uptake period up to 7d. Rs ranged from 0.862 to 3.350 mL day^{-1} and De ranged from 1.01E-7 to 4.12E-07 cm^2 s^{-1} (Table 2).

To our knowledge, this is the first time passive samplers are used to analyze anticancer drugs in water and therefore the results cannot be compared to other studies. However, other compounds such as flame retardants or PAHs have been analyzed using ceramic dosimeters. Specifically, the Rs was of 0.39–3.7 mL day^{-1} (12 °C) and De varied between 8.1E-8 and 7.6E-7 cm^2 s^{-1} for brominated flame retardants and organophosphorous flame retardants, respectively, using commercially available ceramic passive samplers (Cristale et al., 2013). For PAHs, Rs between 1.5 and 2.5 mL day^{-1} were reported and adsorption up to 550 μg when deployed in contaminated groundwater during 1 year (Bopp et al., 2005). The high stability of PAHs allows larger deployment times, which cannot be achieved when analyzing anticancer drugs due to their degradability. Also, low deployment times can be used when sampling groundwaters with very clean waters, but not for wastewaters due to fouling, This is why the sampler has to be highly porous, to enable a quick uptake of contaminants.

Considering that cytostatic drugs are present al low concentration and that wastewater is a complex matrix, several considerations have to be done to ensure the right performance of the MCPS and field deployment. First, cytostatic compounds are not always stable, rather most of them are unstable in water and this has an obvious influence in their uptake; therefore, knowledge on the stability of target compounds must be evaluated prior to testing with any passive sampler to determine uptake kinetics. Secondly, triplicate analysis is needed to perform an accurate calibration. The third consideration is that the uptake is not fast for these compounds, and the time needed to diffuse through the ceramic barrier and accumulate in the sorbent at levels that can be measured is of minimum 2 d. At t = 1d (dosimeter lag time), cytostatics were not detected (data not shown). Taken into account the above mentioned considerations, a period of 6 d is suggested to ensure the uptake of contaminants and to guaranty that MCPS were deployed during a period where uptake is linear with relatively short exposure time.

3.4. MCPS analysis of cytostatic compounds in wastewater and comparison with grab sampling

Due to the very poor wastewater quality entering the plant, which is characterized by high suspended solids and organic matter, the MCPS installed in influent waters were completely covered with slime and dirt and could not be analyzed. Therefore, in the presence of large amounts of particles and organic matter, as is the case of WWTP influents, MCPS are easily obstructed and this prevents the free flow of chemicals across the ceramic barrier. Contrarily, grab sampling of influent WWTP waters and extraction using SPE permitted to identify the presence of 4 drugs (CYC, CAP,

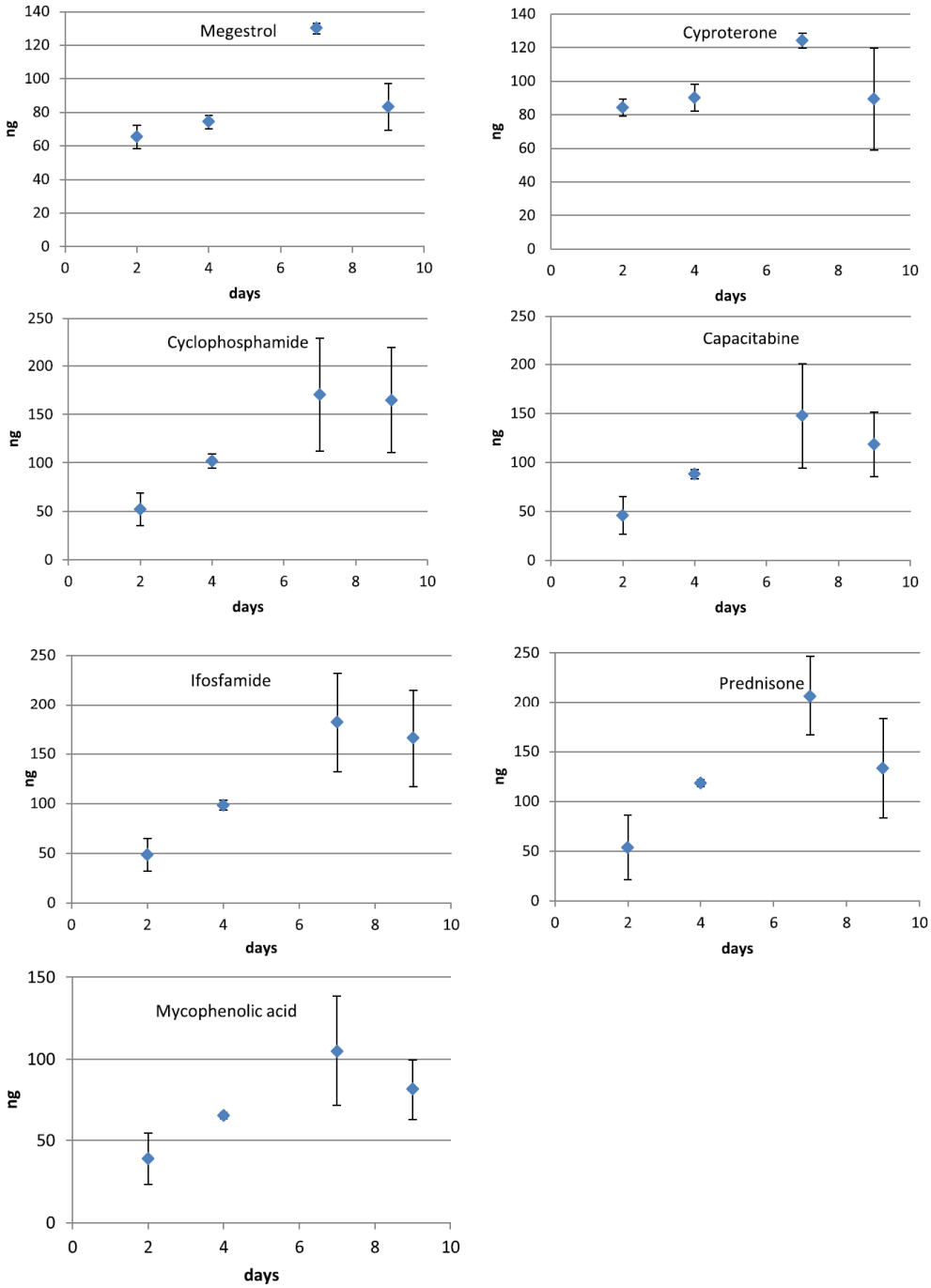

Fig. 3. Calibration indicating the mass adsorbed in the ceramic passive sampler as a function of time (2, 4, 7 and 9 days).

PRE and MPA) at mean concentrations from 15 ± 9 up to 3099 ± 500 ng L^{-1} (n = 4 days) (Fig. 4). These drugs were systematically detected all four days that grab samples were collected, thus indicating that their presence is significant in the WWTP under study.

The MCPS was only validated in WWTP effluent waters using a deployment time of 6 days. This sampling period was chosen as the most suitable one according to previous calibration results, and

Table 2
Calibration parameters obtained with the MCPS: slope of the MCPS calibration (k), sampling rate (Rs) and diffusion coefficient (D_e).

Compounds	MCPS		
	k (ng d^{-1})	Rs (mL day^{-1})	D_e (cm^2 s^{-1})
MEG	13.4	3.350	4.12E-07
CYP	8.25	0.825	1.01E-07
CYC	23.6	1.028	1.27E-07
CAP	20.3	0.862	1.08E-07
IFO	26.8	1.165	1.43E-07
PRE	30.5	1.425	1.78E-07
MPA	13.2	0.943	1.16E-07

because it was expected that longer exposure time in wastewaters would cause fouling, as previously observed in river water (Cristale et al., 2013). To validate the results obtained with the MCPS, grab water samples were collected during the same period and contaminants were extracted using SPE.

In the WWTP effluent, CYC and MPA were detected using MCPS at a net amount of 0.12 and 0.77 ng respectively. Considering the mass adsorbed and the Rs, a concentration of 19.3 ± 3 ng L^{-1} and 136 ± 28 ng L^{-1} was calculated for CYC and MPA, respectively. Using SPE, the mean concentration detected with grab samples (n = 4 days) was of 17 ± 4 ng L^{-1} for CYC and 195 ± 7 ng L^{-1} for MPA. Although statistical analysis could not be performed given the small amount of samples analyzed, there was a good agreement between both sampling methods, indicating the appropriateness of the MCPS method for measuring the concentration of cytostatics in WWTP effluents, as it was able to detect the 2 compounds present at the highest concentration.

However, using MCPS we were not able to detect CAP in effluents, which was only detected using SPE at a concentration of 13 ± 3 ng L^{-1}. This could be explained because, among studied compounds, CAP is the one with the lowest R_s which prevents detection when the concentration is low.

Finally, PRE was detected in the influent by grab sampling but it was not detected in the effluent neither with MCPS nor with grab sampling. PRE is most likely biodegraded during aerobic treatment in the plant, as proved in a recent study using controlled conditions (Franquet-Griell et al., 2017b).

The capacity of the MCPS to detect concentrations at the ng L^{-1} level demonstrate their applicability as levels of cytostatic drugs in Spanish WWTP are typically within the range of ng and μg L^{-1}. For instance, CYC was detected at <4–10 ng L^{-1} (Gómez-Canela et al., 2014) and nd-25.5 ng L^{-1} (Ferrando-Climent et al., 2013) and CAP at concentrations up to 27 ng L^{-1} (Negreira et al., 2013), whilst PRE was detected at lower concentration (<12 ng L^{-1}) (Gómez-Canela et al., 2014) in WWTP influents. Concentrations of CYC in WWTP effluents were lower, in the range of <4–5 ng L^{-1} (Gómez-Canela et al., 2014) and were not detected by Martín et al. (2011, 2014). MPA was the compound detected at the highest concentration, although its presence in WWTPs has not been reported before. However, its presence in surface waters has recently been reported at concentrations up to 200 ng L^{-1} (Franquet-Griell et al., 2016; Giebułtowicz and Nałęcz-Jawecki, 2016).

When comparing the results obtained with grab sampling and MCPS, grab sampling was able to detect 4 cytostatics in influent waters whereas the MCPS were clogged due to slime and dirt. In effluent waters, both methods are adequate to monitor the presence of cytostatics. Whereas grab sampling can provide more variable information depending on the sampling period, the MCPS represents an efficient and cost-effective method for the time integrated control of the 7 stable cytostatics in effluent waters. MCPS has the additional advantage of easy deployment, which facilitates the monitoring and control of cytostatic drugs in water.

4. Conclusions

This study proposes a macroporous passive sampling device for efficient monitoring of some cytostatic compounds in effluent wastewaters with relatively short deployment times. MCPS are made of alumina and its morphology shows a hierarchical pore size

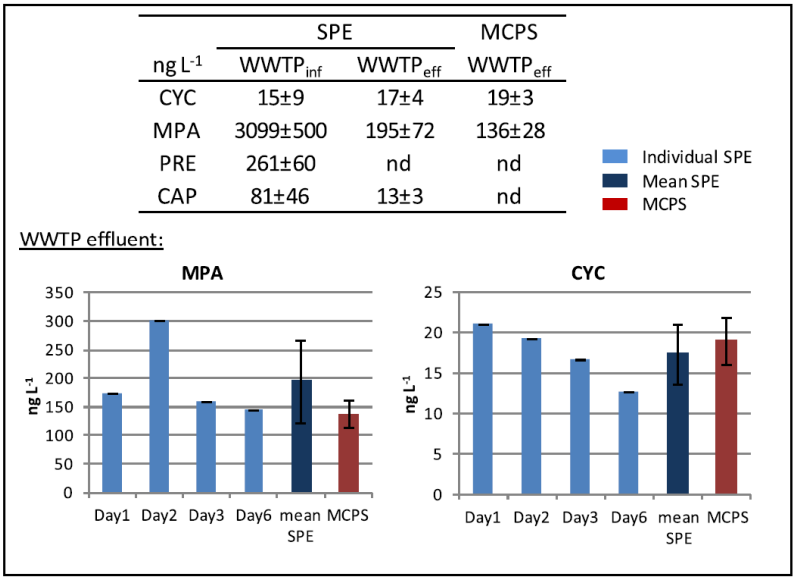

Fig. 4. Concentration of the cytostatic drugs detected in the WWTP influent and effluent (ng L^{-1}). Comparison between grab sampling with SPE (blue columns) and time-weighted averaged concentration for the MCPS (n = 3, red column). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

distribution of large cavities of 10–15 μm diameter connected by pores of 80–200 nm diameter. The MCPS was tested to monitor a suite of 16 cytostatic compounds and only the 7 most stable compounds (IFO, CYC, CAP, PRE, MEG, CYP and MPA) were efficiently retained. The sampling rates and diffusion constants were up to 3.350 mL day^{-1} and 4.12E-07 cm^2 s^{-1}, respectively. Upon deployment in WWTP, the MCPS was not able to detect cytostatic contaminants in influents due to fouling. However, in WWTP effluents, a good agreement was observed between the concentrations of CYC and MPA detected with the MCPS and the mean concentration obtained from grab samplers. Their presence in effluents indicates that their elimination is not complete and they are released to surface waters. In this sense, the MCPS herein developed would allow the monitoring of stable cytostatic compounds in an efficient, low cost and easy to use way so that they could be implemented in WWTP effluents to control the discharge of these genotoxic compounds to receiving waters. Efforts in order to obtain different pore morphologies, membrane thickness and compositions aiming for different diffusion rates are being currently carried out in our laboratory.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.05.051.

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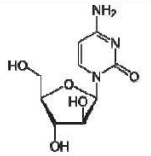
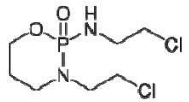
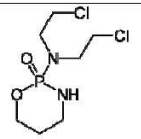
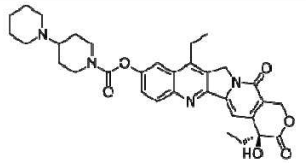
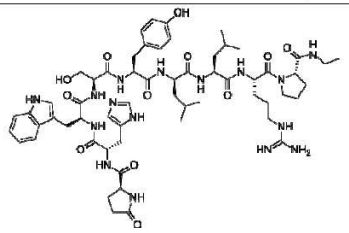
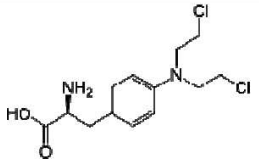
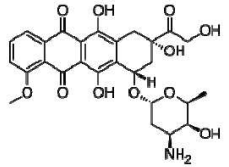
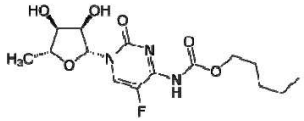
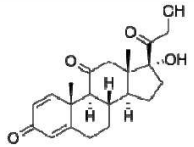
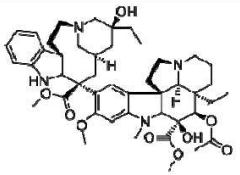
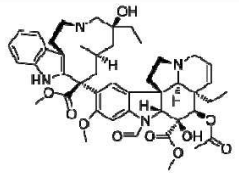
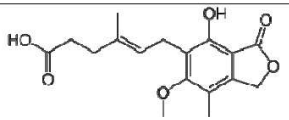
Supplementary information

Table S1. Physicochemical properties of target compounds.

