Leesuriales 2022

11 - 12 juillet 2022

Evaluation écotoxicologique d'un polluant pharmaceutique et de ses produits de dégradation par une approche multi-modèle

Fidji SANDRÉ (doctorante) ; Christophe MORIN (Pr.) ; Laure GARRIGUE-ANTAR (Pr.)

sandrefidji@gmail.com

laboratoire eau environnement systemes urbains

École des Ponts ParisTech

UPEC UNIVERSITÉ PARIS-EST CRÉTEIL

OPUR

(le vrai logo)

Directive Cadre sur l'eau 2000/60/EC

- Bon état écologique
- Bon état chimique

Directive Cadre sur l'eau 2000/60/EC

Bon état écologique

Bon état chimique

Solvents

Usages industriels
(synthesis intermediates, plasticizers, surfactants, additives)

Métaux

Retardateurs de flamme

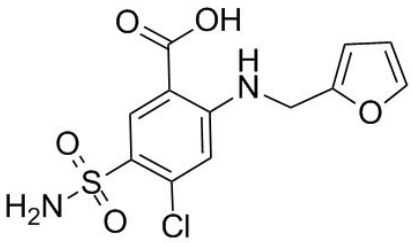
Biocides

Composés pharmaceutiques ?

Priority substances listed in Annex X of the WFD					
1	Alachlore	18	Hexaclorocyclohexane	30	Composés du tribultylétain
2	Anthracène	19	Isoproturon	31	Trichlorobenzènes (tous isomères)
3	Atrazine	20	Plomb et ses composés	32	Trichlorométhane
4	Benzène	21	Mercure et ses composés	33	Trifluraline
5	Pentabromodiphényléther (PBDE)	22	Naphtalène	34	Dicofol
6	Cadmium et ses composés	23	Nickel et ses composés	35	PFOS
7	C10-13-chloroalcanes	24	Nonylphénols	36	Quinoxyfène
8	Chlorfenvinphos	25	Octylphénols	37	Dioxines
9	Chlorpyrifos	26	Pentachlorobenzène	38	Aclonifène
10	1,2-Dichloroéthane	27	Pentachlorophénol	39	Bifénox
11	Dichlorométhane	28	Hydrocarbures Aromatiques Polycycliques	40	Cybutryne
12	Di(2-éthylhexyl)phtalate (DEHP)		Benzo-[a]-pyrène	41	Cypermethrine
13	Diuron		Benzo-[b]-fluoranthène	42	Dichlorvos
14	Endosulfan		Benzo-[k]-fluoranthène	43	Haxabromocyclododécanes
15	Fluoranthène		Benzo-[g,h,i]-perylène	44	Heptachlore et eoxydes d'heptachlore
16	Hexachlorobenzène		Indeno-[1,2,3-cd]-pyrène	45	Terbutryne
17	Hexachlorobutadiène	29	Simazine		

Quelles molécules ?

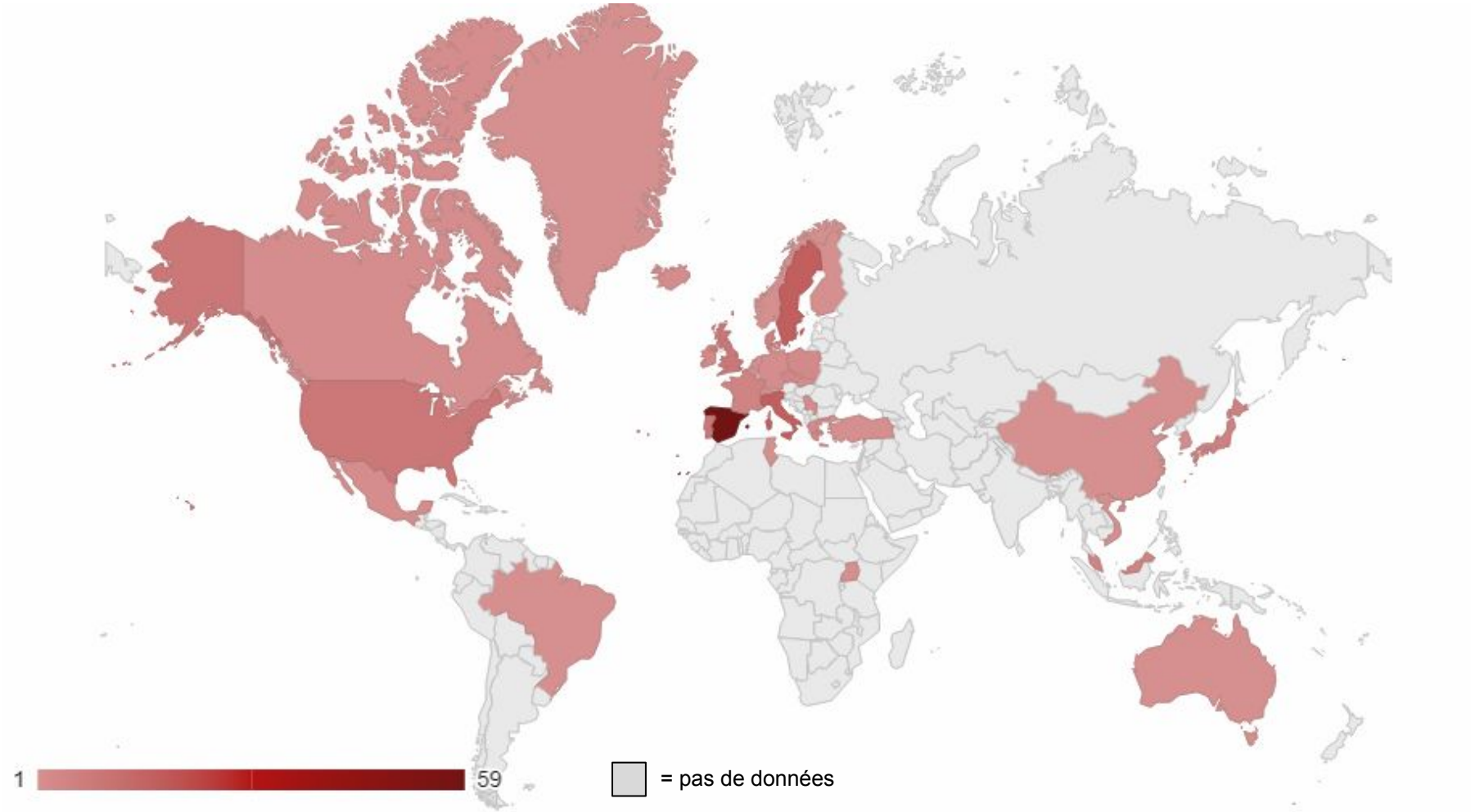
Furosemide
FUR



- Diurétique largement utilisé depuis 1964 [1]
- Parmi les médicaments les plus prescrits au monde [2]
- Fréquemment détecté dans l'environnement aquatique

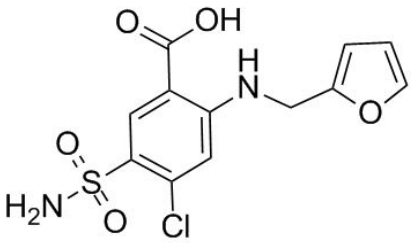
[1] Stokes & Nunn, 1964 [2]ClinCalc DrugStats Database 2018

Nombre d’études dans lesquelles le furosémide a été quantifié



Quelles molécules ?

Furosemide
FUR



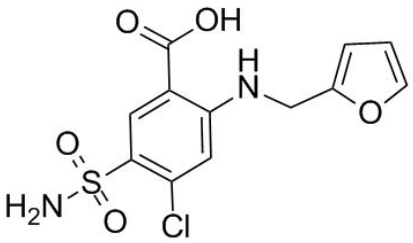
- Diurétique largement utilisé depuis 1964 [1]
- Parmi les médicaments les plus prescrits au monde [2]
- Fréquemment détecté dans l'environnement aquatique

	[C]mean	[C]max.	Refs
Pharmaceutical facilities	**1 250 000** ng.L^{-1}	**1 300 000** ng.L^{-1}	2
Hôpital effluents	**19 000** ng.L^{-1}	**392 000** ng.L^{-1}	26
WWTP Influents	**4 000** ng.L^{-1}	**72 000** ng.L^{-1}	52
WWTP Effluent	**2 000** ng.L^{-1}	**26 000** ng.L^{-1}	73
Surface water	**250** ng.L^{-1}	**7 000** ng.L^{-1}	42
WWTP sludge	**150** µg/kg	**700** µg/kg	17
Sediments	**70** µg/kg	**350** µg/kg	7

[1] Stokes & Nunn, 1964 [2] ClinCalc DrugStats Database 2018

Quelles molécules ?

