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Ecotoxicological tools to assess cytostatics effects in freshwater environments: in the aid of drugs prioritization

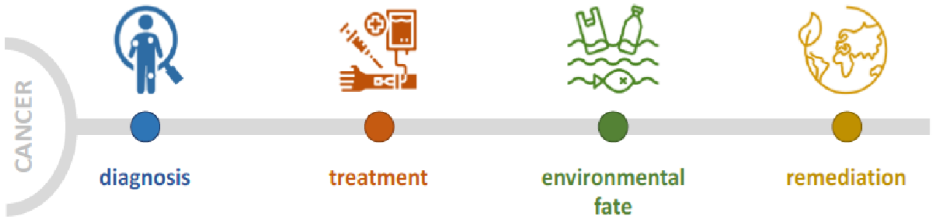
Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

Cátia Venâncio[1*], Bruna Monteiro[2], Rafael Francisco[3], Márcia Neves[3], Mara G. Freire[3], Ana Catarina Sousa[3], Isabel Lopes[1*]

[1]CESAM-Centre for Environmental and Marine Studies, University of Aveiro, Aveiro, Portugal; [2]Department of Biology, University of Aveiro, Aveiro, Portugal; [3]CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

IonCytDevice

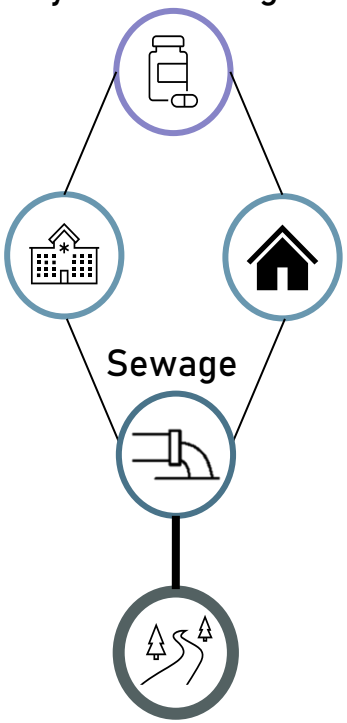


*catiavenancio@gmail.com
*ilopes@ua.pt

Ecotoxicological tools to assess cytostatics effects in freshwater environments: in the aid of drugs prioritization

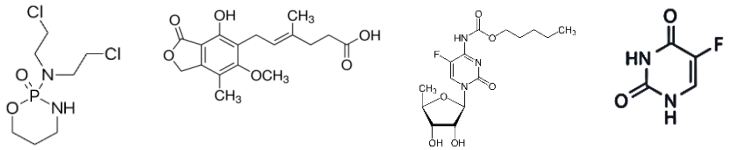
The problem…

Cytostatic drugs



The workplan…

CYP | MPA | CAP | 5-FU



$LC(E)_{50}$/AF as PNEC

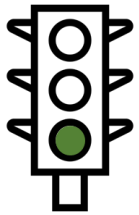
MEC → ↓

Risk Quotient (RQ)

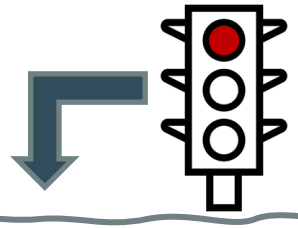
The result…

RQ

CYP 0.003

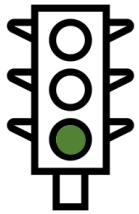


MPA 970.6

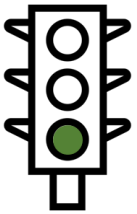


Need to prevent or mitigate their entrance in the environment

CAP 0.068



5-FU 0.449



Abstract| Keywords

Given the growing number of cancer diseases, new cytostatic drugs are approved daily, often with concomitant development or refinement of some of these drugs aiming at decreasing patient discomfort during administration period (e.g. prodrugs). Classified as highly toxic, they represent a major environmental problem that may potentiate disease occurrences. For newer cytostatics and pro-drugs there are no (or few) reported effects to aquatic organisms, which constraints their prioritization.

