Results of the FP7 project CYTOTHREAT - Fate and effects of cytostatic pharmaceuticals in the environment and identification of biomarkers for an improved risk assessment on environmental exposure

Summary of the presentation at Science-Policy Event on Pharmaceuticals in the Environment (21.11.2013, Brussels, Belgium) by Metka Filipic, National Institute of Biology (Slovenia)

(Please scroll down in this document for presentation slides.)

The consumption and consequently environmental exposure to anticancer drugs is much lower from that of many other pharmaceuticals. However, the mechanisms of action of most of the anticancer drugs are by interference with genetic material and cell signalling, which are very similar in all organisms. Theoretically exposure to anticancer drug residues may affect also non-targeted organisms. The project CytoThreat (www.cytothreat.eu) is emphasized on the evaluation of the environment and human health risks posed by residues of anticancer drugs released into environment. The aims are to provide new analytical methods needed for to determine the actual environmental exposure of these drugs, their metabolites and transformation products detection, to provide missing ecotoxicity data needed for accurate environmental risk assessment and identify biomarkers of delayed effects that may be used for development of early warning systems.

The partners developed a multi-residue method based on on-line SPE-LC-MS/MS and direct sample injection LC-MS/MS method for analysis of up to 43 compounds and metabolites with detection and quantification limits in the range of nano to pico gram per liter. In degradation/transformation studies they evaluated different methods and conditions, and also identified a number of transformation products including 13 not known before. The analysis of real samples confirmed the presence of certain anticancer drugs and metabolites in wastewaters, but except cis-platin, not in recipient surface waters.

The ecotoxicity studies were performed with four selected drugs (5-fluorouracil, etoposide, cis-platin and imatinib mesilate) with aquatic organisms at three trophic levels (phytoplankton, crustacean and fish) and were coupled to the determination of genotoxic effect and gene expression analysis. In D. magna and C. dubia significant reduction of reproduction was observed at concentrations of several hundreds of ng/L (5-fluorouracil and cis-platin) to µg/L concentration range (etoposide and imatinib mesilate), whereas significant increase in DNA damage (comet assay) was already after 24h exposure for all four compounds observed at lower concentrations than inhibition of reproduction. This indicates association of DNA damage with the reduced reproduction. Therefore in crustacean detection of DNA damage may be proposed as potential early biomarker of adverse reproductive effects. Zebrafish were relatively insensitive to the acute exposure as well as sub-chronic exposure to the selected anticancer drugs. The chronic two generation toxicity study with 5-fluorouracil revealed histopathological changes in liver and kidney, induction of micronuclei in blood cells and changes in gene expression in liver in fish exposed to 10 ng 5-fluorouracil/L. This result indicates that the Early life stage toxicity test in fish, that is currently proposed by EMA may not be appropriate for detection of the effects of chronic exposure to pharmaceuticals with genotoxic potential.

Anticancer drugs in aquatic environment: occurence and toxicity to aquatic organisms

prof. dr. Metka Filipič

National Institute of Biology

Science-Policy Event

Pharmaceuticals in the Environment: Current scientific developments and policy responses

Brussels, November 21, 2013

This project received funding from the European Community's Seventh Framework Programme ([FP7/2007-2013]) under grant agreement n° 265264.

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CYTOTHREAT:

Fate and effects of cytostatic pharmaceuticals in the environment and identification of biomarkers for an improved risk assessment on environmental exposure

Work programme topics addresed:

- Activity 6.1 “Climate Change, Pollution and Risks”;
- Sub-Avtivity 6.1.2 “Environment and Health”;
- Area 6.1.2.2 “Health effects of environmental stressors other than climate change”
- **Topic ENV.2010.1.2.2-2: “Human health and environmental effects of exposure to pharmaceuticals released into the environment”**

Funding scheme: Collaborative Project: Small and medium-scale focused research project

Project start: January 2011 for 48 month

Grant agreement nº: 256264

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	Partner		Role
1	**National Institute of Biology**		coordination, in vitro studies, toxicogenomic analysis of in vivo and in vitro experiments
2	**Jožef Stefan Institute**		new analytical methods, degradation simulation, degradation products,
3	**Medical University Vienna**		toxicity in higher plant systems, risk assessment
4	**Szent István University**		in vivo acute and chronic studies in zebrafish
5	**Seconda Universita di Napoli**		acute and chronic toxicity, and genotoxicity studies in crustacea
6	**Spanish Council of Scientific Research**		new analytical methods, chem. anal. of traces of cytostaics in water samples, occurrence…
7	**Institute for Medical Research and Occupational Health**		in vitro and in vivo genotoxicity determination (comet assay, MN assay)
8	**Institute for Multidisciplinary Research, University of Belgrade**		genotoxic effects in mussel
9	**RR & CO. Knowledge Centre Ltd.**		administrative and financial coordination

Context

- Potential risks associated with the release of pharmaceuticals into the environment are an important issue for environmental regulators and for the pharmaceutical industry.
- Cytostatic drugs are compounds with a very potent mechanism of action; therefore, they are of particular environmental concern, even though consumption rates and PEC may be low compared to many other drugs.
- Cytostatic drugs are highly hazardous compounds due to their genotoxic properties, which may cause unexpected long-term effects.
- At present there is a nearly complete gap of knowledge that would allow for the assessment of the risks when these compounds are released to the environment.

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Objectives

To fill in the knowledge gaps to allow for the risk assessment of these compounds when released into environment.

1. To assess the occurrence and fate of cytostatic pharmaceuticals, their metabolites and transformation products in wastewater treatment systems and in the environment.
2. To explore potential delayed and irreversible effects of cytostatic pharmaceuticals at environmentally relevant concentrations in aquatic experimental models, and compare the data to those obtained in human experimental models.
3. To explore combined effects of mixtures of cytostatic pharmaceuticals, their excreted metabolites and transformation products formed in the environment and/or waste water treatment.
4. To develop, based on the obtained results, guidance on how to improve the environmental and human risk assessment of cytostatics released into the environment.