Furosemide
FUR



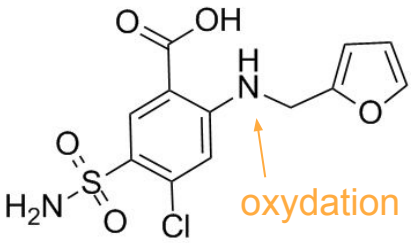
- Diurétique largement utilisé depuis 1964 [1]
- Parmi les médicaments les plus prescrits au monde [2]
- Fréquemment détecté dans l'environnement aquatique

	[C]mean	[C]max.	Refs
Pharmaceutical facilities	**1 250 000** ng.L^{-1}	**1 300 000** ng.L^{-1}	**2**
Hôpital effluents	**19 000** ng.L^{-1}	**392 000** ng.L^{-1}	26
WWTP Influents	**4 000** ng.L^{-1}	**72 000** ng.L^{-1}	52
WWTP Effluent	**2 000** ng.L^{-1}	**26 000** ng.L^{-1}	73
Surface water	**250** ng.L^{-1}	**7 000** ng.L^{-1}	42
WWTP sludge	**150** µg/kg	**700** µg/kg	17
Sediments	**70** µg/kg	**350** µg/kg	7

[1] Stokes & Nunn, 1964 [2] ClinCalc DrugStats Database 2018

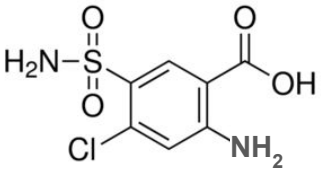
Quelles molécules ?

Furosemide
FUR



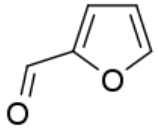
(1)

Saluamine
SAL



+

Furfural
FRF



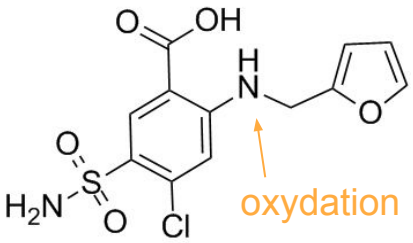
- Connus comme métabolites du furosémide depuis 1981[1] mais très peu étudiés
- Saluamine: génotoxique & cytotoxique [3]

[1] Andreasen et al. 1981 [2] Thermo Fisher Safety Data Sheet [3] Al-Omar et al. 2009

Quelles molécules ?

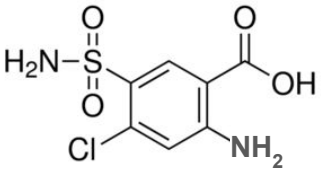
* rapidement biodégradé

Furosemide
FUR

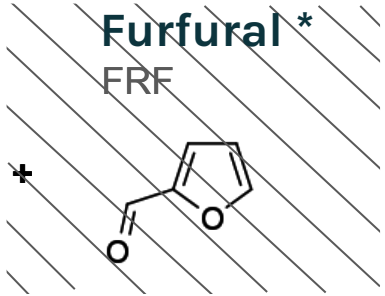


(1)

Saluamine
SAL



Furfural *
FRF



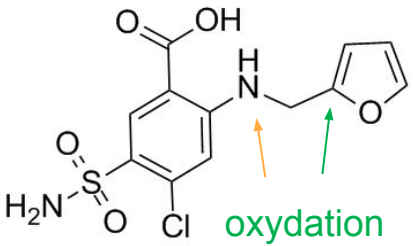
- Connus comme métabolites du furosémide depuis 1981[1] mais très peu étudiés
- Saluamine: génotoxique & cytotoxique [3]

[1] Andreasen et al. 1981 [2] Thermo Fisher Safety Data Sheet [3] Al-Omar et al. 2009

Quelles molécules ?

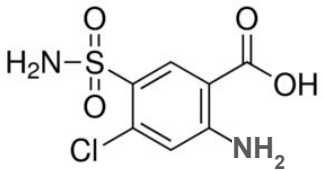
* rapidement biodégradé

Furosemide
FUR

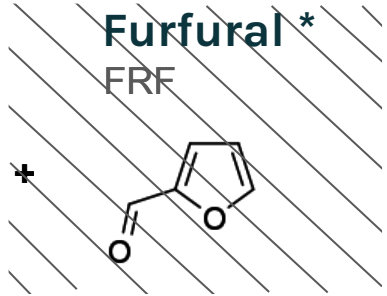


(1)

Saluamine
SAL

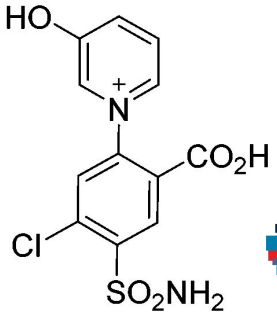


Furfural *
FRF



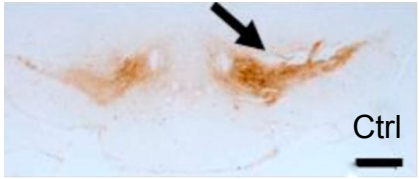
(2)

Pyridinium du Furosemide
PYR



- Produit par électrochimie [1] et bioconversion [2 3]
- Identifié comme métabolite humain [4]
- Possible inducteur de neurodegeneration [4]

Mort des neurones dopaminergiques [4]

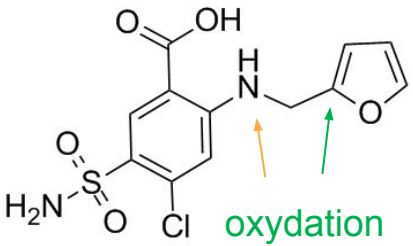


[1] Laurencé et al. 2011 [2] Olvera-Vargas et al. 2016 [3] Laurencé et al. 2014 [4] Laurencé et al. 2019

Quelles molécules ?

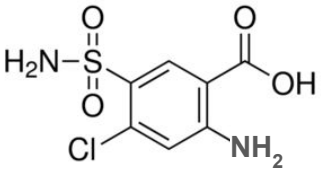
* rapidement biodégradé

Furosemide
FUR

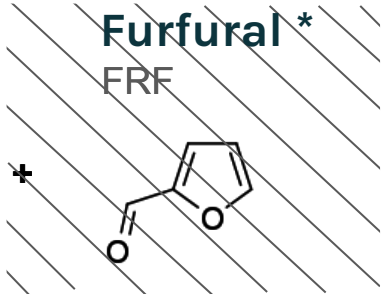


(1)

Saluamine
SAL

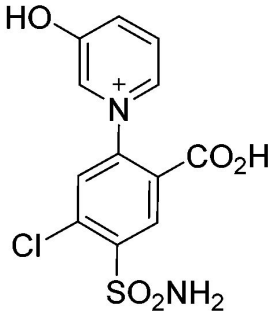


Furfural *
FRF



(2)

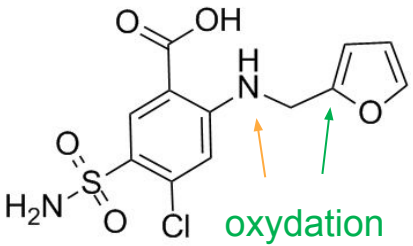
Pyridinium du Furosemide
PYR



Quelles molécules ?