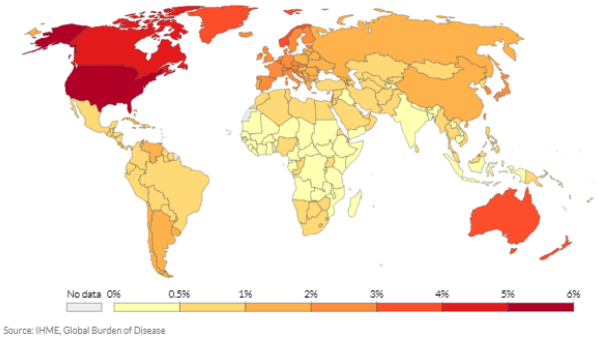
In face of the points raised, the IonCytDevice project intended to bridge some of these knowledge gaps and has delivered very important benchmarks. Predictions have been obtained on the environmental impacts of three cytostatics (cyclophosphamide: CYP; 5-fluoroucil: 5-FU; and mycophenolic acid: MPA) and one prodrug (capecitabine: CAP) on freshwater biota, with focus on new species and endpoints likely to be framed in meta-analysis studies as well. The results revealed that, for now, CYP, 5-FU, and CAP (prodrug) pose no risk, whilst MPA was flagged as of high environmental risk.

Keywords: Risk quotient, anticancer drugs, *Raphidocelis subcapitata, Brachionus calyciflorus*, *Hydra viridissima*, *Danio rerio*

Introduction |Cancer and cytostatics drugs in numbers

WORLWIDE
17 million new cases

~ 27.5 million new cases of cancer each year by 2040



Cytostatics consumption

Introduction |Cancer and cytostatics drugs in numbers

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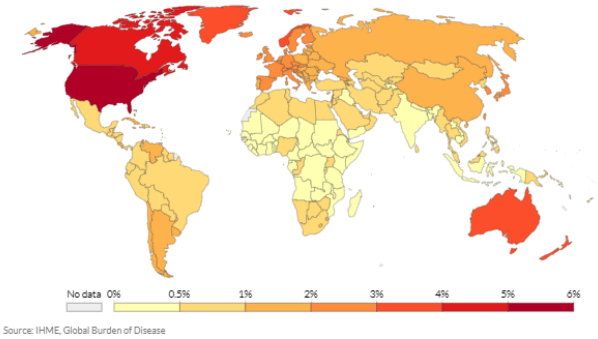
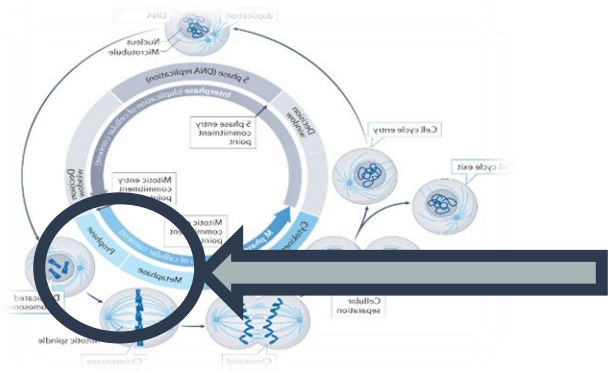