Target Compound	CAS Nº	Molecular formula	MW (g mol^{-1})	Watersolubility (mg L^{-1})	pKa	Log Koc	Structure
Cytarabine	69-74-9	$C_9H_{13}N_3O_5$	243.2	$1.76 \cdot 10^5$	4.22	-2.51	
Ifosfamide	3778-73-2	$C_7H_{15}Cl_2N_2O_2P$	261.1	3780	4.75	0.86	
Cyclophosphamide	6055-19-2	$C_7H_{15}Cl_2N_2O_2P$	261.1	40	nd	0.76	
Irinotecan	100286-90-6	$C_{33}H_{38}N_4O_6$	586.6	107	nd	3.2	
Leuprolide	53714-56-0	$C_{59}H_{84}N_{16}O_{12}$	1209.4	0.0045	nd	1.16	

Melphalan	148-82-3	$C_{13}H_{18}Cl_2N_2O_2$	305.2	45.7	-0.432	-0.52	
Doxorubicin	25316-40-9	$C_{27}H_{29}NO_{11}$	543.5	10	11.02	1.27	
Capecitabine	154361-50-9	$C_{15}H_{22}FN_3O_6$	359.3	26	1.9	0.56	
Prednisone	53-03-2	$C_{21}H_{26}O_5$	358.4	312	13.90	1.46	
Vinblastine	143-67-9	$C_{46}H_{58}N_4O_{19}$	810.9	0.0446	14.41	4.32	
Vincristine	2068-78-2	$C_{46}H_{56}N_4O_{10}$	824.9	0.26	14.41	2.82	
Mycophenolicacid	24280-93-1	$C_{17}H_{20}O_6$	320.3	22	3.57	2.8-4.2	

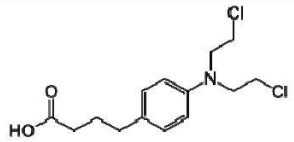
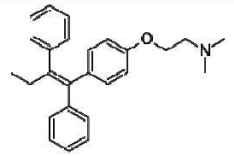
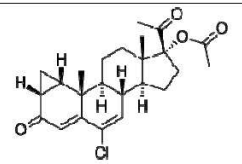
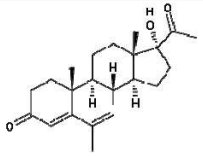
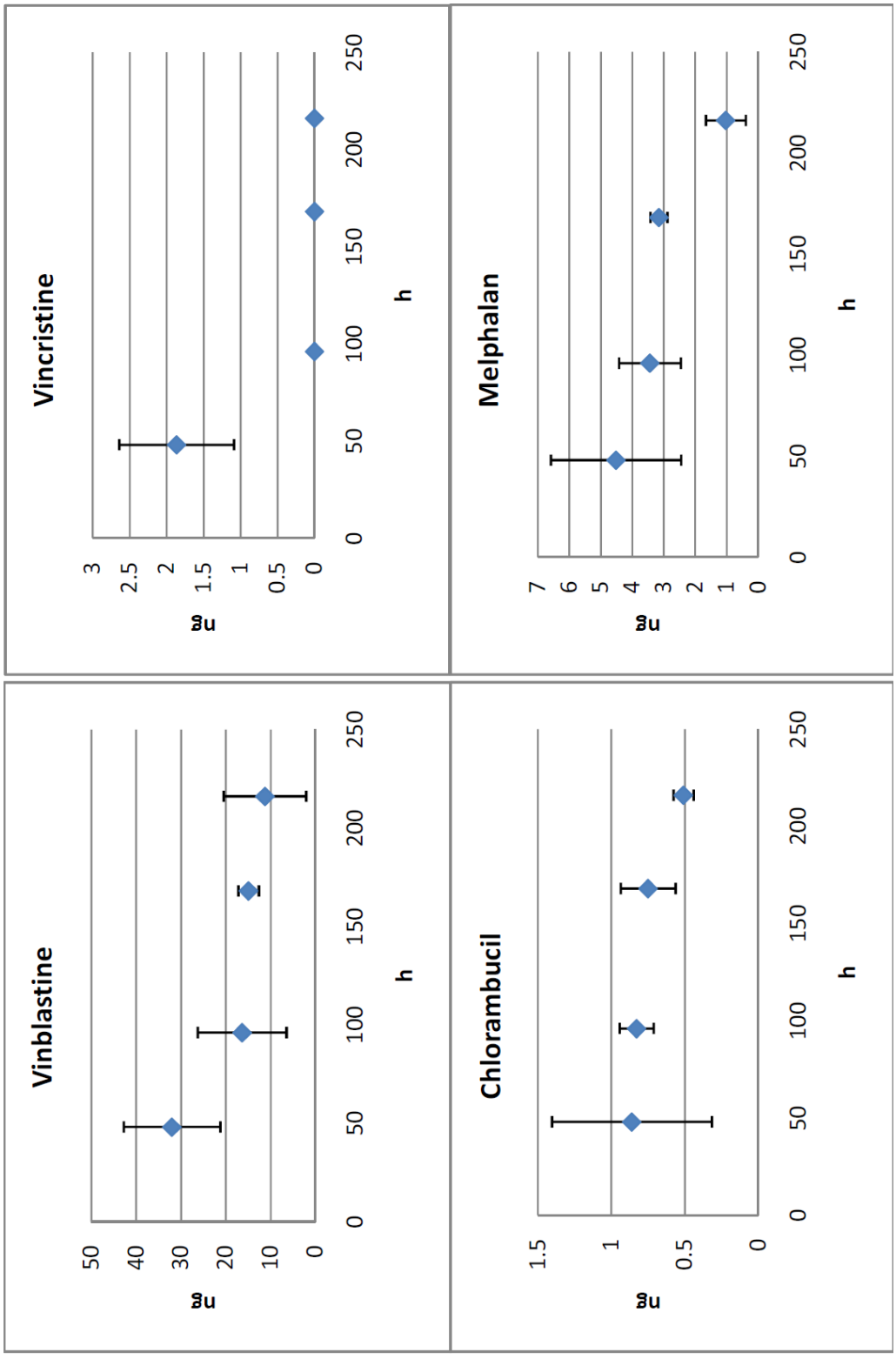
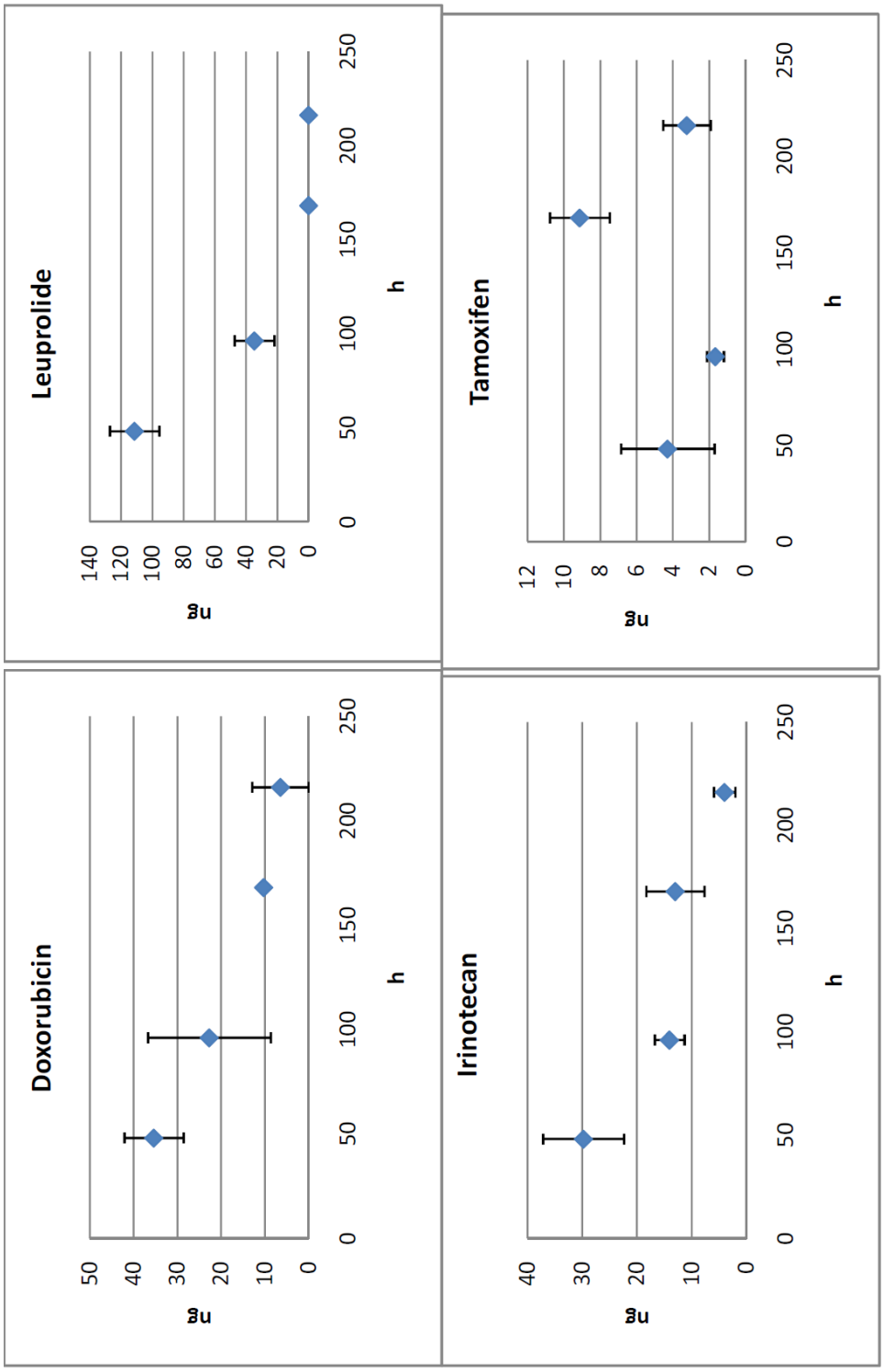
Chlorambucil	305-03-3	$C_{14}H_{19}NO_2Cl_2$	304.2	$1.24 \cdot 10^{+4}$	5.75	1.7	
Tamoxifen	10540-29-1	$C_{26}H_{29}NO$	371.5	0.1936	14.0	6.30	
Cyproterone	427-51-0	$C_{24}H_{29}ClO_4$	416.94	0.649	3.28	4.18	
Megestrol	595-33-5	$C_{22}H_{30}O_3$	342.4	2	17.61	4	

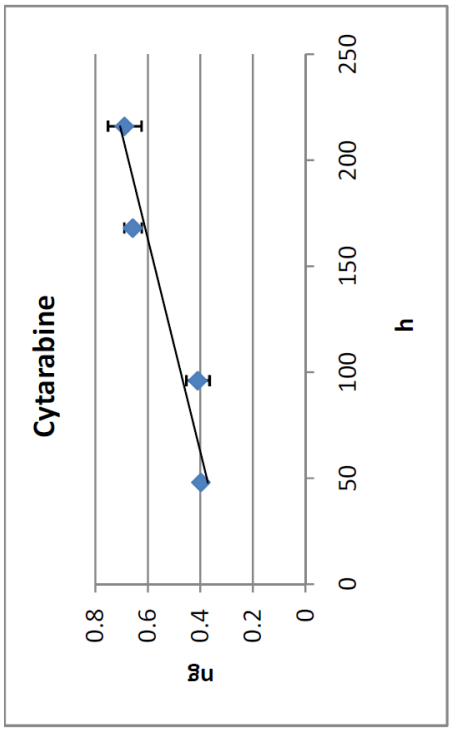
Table S2. Recovery tests

	Recoveries MCPS (%, n=3)		
	REC1	**REC 2**	**REC3**
CYT	27±6	23±0.3	40±3
IFO	107±23	87±33	107±3
CYC	96±25	82±19	113±7
IRI	nd	nd	38±13
LEU	nd	nd	nd
MEL	88±33	37±5	86±6
DOX	60±16	49±15	95±23
CAP	119±32	96±14	134±8
PRE	98±23	83±11	102±4
VINC	nd	nd	nd
VINB	nd	nd	nd
MPA	119±25	122±23	104±14
CHL	64±26	57±11	51±7
TAM	46±27	51±18	69±8
CYP	129±64	74±25	93±11
MEG	103±30	92±11	73±7



SI3. Adsorption in MCPS of less stable cytostatic drugs


Leuprolide
ng
h
0
20
40
60
80
100
120
140
0
50
100
150
200
250
Tamoxifen
ng
h
0
2
4
6
8
10
12
0
50
100
150
200
250
Doxorubicin
ng
h
0
10
20
30
40
50
0
50
100
150
200
250
Irinotecan
ng
h
0
10
20
30
40
0
50
100
150
200
250


Cytarabine
ng
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
h

4.2.2. Article científic VII:

Laboratory calibration of o-DGT for analysis of cytostatic drugs. Helena Franquet-Griell, Chang-Er L. Chen, Silvia Lacorte, Hao Zhang, Kevin C. Jones(en preparació)

LABORATORY CALIBRATION OF O-DGT FOR ANALYSIS OF CYTOSTATIC DRUGS

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ABSTRACT

Cytostatic compounds have emerged as a new group of water contaminants due to the high incidence of cancer in the population. Sampling techniques have a great influence when performing sampling campaigns, as the preservation of the samples is of high importance and directly influences the results obtained. However, little information is available on passive sampling methodologies for cytostatics. In the present study calibration of o-DGT for cytostatic drugs has been performed for the first time. The most recalcitrant compounds were selected to perform a step-by-step optimization which includes the study of the adsorption of cytostatics in the membrane filters and diffusive and binding gels and we have tested the extraction efficiency, the capacity, kinetics and the diffusion coefficients. After obtaining promising results, o-DGT proved to be ready for field deployment.

Keywords: Diffusive Gradient in Thin-film (DGT); passive sampler; pharmaceuticals; water

1. INTRODUCTION

Passive sampling techniques have been extensively studied because of their advantages over active sampling methods. The *in situ* uptake of the analytes provides time integrating results, which can represent a better characterisation of environmental contamination in areas where analyte concentration is not constant in time (Chen et al., 2013; Seethapathy et al., 2008). Devices used for passive sampling are easy to deploy in many sites simultaneously and do not need external power to operate. However, one of the main drawbacks is that the uptake of chemicals can be affected by external parameters, such as temperature, salinity, pH or water flow. These variations have to be studied before any field deployment of passive samplers and sometimes, it implies laboratory and *in situ* calibration (Zhang et al., 2008).

The high versatility of passive samplers makes them suitable for monitoring a wide range of chemicals in different environmental compartments including air (Farrar et al., 2005), water (Sanchez-Hernandez et al., 2004) and soils (King et al., 2007). Focused on polar organic chemicals in water monitoring, Polar Organic Chemical Integrative Samplers (POCIS) (Harman et al., 2012) and Chemcather (Moschet et al., 2015) have been used to analyse many pharmaceuticals in surface waters. Recently, the diffusive gradients in thin films (DGT) for organics (o-DGT) (Chen et al., 2012; Chen et al., 2013) have been introduced for antibiotics as model compounds to overcome one of the limitations of above mentioned passive samplers: the water flow effect on the sampling rate.

The DGT was first developed for the analysis of inorganic chemicals in 1994 (Davison and Zhang, 1994). The principle of DGT performance has been widely described (Davison and Zhang, 2012; Zhang and Davison, 1995). Briefly, DGT consists of three layers: a filter, a diffusive gel and a resin binding gel overlapped in a plastic holder (Figure 1). During deployment time, analytes from the external solution diffuse through the filter and diffusive gel according to Fick's first law and are retained in the binding layer. In general, the thickness of the diffusive layer (filter and diffusive gel) is much higher than the thickness of aquatic diffusive boundary layer (DBL), which is the key feature of DGT. For this reason, DGT are not dependent on hydrodynamic conditions, which is a great advantage compared to other passive samplers (Zhang and Davison, 1995) leading to the introduction of o-DGT.

Since its first introduction, o-DGT has been extended to sample many other polar organic contaminants, such as phenols (Dong et al., 2014; Zheng et al., 2015) and some pharmaceuticals and pesticides (Challis et al., 2016). o-DGTs were calibrated using different binding layers according to each group of chemicals, such as molecularly imprinted polymers (Dong et al., 2014), charcoal (Zheng et al., 2015) and HLB (Challis et al., 2016), and good performance in field deployment were obtained. Whether o-DGT can be used for cytostatic drugs, another critical group of pharmaceuticals and yet not tested, would be of great interests.

Cytostatic drugs are a wide group of chemicals used in cancer treatments. High consumptions have recently been reported due to the increasing cancer incidence (17.5 or 20.7 tons in France and Germany respectively) (Besse et al., 2012; Kümmerer et al., 2016). After administration, they are partially excreted as the unchanged drug and they can finally reach surface waters, where the presence of these compounds at ng or µg/L has been confirmed by several authors using active sampling (Franquet-Griell et al., 2017a; Giebułtowicz and Nałęcz-Jawecki, 2016; López-Serna et al., 2012; Martín et al., 2011; Valcárcel et al., 2011). These studies

reveal the importance of water monitoring given that these compounds are discharged to the environment at a continuous rate.

The aim of this study was develop a method based on o-DGT passive sampling for the analysis of 5 cytostatic drugs in water. Compounds studied were selected based on their stability in water (Franquet-Griell et al., 2017c). This paper presents a systematic laboratory calibration for o-DGT before field deployment can be implemented.