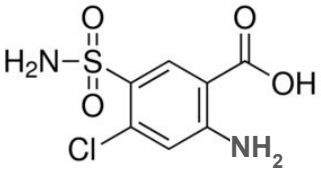
* rapidement biodégradé

Furosemide
FUR

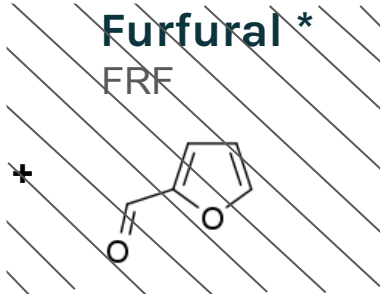


(1)

Saluamine
SAL

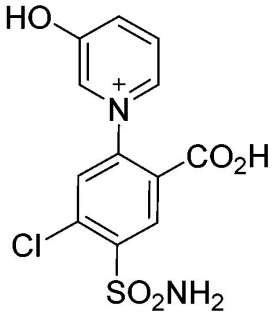


Furfural *
FRF



(2)

Pyridinium du Furosemide
PYR



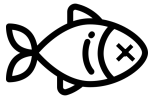
Présentent-ils un risque à des concentrations environnementales ?

Caractérisation du risque

Caractérisation du risque = ?

Caractérisation du risque

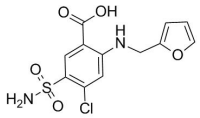
Caractérisation du risque = $\frac{\text{Concentrations environnementales}}{\text{Concentrations toxiques}}$



Concentrations environnementales ?

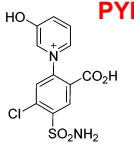
Ultra Performance Liquid Chromatography
+ mass spectrometer

FUR



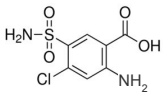
m/z 329,74

PYR



m/z 328,73

SAL

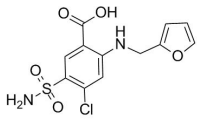


m/z 250,66

Concentrations environnementales ?

Ultra Performance Liquid Chromatography
+ mass spectrometer

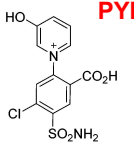
FUR



m/z 329,74

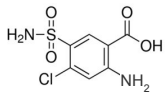
✅

PYR



m/z 328,73

SAL



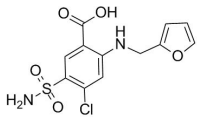
m/z 250,66

?

Concentrations environnementales ?

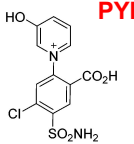
Ultra Performance Liquid Chromatography
+ mass spectrometer

FUR



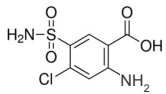
m/z 329,74

PYR



m/z 328,73

SAL



m/z 250,66

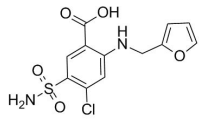
Analyse d'échantillons potentiellement très concentrés

Effluents d'EHPAD

Concentrations environnementales ?

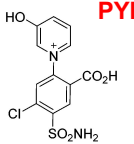
Ultra Performance Liquid Chromatography
+ mass spectrometer

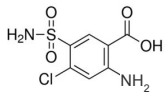
FUR



m/z 329,74 ✅

PYR **SAL**





m/z 328,73 m/z 250,66 ?

Analyse d'échantillons potentiellement très concentrés

Effluents d'EHPAD

FUR → 58 000 ng/L

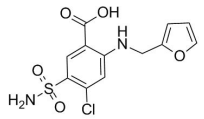
PYR → 2 000 ng/L

SAL → 6 500 ng/L ✅

Concentrations environnementales ?

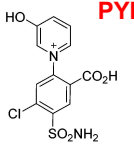
Ultra Performance Liquid Chromatography
+ mass spectrometer

FUR



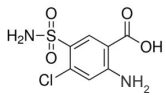
m/z 329,74

PYR



m/z 328,73

SAL



m/z 250,66

Analyse d'échantillons potentiellement très concentrés

Effluents d'EHPAD

FUR → 58 000 ng/L

PYR → 2 000 ng/L

SAL → 6 500 ng/L

Entrée de STEU

FUR → 3 000 ng/L

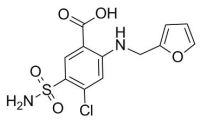
PYR → 2 500 ng/L

SAL → 3 250 ng/L

Concentrations environnementales ?

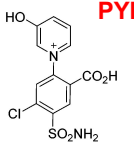
Ultra Performance Liquid Chromatography
+ mass spectrometer