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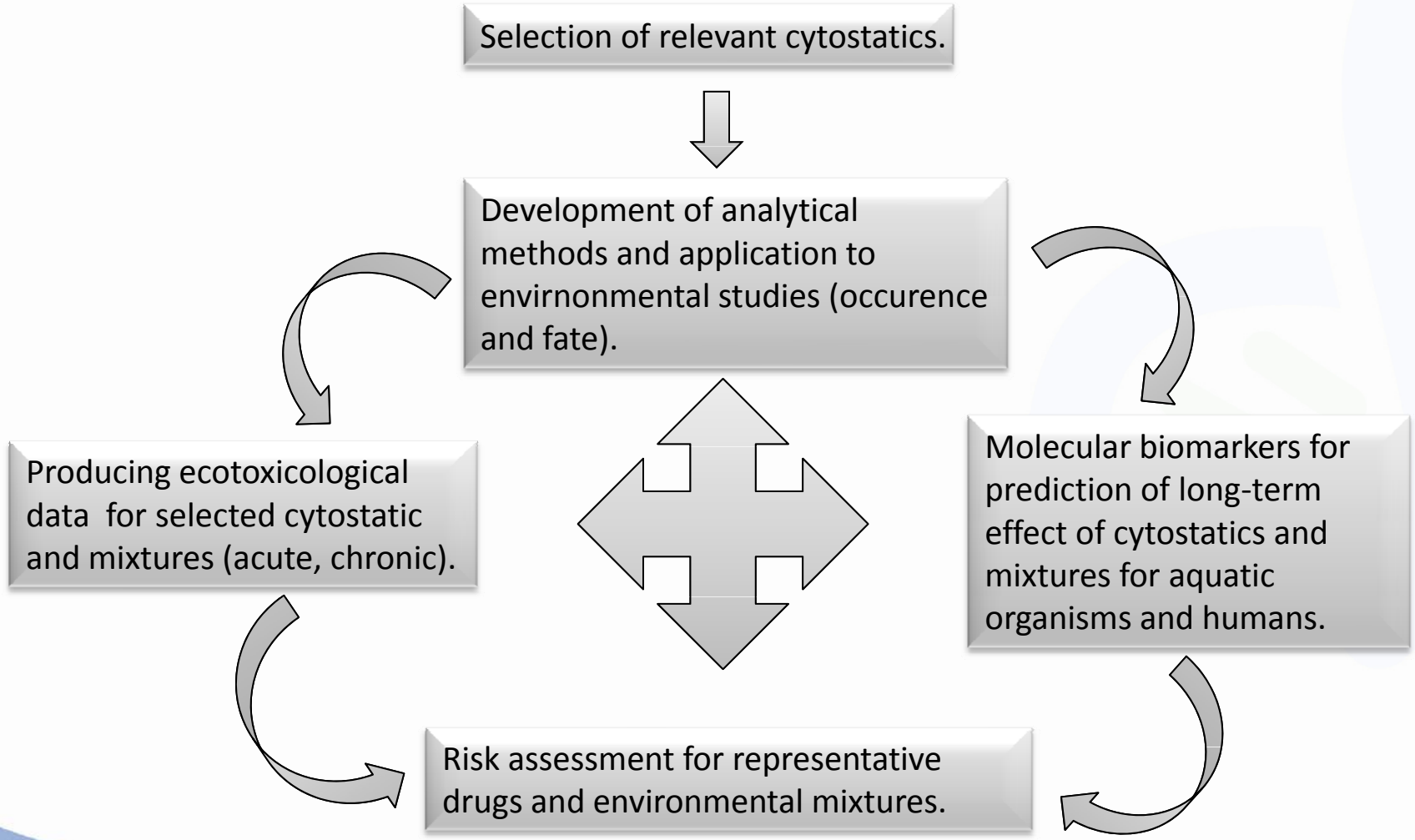
Approach



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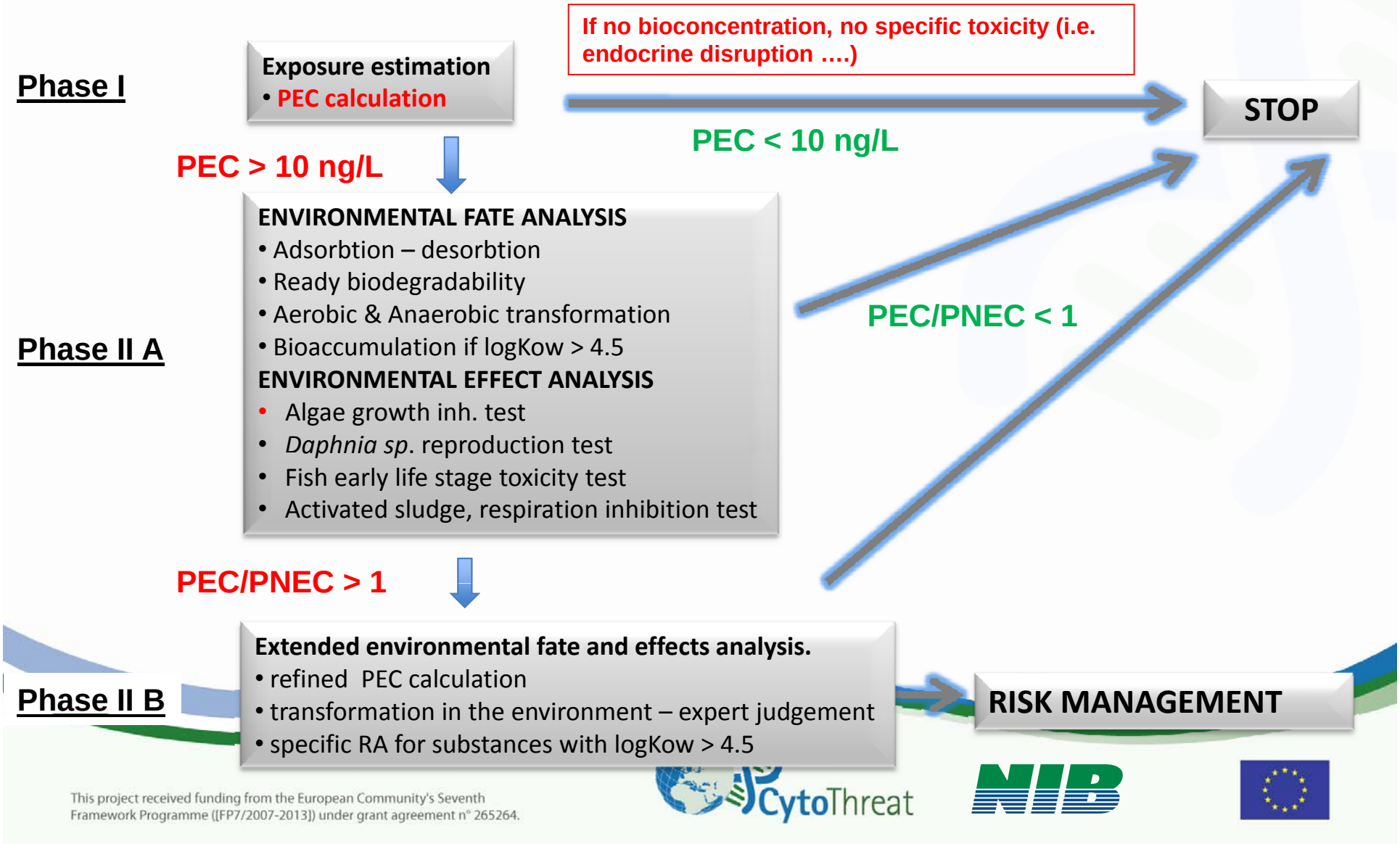
EMA (2006) guidelines for environmental risk assessment of medicinal products for human use



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Exposure estimation based on consumption

FRANCE (60 MIO inhab)

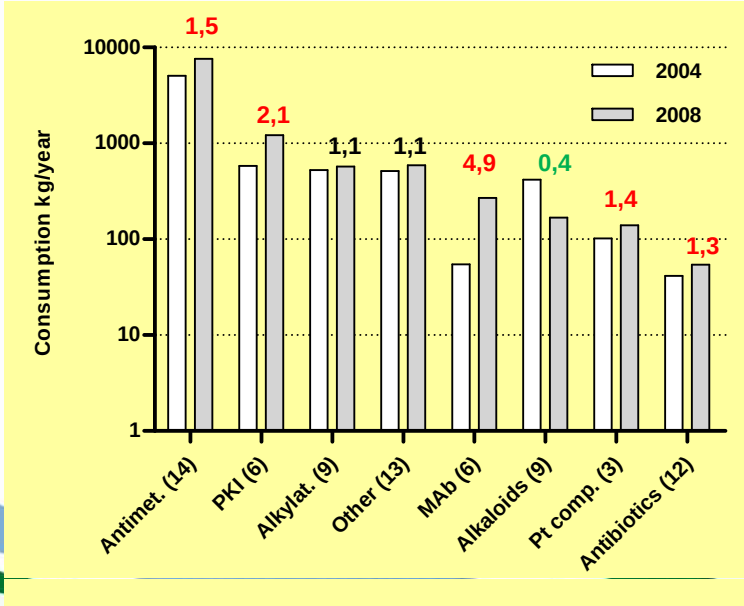
Total:	Per capita:
2004: 13 t	218 mg
2008: 17.5 t	290 mg