Cytostatics consumption

- Also known as antineoplastic drugs
- Synthetic or natural origin

Mode of action



DNA replication blocking in the tumor cells

Metastasis

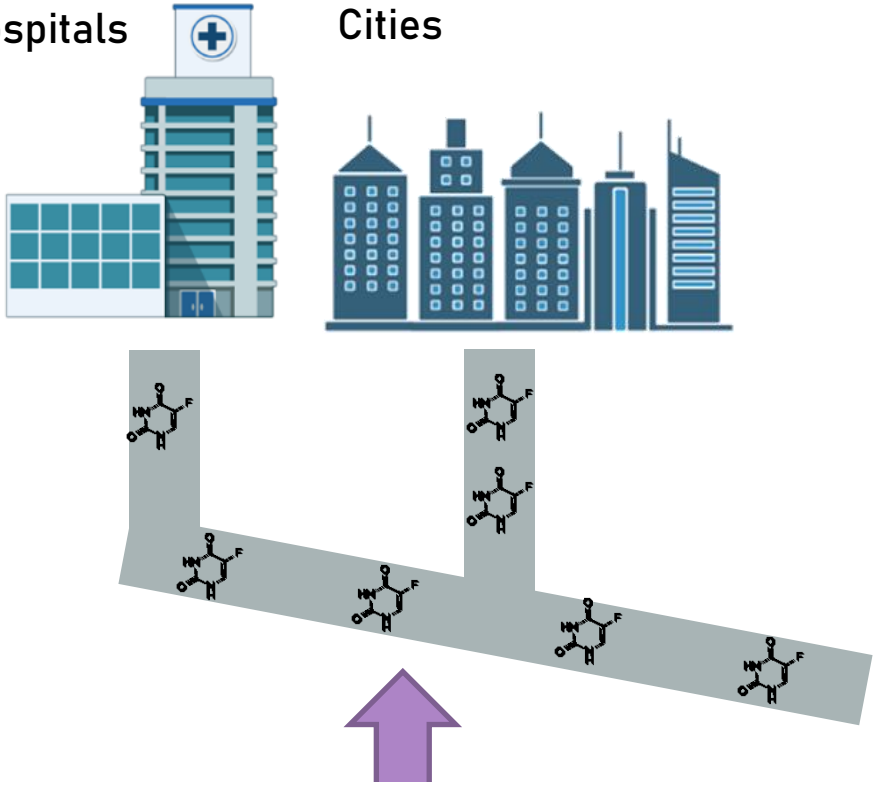
Upon administration: fate of cytostatics?

Introduction |Upon administration: fate of cytostatics?

Key Emission Sources

Hospitals

Cities



Cytostatics unmetabolized by patients

(Ort et al., 2010)

Introduction |Upon administration: fate of cytostatics?

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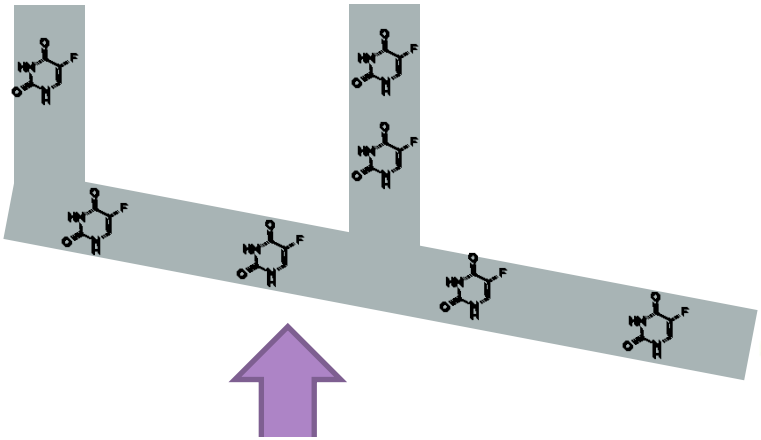
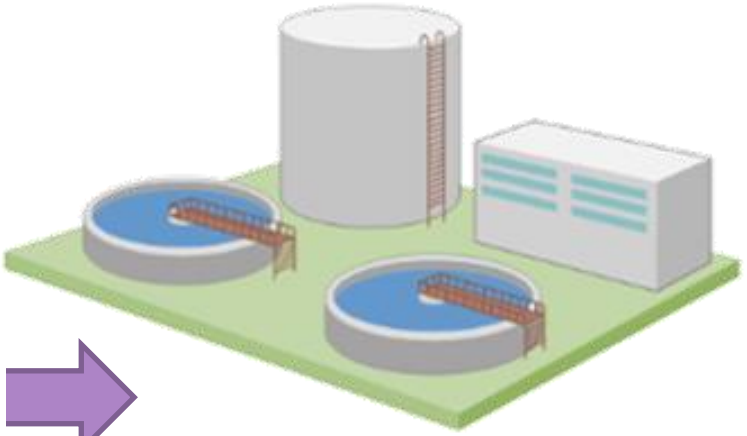