FUR



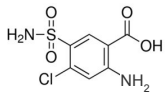
m/z 329,74

PYR



m/z 328,73

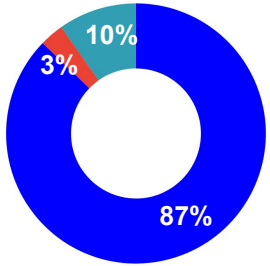
SAL



m/z 250,66

Analyse d'échantillons potentiellement très concentrés

Effluents d'EHPAD

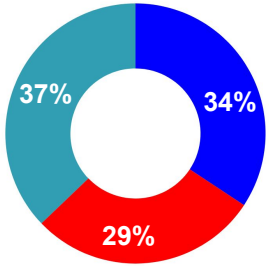


FUR → 58 000 ng/L

PYR → 2 000 ng/L

SAL → 6 500 ng/L

Entrée de STEU



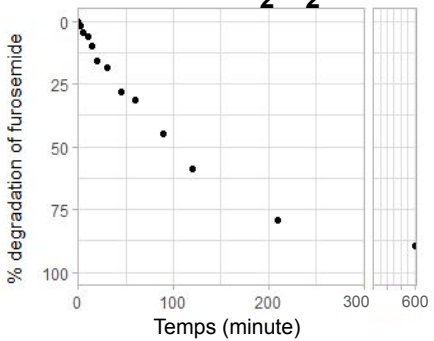
FUR → 3 000 ng/L

PYR → 2 500 ng/L

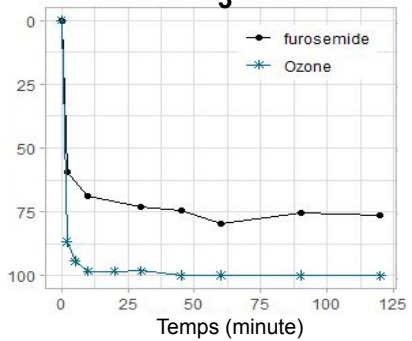
SAL → 3 250 ng/L

Dégradation du furosémide

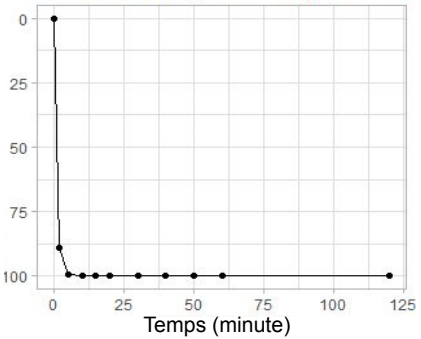
$UV+H_2O_2$



O_3



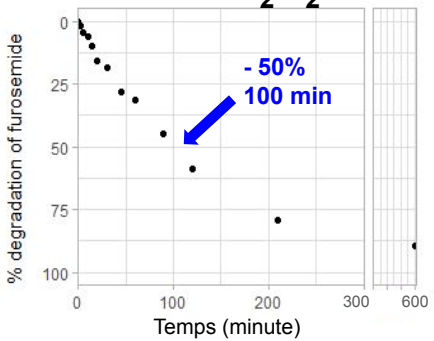
Cl



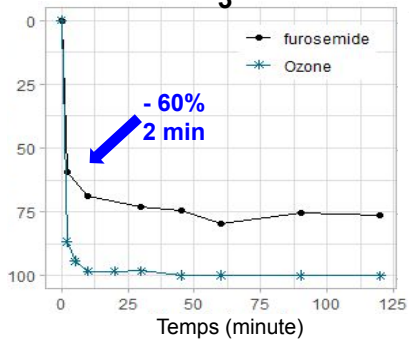
Expériences avec Nina

Dégradation du furosémide

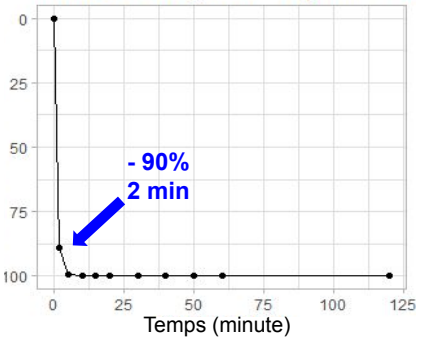
$UV+H_2O_2$



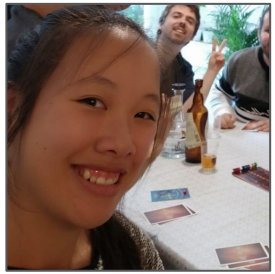
O_3



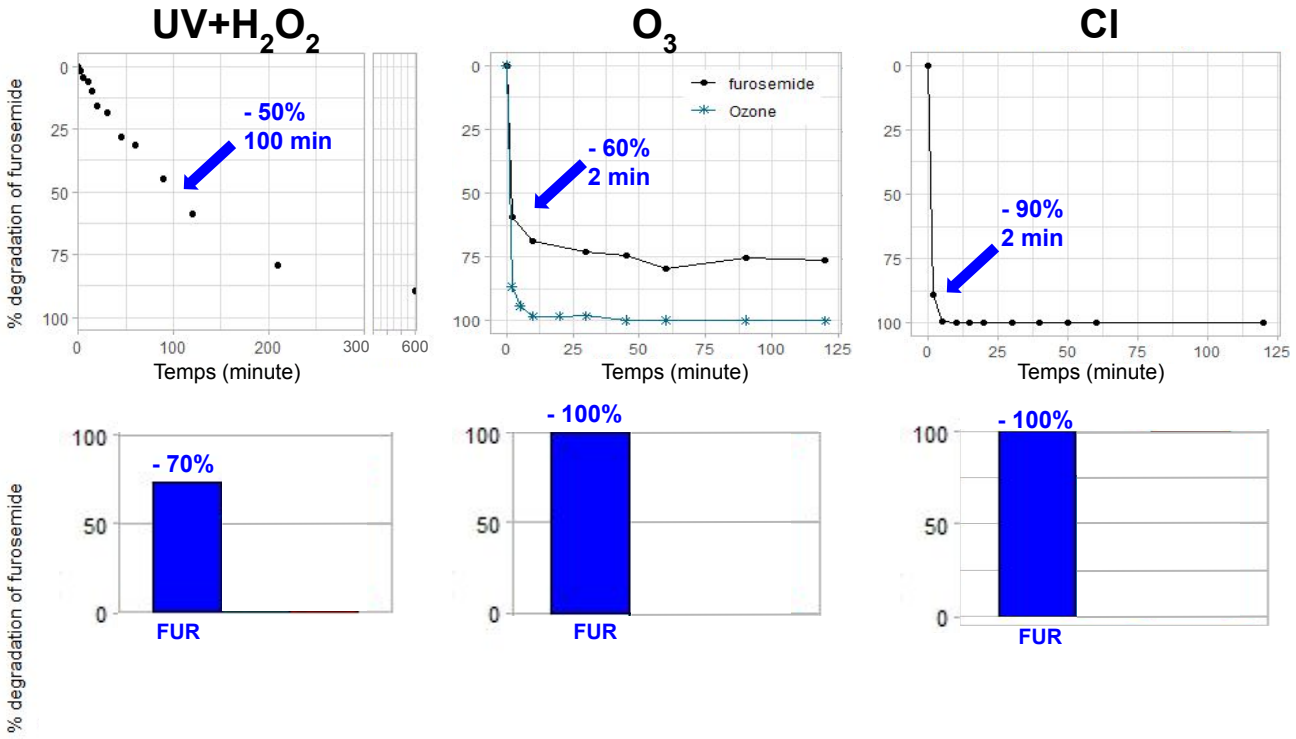
Cl



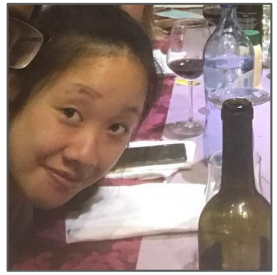
Expériences avec Nina



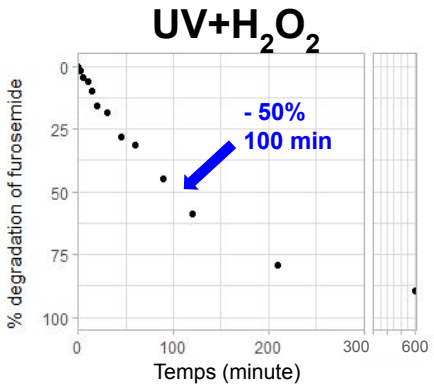
Dégradation du furosémide

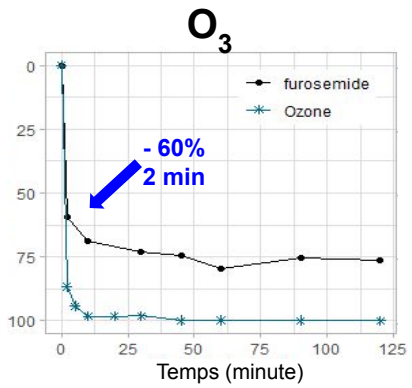


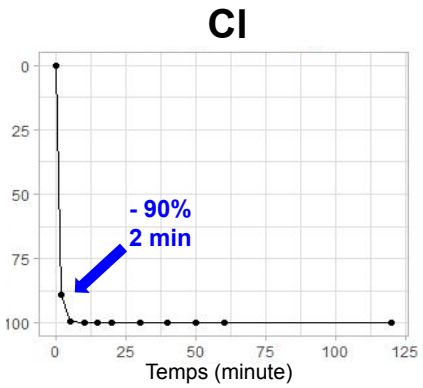
Expériences avec Nina

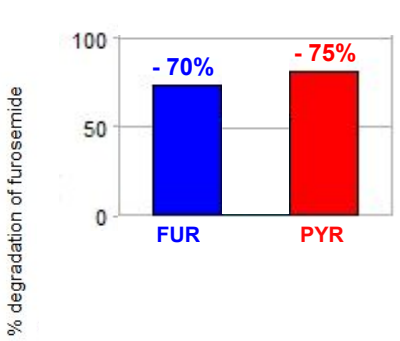


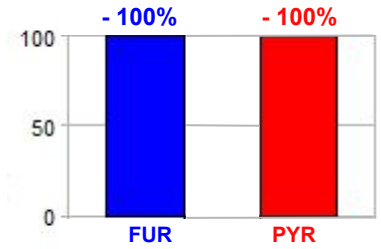
Dégradation du furosémide