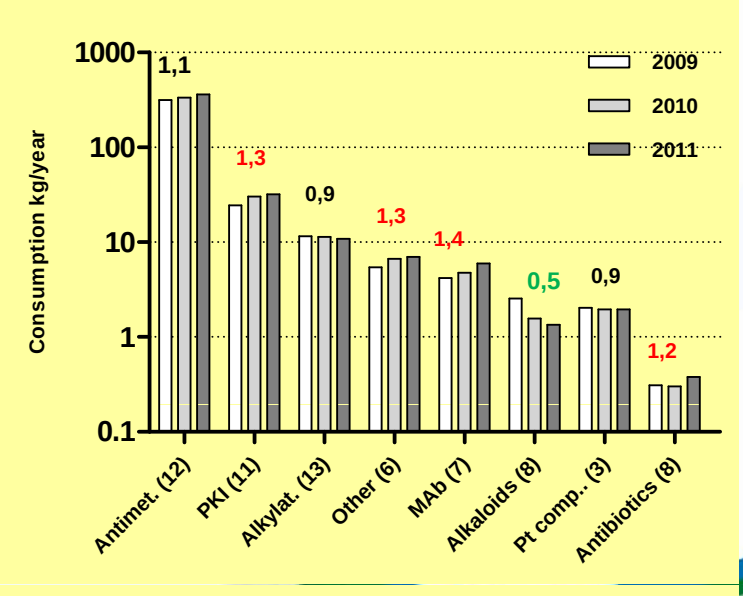
Increase 2004 - 2008: 1.34 fold

SLOVENIA (2 MIO inhab.)

Total:	Per capita
2009: 500 kg	250 mg
2010: 536 kg	268 mg
2011: 570 kg	285 mg

Increase 2009 - 2011: 1.14 fold





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Top 30 most consumed anti-cancer drugs (PEC ng/L)

	Compound	MoA	PEC		F_{ex}	R-PEC	
			SLO	FR		SLO	FR
1	CAPECITABINE	Antimet.	232,1	117,2	0,0	7,0	3,5
2	HYDROXYCARBAMIDE	Alkylat.	102,3	156,1	0,5	51,1	78,1
3	**FLUOROURACIL**	**Antimet.**	**9,3**	**39,6**	**0,2**	**1,9**	**7,9**
4	**IMATINIB**	**PKI**	**5,0**	**20,0**	**0,3**	**1,3**	**5,0**
5	MITOTANE	Immunosup.	4,6	5,3	0,6	2,7	3,2
6	*DACARBAZINE*	Alkylat.	4,4	0,7	0,5	2,2	0,3
7	*SORAFENIB*	PKI	3,9		0,5	2,0	
8	LAPATINIB	PKI	3,9	2,7	0,7	2,7	1,9
9	NILOTINIB	PKI	2,8	1,3	0,6	1,7	0,8
10	ERLOTINIB	PKI	2,4	3,4	0,0	0,0	0,1
11	GEMCITABINE	Antimet.	2,3	7,7	0,1	0,2	0,9
12	*GEFITINIB*	PKI	1,9		n.a.	1,9	
13	TEMOZOLOMIDE	Alkylat.	1,5	1,2	0,1	0,1	0,1
14	METHOTREXATE	Antimet.	1,4	1,7	0,9	1,3	1,5
15	BEVACIZUMAB	Mab	1,4	2,0	n.a.		
16	RITUXIMAB	Mab	1,4	1,7	n.a.		
17	*PAZOPANIB*	PKI	1,3		0,7	0,9	
18	TRASTUZUMAB	Mab	1,3	1,3	n.a.		
19	IFOSFAMIDE	Alkylat.	1,2	2,4	0,5	0,6	1,2
20	MERCAPTOPURINE	Antimet.	0,8	2,2	0,1	0,1	0,2
21	CARBOPLATINUM	Pt alkylat.	0,7	1,9	1,0	0,7	1,9
22	CYTARABINE	Antimet.	0,7	3,1	0,1	0,1	0,3
23	**ETOPOSIDE**	**Topo. inh.**	**0,5**	**0,9**	**0,9**	**0,5**	**0,9**
24	SUNITINIB	PKI	0,4	0,5	n.a.	0,4	0,5
25	OXALIPLATIN	Pt alkylat.	0,3	0,8	n.a.		
26	IRINOTECAN	Topo. inh.	0,3	1,1	0,5	0,2	0,5
27	**CISPLATIN**	**Pt alkylat.**	**0,3**	**0,5**	**n.a.**		
28	PACLITAXEL	Mitotic inh.	0,2	0,9	0,2	0,0	0,2
29	CYCLOPHOSPHAMIDE	Alkylat.	0,2	7,0	0,3	0,1	1,7
30	PROCARBAZINE	Alkylat.	0,2	0,8	0,2	0,0	0,2

MoA	PEC		R-PEC	
	SLO	FR	SLO	FR
Antimetabolites	**246,7**	**171,5**	**10,5**	**14,3**
Protein kinase inh.	**21,8**	**27,8**	**10,9**	**8,2**
MAB	4,01	4,92	-	-
Alkylating ag. w/o HYD	2,7	3,6	0,7	1,3
Pt based alkyl. agents	**1,4**	**3,2**	**-**	**-**
Topoisomerase inh.	**0,8**	**2,0**	**0,6**	**1,4**

Data:
France - 2008: Besse et al., Env. Int. 39,73–86, 2012
Slovenia – 2011: Institute of Oncology Ljubljana.

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ANALYTICAL METHODS

❑ LC-MS/MS based methods:

- Multi-residue method based on on-line SPE-LC-MS/MS for analysis of **17 cytostatics and metabolites** in different water matrices*,

PICO (Spark Holland) + 4000 Q-Trap (AB Sciex) = 40 min

- Multi-residue LC-MS/MS method based on direct sample injection for analysis of **up to 26 compounds** (including metabolites) in stability studies**

**N. Negreira et al. (2013) J. Chromatogr. A 1280, 64-74.*
*** N. Negreira et al. (2013) Talanta. 116, 290-299.*

On-line SPE-LC-MS/MS - Method performance

Compound	R^2	LOD		Ldet		Rel. recovery (%) ± relative standard deviation			
	HPLC	WWE	WWI	WWE	WWI	WWE		WWI	
						[a]20 ng L^{-1}	[a]500 ng L^{-1}	[a]20 ng L^{-1}	[a]500 ng L^{-1}
GEM	0.9999	0.7	0.7	9.3	9.3	96±15	93±1	96±2	114±2
TMZ	0.9934	1.0	1.1	42	50	98±12	94±2	95±12	103±4
MET	0.9970	0.5	0.6	1.8	2.0	87±15	99±1	94±14	104±8
OH-MET	0.9995	1.3	1.6	4.3	5.2	88±14	88±1	83±5	99±2
IRI	0.9996	0.4	1.4	1.2	4.5	nq	111±6	nq	108±10
IMA	0.9945	36	54	120	180	82±2	95±2	72±11	88±1
IF	0.9996	0.6	0.6	2.0	2.0	107±13	95±2	92±4	107±1
CP	0.9998	0.5	0.6	1.5	2.0	115±12	96±2	102±11	99±1
ERL	0.9989	1.0	0.5	3.4	1.5	91±2	95±1	111±12	111±1
ETP	0.9981	12	20	40	65	nq	94±4	nq	81±3
DOX	0.9998	0.7	0.8	2.4	2.5	98±10	95±5	75±4	71±1
CAP	0.9989	0.5	0.7	1.5	2.4	107±8	104±12	88±8	119±12
OH-D-TAM	0.9986	1.5	1.5	5.0	5.0	76±5	99±17	79±14	96±7
OH-TAM	0.9991	0.3	0.7	1.1	2.4	89±4	99±11	103±12	100±8
TAM	0.9978	0.9	1.0	3.0	3.4	101±2	117±1	104±11	107±2
OH-PAC	0.9990	1.1	1.1	3.6	3.6	92±11	94±10	113±6	111±9
PAC	0.9999	1.2	1.3	4.0	4.4	102±2	99±1	108±12	101±7

[a] spiking level

→ High reliability of results due to the use of isotopically labelled compounds as surrogate standards for quantification by the isotope dilution method.