2. METHODS AND MATERIALS

2.1. Chemicals

Five cytostatic drugs were selected as test compounds for o-DGT calibration, namely cyclophosphamide, ifosfamide, capecitabine, prednisone and mycophenolic acid. These drugs were selected because of their high stability in water (Franquet-Griell et al., 2017d; Gómez-Canela et al., 2013; Negreira et al., 2014). Pure analytical standards (98-99%) were acquired from Sigma-Aldrich (St. Louis, MO, USA). The internal standard (IS) cyclophosphamide-d_4 was purchased from Santa Cruz Biotechnology, USA. Sodium chloride (NaCl), sodium hydroxide (NaOH), formic acid (HCOOH), methanol (MeOH) and acetonitrile (ACN) HPLC grade were supplied by Fischer Scientific UK limited. Amberlite XAD18^{TM} was acquired from Rohm and Haas Company (Pennsylvania, USA), and the hydrophilic-lipophilic balance polymer (HLB) resin as SPE cartridges from Waters (UK limited). Before use, both resins were conditioned with MeOH and milliQ water before use for making binding gels. Hydrophilic polypropylene filter (GHP) and polyethenesulfone filter (PES) were obtained from Whatman (UK), agarose powder was purchased from Fisher Scientific (UK limited) and DGT gel solutions were obtained from DGT Research Ltd.

2.2 Gel preparation

Diffusive and binding gels were prepared following previous procedures (Zhang and Davison, 1999), using agarose and HLB or XAD18 resins, respectively. Agarose diffusive gel (1.5%) was prepared by mixing 0.9 g of agarose in 60 mL of boiling water until all the agarose was dissolved and the solution became transparent. Then, the hot solution was pipetted into a preheated, gel-casting assembly and left at room temperature to reach gelling temperature (≤36ºC).

HLB and XAD18 sorbents are usually used for the analysis of other organic compounds (Chen et al., 2012) thus they were selected to test the adsorption. HLB is a hydrophilic-lipophilic polymer. On the other hand, XAD18 is highly cross-linked, polystyrenic adsorbent. Both materials were tested to prepare the binding gels and to select the most appropriate for these chemicals. Binding gels with HLB and XAD18 were prepared according to well-documented procedures

(Zhang and Davison, 1999). In brief, 4 g (weight wet) of HLB or XAD18 resins were spiked into the gel solution and mixed well. The solution was then pipetted into the space between two glass plates and kept at 45ºC about 1 hour. All gels were washed with milliQ water and cut into a disc shape and kept in milliQ water with 0.01M NaCl until use.

2.3 Chemical analysis

The analytical method was developed and validated in a previous study (Franquet-Griell et al., 2017d). Shortly, analysis was performed by ultra high performance liquid chromatography coupled to a triple quadrupole mass analyser (Acquity UPLC-TQD, Waters, USA) in positive electrospray mode (ESI+). The column was an Acquity UPLC BEH C18 (100 mm x 2.1 ID, 1.7 µm), and ACN (0.1% HCOOH) and water (0.1% HCOOH) were used as mobile phase at a flow rate of 0.4 mL/min.

2.4. DGT optimization

Figure 1 shows the step-by-step calibration of the DGT samplers for the cytostatics. These tests are described in the following subsections.

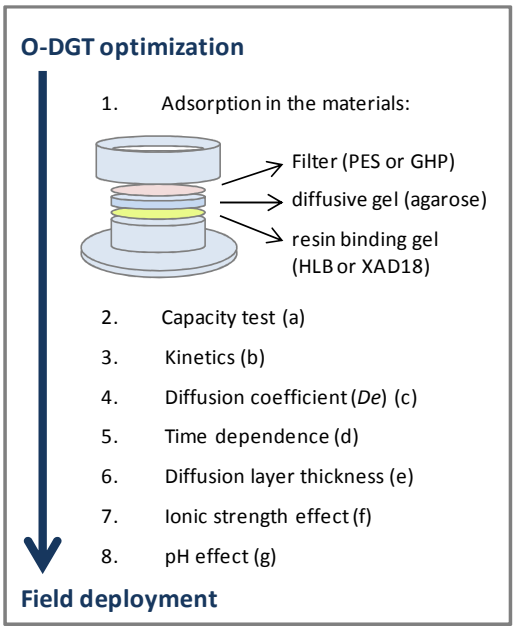

Figure 1. Workflow for the calibration of o-DGT for cytostatic drugs followed in the present study (a-g refers to figure 3 for the different tests performed).

2.4.1 Adsorption by membrane filters, diffusive gel and binding gels.

This test was performed to ensure that cytostatic drugs are not adsorbed in the filter nor in the diffusive gel but only in the binding gel. GHP and PES filters (n=3, respectively), the agarose diffusive layer (n=3) and the binding gel (HLB or XAD18) (n=6, respectively) were individually placed in 10 mL of a solution containing the mixture of cytostatic drugs at 50 µg/L each and placed in a shaker (Orbital DOS-20L Skyline ELMI, USA) for 5 hours. Final concentration in the solution was compared with a control, which consisted in the same solution of cytostatics without containing any material.

2.4.2 Extraction procedure and recoveries

To select the appropriate solvent to recover cytostatic drugs from the binding gels, MeOH and ACN were tested. After the adsorption experiment, HLB and XAD18 gels were collected and inserted in a new vial, to which 5 mL of solvent (MeOH or ACN) were added and left in an ultrasonic bath (for 30 minutes) (n=3 for each solvent and resin, respectively). The extract was transferred to an amber vial. This procedure was repeated with another 5 mL of the same solvent. Finally, an aliquot of the combined two extracts were transferred to an HPLC-vial, dried under N_2 and reconstituted using a water solution (0.2% HCOOH) and 25 ng of IS prepared in ACN.

2.4.3 Capacity

To test the maximum capacity of the resins, gels (HLB and XAD18) were individually exposed to different concentrations of the cytostatic solutions (1, 2, 4, 8 and 10 mg/L) for 23 h (n=2). Concentration in the solution was compared before and after this time. A control sample was prepared at 1 mg/L without the resin gel to control possible degradation. This concentration was selected because lower concentrations are easily degraded and analytes could not be detected.

2.4.4 Kinetics

Adsorption has to be fast in order to retain the compounds when they reach the binding gel. Binding gels (HLB and XAD18) were exposed to 10 mL of solution spiked at 30 µg/L of cytostatic drugs. They were shaken and two gels were periodically removed, at 0, 3, 10, 15, 20, 25, 30, 45, 60, 90 min and 2, 2.5, 3, 4, 5, 6, 21, 23, 25 and 26 hours. Aliquots from a control solution, without any gels, were also taken at the same time to control the possible degradation.

2.4.5 Diffusion coefficient measurements

A two-compartment diffusion cell was used to measure the diffusion coefficient (De) (Figure 2). This parameter will be used to calculate the concentrations in the environment of a particular chemical according to the adsorbed mass (see equation 1). The donor compartment (A) was filled with 0.01M NaCl and the mixture of cytostatics at 2 mg/L and the receiving compartment (B) was only filled with 0.01M NaCl. Both cells were separated by a hole (1.8 cm^2 surface) covered with a GHP filter and an agarose diffusive gel (0.8 mm). Both solutions were stirred by a magnetic stir bar and kept at room temperature (23±1ºC). An aliquot was taken every 20 min from both cells to maintain the same volume. All samples from compartment B and 6 samples from compartment A were analysed, after adding 25 ng of IS. Equation 3 was used to calculate De, as described in section 2.5.

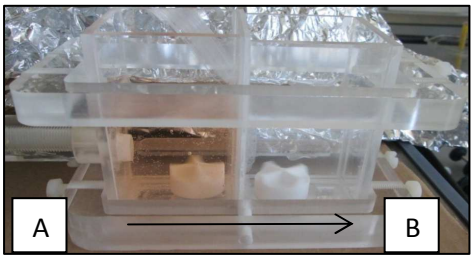

Figure 2. Diffusion cell, containing 2 mg/L cytostatic drugs in the donor compartment.

2.4.6 Time dependence

To evaluate if the adsorption in the device is linear with time, assembled DGTs using HLB and XAD18 resins were prepared and deployed in 8L milliQ water (0.01M NaCl, pH=6.4) spiked at 30 µg/L. They were kept up to three days at room temperature (25±1ºC) covered with aluminium foil to avoid degradation. The solution was constantly stirred and four devices (two HLB and two XAD18) were removed at different time intervals (5h, 22h, 29h, 46h and 70h). After removal, they were rinsed with milliQ water and disassembled. The binding layer was placed in a vial and extracted as described in section 2.3.2 using ACN. An aliquot of the exposed solution was also taken to measure its concentration at the time of DGT retrievals. This test allowed calculating the sampling rate (Rs) using equation 3 (section 2.5).

2.4.7 Diffusion layer thickness dependence

Assembled DGTs using XAD18 and different thicknesses of agarose diffusive gels (0.5, 0.8, 1 and 1.5 mm) were prepared. They were immersed in 2 L of milliQ water (0.01 M NaCl, pH = 6.6) and spiked at 20 µg/L. The tank was left at room temperature (22 ± 1ºC) covered with aluminium

foil to avoid degradation and the solution was stirred with a magnetic stir bar. After 20 hours, the device was retrieved and rinsed with milliQ water and the binding layer was extracted as previously described. Aliquots from the solution were also collected at the beginning and at the end of the experiment to measure the concentrations directly in the solution.

2.4.8 Effect of pH and ionic strength

The different characteristics of the exposed media can affect the sampling of target contaminants in the o-DGT. Thus, the effects of the solution pH and ionic strength (two key potential factors) were tested for DGTs prepared with both HLB and XAD18 resins. Assembled devices were deployed in 2 L tanks with milliQ water spiked at 20 µg/L and left at different conditions for 20 h. Four tanks were prepared to test pH, at 5, 6, 7 and 8 (with 0.01 M NaCl, at 24 ± 2 ºC). To test the ionic strength effect, four solutions were prepared at 0.001M, 0.01 M, 0.1 M and 0.5 M using NaCl (pH was kept between 6 and 7, at 23 ± 2 ºC). Aliquots from the solutions were also sampled at the beginning and at the end of the experiment to control the stability of cytostatics.

2.5 DGT calculations

The mass (M) of target chemical accumulated in the resin gel can be expressed by equation 1 when we assume the aqueous diffusive boundary layer is negligible (Chen et al., 2012):

$$M = \frac{D_e \cdot C_b \cdot A \cdot t}{\Delta g} \quad (1)$$

where, D_e (cm^2/s) is diffusion coefficient of each compound, C_b (µg/L) is the concentration of the analyte in the external solution, A (cm^2) is the exposure area of the DGT, t (s) is the deployment time and Δg (cm) is the diffusion layer thickness, which corresponds to the thicknesses of diffusion gel plus the filter.

Cb that in many cases we are interested in can be obtained by rearranging eq. (1) to (2) when we know the *De* and M:

$$Cb = \frac{M \cdot \Delta g}{De \cdot A \cdot t} \quad (2)$$

To obtain de diffusion coefficient (De) using the diffusion cell (described in section 2.3.5), equation 2 was used:

$$D_e = \frac{k \cdot \Delta g}{C_s \cdot A_s} \quad (3)$$

where, *k* (ng/min) is the slope of the linear plot of the measured mass in the receptor compartment versus time, Cs (µg/L) is the concentration of the analyte in the source compartment and As (cm^2) is the area of the connecting window between cells.

Finally, to calculate the sampling rate (Rs), equation 4 was used:

$$Rs = \frac{De \cdot A}{\Delta g} \quad (4)$$

3. RESULTS AND DISCUSSION

3.1 Adsorption by membrane filters, diffusive geland binding gel

Adsorption of anticancer drugs was tested individually for the different parts that are used to assemble DGTs (SI1). First, two different filters were tested: PES and GHP. PES filter was good for all drugs except for mycophenolic acid, which was adsorbed 33% of the initial concentration. On the contrary, GHP filter did not adsorb any drug significantly. Solutions containing the agarose diffusive layer were 92-107 % of the initial concentration after 5h, meaning that cytostatics were not adsorbed in the agarose and it is suitable for DGT assembling for these drugs, similar to previous results for antibiotics (Chen et al., 2012; Chen et al., 2013). And finally, HLB and XAD18 binding gels were tested. After 5h, the concentration of cytostatics remaining in the solution was ≤10% of the initial concentration, and therefore both resins are potentially capable of retaining these compounds. Therefore, GHP filter and agarose gel were suitable for o-DGT for these chemicals and were selected for further testing with both HLB and XAD18 resins.

3.2 Extraction procedure and recoveries

After adsorption of cytostatic drugs in the binding layer, MeOH and ACN were tested as elution solvents. 56-76% of the adsorbed mass was recovered from HLB resins and 32-43% from XAD18 (SI2) when MeOH was used as the solvent, while with ACN, 89-102% was recovered from HLB and 71-103% from XAD18. Therefore, ACN was selected as extraction solvent for the following tests.

3.3 Capacity

Resin gels showed high capacity to accumulate cytostatic drugs, which increased when increasing the exposure concentration. Mycophenolic acid has been selected as an example, and the different tests performed are shown in Figure 3a.

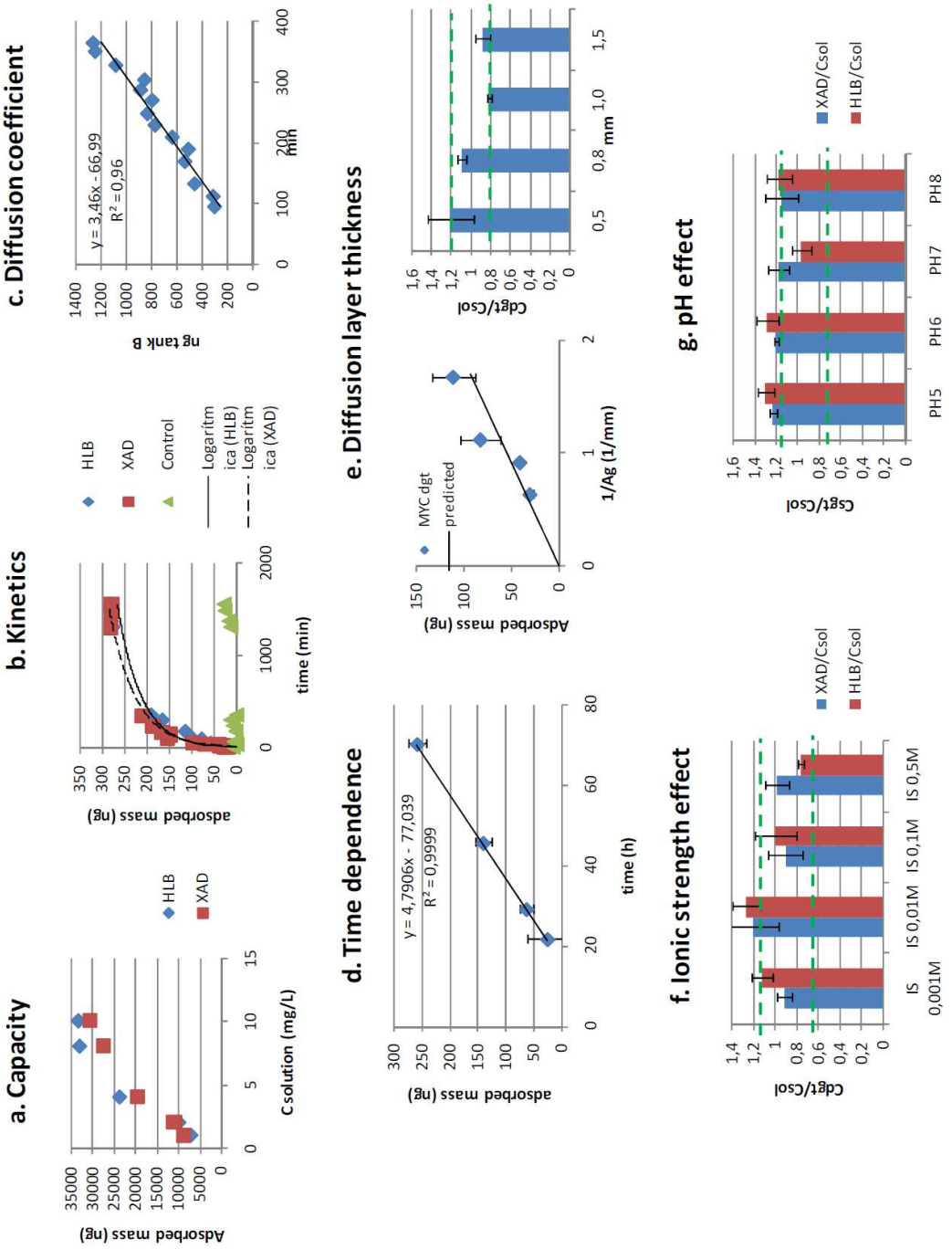

Figure 3. o-DGT optimization for mycophenolic acid.