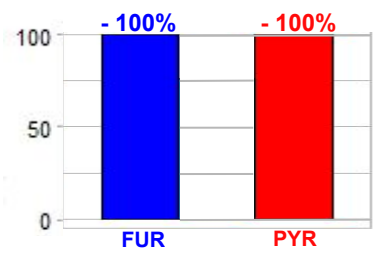












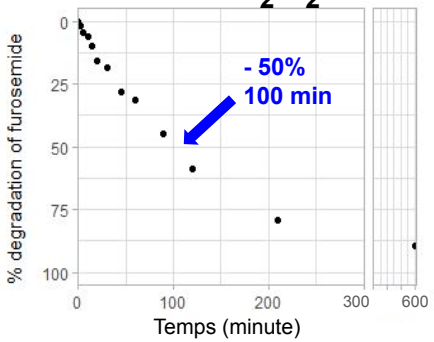
Expériences avec Nina

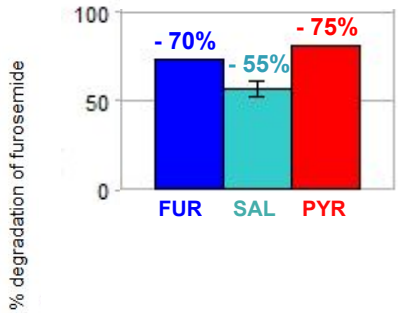
Dégradation du furosémide

EUP +FUR

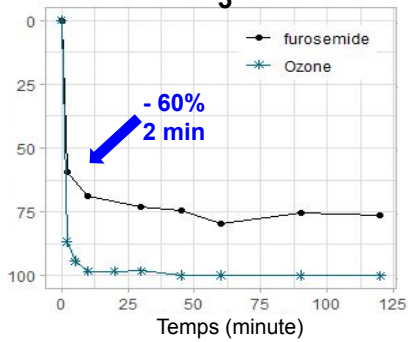
EHPAD

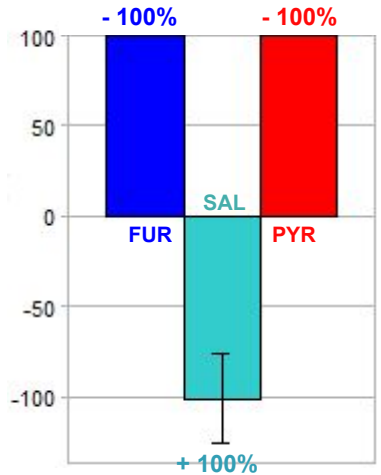
$UV+H_2O_2$



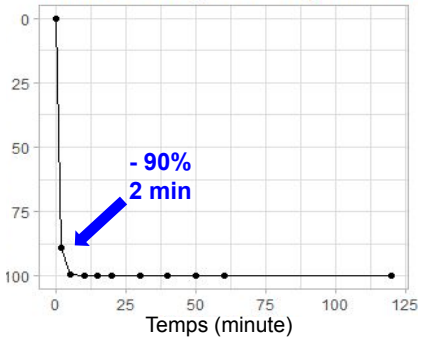


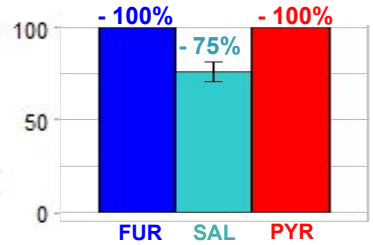
O_3





Cl

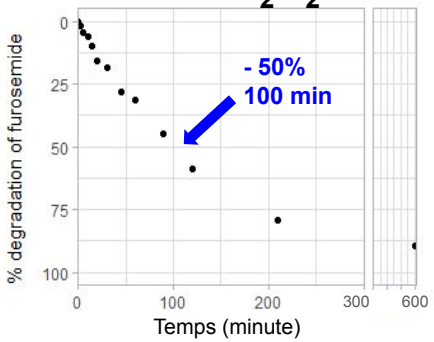


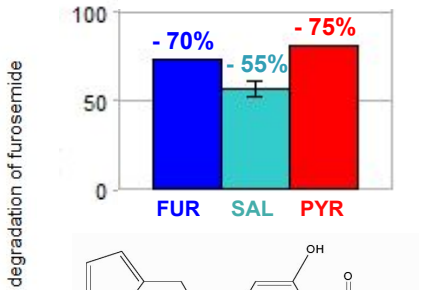


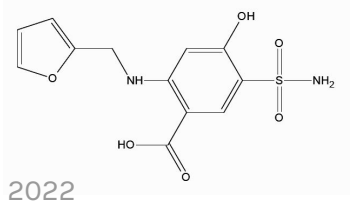
Expériences avec Nina

Dégradation du furosémide

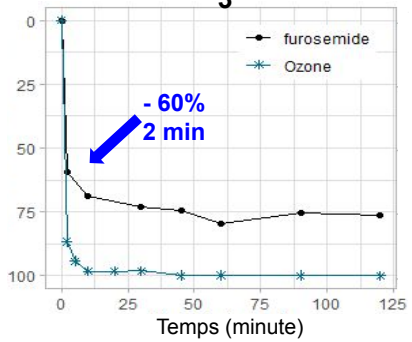
$UV+H_2O_2$

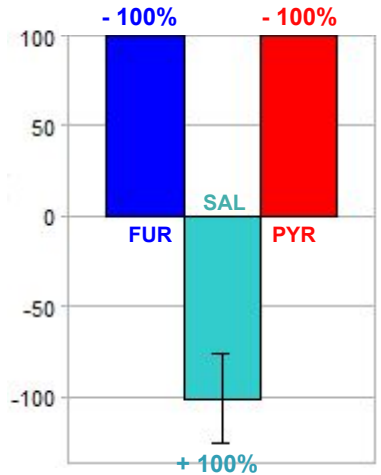




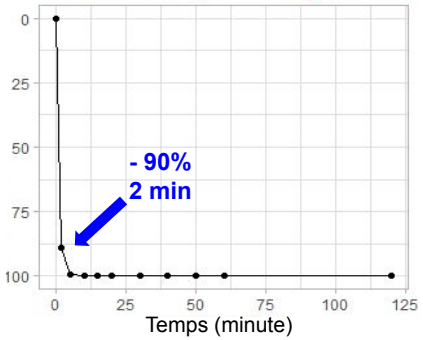


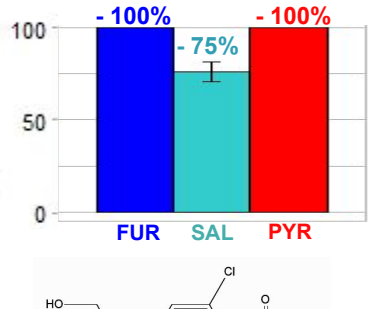
O_3

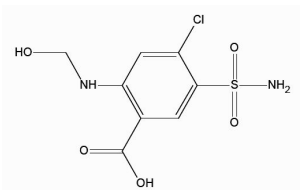




Cl





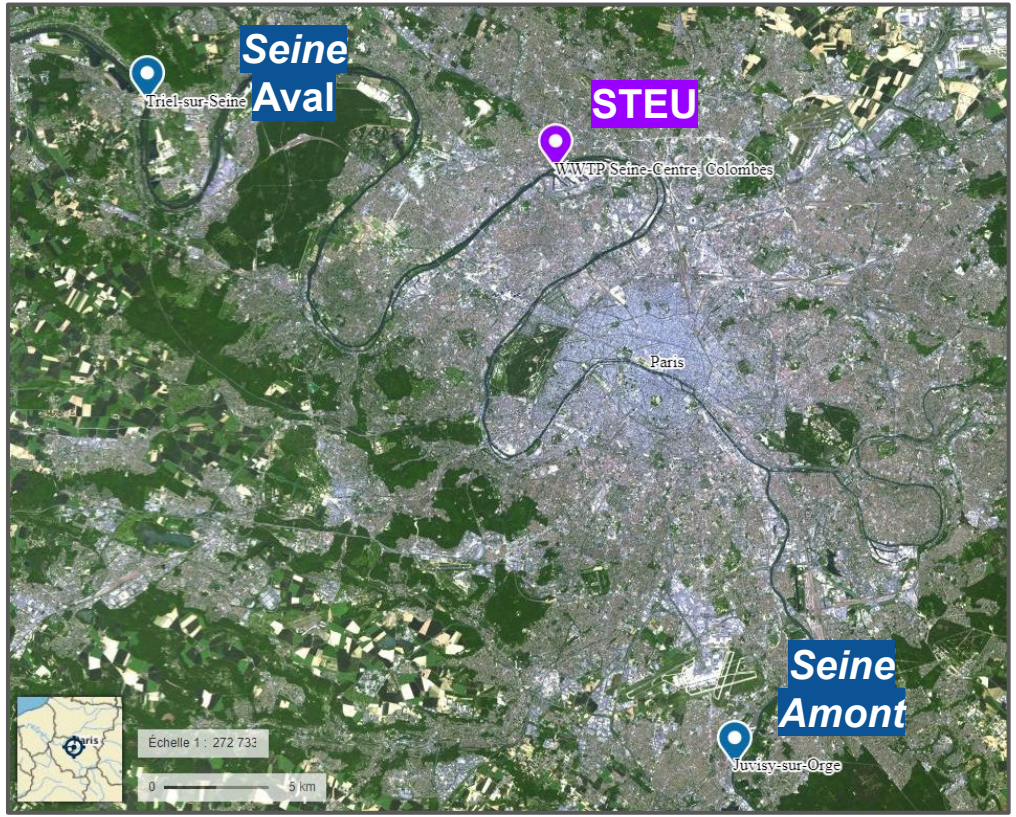


Expériences avec Nina

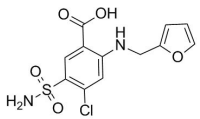
Recherche des sous produits avec Lamyae

Concentrations environnementales ?

Thèse de Sadia OPUR

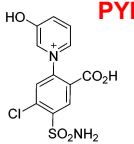


FUR



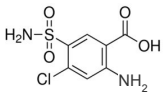
m/z 329,74 ✅

PYR



m/z 328,73

SAL



m/z 250,66 ?

Concentrations environnementales ?