Results of Spanish samples

		Concentration (ng L^{-1})									
	Sampling period	MET	IRI	IF	CP	DOX	CAP	OH-D-TAM	OH-TAM	TAM	OH-PAC
Influent WWTP (Catalonia)	04/2012	2.1-20.1	nd	7.3-43.3			8.2-27.0			4.3-17.2	4.4
Hospital effluent (Catalonia)	10/2012	2.0-19.4			5.9-100.0			nd	nd	nd	nd
Influent WWTP (Catalonia)	01/2013	nd	8.8-21.3	nd	7.1-17.3			nd	nd	177.6-180.6	
Effluent WWTP (Catalonia)	01/2013	nd	16.8	nd	4.1-11.3			91.6	37.4-164.0	102.0-147.0	
Influent WWTP (Spain)	10/2011-01/2012	2.6-18.1		2.2-27.9	2.4-43.8	2.5-2.7	5.6-72.6				3.7-18.5
Effluent WWTP (Spain)	10/2011-01/2012			2-5-15.9	2.5-25		5.2-36.0		5.8		3.7

GEM; OH-MET; IMA; ERL; ETP and PAC were not detected in these samples

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Occurrence of 5-FU and cis-Pt and IF and CP in

5-FU was determined in 4 out of 12 water samples. All positive samples were wastewaters where 5-FU was determined in concentrations of several ten ng/L (35 – 92 ng/L) in wastewaters from oncological wards, whereas its concentrations were lower in municipal wastewater treatment plant influents (4.7 – 14 ng/L)*.

cis-Pt was determined in almost all samples. Generally, hospital effluents contained higher amounts of detected compound (up to 639 ng/L). Large receiving surface water (case A) contained cis-platinum in ng/L, probably due to dillution factor, while small stream after WWTP B reached up to 275 ng/L cis-Pt when sampled in dry period and 5.5 ng/L when sampled in rainy period.

CP and IF were detected only in hospital effluent A (12.1 $\mu g L^{-1}$ and 10.5 $\mu g L^{-1}$ for CF and IF, respectively). **Selected metabolites** were not shown to be present in any of the samples analysed so far.

Kosjek et al., Journal of Chromatography A, 1290 (2013) 62– 72

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Degradation/transformation studies

1.Chlorination experiments:

- **19 cytostatics**
- **detailed study of the reactivity of ETO, 5-FU, IMA**

2.UV treatment/photodegradation:

- **IF, CP**
- **5-FU, CAP**

3.Biodegradation study:

- **5-FU, CAP**

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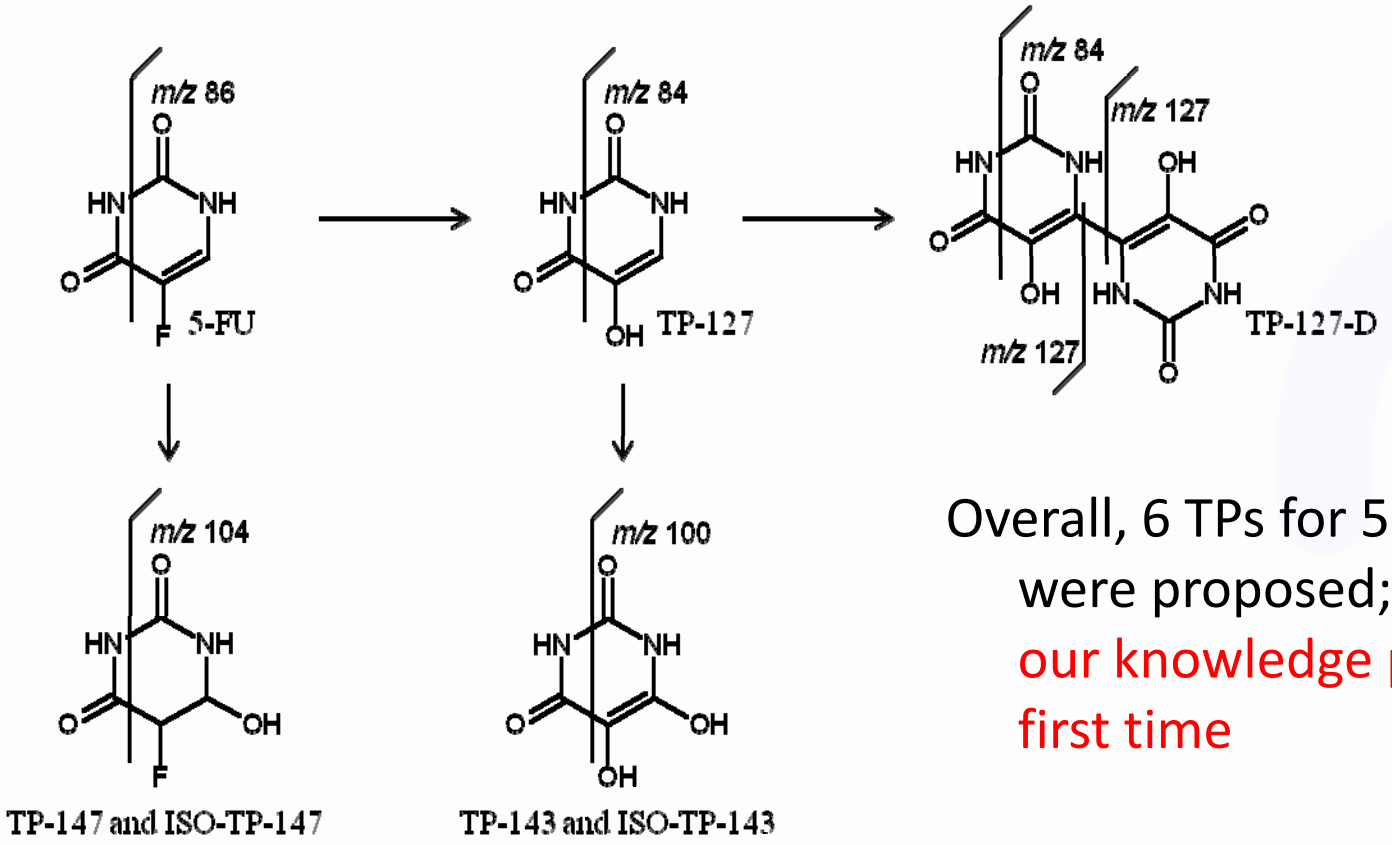
UV treatment / photodegradation: 5-FU, CAP



Overall, 6 TPs for 5-FU and 10 for CAP were proposed; 13 of these are to our knowledge published for the first time

cis and *trans* positions

"Fluorouracil in the environment: analysis, occurrence, degradation and transformation", Tina Kosjek, Silva Perko, Dušan Žigon, Ester Heath, *Journal of Chromatography A*, *Volume 1290* *17 May 2013, Pages 62-72*

Kosjek et al., Journal of Chromatography A, 1290 (2013) 62– 72

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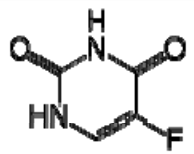
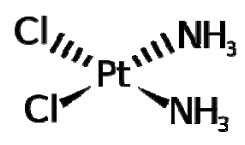
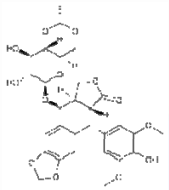
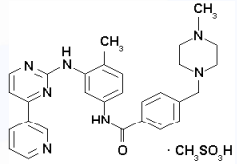
Framework Programme ([FP7/2007-2013]) under grant agreement n° 265264.