Figures with the results for the other tested compounds are shown in the supplementary material (SI3). The highest adsorption was obtained for mycophenolic acid using HLB resin (up to 33.3 µg for a gel disc) and it was 30.6 µg/disc for XAD18 binding gel. For the other compounds, HLB also had higher adsorption, with 15-16 µg/disc accumulated, whereas XAD18 retained 8.8-11 µg/disc. Capacity for cytostatics is lower than reporter for other drugs, such as the antibiotic sulfamethoxazole with 180-360 µg/disc in XAD binding gels (Chen et al., 2012) or 140-194 µg/disc for bisphenols using activated charcoal (Zheng et al., 2015).

Although the capacity of HLB is slightly higher than XAD18, both may be used in the o-DGT. Considering the capacity for mycophenolic acid and ifosfamide, the drugs that showed the highest and lowest capacity, if deployed for two weeks, their maximum concentration would be 385140 ng/L and 205 ng/L respectively. The maximum concentrations detected for these two drugs were 656 ng/L for mycophenolic acid in surface water (Franquet-Griell et al., 2017b) and 130 ng/L for ifosfamide in a WWTP influent (Ferrando-Climent et al., 2013). Therefore, even HLB offers better results in the present calibration study, XAD18 showed also enough capacity to accumulate cytostatics. Therefore, further tests were performed using both resins.

3.4 Kinetics

The uptake kinetics has to be fast in order to retain the compounds quickly when they reach the binding layer, otherwise, the interfacial concentration between diffusive gel and binding gel would be non-zero. Adsorption of cytostatic drugs in the binding resin is fast using either HLB or XAD18 (Figure 3b and SI4). During the first 60 minutes, adsorption is quicker and then uptake rate decreases following a logarithmic curve. This decrease of uptake is probably attributed to a decrease of the available cytostatics in solution.

3.5 Diffusion coefficient measurements (*De*)

The diffusion coefficient measured in the diffusion cell will allow the calculation of the water concentration when deploying o-DGT. Figure 3c represents the mass detected in donor cell vs. time for mycophenolic acid (see SI5 for the other compounds). Using the slope of the curve and equation 3, *De* was calculated for each compound. The *De* Values are 2.78E-6 and 1.02E-5 cm^2/s (Table 1). There are no previous studies using o-DGT for cytostatics, but these values are in the similar range with those for antibiotics (0.80 – 6.24 cm^2/s) (Chen et al., 2013), bisphenols (4.44-5.64e-6 cm^2/s) (Zheng et al., 2015) and other pharmaceuticals and pesticides (1.67e-6 - 4.74E-6 cm^2/s) (Challis et al., 2016). These *De* values for cytostatics in o-DGT are higher than those in the recently developed ceramic passive samplers (1.01E-07 to 4.12E-07

cm^2/s)(Franquet-Griell et al., 2017d), which probably due to that the restricted pore in the ceramic sampler.

Table 1. Diffusion coefficient (*De*) and sampling rate (Rs) obtained for the cytostatics studied.

	De (cm^2/s)	Rs (mL/day)
Ifosfamide	1.02E-05	30
Cyclophosphamide	7.10E-06	21
Capecitabine	2.22E-06	6.1
Prednisone	2.78E-06	6.6
Mycophenolic acid	2.05E-06	8.3

3.6 Time dependence

Figure 3d shows the adsorbed mass for mycophenolic acid (as an example) vs. time (See SI6 for the other drugs). In general, the mass accumulated by o-DGT increased linearly with the deployment time, validating the equation (1) as expected. Slightly decline at the end of the experiments might be attributed to the lower solution concentration. After a deployment time of 5h, none of the studied drugs was detected using o-DGT, probably because the gradient in the diffusive layer has not yet been established. But longer deployment periods (29 or 46h) provide results within the acceptable range.

This experiment is set to validate the equation (1) about the mass dependence on the deployment time, and we aim to work at the linear uptake regime for this kinetic passive sampler. In the field deployment, this is also very helpful. If we deploy DGT samplers for two different times (at least), we can check if the sampler has reached the capacity based on that the results that from these two time intervals are close to each either or not. Otherwise, we might have a risk of underestimation of the water concentration if capacity already reached at the time of retrieving the samplers (Chen et al., 2013)

Rs were calculated using equation 4 and ranged between 6.1 and 30 mL/day (Table 1). The Rs value for sulfamethoxazole was 11 mL/day (Chen et al., 2012), within the range obtained in the present study. Challis et al. (2016) reported Rs between 8.8 and 16.1 mL/day. Rs for DGT are higher than Rs obtained for cytostatic drugs using MCPS (0.8-1.4mL/day)(Franquet-Griell et al., 2017d), which could be explained because the diffusion layer is thinner than the ceramic wall

3.7 Diffusion layer thickness dependence

Figure 3e and SI7 represent the adsorbed mass according to the thickness of the diffusion layer (1/Δg), which is inversely proportional.

In the same figure Cdgt/Csol was represented and the results were within the accepted range, meaning that all thicknesses are appropriate to be used for DGT assembling.

3.8 Effect of ionic strength and pH

Ionic strength in surface water and seawater typically range between 0.001 and 0.5 M, and pH can usually vary between acidic and basic conditions. Therefore, tests were performed varying these conditions to confirm they can be deployed in different water compartments.

Figures 3f and SI8 represent Cdgt/Csol ratio according to the different ionic strength tested (0.001, 0.01, 0.1 and 0.5 M NaCl). Only at 0.5M, which represented sea water, adsorption was slightly lower than the other ionic strengths tested. However, for all experiments, the five drugs were within the acceptable range of 0.8 and 1.2.

The effect of pH is represented in Figures 3g and SI9, showing the ratio Cdgt/Csol vs. pH (5, 6, 7 and 8). For prednisone and capecitabine, results at pH5 are slightly lower than values using the other conditions. These two drugs have pKa values of 12.6 and 8.23, similar to the pKa of the other studied drugs, meaning that they will be in its neutral form at any of the tested pH and it should not affect its adsorption. In fact, results are in most conditions within the acceptable range (0.8 - 1.2). Therefore, DGT for the analysis of anticancer drugs could be used in different conditions and deployed in different types of waters.

4. CONCLUSIONS

The use of passive sampling techniques has several advantages for the integrated time monitoring of contaminants in water and this is the first time o-DGT samplers have been tested for cytostatic drugs. After optimization tests, GHP filter and 0.8 mm agorose gel were selected as the most appropriate configuration for o-DGT assembling. Concerning the binding gel, both HLB and XAD18 showed to be suitable, as no major differences were found on their performance. Both resins showed high capacity to retain cytostatics, fast kinetics and linear adsorption. Deployment at different ionic strength and pH conditions did not affect the adsorption of the tested drugs, making the system suitable to be deployed in different water compartments. So, o-DGT is ready to be deployed in the field to monitor recalcitrant anticancer drugs.

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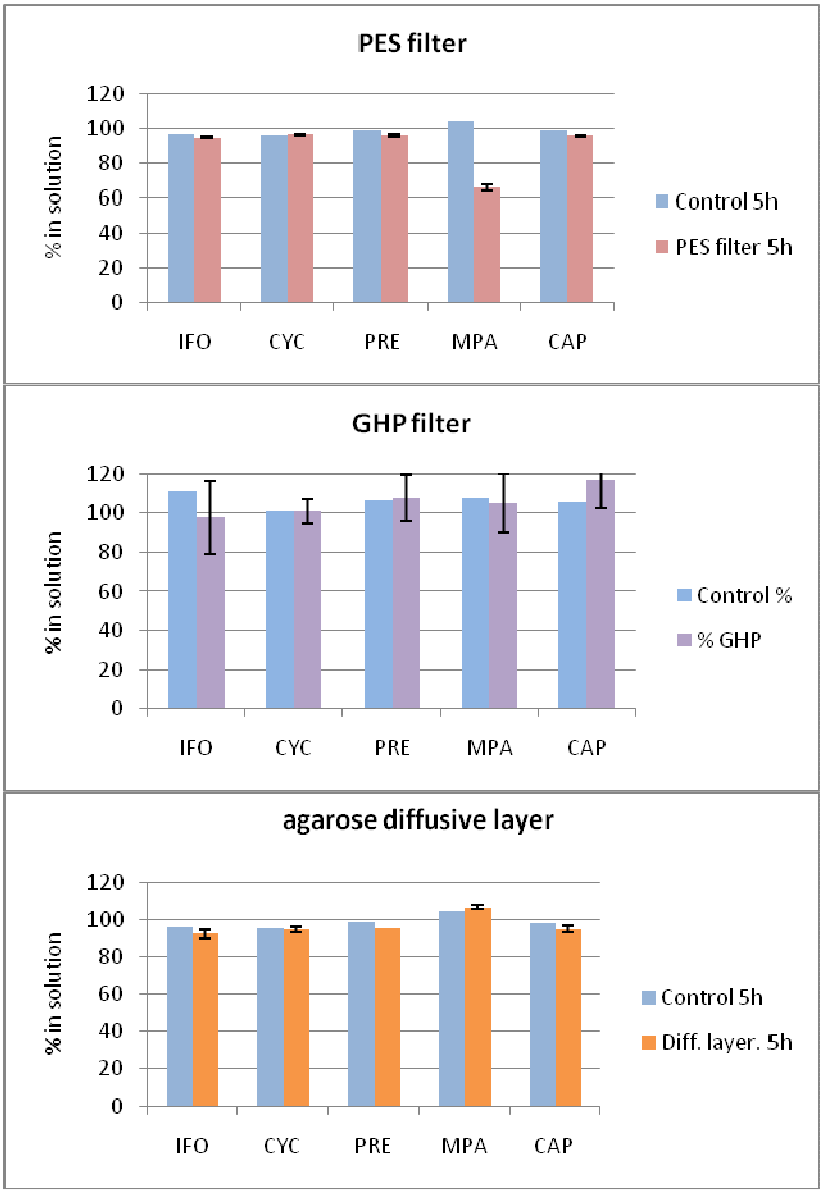
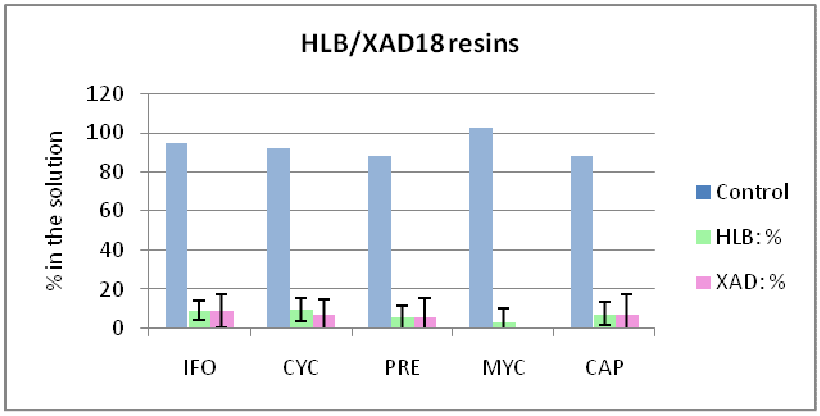
Supplementary Information

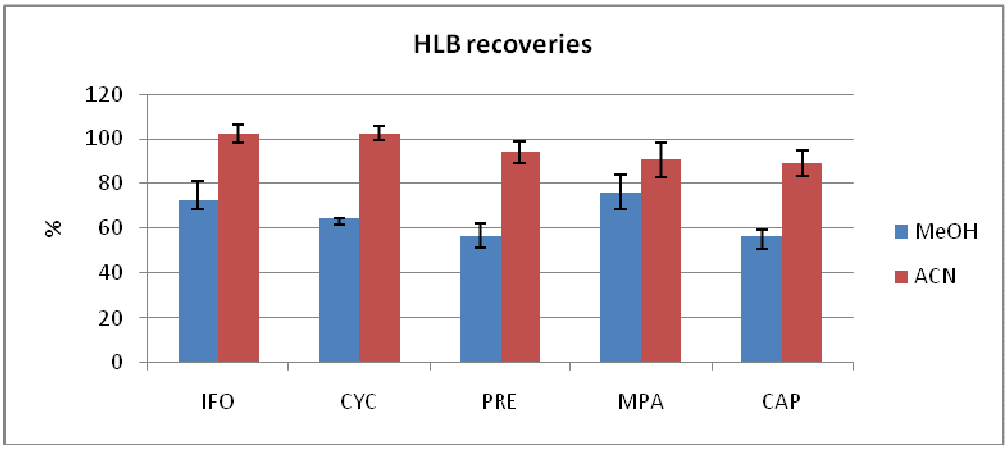
*IFO: ifosfamide; CYC: cyclophosphamide; PRE: prednisone; MPA: mycophenolic acid; CAP: capecitabine

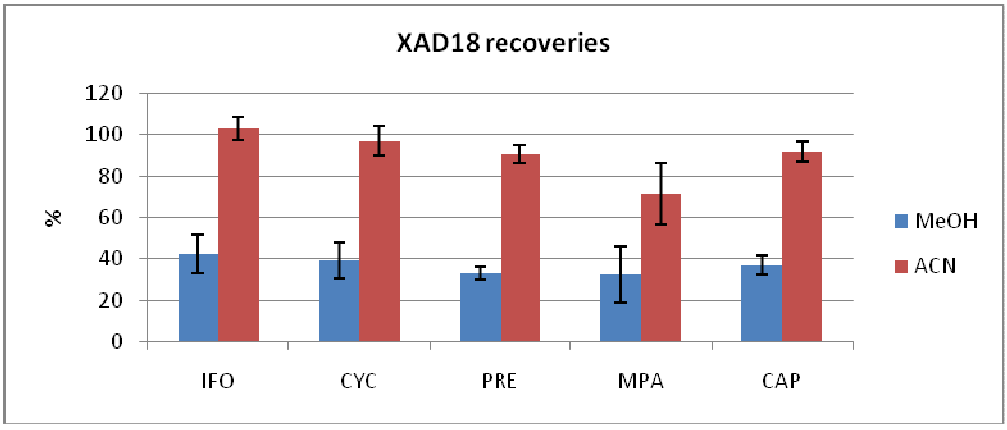
SI1. Adsorption by binding gel (HLB and XAD18), agarose diffusive gel and membrane filters (GHP and PES). Graphics represents the % of each drug present in the solution after exposure time.