Thèse de Sadia OPUR

Seine Amont, Paris

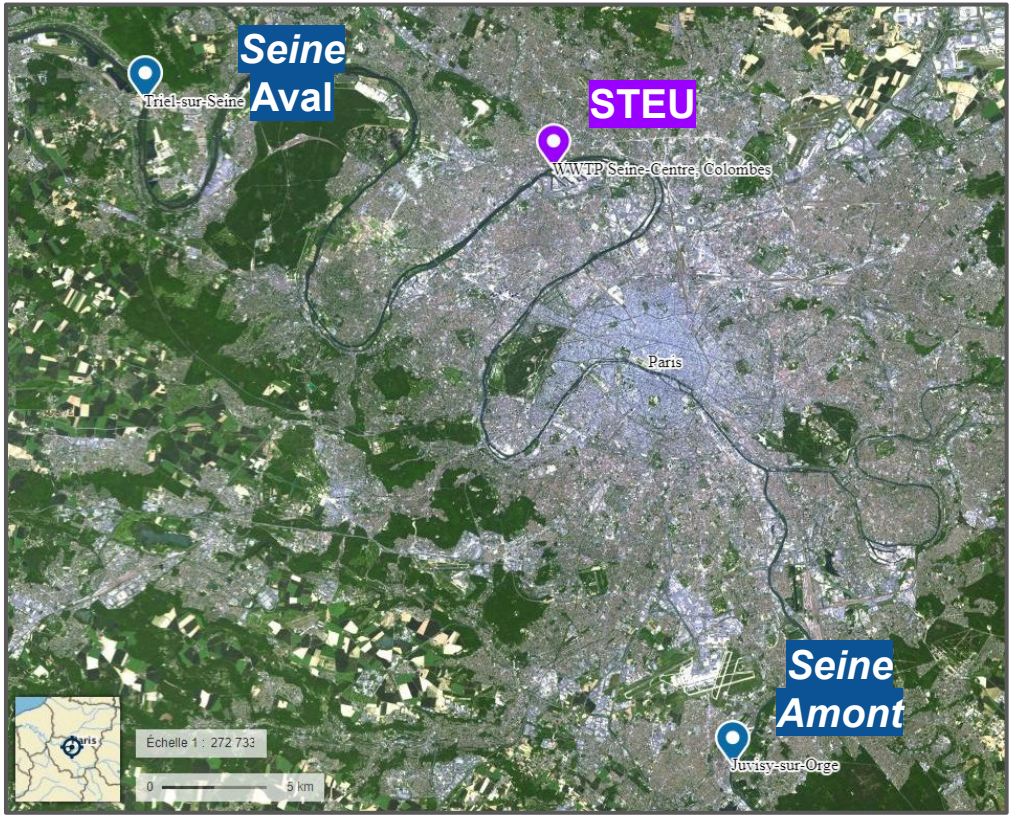
FUR
30 ng.L^{-1}

STEU *"Seine-Centre"*, Paris

FUR
900 ng.L^{-1}

Seine Aval, Paris

FUR
200 ng.L^{-1}



géoportail.fr

Concentrations environnementales ?

Thèse de Sadia OPUR

Seine Amont, Paris

FUR	PYR	SAL
30 ng.L^{-1}	60 ng.L^{-1}	50 ng.L^{-1}

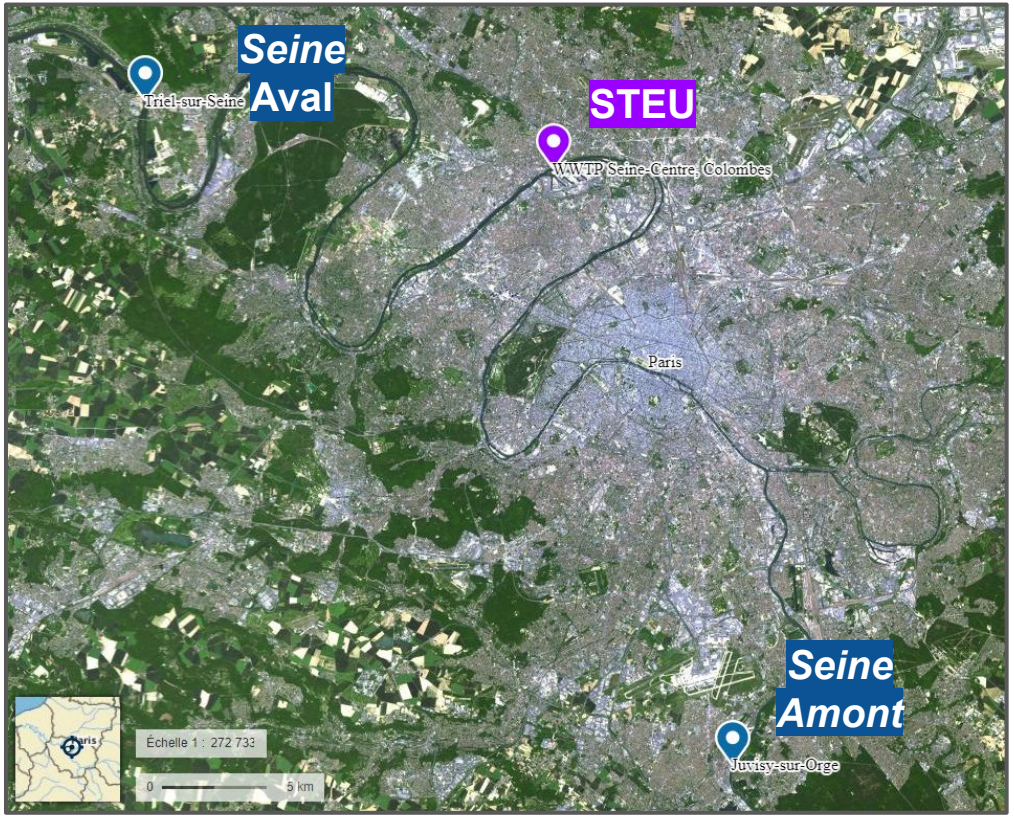
STEU *"Seine-Centre"*, Paris

FUR	PYR	SAL
900 ng.L^{-1}	250 ng.L^{-1}	500 ng.L^{-1}

Seine Aval, Paris

FUR	PYR	SAL
200 ng.L^{-1}	250 ng.L^{-1}	100 ng.L^{-1}

→ Première évidence de SAL et PYR

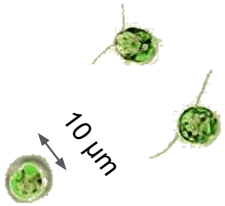


géoportail.fr

Quels organismes et bioessais?

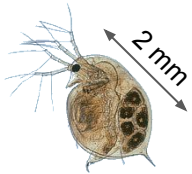
Algue verte

Chlamydomonas reinhardtii



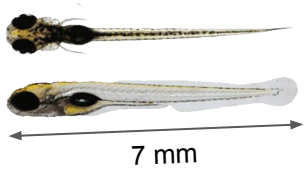
Daphnie

Daphnia magna



Poisson zèbre

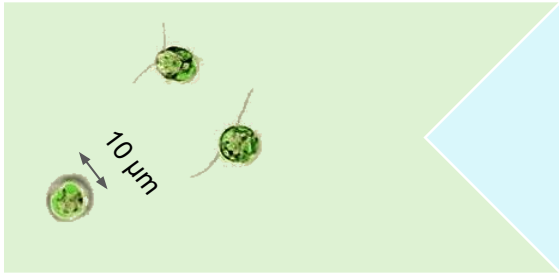
Danio rerio



Quels organismes et bioessais?

Algue verte

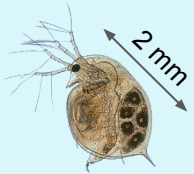
Chlamydomonas reinhardtii



- Producteur primaire

Daphnie

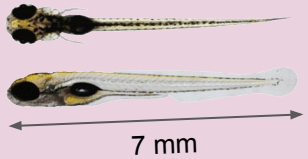
Daphnia magna



- Organisme filtreur

Poisson zèbre

Danio rerio

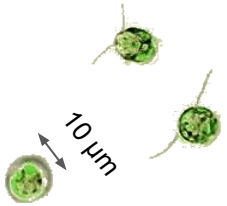


- Prédateur

Quels organismes et bioessais?