Cytostatics unmetabolized by patients

(Ort et al., 2010)

Wastewater treatment plants (WWTP)

- Not significantly removed
- Low-efficiency removing methods
- Interference with other matrices
- High persistence



Introduction |Upon administration: fate of cytostatics?

IonCytDevice

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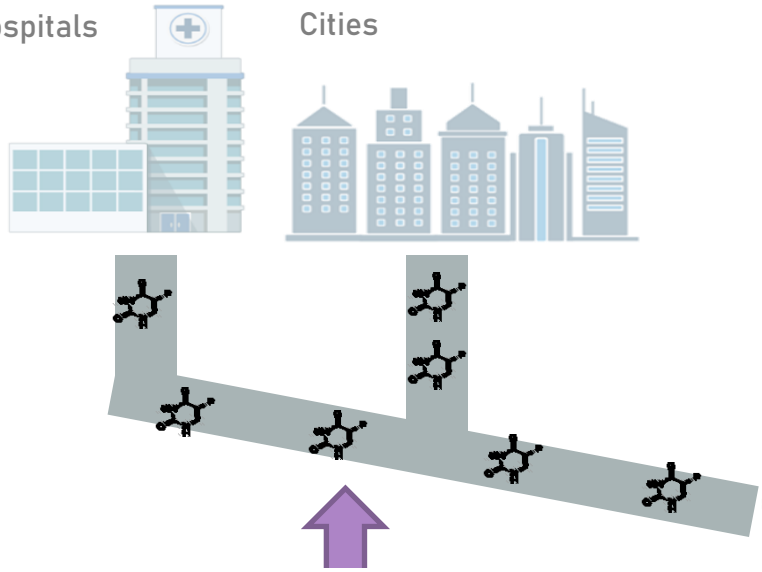
Cities



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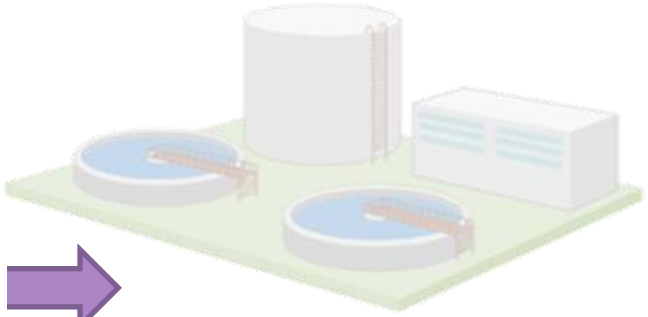