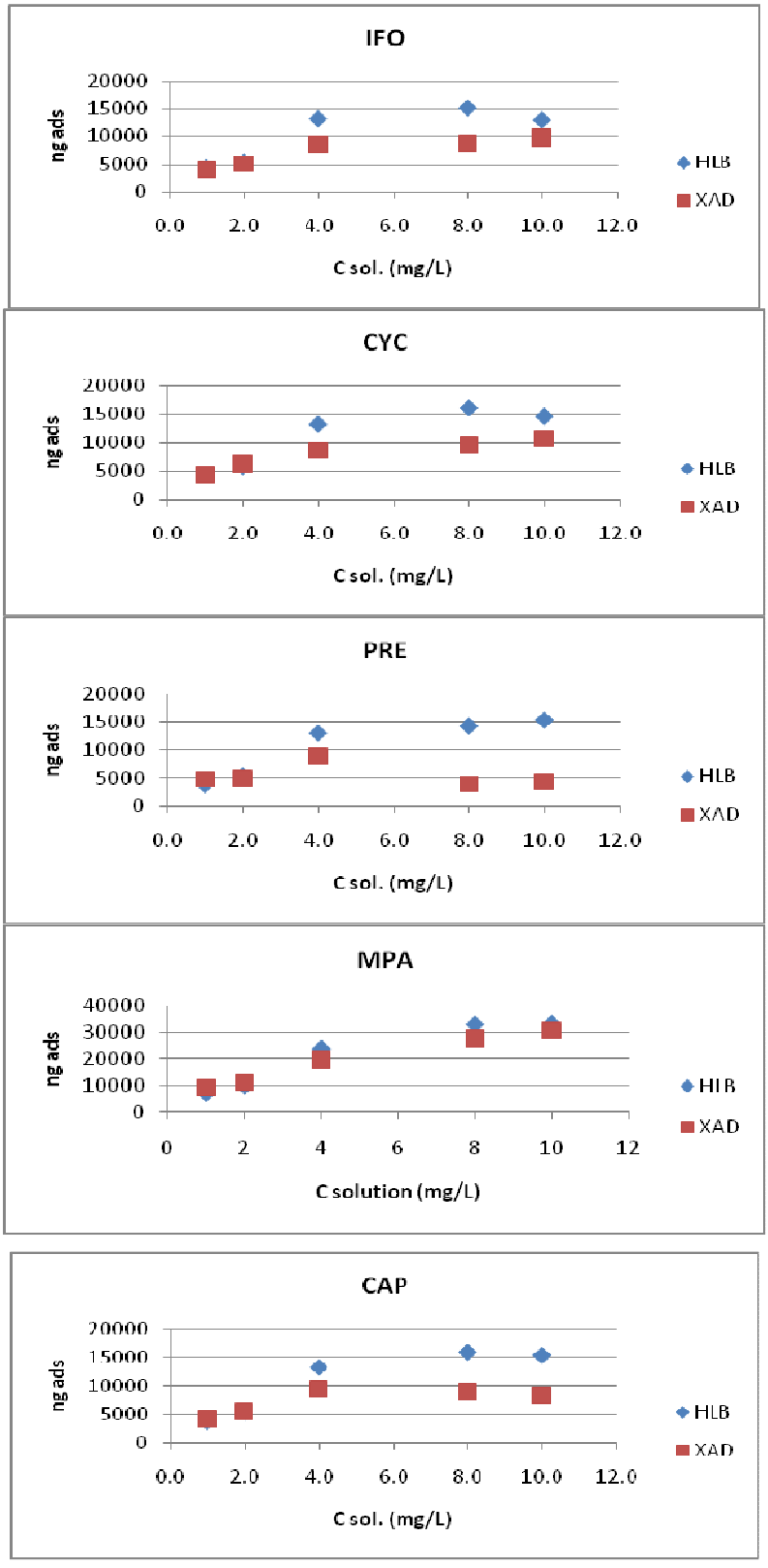


SI2. Recoveries obtained using two extraction solvents (MeOH and ACN) for the two binding gels tested, HLB and XAD18.

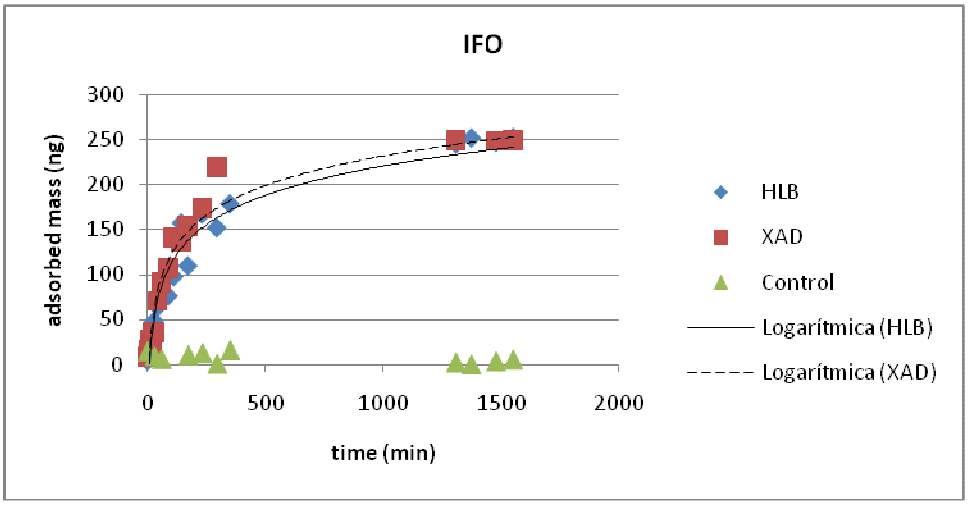


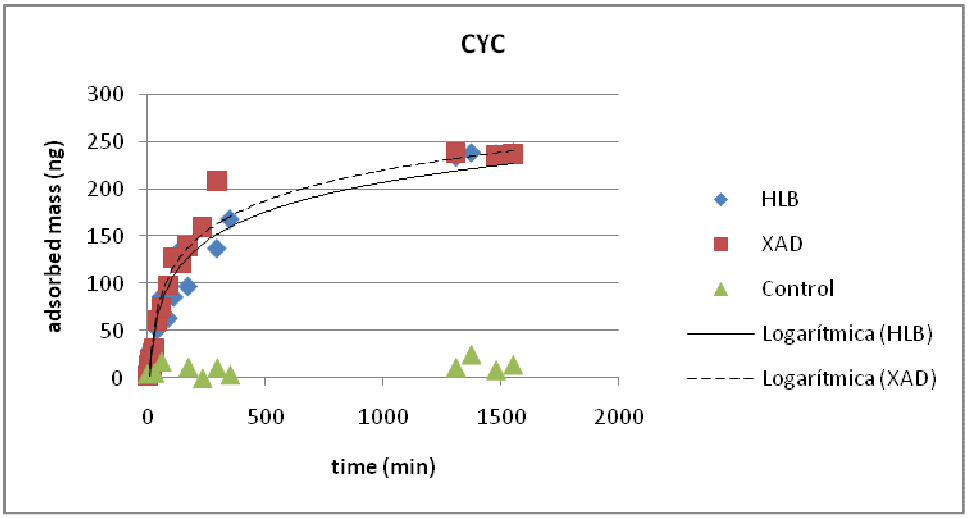


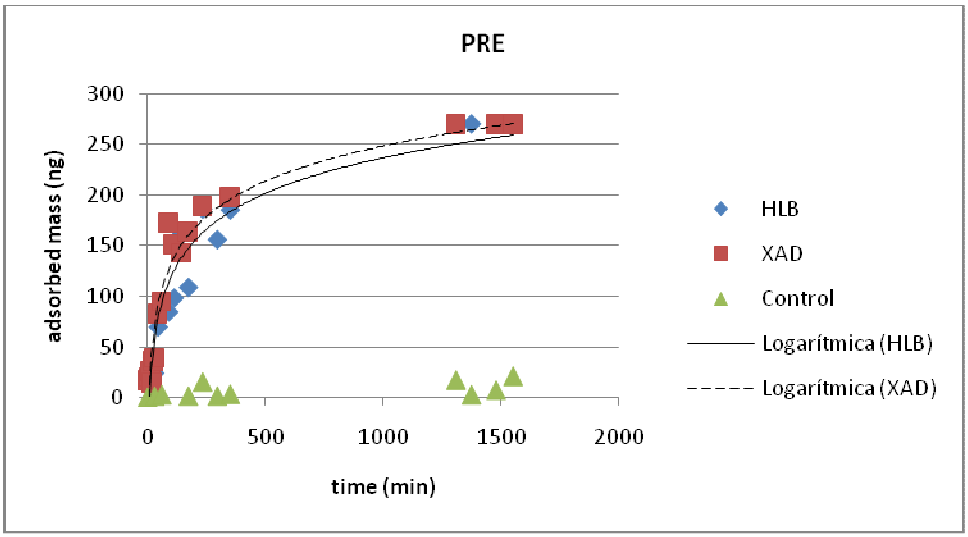
SI3. Capacity test: adsorbed mass (ng) in the resin gel depending on the external concentration (Csol.).

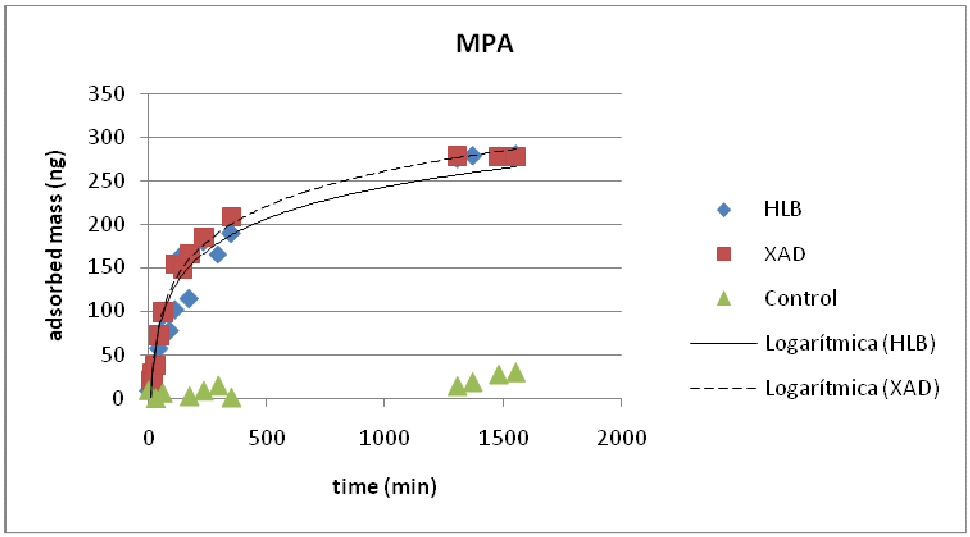


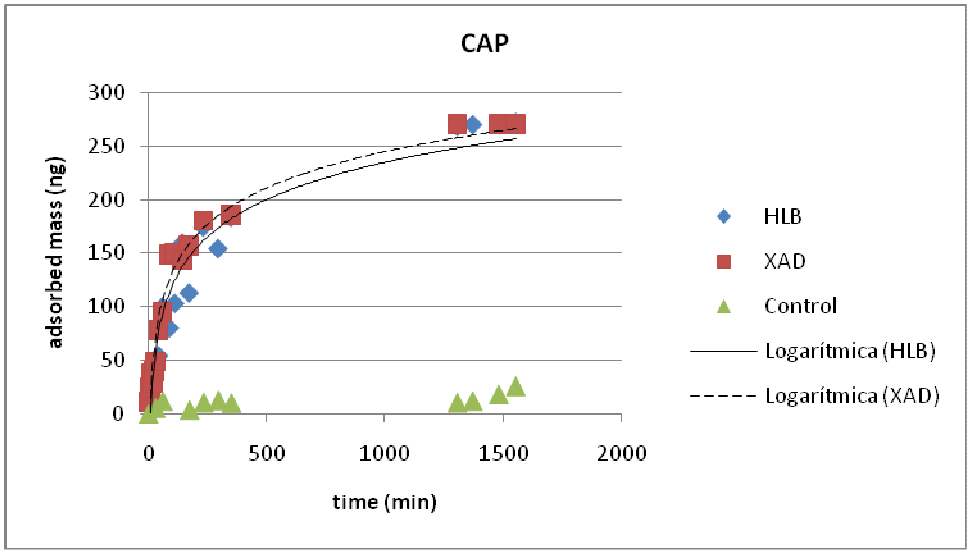
SI4. Kinetics: adsorbed mass (ng) in the resin gel (HLB or XAD18) vs. time