Four cytostatics were selected for ecotoxicity and genotoxicity studies

name	5-fluorouracil	cisplatin	etoposide	imatinib-mesylate
				
MoA	**animetabolite**	**alkylating-like agent**	**topoisomerase inhibitor**	**tyrosin kinase inhibitor**
		Genotoxicity data		
Bact . mutagenicity	Positive	Positive	Negative	No data
CA aberr. *in vitro*	Positive	Positive	Positive	Positive/Negative
CA aberr. *in vivo*	Positive	Positive	Positive	No data
DNA damage	Positive	Positive	Positive	No data
Carcinogenicity (IARC)	Group 3	Group 2A	Group 1	No data

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Eco(geno)toxicity testing

	Alga: *P. subcapicata* & Cyanobacteria *S. leopoliensis*	**Crustacea: *D. magna* & *C. dubia***	**Fish: *D. rerio* (zebrafish)**
Acute toxicity	Freshwater Alga and Cyanobacteria, Growth Inhibition Test (OECD 201)	*D. magna*: Acute Immobilisation Test (OECD 202, 2004): *C.dubia*: Acute mortality assay (EPA-600-4-90/027F, 1993)	Fish, Acute Toxicity Test – limit test (OECD 203) Fish Embryo Toxicity (FET) Test (OECD draft GD)
Chronic toxicity		Reproduction inh. *D. magna*: (OECD 211, 2008); *C. dubia* (ISO/FDIS 20665, 2008 EPA-600/4-91-002, 1994)	Fish, Early-Life Stage Toxicity Test (OECD 210) **Fish two-generation toxicity test (EPA draft)**
Genotoxicity		**Comet assay**	**Comet assay,** **MN assay**
Gene expression			**Gene expression analysis: microarrays, QRT-PCR**

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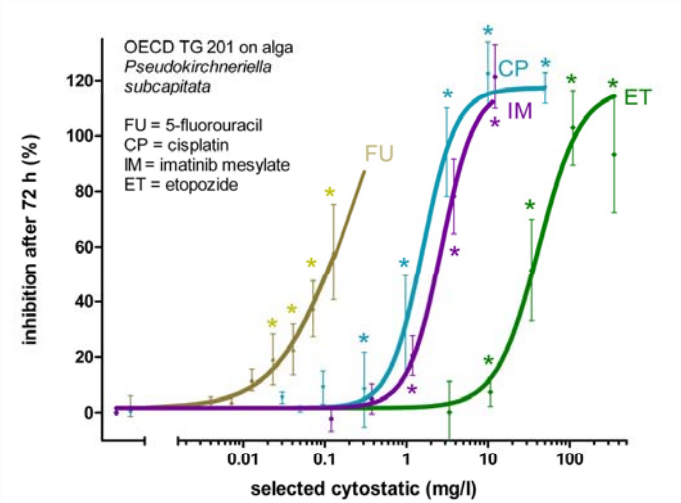
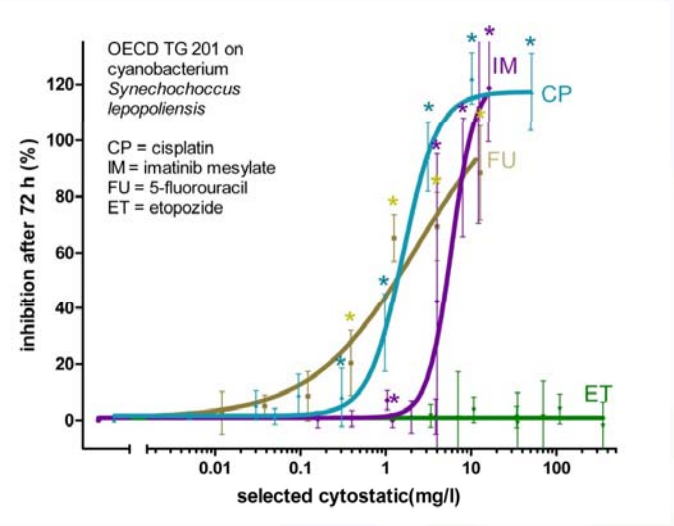
Alga and cyanobacteria growth inhibiton





P. subcapitata			
mg/L	EC_{50}	EC_{10}	NOEC
5-FU	0,13	0,02	0,01
DCCP	1,52	0,61	0,50
IM	2,29	0,79	0,38
ET	30,43	13,61	10,74

S. leopoliensis			
mg/L	EC_{50}	EC_{10}	NOEC
5-FU	1,20	0,13	0,12
DCCP	0,67	0,05	0,10
IM	5,36	3,21	3,84
ET	ND	ND	351,05

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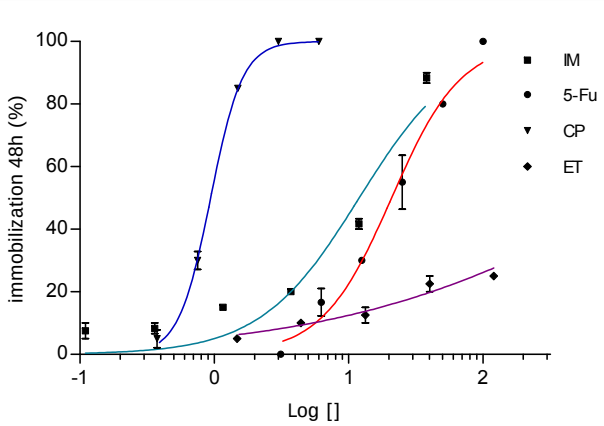
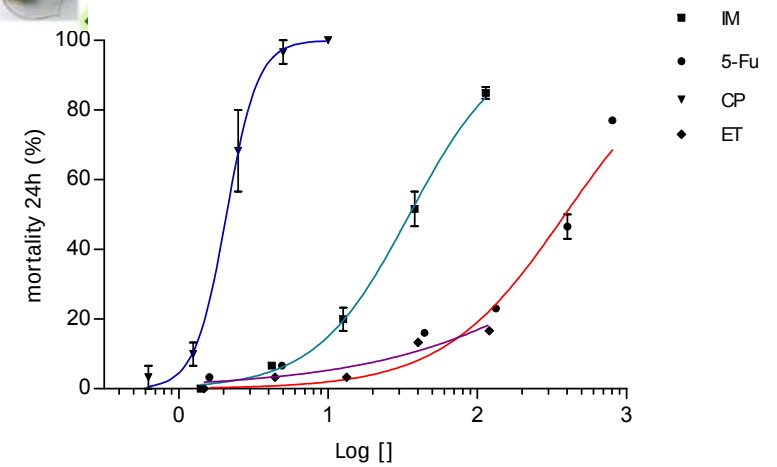
Acute toxicity in crustacea