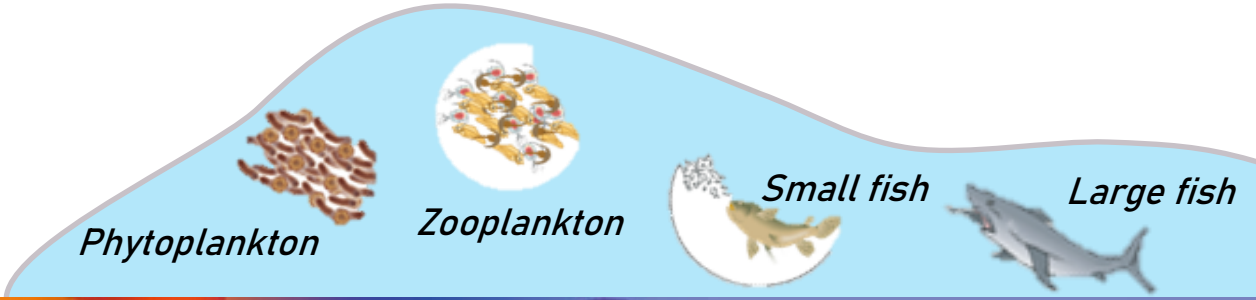
FINAL FATE: ENVIRONMENT

Up to 20% of unmetabolized compound, depending on the cytostatic

Introduction |Ecotoxicology in the aid of cytostatics prioritization

SMALL DOSAGES for effect

UNSELECTIVE potentially targeting any living organism/cell



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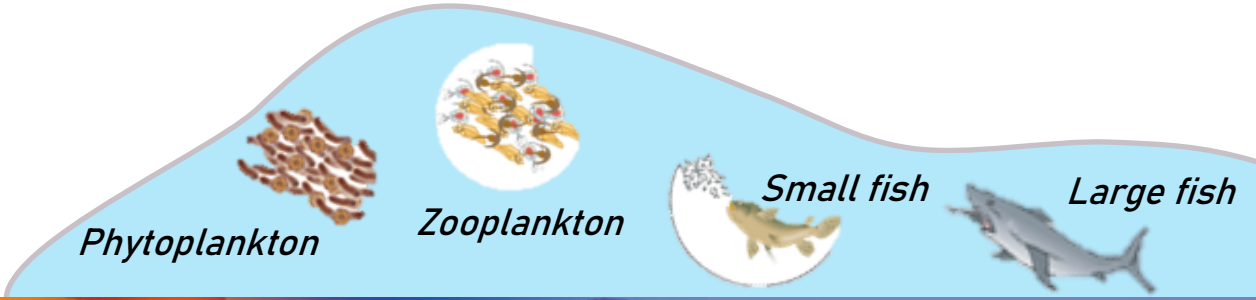
- Cytotoxic
- Genotoxic
- Mutagenicity
- Teratogenicity



Introduction |Ecotoxicology in the aid of cytostatics prioritization

SMALL DOSAGES for effect

UNSELECTIVE potentially targeting any living organism/cell

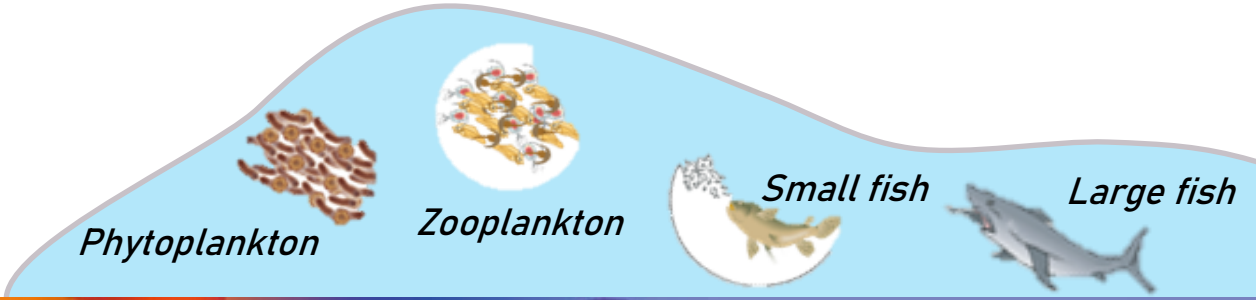
Cytotoxic

Genotoxic

Mutagenicity

Teratogenicity

- Major environmental threat
- Household consumption



Objectives

Predict the environmental impacts of four cytostatics in freshwater biota using key trophic level species

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Predict the environmental impacts of four cytostatics in freshwater biota using key trophic level species

Deliver updated information on the potential environmental hazard of two widely used cytostatic drugs (1st case study) and a cytostatic drug and its pro-drug (2nd case study)

Methodology | Median lethal/sublethal concentrations LC_{50}/EC_{50}

Producers

Raphidocelis subcapitata
OECD (2011) 201: Freshwater alga and cyanobacteria, growth inhibition test. OECD Guidelines for the Testing of Chemicals