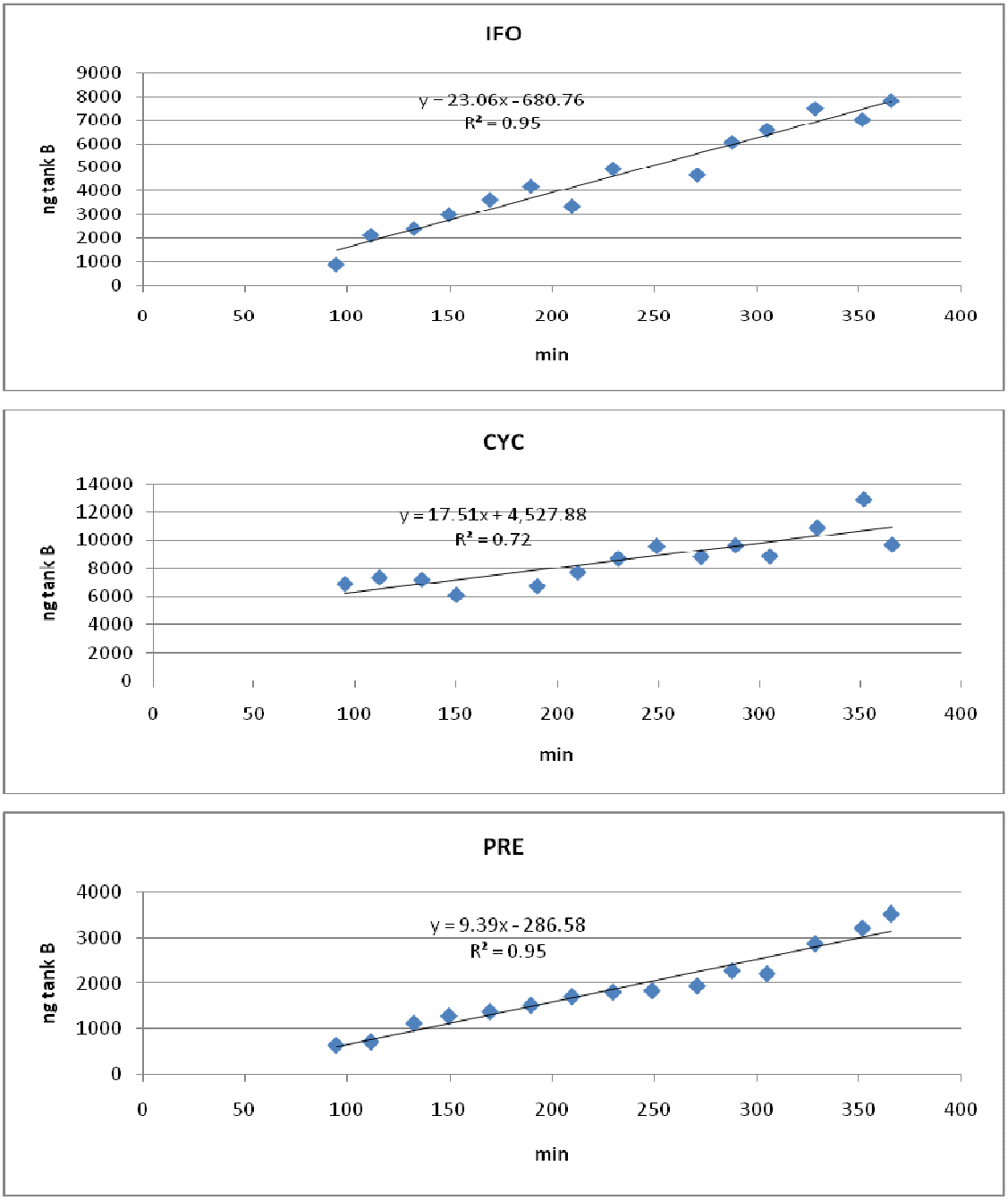


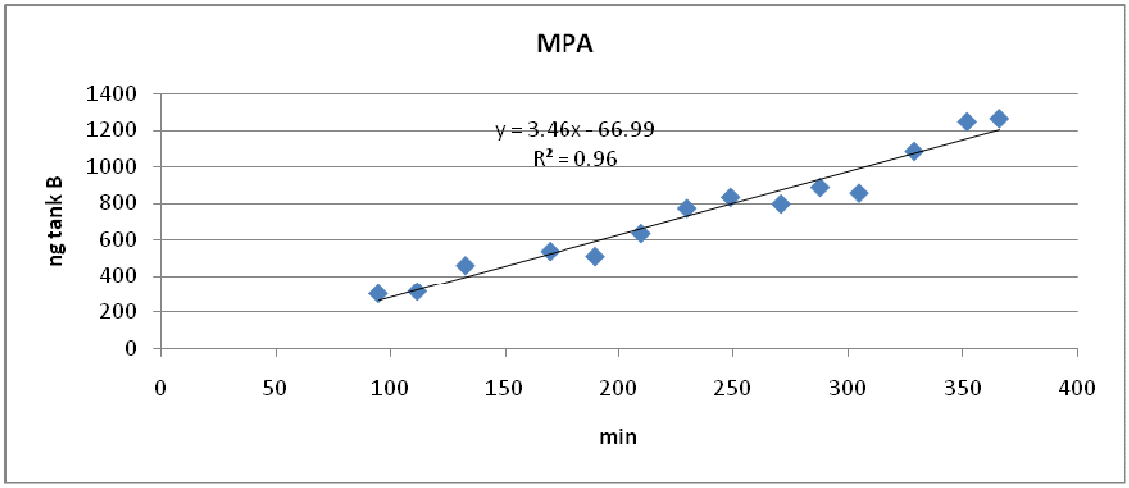


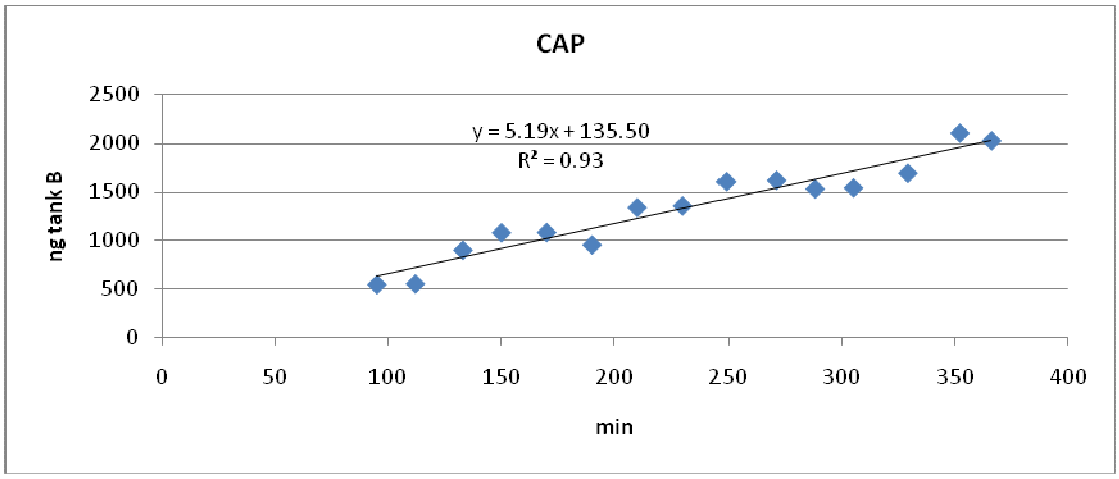

MPA
adsorbed mass (ng)
350
300
250
200
150
100
50
0
0
500
1000
1500
2000
time (min)
HLB
XAD
Control
Logarítmica (HLB)
Logarítmica (XAD)


CAP
adsorbed mass (ng)
300
250
200
150
100
50
0
0
500
1000
1500
2000
time (min)
HLB
XAD
Control
Logarítmica (HLB)
Logarítmica (XAD)

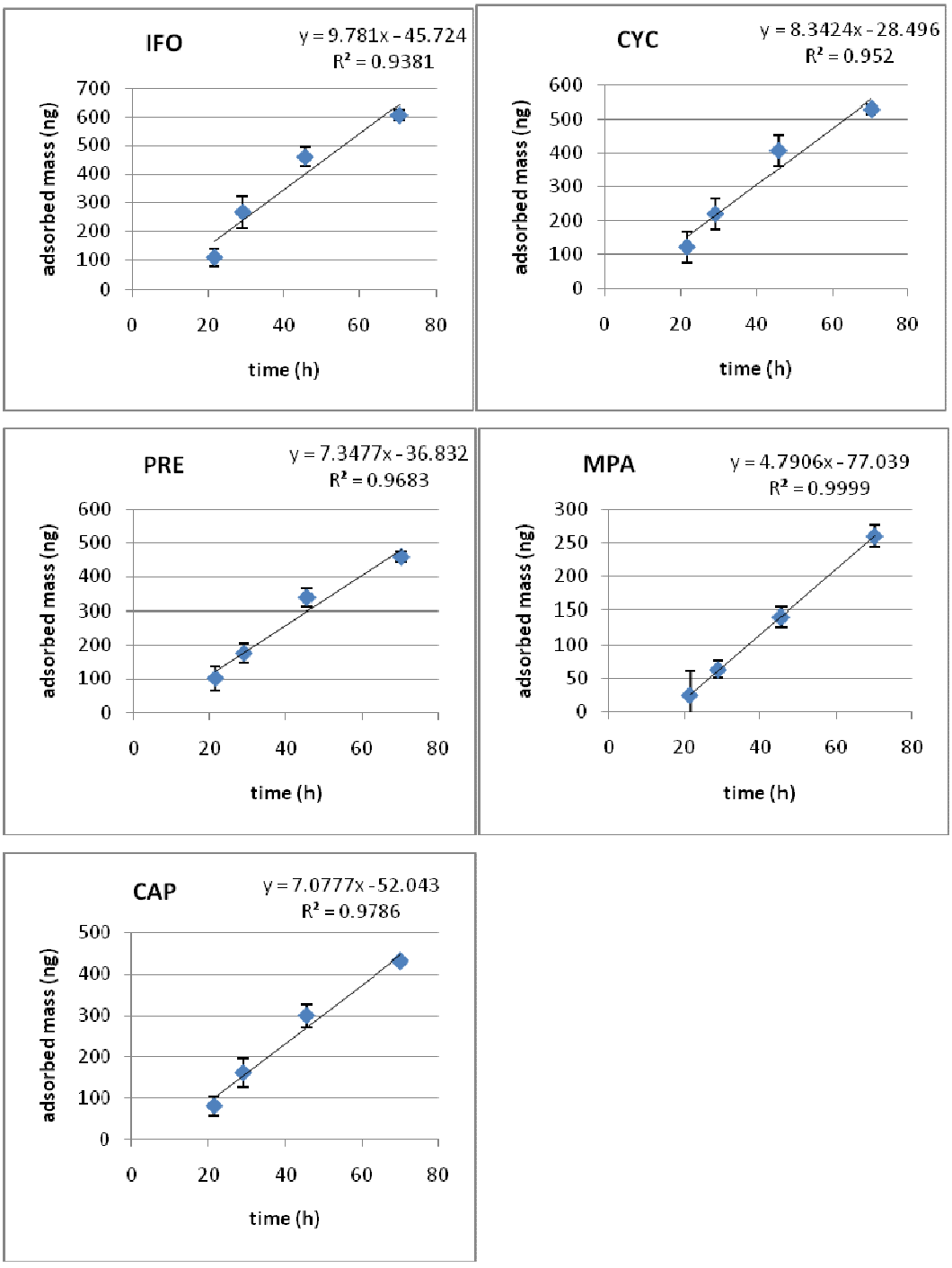
SI5. Diffusion coefficient measurements: ng in tank B from the diffusion cell vs. time.



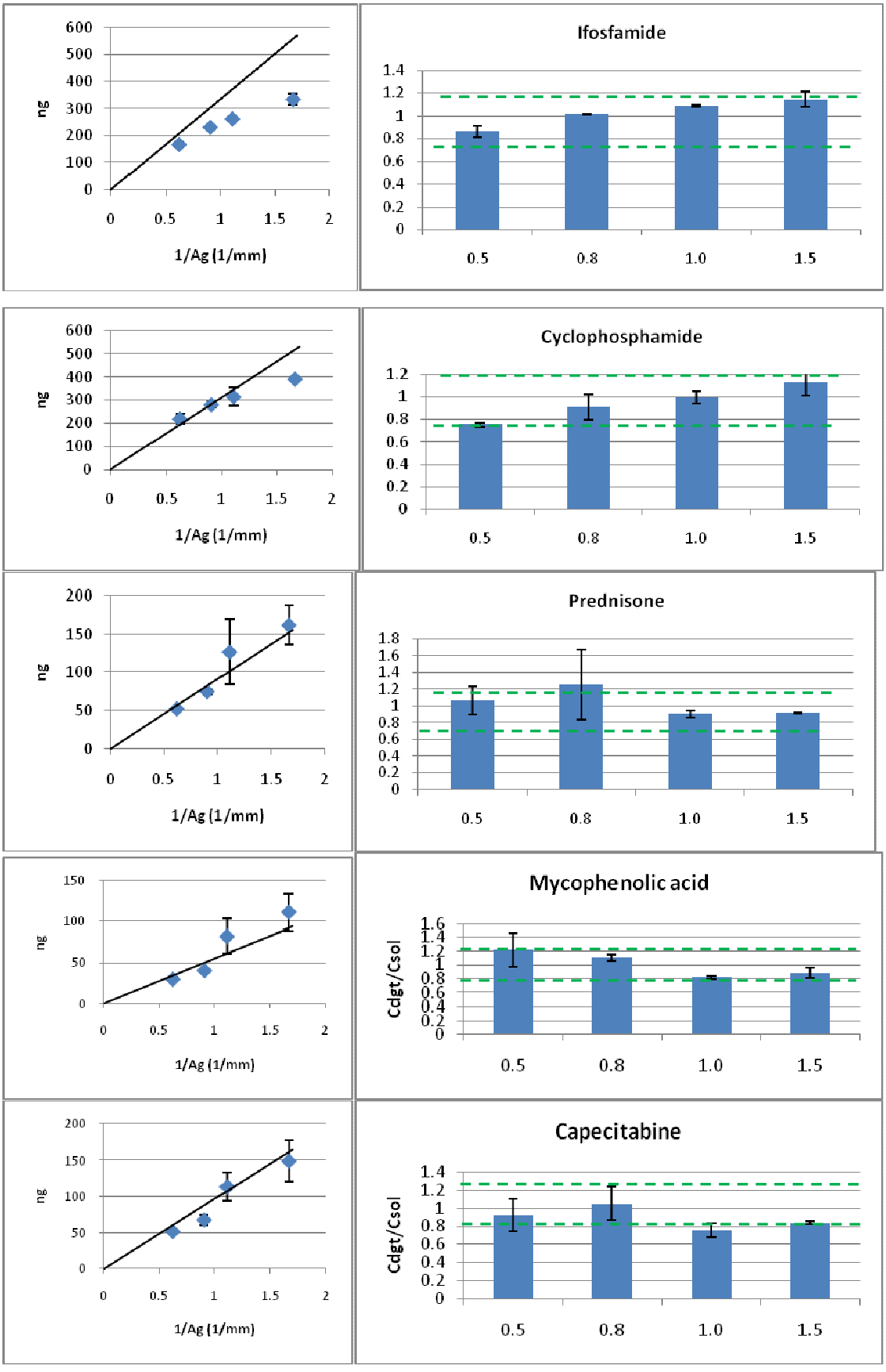

MPA
y = 3.46x - 66.99
R² = 0.96
ng tank B
min
0
200
400
600
800
1000
1200
1400
0
50
100
150
200
250
300
350
400


CAP
y = 5.19x + 135.50
R² = 0.93
ng tank B
min
0
500
1000
1500
2000
2500
0
50
100
150
200
250
300
350
400

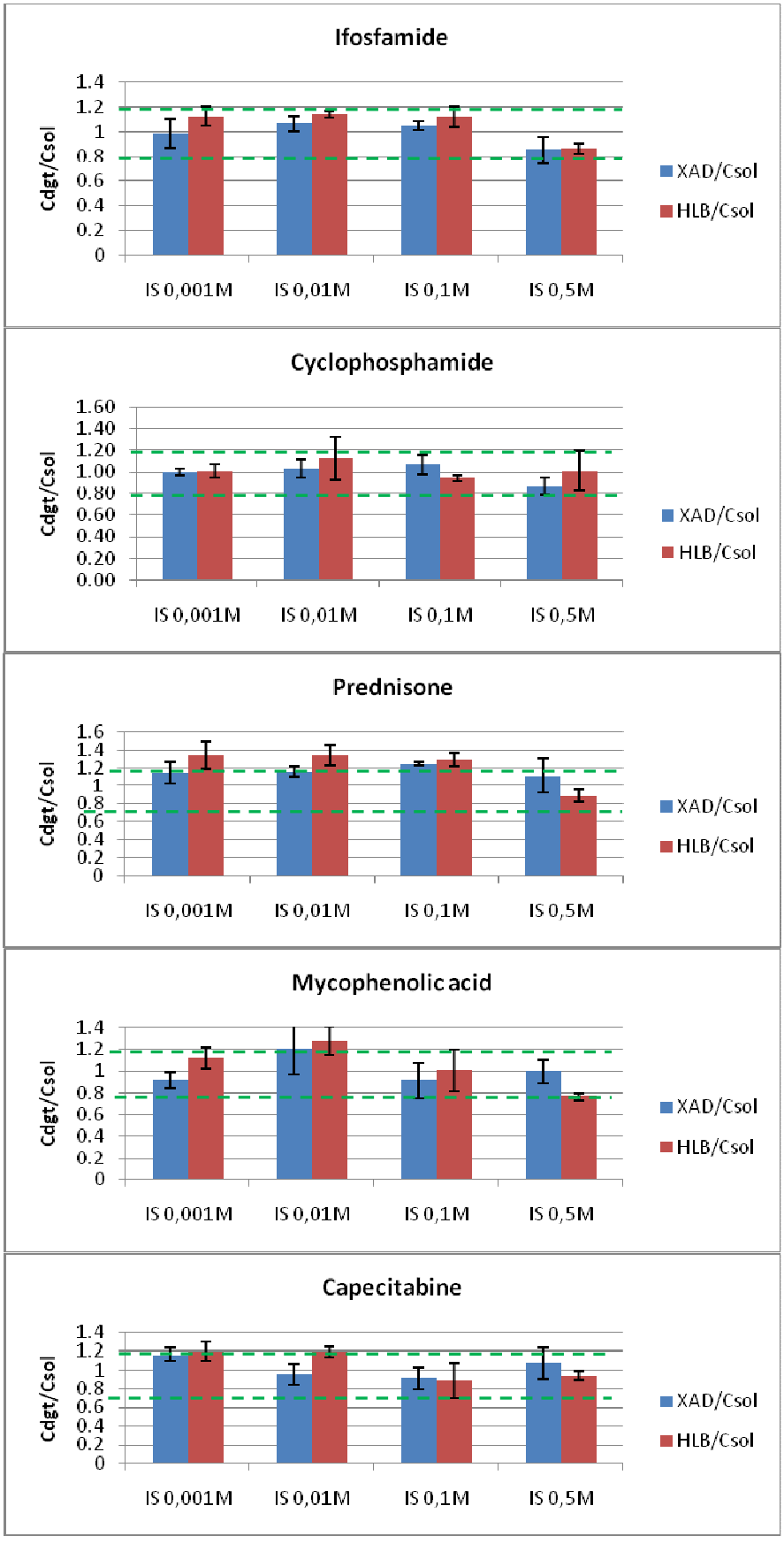
SI6. Time dependence: adsorbed mass (ng) in function of time (h) (n=2).