Algue verte

Chlamydomonas reinhardtii

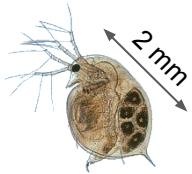


- Producteur primaire

Phytotoxicité
…

Daphnie

Daphnia magna

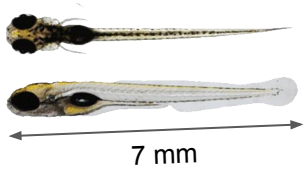


- Organisme filtreur

Toxicité aiguë
Cardiotoxicité
…

Poisson zèbre

Danio rerio



- Prédateur

Stress oxydant
Test de comportement
…

Algue verte

Chlamydomonas reinhardtii

Croissance des algues

24h 48h 72h 96h 120h

Début de l'expérience à 250 000 cells/mL

Croissance des algues

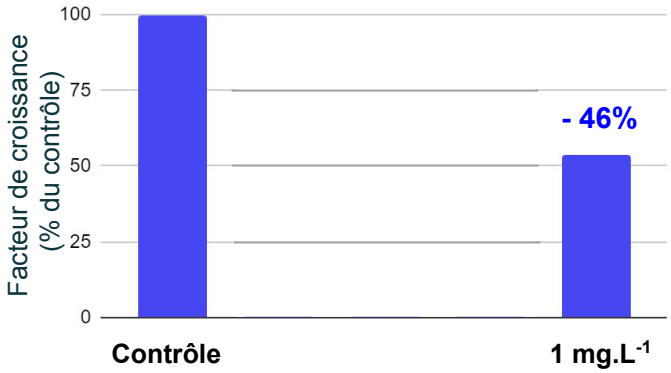
Algue verte

Chlamydomonas reinhardtii

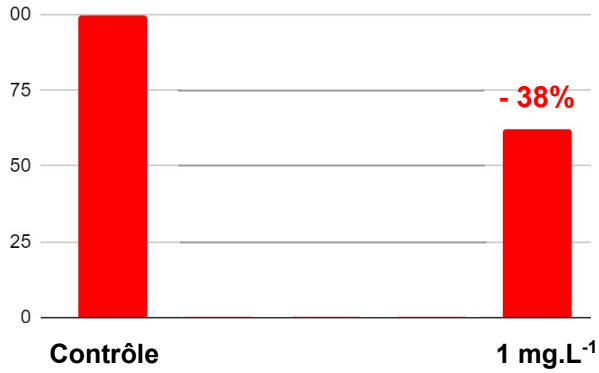
24h 48h 72h 96h 120h

Début de l'expérience à 250 000 cells/mL

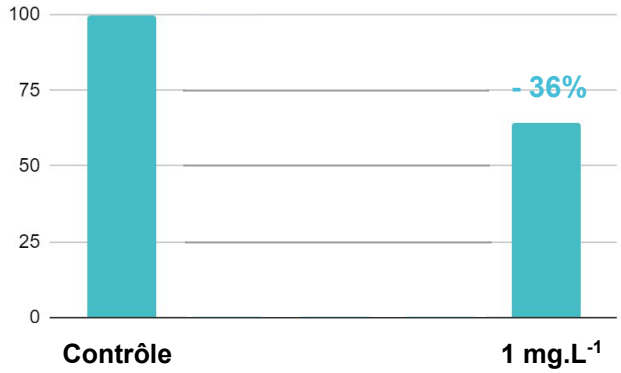
Furosemide



Pyridinium du Furosemide



Saluamine



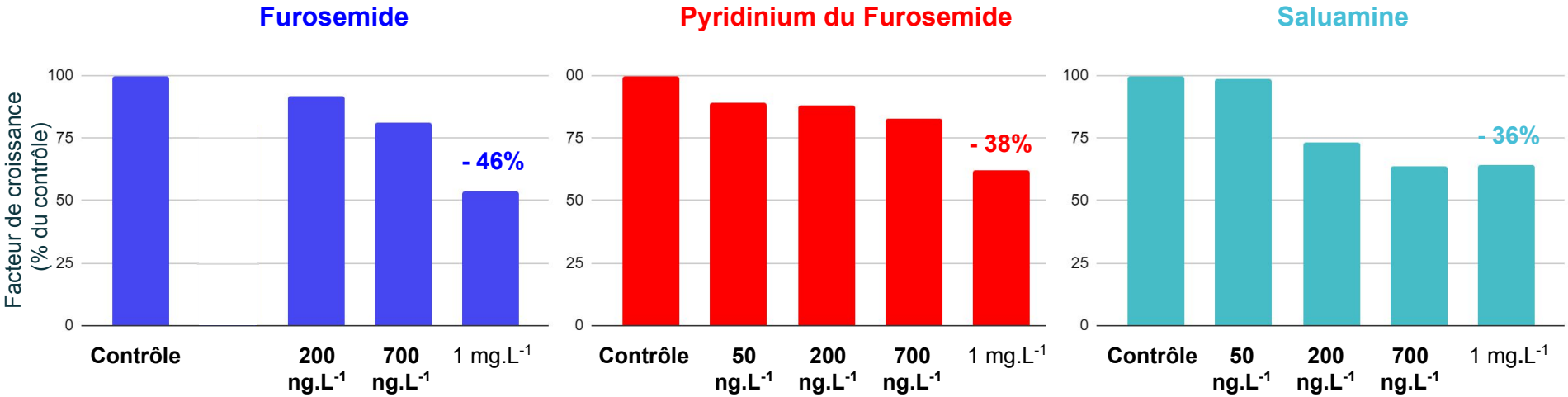
Croissance des algues

Algue verte

Chlamydomonas reinhardtii

24h 48h 72h 96h 120h

Début de l'expérience à 250 000 cells/mL

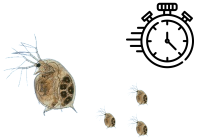


→ Inhibition de la croissance à des concentrations environnementales pour FUR, PYR et SAL