Primary consumers

Brachionus calyciflorus
MicroBioTests Inc.
Standard Operation Procedure

Secondary consumers

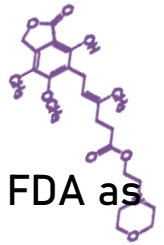
Danio rerio
OECD (2013) 236: Fish embryo acute toxicity (FET) test. OECD Guidelines for the Testing of Chemicals
Hydra viridissima
Trottier et al., 1997; Quinn et al., 2012

1st CS | Contributions towards the hazard evaluation of two widely used cytostatic drugs*

Cyclophosphamide (CYP)

- Model cytostatic; used in clinical context since the 40's
- Widely used

Mycophenolic acid (MPA)



- Novel drug; classified by the US FDA as a priority drug
- Increasing administration rates

1st CS |Contributions towards the hazard evaluation of two widely used cytostatic drugs*

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- 5-25% excretion as parent compound

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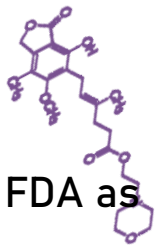

- Novel drug; classified by the US FDA as a priority drug
- Increasing administration rates
- < 1% excretion as parent compound

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- Probably the largest dataset available regarding its ecotoxicity

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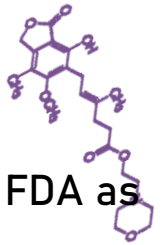

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- Low threat to the environment

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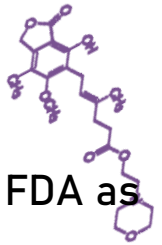

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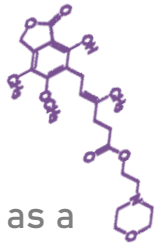

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BENCHMARKS difficult to obtain data reported as > X mg/L or, based on qualitative annotations

NO DATA
No clear conclusion on hazard assessment

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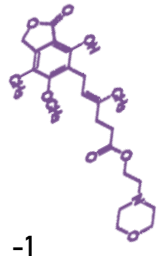
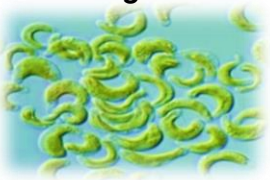
Mycophenolic acid (MPA)



algae



- Yield, $EC_{50,72h}$: 593.0 mg L^{-1}
- Growth rate, $EC_{50,72h}$: 1108 mg L^{-1}

- Yield, $EC_{50,72h}$: 0.00068 mg L^{-1}
- Growth rate, $EC_{50,72h}$: 0.00167 mg L^{-1}

rotifer

- Mortality, $LC_{50,24h}$: 6397 mg L^{-1}

- Mortality, $LC_{50,24h}$: not determined; > 30 mg L^{-1}

fish

- Mortality, $LC_{50,96h}$: 1306 mg L^{-1}
- Abnormalities, $EC_{50,96h}$: 1030 mg L^{-1}

- Mortality, $LC_{50,96h}$: 1.410 mg L^{-1}
- Abnormalities, $EC_{50,96h}$: 0.160 mg L^{-1}
- Hatching, $EC_{50,96h}$: 0.945 mg L^{-1}

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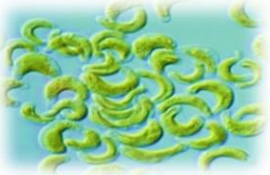

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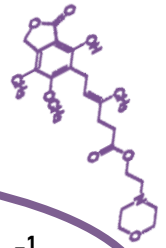

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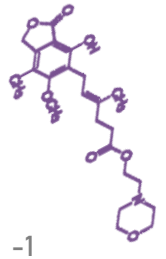

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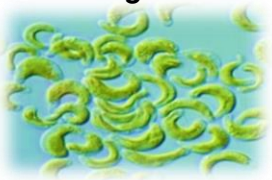