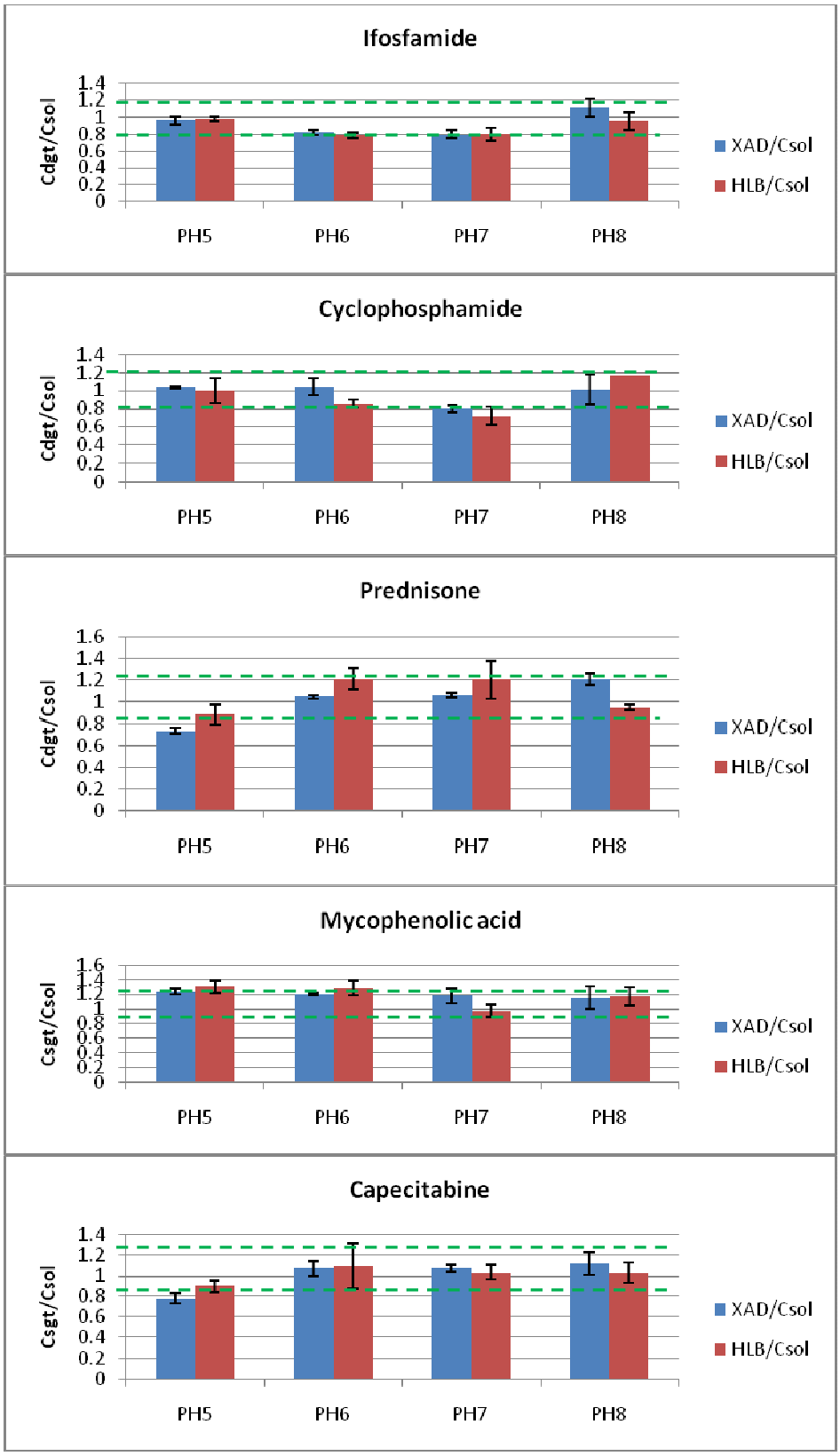
SI7. Diffusion layer thickness dependence: concentration of the solution using DGT calculated using equation 1 (Cdgt) divided by the real concentration in the solution (Csol) in function of diffusion layer thickness. Theoretical adsorption: ———



SI8. Effect of ionic strength



SI9. Effect of pH.



4.3. Discussió de resultats

Els mostrejadors passius s'han postulat com a alternativa al mostreig actiu ja que són capaços d'obtenir concentracions representatives de la presència de contaminants orgànics al medi ambient. A l'article científic IV, les concentracions detectades en el riu Besòs durant les dues campanyes de mostreig va ser molt diferents, indicant que els nivells d'aquests fàrmacs en aigües superficials varien significativament. Per tant, l'ús de mostrejadors passius pot representar una millora a l'hora de determinar la presència de citostàtics al llarg del temps. Malgrat els nombrosos avantatges d'aquesta tècnica, els articles presentats en aquesta tesi recullen per primera vegada el desenvolupament i el calibratge de mostrejadors passius per a l'anàlisi de citostàtics. La optimització del mostrejador ceràmic i el calibratge dels DGT s'ha recollit en els articles científics VI i VII respectivament.

Abans de poder aplicar aquests mostrejadors, es va determinar en primer lloc la configuració adequada per a cadascun (mida, material,porositat,...). Els mostrejadors passius ceràmics utilitzats en aquesta tesi van ser dissenyats i optimitzats conjuntament amb l'Institut de Ciència de Materials d'Aragó (ICMA-CSIC). Aquests mostrejadors es van desenvolupar seguint el model de dosímetre ceràmic creat per Martin et al. (2001), de 50mm de llargada, 1,5 mm de gruix i porus de 5 nm.Aquests dosímetres s'han utilitzat amb èxit per a la determinació d'hidrocarburs aromàtics policíclics (PAHs) en aigües subterrànies (Bopp et al., 2005) i retardants de flama (Cristale et al., 2013) però es van obtenir valors de velocitat de mostreig (Rs) no gaire elevats (1,5-2,5 mL/dia i 0,39-3,7 mL/dia respectivament) i calien llargs períodes de temps per preconcentrar els analits. Degut a la poca estabilitat d'alguns citostàtics, es requereixen mostrejadors més ràpids, que permetin la captació dels analits en menys temps. A més, si es vol determinar llur presència en aigües residuals, caldrà mostrejadors passius que siguin més permeables, i per tant també ràpids, per evitar problemes d'obturació i *fouling*. Per aquest motiu es va voler desenvolupar un mostrejador que complís aquests requisits. La diferència principal entre els dosímetres ceràmics i el desenvolupat en aquesta tesi rau en l'estructura interna de la membrana ceràmica. Els nous mostrejadors es van dissenyar de manera que els porus de la membrana d'alúmina fossin grans per afavorir al màxim la captació dels analits, però més petits que la mida de partícula del polímer adsorbent del seu interior, generalment d'uns 30 µm. Així, es van dissenyar aquests nous mostrejadors amb cavitats de 10-15 µm connectades per porus de menys de 200 nm de diàmetre. A la Figura 1 de l'Article VI es mostren les imatges en microscopi electrònic de la membrana, on es poden observar aquestes cavitats. La major

porositat ha de permetre més facilitat de pas dels analits a través de la ceràmica, que implica una major captació. Per aquest mateix motiu se'ls ha anomenat MCPS (*macroporous ceramic passive samplers*). Un cop preparada la ceràmica es van provar diferents configuracions del mostrejador, que es mostren en la Figura 4.3. Es van elaborar diferents gruixos (a), entre 0,8 i 2,5 mm, i es van provar els sistemes de tancament (b-c) fins obtenir la configuració final (d): 45 mm de llargada, 13 mm de diàmetre extern i 1,5 mm de gruix, amb taps cònics.

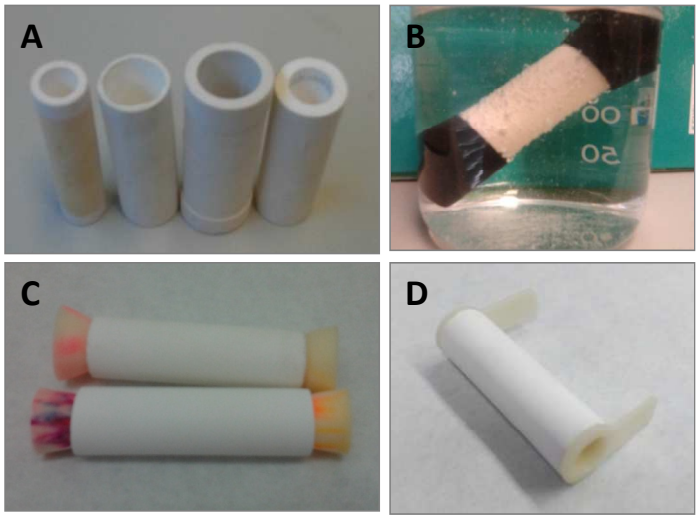


Figura 4.3. Proves per optimitzar la configuració del mostrejador ceràmic MCPS. a) diferents gruixos del mostrejador; b) tancament amb taps exteriors; c) tancament amb taps interiors; d) configuració final.

L'optimització dels DGT es va dur a terme a l'*Environment Centre*, de la Universitat de Lancaster, al Regne Unit, durant l'estada predoctoral de quatre mesos. L'elecció de la seva configuració (suport i materials) va ser més senzilla, ja que la seva versió per al mostreig de compostos inorgànics és comercial (www.dgtresearch.com). No obstant, les resines adsorbents per a compostos orgànics no estan disponibles i es van preparar al laboratori. La Figura 1 de l'Article VII mostra l'esquema de les diferents part de que consta aquest mostrejador, de les quals es va preparar el gel de difusió d'agarosa i la capa receptora de resina HLB o XAD. L'elaboració d'aquests gels és més complexa que el condicionament de l'adsorbent de l'MCPS, que es va activar amb MeOH i aigua abans de ser col·locat a l'interior del mostrejador. Per complementar la descripció de la preparació dels gels pel DGT de l'Article VII (secció 2.2), la Figura 4.4a mostra el procediment utilitzat. Per preparar el gel de difusió d'agarosa es mostren els vidres, separats per una peça blanca del gruix del gel desitjat. Els vidres es van subjectar amb les peces negres per evitar el seu desplaçament. Un cop a temperatura ambient, la solució gelifica i es van poden tallar els discs que van servir per muntar els mostrejadors. A la Figura 4.4b es mostren les plaques de vidre per a la preparació de la resina receptora, que va ser d'HLB o

XAD. Aquest material s'ha de preparar amb uns dies d'antelació amb previsió del nombre de mostrejadors que es necessitaran, ja que han d'estar unes hores en aigua per hidratar-se abans de poder-se utilitzar. Això representa un desavantatge en front els mostrejadors ceràmics, que poden preparar-se i ser utilitzats en un mateix dia.

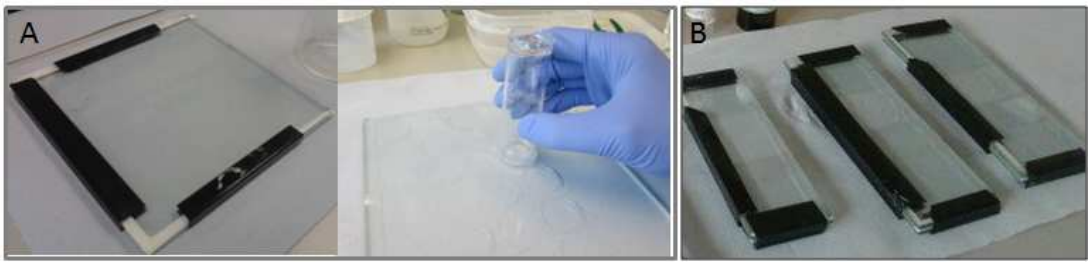


Figura 4.4. a) Preparació i tall de la capa de difusió b) Preparació dels gels receptors

Un cop preparades les diferents capes que conformen el DGT, es va fer el muntatge un a un (Figura 4.5). Aquest procediment també és més delicat que el muntatge dels MCPS i pot durar entre 1 i 10 min per mostrejador, depenent de l'habilitat de l'operador. En primer lloc es va col·locar el gel receptor sobre la base del DGT. Les partícules de resina es troben majoritàriament en una de les cares del gel, i per tant és important col·locar-lo en la direcció adequada. D'aquesta manera però, el gel té tendència a cargolar-se sobre si mateix (Figura 4.5a) i cal deixar-lo ben pla amb l'ajuda d'unes pinces, abans de posar la següent capa.

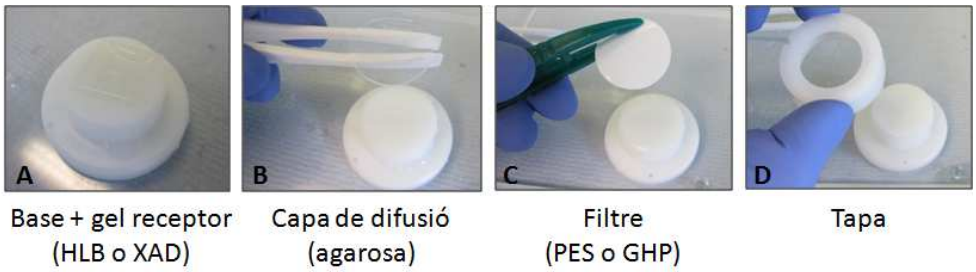


Figura 4.5. Procés de muntatge d'un mostrejador DGT. a) Col·locació de la base i el gel receptor, b) gel d'agarosa, c) filtre, d) tapa.

En segon lloc es va col·locar la capa de difusió d'agarosa (Figura 4.5b). En agafar-la amb les pinces s'ha d'anar en compte de no subjectar-la massa fort per no trencar-la, ja que és molt fràgil. Finalment, es va col·locar el filtre i la tapa que subjecta les diferents capes (Figura 4.5c-d). Quan es van preparar els DGTs per optimitzar el gruix de la capa de difusió (Article VII, secció 2.4.7) es va tenir en compte que entre la base i la tapa hi ha un espai determinat, pensat per subjectar les diferents capes sense que es moguin ni quedin massa comprimides. En les altres

proves de calibratge es va utilitzar la configuració més habitual per aquests mostrejadors: un gel receptor de 0,5 mm, una capa de difusió de 0,8 mm i un filtre de 0,1 mm, que sumen 1,4 mm. Per tant, en muntar els DGT amb un gel de difusió de 0,5 mm, es va col·locar també un gel de difusió més prim sota el gel receptor per suplir el gruix que faltava i evitar que quedés balder. Si no estigués ben ajustat, l'aigua passaria pels laterals enlloc de travessar la capa de difusió i l'adsorció dels analits no estaria controlada. En canvi, en utilitzar gels més gruixuts, de 1 o 1,5 mm, es va col·locar una petita peça entre la base i la tapa que va impedir que aquesta tanqués massa fort.

Un cop preparats els mostrejadors, el següent pas consisteix a fer-ne el calibratge. No obstant, per als MCPS, previ a la seva aplicació amb citostàtics es va provar l'eficàcia de les membranes amb blau de metilè ($C_{16}H_{18}ClN_3S$), un compost que se sol utilitzar per tenyir cèl·lules en estudis de citologia. Dissolt en aigua te un color blau intens que permet mesurar amb facilitat el seu pas a través de la membrana ceràmica mitjançant espectrofotometria. Per aquesta prova es van col·locar els mostrejadors sense adsorbent, plens d'aigua milliQ, en una solució amb blau de metilè i es van retirar després de 2, 5, 6 i 9 dies. A la Figura 4.6 es mostra com la concentració a l'interior de l'MCPS augmenta linealment amb el temps i que la concentració de la solució externa es manté estable després de decaure lleugerament. Amb aquesta prova es va calcular un Rs de 0,17 mL/dia, equivalent a una difusió de 1505 ng/dia, i un De de 2.59E-07 cm^2/s. El valor de Rs és menor que els descrits pel dosímetre ceràmic de Bopp et al. (2005) (1,5-2,5 mL/dia) però s'ha de tenir en compte que al no tenir adsorbent a l'interior no es va produir la preconcentració de l'analit. En aquesta prova, si els mostrejadors es deixessin més temps en solució, la concentració del blau de metilè a l'interior augmentaria fins arribar a igualar-se amb la concentració externa.

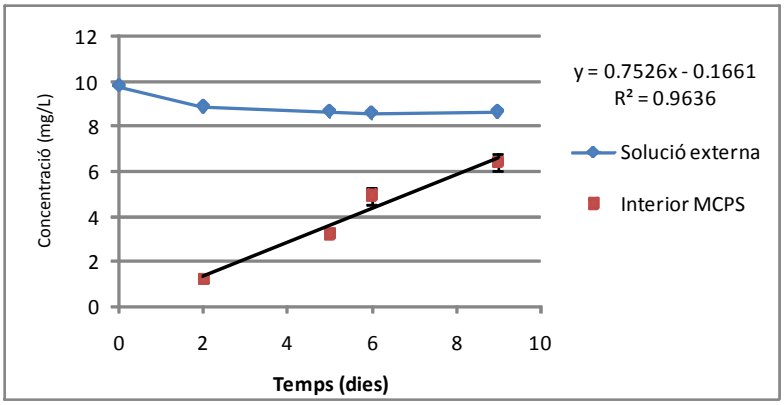


Figura 4.6. Concentració de blau de metilè a l'interior de l'MCPS i a la solució externa.