Immobilization *Daphnia magna*



Mortality *Ceriodaphnia dubia*



Compound	*Daphnia magna 48h*		*Ceriodaphnia dubia 24h*	
mg/L	EC_{50}	EC_{10}	LC_{50}	LC_{10}
5-FU	20.84	5.64	~ 500	16.83
CDDP	0.94	0.51	2.50	1.35
ET	nd	5.25	nd	30.20
IM	11.97	1.87	31.92	6.79

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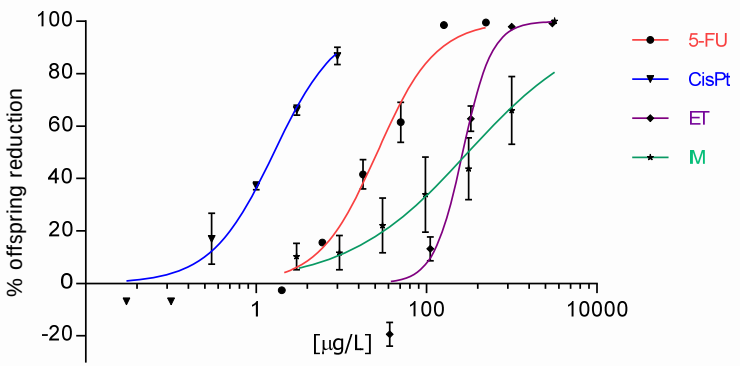
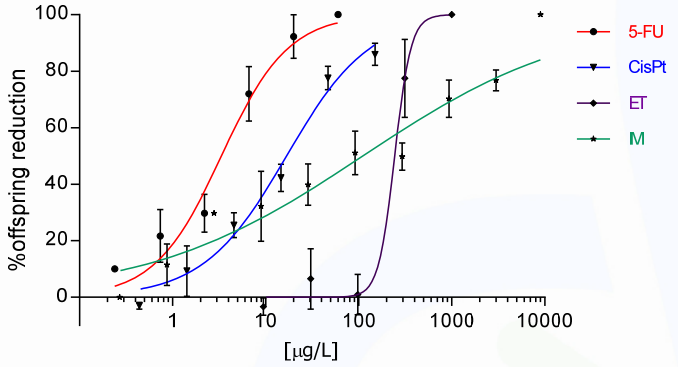
Chronic toxicity in crustacea

Daphnia magna: 21 days



Ceriodaphnia dubia: 7 days



Compound	*Daphnia magna 21 days*		*Ceriodaphnia dubia 7 days*	
µg/L	EC_{50}	EC_{10}	LC_{50}	LC_{10}
5-FU	26.42	4.58	3,35	0.55
DCCP	1.63	0.25	16.83	1.75
ET	239	98	204	96
IM	308	8.34	115	0.43

Toxic effect at µg/L concentration levels.
Differences in susceptibility of the two crustacea test species.

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Acute and chronic toxicity in zebrafish

Compound	Fish, Acute Toxicity Test – limit test (OECD 203)	Fish Embryo Toxicity (FET) Test (OECD draft GD) – 120 h	Fish, Early-Life Stage Toxicity Test (OECD 210)
	LC_{50} mg/L	LO(A)EC mg/L	LO(A)EC mg/L
5-FU	> 100	2000*	1
DCCP	64.45	50	-
ET	> 100	200	-
IM	70.8	76,7	10

*NOEC 48h

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5-FU: zebrafish 2 generation assay

Treatment:
0.01, 1, 100 µg/L
2 replicates/conc.

P generation
pre-treatment 2 weeks

2º sex characteristics
Reproduct. Behaviour
Spawing activity
Fecundity
Fertilizations success

F1 generation
(min. 100 embryos/replicate)

Hatching success
Time to hatch
Normal/abnormal

3-4 mpf

Termination:
comet assay: gill, liver gonads, blood
MN assay: blood
Liver mRNA isolation

Termination:
Body characteristics
2º sex characteristics
Histology

F2 generation
(according to OECD 210-Early-Life Stage Toxicity Test)

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Survival, body parameters and histopathology

- No effect on survival of F1 and F2 generation.
- No effect on body weight and length of F1 and F2 generation.
- No changes during the early stage development of F1 and F2.
- No effect on fecundity of fish or fertilization percentage of eggs

- Histopathology:
  - Liver: lipidosis and regressive degeneration at all 5-FU treated groups; atrophy in individuals treated with the highest 5-FU concentration.
  - Kidney: hyperplasia of the hematopoietic tissue of the kidney in fish treated with 10 ng/l and 1 µg/l 5-FU; depletion of hematopoietic tissue and tubulonephrosis in the 100 µg/l group.

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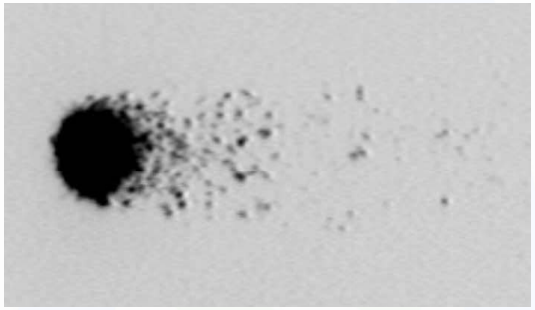
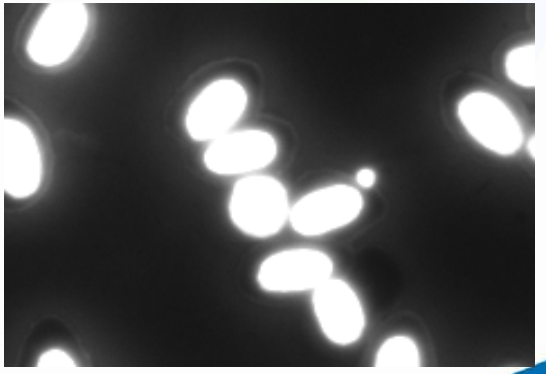
Genotoxicity endpoints

- DNA damage: comet assay
  - *D. magna* (24 h exposure)
  - *D.rerio* embryos *(24 h exposure)*
  - *D.rerio* F1, liver, gill, gonads, blood cells (2 generation exposure)
  - Mussel (data not shown)



- Chromosomal aberrations: micronucelus assay
  - *D.rerio* F1 blood cells (2 generation exposure)
  - Higher plants (data not shown)



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Crustacea – comet assay (24h expopsure)

D. magna

Compound	LOAEC (ug/L) DNA strand breaks	EC_{10} (µg/L) 21 day exp.
5-FU	0.5	4.6
ET	3.0	98
CDDP	0.1	0.25
IM	2.0	8.34

C. dubia

Compound	LOAEC (ug/L) DNA strand breaks	EC_{10} (µg/L) 7 day exp.
5-FU	0.06	0.55
ET	0.1	96
CDDP	0.3	1.75
IM	0.3	0.43

DNA damage after 24 h exposure at concentrations < EC10 for reproduction inhibition.

DNA damage after 24 h is an early biomarker of reproductive effects.