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1000-fold difference

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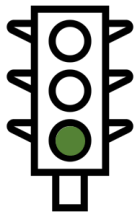
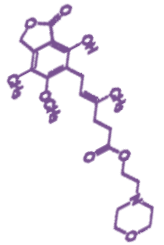
<u>Environment:</u>
concentrations up to 0.0019 mg L^{-1}



NEGLIGIBLE RISK

Mycophenolic acid (MPA)



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<u>Environment:</u>
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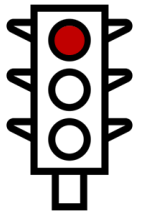


HIGH RISK

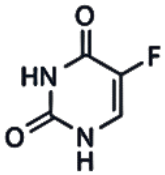
2nd CS | Comparing the pro-drug capecitabine (CAP) with its active metabolite 5-fluorouracil (5-FU)

IonCytDevice

Capecitabine (CAP)

- Pro-drug developed to reduce patient discomfort upon administration
- Second most prescribed cytostatic

5-fluorouracil (5-FU)



- Highly prescribed cytostatic
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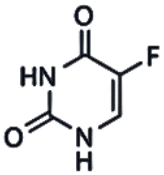
5-fluorouracil (5-FU)



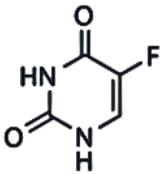
- Highly prescribed cytostatic
- Intravenous administration
- 7-20% excretion as parent compound

2nd CS | Comparing the pro-drug capecitabine (CAP) with its active metabolite 5-fluorouracil (5-FU)

Capecitabine (CAP)

- Pro-drug developed to reduce patient discomfort upon administration
- Second most prescribed cytostatic
- 3% excretion as parent compound
- One of the least cytostatics studied

5-fluorouracil (5-FU)



- Highly prescribed cytostatic
- Intravenous administration
- 7-20% excretion as parent compound
- Large dataset on ecotoxicological effects but reported data with several orders of magnitude difference

2nd CS | Comparing the pro-drug capecitabine (CAP) with its active metabolite 5-fluorouracil (5-FU)

IonCytDevice

Capecitabine (CAP)

- Pro-drug developed to reduce patient discomfort upon administration
- Second most prescribed cytostatic
- 3% excretion as parent compound
- One of the least cytostatics studied
- Yet to be defined

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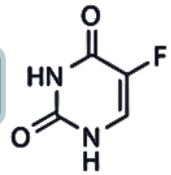

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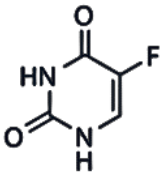

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Urgent delivery of ecotoxicity data and risk assessment

Further evidence is necessary to draw solid conclusions on whether it poses risk or not

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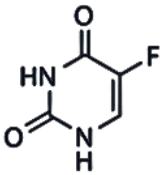
Capecitabine (CAP)

5-fluorouracil (5-FU)

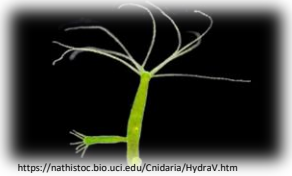


algae

- Yield, $EC_{50,72h}$: 0.077 mg L^{-1}
- Growth rate, $EC_{50,72h}$: 0.630 mg L^{-1}

- No effect

cnidarian



https://nathistoc.bio.uci.edu/Cnidaria/HydraV.htm

- Mortality, $LC_{50,24h}$: no mortality
- Malformations, $EC_{50,96h}$: 1155.6 mg L^{-1}
- Feeding rate, $EC_{50,96h}$: 22.0 mg L^{-1}

- Mortality, $LC_{50,24h}$: 55.4
- Feeding rate, $EC_{50,96h}$: 67.94 mg L^{-1}

fish

- No effect

- Mortality, $LC_{50,96h}$: 4546 mg L^{-1}
- Abnormalities, $EC_{50,96h}$: 2459 mg L^{-1}
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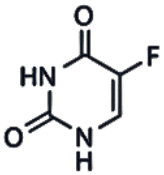
Capecitabine (CAP)