Havent comprovat que el blau de metilè, que simula els compostos orgànics, va poder travessar la membrana ceràmica, es va preparar un grup de mostrejadors amb adsorbent al seu

interior, tal com s'han de preparar per fer el calibratge i per l'aplicació en el medi i es van posar en la solució. En aquesta segona prova, el blau de metilè va quedar acumulat a l'adsorbent i l'aigua de l'interior del mostrejador no estava acolorida. Així, va quedar demostrat que els analits podien quedar retinguts en l'adsorbent i que es podia utilitzar aquesta configuració per fer el calibratge dels mostrejadors per a fàrmacs citostàtics. Aquest calibratge es va fer avaluant la captació dels analits en funció del temps, tal com s'ha descrit a l'Article VI, i va permetre calcular el De i el Rs per a 7 citostàtics (la ifosfamida, la ciclofosfamida, la capecitabina, la prednisona, el megestrol, la ciproterona i l'àcid micofenòlic), ja que la resta van hidrolitzar significativament en el temps de mostreig. Aquests compostos coincideixen amb els que s'han identificat com a compostos més estables envers a la hidròlisi en l'estudi de degradació de l'Article III.

En el calibratge, els mostrejadors DGT es van optimitzar per a 5 citostàtics. Aquests compostos es van escollir entre els més estables (com la ifosfamida i la ciclofosfamida) i entre els de PEC més elevat (com l'àcid micofenòlic, la capecitabina i la prednisona), i es van obviar els compostos que s'hidrolitzen ràpidament en aigua. En el seu calibratge, a més de determinar la captació de citostàtics en funció del temps, també es va avaluar l'efecte del pH i de la força iònica de la solució. Es va veure que aquests dos paràmetres no afecten significativament la retenció dels citostàtics, com es mostra a les imatges de la Figura 3, SI8 i SI9 de l'Article VII . Aquestes proves es van dur a terme al mateix temps que s'estaven posant a punt els MCPS i per tant, no va ser necessari avaluar els mateixos paràmetres amb el mostrejador ceràmic. Per a cap d'ambdós mostrejadors tampoc es va avaluar com afecta l'agitació del medi perquè, tant per al'MCPS i com pel DGT, la captació dels analits es basa en processos de difusió i, per tant, no hi influeix significativament.

En l'Article VI, a la Figura SI3, s'evidencia que en un període de 7 dies els MCPS poden retenir més de 100 ng. El calibratge es va fer durant un període de 11 dies però es va constatar que a partir del dia 7, la captació disminuïa. Aquest valors són més alts que els obtinguts per als retardants de flama bromats (2-3 ng) en un temps equivalent, però inferiors al obtinguts per als retardants de flama organofosforats (300-400 ng) (Cristale et al., 2013). En canvi, els DGT van poder retenir entre 250 i 600 ng en només tres dies (SI6, Article VII), demostrant una captació més ràpida dels analits. L'ús de mostrejadors passius s'ha utilitzat per a llargs períodes, 12 mesos per PAHs (Bopp et al., 2005) o almenys 3 setmanes per retardants de flama (Cristale et al., 2013). Malgrat fer la preconcentració *insitu*, la resina adsorbent continua estan en contacte amb l'aigua i per tan els compostos retinguts encara són susceptibles de ser hidrolitzats. Tant els PAHs com els retardants de flama són compostos molt estables en aigua i per tant, no es degraden un cop

captats en el mostrejador. En canvi, els fàrmacs citostàtics són, en comparació, més inestables i per això es recomanen períodes de mostreig més curts (1 setmana).

Els paràmetres de calibratge obtinguts per al blau de metilè i per als compostos citostàtics amb els dos mostrejadors es recullen a la Taula 4.2. Amb el blau de metilè es van obtenir els Rs i De més baixos, però es tracta d'un compost de prova que es va utilitzar sense adsorbent a l'interior del mostrejador només per observar si els analits podien travessar la membrana ceràmica.

Taula 4.2. Paràmetres de calibratge, Rs (mL/dia) i De (cm^2/s) dels mostrejadors passius MCPS i DGT

	MCPS		**DGT**	
	Rs (mL/dia)	De (cm^2/s)	Rs (mL/dia)	De (cm^2/s)
Blau de metilè*	0,170	5,70E-08		
Ifosfamida	1,165	1,43E-07	30	1,02E-05
Ciclofosfamida	1,028	1,27E-07	21	7,10E-06
Prednisona	1,425	1,78E-07	6,6	2,78E-06
Capecitabina	0,862	1,08E-07	6,1	2,22E-06
Àcid micofenòlic	0,943	1,16E-07	8,3	2,05E-06
Megestrol	3,35	4,12E-07		
Ciproterona	0,825	1,01E-07		

*sense adsorbent

Per als citostàtics, els valors van ser 10 vegades més grans (Rs de 0,82-3,3 mL/dia i De de 1,1E-07-4,1E-7 cm^2/s). Els millors resultat però, es van obtenir amb els DGT, amb Rs de 6,1-30 mL/dia i De de 2,05E-6-1,02E-5 cm^2/s. El gruix dels DGT és menor que el dels MCPS (0,9 i 1,5 mm respectivament), però l'àrea de contacte amb el medi és també menor (3,14 i 14,12 cm^2 respectivament), per tant no són valors suficients per explicar aquestes diferències. L'explicació a aquest comportament pot raure en la pròpia configuració del mostrejador. Mentre que l'MCPS té la resina adsorbent dipositada al seu interior, la resina al DGT es troba subjecte amb el gel i completament en contacte amb la superfície del gel de difusió d'agarosa, fet que maximitza el contacte i per tant, la captació dels analits. Amb aquests valors de calibratge sembla que els mostrejadors DGT poden ser més adequats per al mostreig de citostàtics en aigües, ja que la velocitat de mostreig és més elevada.No obstant, la seva aplicació real encara no s'ha pogut provar.

L'aplicabilitat per a l'anàlisi de citostàtics al medi es va provar per als MCPS en una EDAR. Malgrat que les aigües de l'influent van obturar els mostrejadors, a la sortida de la planta es va poder detectar l'àcid micofenòlic i la ciclofosfamida, a concentracions comparables a les detectades mitjançant l'extracció per SPE, com es mostra a la Figura 4 de l'Article VI. Altra

vegada, l'àcid micofenòlic va ser el citostàtic detectat a concentracions més elevades, fet que confirma una vegada més l'efectivitat de la priorització mitjançant el càlcul de PECs.

A l'hora d'escollir un mostrejador també és important tenir en compte el seu cost. L'adsorbent de l'interior de cada MCPS te un cost de 2,5 €, la ceràmica al voltant d'1 € i els taps 0,5 €. Per tant, un MCPS tindria un cost total d'aproximadament 4 €, al quals caldria afegir el cost de fabricació i de l'operari. D'altra banda, els DGT comercials per compostos inorgànics tenen un cost de £13,50 (uns 16 €), molt més elevat que els MCPS però permeten l'anàlisi de 30 metalls diferents. Per a compostos orgànics, caldria preparar els DGT al laboratori obtenint els diferents materials per separat, però el preu seria del mateix ordre, tot i que caldria afegir-hi el temps de preparació.

Els mostrejadors passius han demostrat un bon potencial per a l'anàlisi de citostàtics en aigües. Aquest sistema de mostreig ha donat més bons resultats per aquells compostos més estables, pels quals s'han pogut calcular els paràmetres de calibratge. I encara que els resultats en aigües d'influents de les EDAR per als MCPS s'han vist afectats per la presència de matèria orgànica aquest problema no s'ha donat en els efluents i s'espera que la seva aplicació en aigües superficials sigui igual de bona. A més, actualment s'està treballant en millorar les característiques de la membrana ceràmica amb l'objectiu de maximitzar la captació dels analits i obtenir uns mostrejadors encara més ràpids. Els mostrejadors DGT també han demostrat molt bones qualitats, que els fan uns bons candidats per a l'anàlisi de citostàtics. No obstant, també caldria veure com afecta la presència de matèria orgànica a aquest mostrejador.

CONCLUSIONS

Els estudis que s'han dut a terme durant el desenvolupament d'aquesta tesi han permès arribar a les següents conclusions:

- El consum dels fàrmacs antineoplàstics amb codi L administrats a Catalunya va ser de 4,7-4,9 tones anuals (2010-2012), mentre que a Espanya va ser de 25,2-21,8 tones anuals (2010-2015). Aquestes dades van permetre calcular les concentracions previstes al medi (PEC) per a cada fàrmac consumit.
    - El càlcul dels PECs ha identificat l'àcid micofenòlic com el compost que s'espera trobar a concentracions més elevades, seguit de la hidroxicarbamida, la capecitabina, la bicalutamida, l'imatinib i la prednisona.
    - Els PECs distribuïts en funció de les conques hidrogràfiques de la península Ibèrica ha identificat la conca del riu Tajo amb uns PECs més elevats.
    - D'acord amb l'avaluació de risc, les concentracions previstes no suposen un risc agut per als organismes aquàtics, però no es poden descartar efectes a llarg termini.

- S'ha desenvolupat un mètode basat en l'extracció en fase sòlida i la cromatografia de líquids acoblada a l'espectrometria de masses per a la determinació de 19 fàrmacs citostàtics en aigua superficial.
    - L'extracció s'ha de realitzar abans de 24h de la recollida de la mostra per evitar pèrdues dels compostos més inestables.
    - L'extracció en fase sòlida ha permès recuperar 14 fàrmacs amb elevada eficiència però la gemcitabina, l'irinotecà, la doxorubicina, el melfalan i el clorambucil van obtenir recuperacions més baixes degut que s'hidrolitzen en aigua.
    - La hidroxicarbamida no ha pogut ser analitzada degut a la seva elevada polaritat.
    - S'ha observat que per a la majoria de citostàtics l'efecte matriu és mínim i no afecta l'extracció dels analits de les mostres de riu.
    - Tant la cromatografia de líquids acoblada a l'espectrometria de masses en tàndem (LC-MS/MS) com a l'espectrometria de masses d'alta resolució (LC-Orbitrap-MS) permeten la detecció de citostàtics a nivells de ng/L, amb bona resolució i selectivitat.

- Degut que els citostàtics es detecten en aigua a nivells de ng/L la quantificació amb patrons marcats isotòpicament ha permès obtenir bona linealitat i reproducibilitat a nivells traça.

- S'ha realitzat un estudi de monitorització ambiental per avaluar la presència de citostàtics al llarg de la conca del riu Besòs.
  - S'ha detectat la presència de set citostàtics (tamoxifè, ifosfamida, ciclofosfamida, erlotinib, megestrol, clorambucil i àcid micofenòlic) amb concentracions més elevades al tram baix del riu.
  - S'han detectat concentracions relativament més elevades durant el mostreig d'estiu, relacionat amb cabals més petis.
  - En aquest estudi s'ha identificat per primera vegada la presència d'àcid micofenòlic en aigua superficial.

- S'ha determinat la presència d'àcid micofenòlic a l'entrada d'una planta potabilitzadora d'aigua, però l'anàlisi de l'aigua dels diferents tractaments ha determinat que aquest no arriba a l'aigua de distribució.
  - L'estudi de degradació de l'àcid micofenòlic amb diòxid de clor ha confirmat la seva ràpida eliminació sense formació de productes de degradació.

- L'estudi de degradació de 16 citostàtics sota diferents tractaments ha permès calcular les constants de degradació i identificar els citostàtics més persistents.
  - Els fàrmacs que degraden en aigua pura són la vincristina, la vinblastina, la daunorubicina, la doxorubicina i l'irinotecà i el clorambucil.
  - La biodegradació va permetre eliminar la prednisona, la citarabina i el megestrol.
  - El tractament amb llum UV va permetre eliminar el melfalan i l'etopòsid.
  - L' UV-H_2O_2va permetre eliminar tota la càrrega de citostàtics en 4 min.
  - El simulador de llum solar no va aconseguir degradar completament els fàrmacs estudiats.
  - Tenint en compte tots els tractaments, els compostos més persistents són la gemcitabina, la citarabina, la capecitabina, la ifosfamida, la ciclofosfamida i l'àcid micofenòlic, i per tant tenen més probabilitat de ser detectats al medi.

- S'ha dissenyat un mostrejador passiu ceràmic per a l'anàlisi de citostàtics en aigua.

- S'han optimitzat les característiques de la membrana ceràmica i la configuració del mostrejadors per obtenir una captació ràpida dels analits.
- El calibratge al laboratori va permetre calcular el coeficient de difusió per als set citostàtics més estables en aigua (ifosfamida, ciclofosfamida, capecitabina, prednisona, megestrol, ciproterona i àcid micofenòlic).
- L'aplicació del mostrejador en aigües residuals d'influent de depuradora no va permetre l'anàlisi de citostàtics degut a la oclusió dels porus.
- L'aplicació en l'efluent de depuradora va donar nivells de citostàtics comparables a les mostres puntuals recollides en el mateix període.

- S'ha fet el calibratge dels mostrejadors passius DGT per els cinc citostàtics més estables.
  - Es van calcular els coeficients de difusió i la velocitat de mostreig per a la ciclofosfamida, la ifosfamida, l'àcid micofenòlic, la capecitabina i la prednisona.
  - S'ha comprovat que les variacions de pH i força iònica del medi no afecten la capacitat del DGT per captar els citostàtics estudiats.
  - L'elevada difusivitat permet preconcentrar els analits amb elevada eficàcia, tot i que el sistema no s'ha provat en condicions reals.

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LLISTAT D'ABREVIATURES I ACRÒNIMS

ACN	Acetonitril
AMI	Aminoglutetimida
AOP	Processos d'oxidació avançada
ATC	*Anatomical Therapeutic Chemical*
AZA	Azatioprina
CE	Electroforesi capil·lar
CHL	Clorambucil
CIP	Ciproterona
CPt	Cisplatí
Cw	Concentració al medi
CYC	Ciclofosfamida
CYT	Citarabina
DAD	Detector de feix de díodes
DAU	Daunorubicina
DCM	Diclorometà
De	Coeficient de difusió
DGT	*Diffusive gradients in thin films*
DF	Factor de dilució
DOC	Docetaxel
DOX	Doxorubicina
EA	Acetat d'etil
EC_{50}	Concentració efectiva
ECHA	*European Chemicals Agency*
EDAR	Estació depuradora d'aigües residuals
EI	Impacte electrònic
EMA	European Medicines Agency
EPI	Epirubicina
ERL	Erlotinib
ESI	Electroesprai
ETAP	Estació de tractament d'aigua potable
ETO	Etopòsid
Fexc	Fracció excretada
FLU	Fludarabina
Fstp	Fracció emesa per les depuradores

5FU	5-fluorouracil
Fwwtp	Fracció eliminada en depuradores
GC	Cromatografia de gasos
GEM	Gemcitabina
GOS	Goserelina
H	Constant d'Henry
HCOOH	Àcid fòrmic
HILIC	*Hydrophilic interaction líquid chromatography*
HPLC	Cromatografia de líquids d'elevada eficàcia
HYD	Hidroxicarbamida
ICP	Plasma d'acoblament inductiu
IFO	Ifosfamida
IMA	Imatinib
IRI	Irinotecà
Kow	Constant octanol-aigua
LC	Cromatografia de líquids
LC_{50}	Concentració letal
LEU	Leuprolida
LLE	Extracció líquid-líquid
LOD	Límit de detecció
LOEC	*Lowest observed effect concentration*
MBR	Bioreactor de membrana
MCPS	*Macroporous ceramic passive sampler*
MDL	Límit de detecció del mètode
MEC	Concentració mesurada al medi
MEG	Megestrol
MEL	Melfalan
MeOH	Metanol
MET	Metotrexat
MITO	Mitomicina
MPA	Àcid micofenòlic
MRM	*Multiple reaction monitoring*
MS	Espectrometria de masses
MS/MS	Espectrometria de masses en tàndem

MTBE	Èter metil tert-butílic
NaOH	Hidròxid de sodi
NCI	Ionització química negativa
NOEC	*No observed effect concentraction*
OECD	*Organisation for Economic Co-operation and Development*
OMS	Organització Mundial de la Salut
PAC	Paclitaxel
PAH	Hidrocarburs aromàtics policíclics
PEC	Concentracions previstes al medi
PEC_{riu}	Concentració prevista en aigües superficials
PEC_{wwtp}	Concentració prevista en l'efluent de depuradora
POCIS	*Polar organic chemical integrative sampler*
PRE	Prednisona
Rs	Velocitat de mostreig
SPE	Extracció en fase sòlida
SPMD	*Semi-permeable membrane devices*
TAC	Tacrolimus
TAM	Tamoxifè
TEM	Temozolomida
UHPLC	Cromatografia de líquids d'ultra elevada eficàcia
UV	Radiació ultraviolada
VINB	Vinblastina
VINC	Vincristina
VINO	Vinorelbina
WW	Consum d'aigua per habitant