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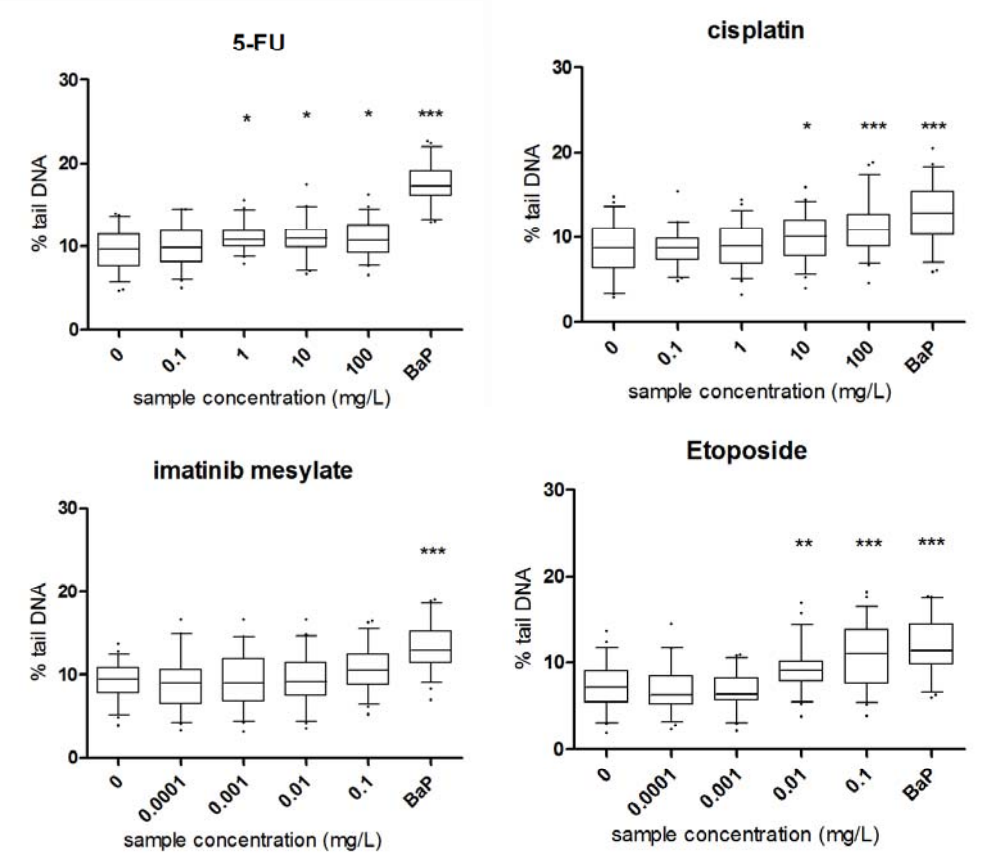
Zebrafish embryos – comet assay (24 h exposure)

Compound	LOAEC (mg/L) DNA strand breaks	LOEC (mg/L) FET test
5-FU	Neg.	2000
ET	0.01	200
CDDP	100	50
IM	Neg.	77



ANOVA, Kruskal–Wallis with Dunnet's post test; ***p<0.001;treated versus control cells

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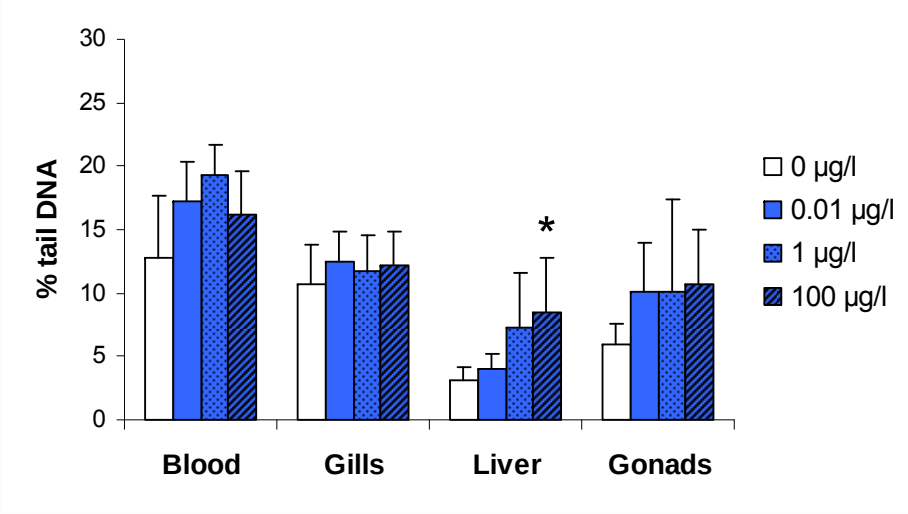
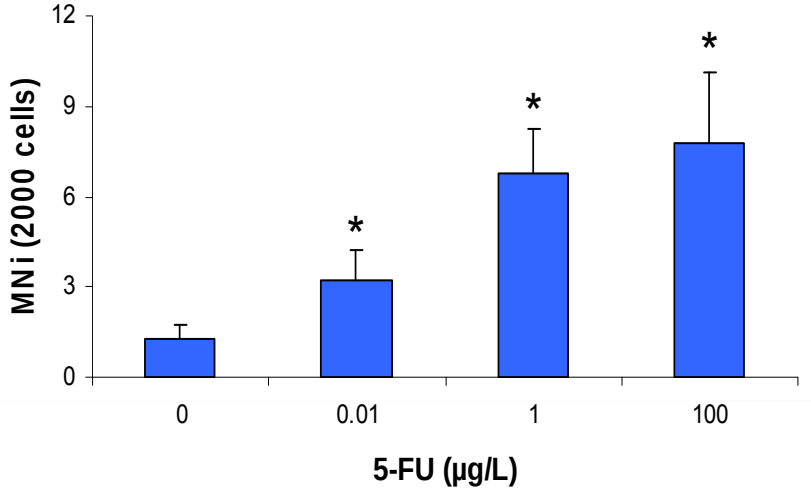
Genotoxicity of 5-FU in F1 zebrafish

Comet assay:



Micronucelus assay:



Although an increase in DNA strand breaks was observed in all tissues, except gill, it was **statisticaly significant (p < 0.05)** only in liver of fish exposed **to 100 µg/L.**

Significant (p < 0.05), dose dependent incrase in micronuclei formation was detected at **all tested concentrations**.

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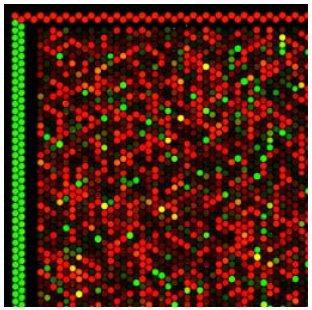
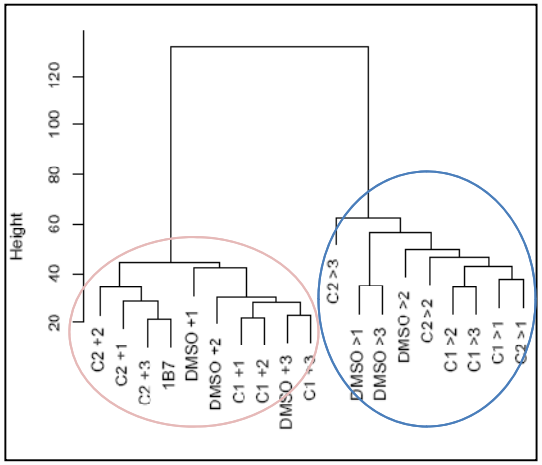
Transctiptome profiling in F1 zebrafish liver