5-fluorouracil (5-FU)



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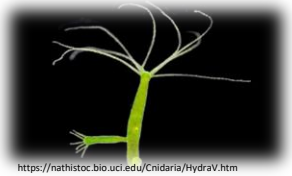

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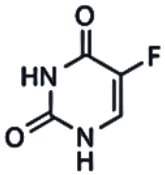

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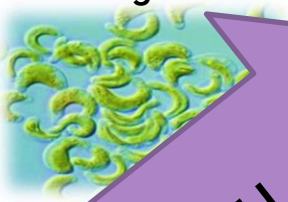

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effect

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MISMATCH

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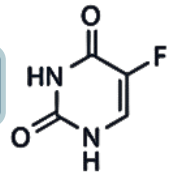

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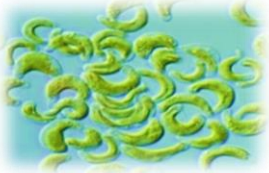
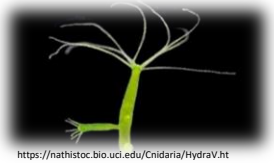


- No effect

cnidarian



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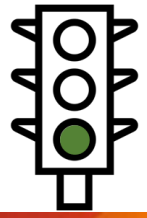
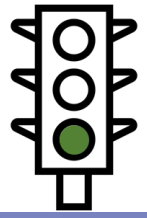
<u>Environment:</u>
concentrations up to
0.00114 mg L^{-1}



NEGLIGIBLE RISK

<u>Environment:</u>
concentrations up to
0.00124 mg L^{-1}



NEGLIGIBLE RISK

Remarks | Aiding on cytostatics prioritization

Cyclophosphamide

- zebrafish provided very important sublethal endpoints likely to provide insights on potential teratogenic effects of this drug
- estimated doses for effect were much higher than those reported for the environment

Mycophenolic acid

- Presented a very high risk to freshwater biota, with an RQ of 965
- Previous studies have reported a distinct classification for MPA: the need of a solid database or a widely accepted guideline with standardized methods and criteria concerning the risk assessment of different pharmaceuticals that could be used worldwide, to minimize the uncertainties associated with these classifications/prioritizations.

Remarks | Aiding on cytostatics prioritization

Capecitabine **5-Fluorouracil**

- First data records on *H. viridissima* and CAP e 5-FU.
- 5-FU was several orders of magnitude more toxic than CAP: suggests CAP development to be a good alternative both for patients and the environment.
- Mismatch between toxic effects posed to different trophic groups highlights the need to deliver these comparisons, with integration of many species and endpoints as possible.

Acknowledgements |

This work was developed within the framework of the projects CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020), CICECO–Aveiro Institute of Materials (UIDB/50011/2020 and UIDP/50011/2020), and CHRC-Comprehensive Health Research Centre (UIDP/04923/2020), financed by national funds through FCT/MCTES and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement, through national funds (FCT/MCTES). Financial support was provided by the project POCI-01-0145-FEDER-031106 (IonCytDevice - PTCD/BTA-BTA/31106/2017) funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. A.C. Sousa acknowledges University of Aveiro, for funding in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. M.C. Neves acknowledges FCT, I.P. for the research contract CEECIND/00383/2017 under the CEEC Individual 2017.

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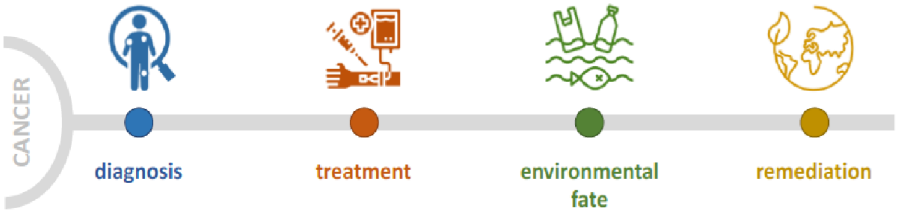

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