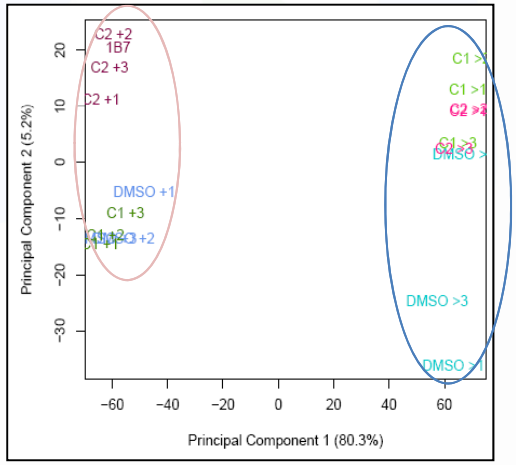
Sample No.	Pool name	Treatment	5FU (µg/L)	Sex	Tank	No. ind.
1	DMSO +1	DMSO	0	F	A	3
2	DMSO +2	DMSO	0	F	A	3
3	DMSO +3	DMSO	0	F	B	3
4	DMSO >1	DMSO	0	M	A	3
5	DMSO >2	DMSO	0	M	B	3
6	DMSO >3	DMSO	0	M	B	2
7	C1 +1	C1	0.01	F	A	3
8	C1 +2	C1	0.01	F	A	3
9	C1 +3	C1	0.01	F	B	3
10	C1 >1	C1	0.01	M	A	2
11	C1 >2	C1	0.01	M	B	3
12	C1 >3	C1	0.01	M	B	2
13	C2 +1	C2	1	F	A	3
14	C2 +2	C2	1	F	B	3
15	C2 +3	C2	1	F	B	2
16	C2>1	C2	1	M	A	3
17	C2>2	C2	1	M	A	3
18	C2 >3	C2	1	M	B	3
19	1B7	C2	1	F	A	1



Hierachical clustering



Principal component analysis



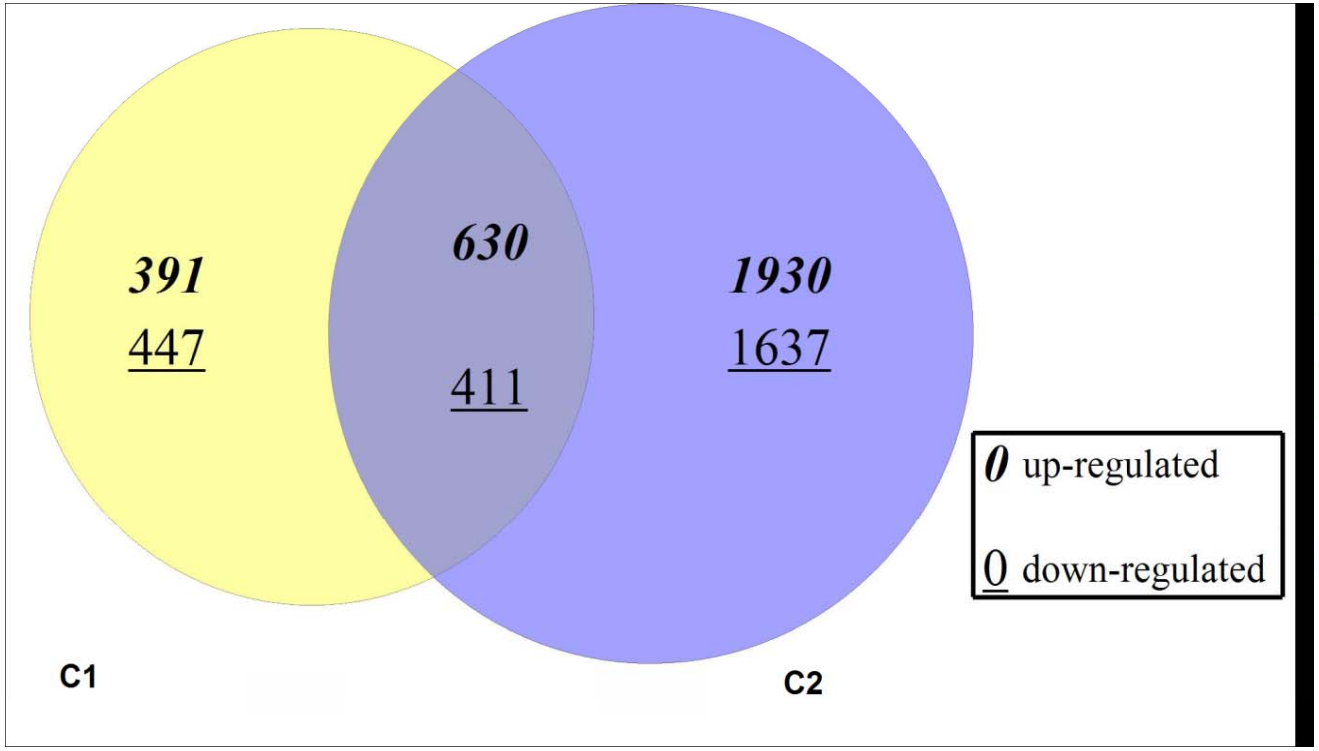
The response of the two genders was very different.

Agilent Zebrafish V3 4x44 Gene Expression Microarrays (4x44K, AMADID 026437): 43.803 *D.rerio* probes

Number of DE genes compared to control

C1 = 10 ng/L

C2 = 1 µg/L



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Chronic toxicity and genotoxicity data (µg/L)

Test	5-FU		Etoposid		Cisplatin		Imatinib	
	EC_{10}	GTOX	EC_{10}	GTOX	EC_{10}	GTOX	EC_{10}	GTOX
P. subcapitata (72 h)	17	-	13610	-	640	-	790	-
S. leopoliensis (72 h)	130	-	nd	-	130	-	3120	-
C. dubia (7 d)	**0,55**	**0,06**	**170**	**0,1**	1.75	0,3	**2,4**	**0,3**
D. magna (21 d)	4,58	0,50	90	3,0	0,25	0,1	46	2
D. rerio (33 d)	1000*	-	-	-	-	-	10000*	-
D. rerio (2 gen. test)	nd	**0,01**	-	-	-	-	-	-

GTOX: genotoxicity – LOAEC (DNA damage in crustacea; MN in F1 zebrafish)
*LOEC;

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Risk quotients (PEC/PNEC)

Compound	PNEC ng/L	Endpoint	PEC ng/L	R-PEC ng/L	PEC/ PNEC	R-PEC/ PNEC
5-FU	1	Zebrafish-MN, histopat.	232,12	6,96	**232,12**	**6,96**
5-FU	6	*C. dubia*-comet	232,12	6,96	**37,7**	**1,16**
ET	10	*C. dubia*-comet	0,94	0,87	0,094	0,087
CP	10	*D. magna*-comet	0,52	-	0,052	-
IM	30	*C. dubia*-comet	19,95	4,99	0,67	0,17

Swedish Prescribing guide (www.fass.se):

- PEC/PNEC ≤ 0.1 insignificant environmental risk.
- 0.1 < PEC/PNEC ≤ 1 low environmental risk.
- 1 < PEC/PNEC ≤ 10 moderate environmental risk
- PEC/PNEC > 10 high environmental risk.

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Impact

A) Contribution to improved risk assessment for human health and ecosystems for phramaceuticals

- **occurence data, new analytical methods, key ecotoxicological parameters, early markers of delayed adverse effects**

B) Contribution to relevant EU policies/strategies

Environmental and Health Action Plan

- Action 7: Develop methodological systems to analyse interactions between environment and health
- Action 8: Ensure that potential hazards on environment and health are identified and addressed

Together for Health: A Strategic Approach for the EU 2008-2013

- Objective 2: Protecting citizens from health threats: Strengthen mechanisms for surveillance and response to health threats.

Water Framework Directive (2000/60/EC)

REACH

- "*development of alternative methods for the assessment of hazards of different (chemical) substances*"

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THANK YOU!

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