Toxicité sur les daphnies

Daphnie

Daphnia magna



daphnies neonates

24h 48h

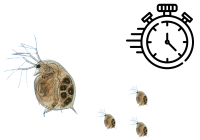
Mortalité

Rythme cardiaque

Toxicité sur les daphnies

Daphnie

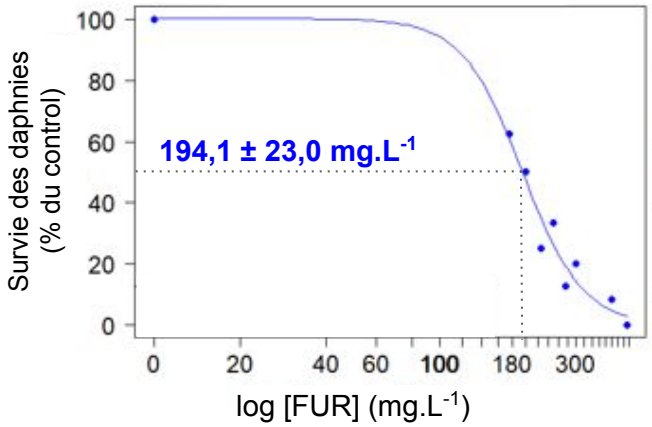
Daphnia magna



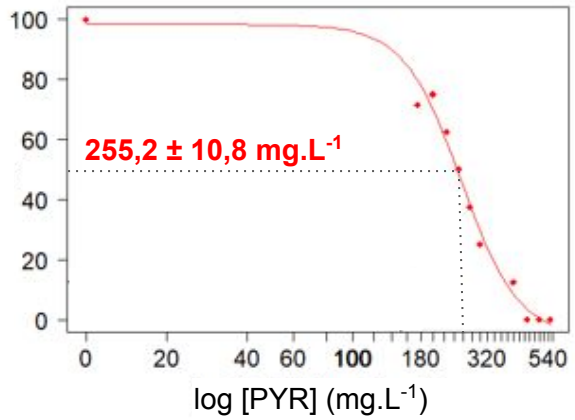
daphnies neonates

24h 48h → Mortalité, Rythme cardiaque

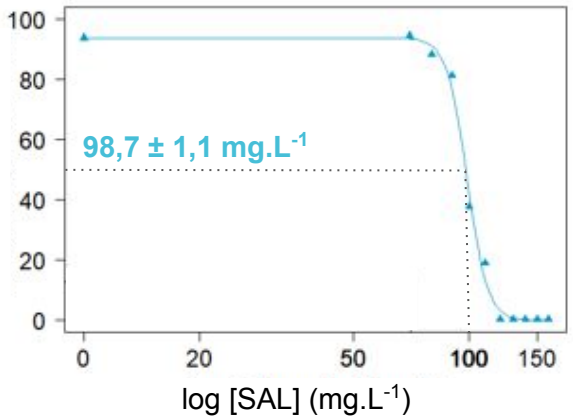
Furosemide



Pyridinium du Furosemide



Saluamine

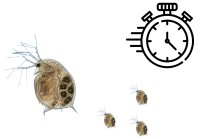


→ La Saluamine est plus toxique que le Furosemide pour les daphnies

Toxicité sur les daphnies

Daphnie

Daphnia magna



daphnies neonates

24h 48h

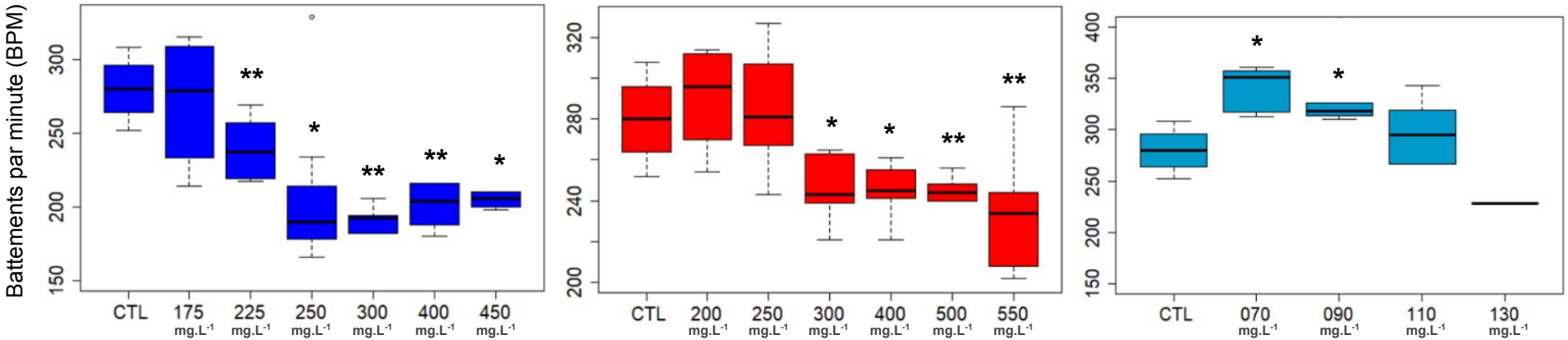
Mortalité

Rythme cardiaque

Furosemide

Pyridinium du Furosemide

Saluamine



→ Diminution significative des BPM pour FUR et PYR et augmentation pour SAL

Significant difference from control : * (p-value<0.05) ** (p-value<0.01) *** (p-value<0.001), kruskal-multiple comparison

Toxicité sur le poisson

Poisson zèbre

Danio rerio

0 - 6 dpf

- Anomalies
- $O_2^{\bullet}$ Stress oxydant ***DCFDA***
- Comportement

Toxicité sur le poisson

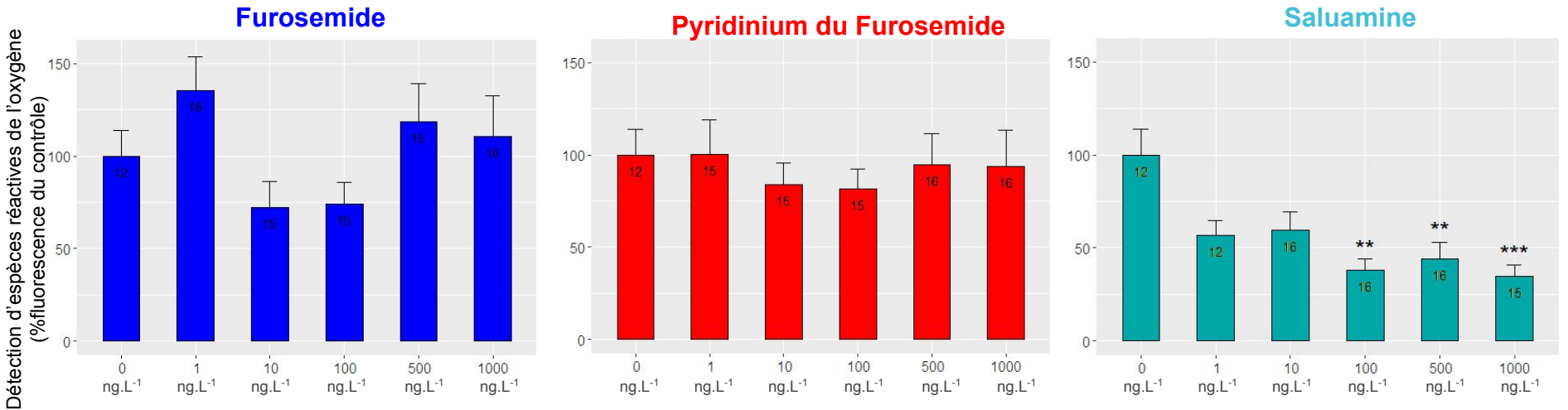
Poisson zèbre

Danio rerio

0 - 6 dpf

- Anomalies
- $O_2^{\bullet}$ Stress oxydant ***DCFDA***
- Comportement

→ Plus d'informations sur le poster d'Aliénor ←



→ Effet antioxydant significatif de la saluamine

Significant difference from control : * (p-value<0.05) ** (p-value<0.01) *** (p-value<0.001), kruskal-multiple comparison

Toxicité sur le poisson

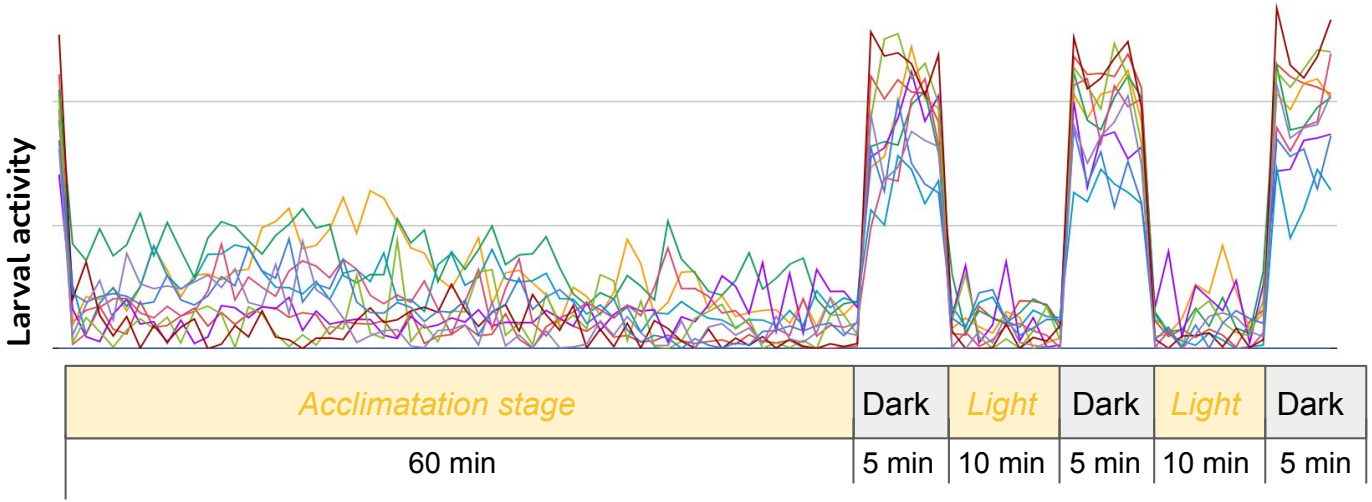
Poisson zèbre

Danio rerio

0 - 6 dpf

- Anomalies
- $O_2^{\bullet}$ Stress oxydant ***DCFDA***
- Comportement

Zebrabox : Appareil permettant la quantification de l'activité locomotrice des larves de poisson zèbre [1]



ViewPoint Behavior Technology

PRAMMICS

[1] Protocole Sandré et al. 2022

Toxicité sur le poisson

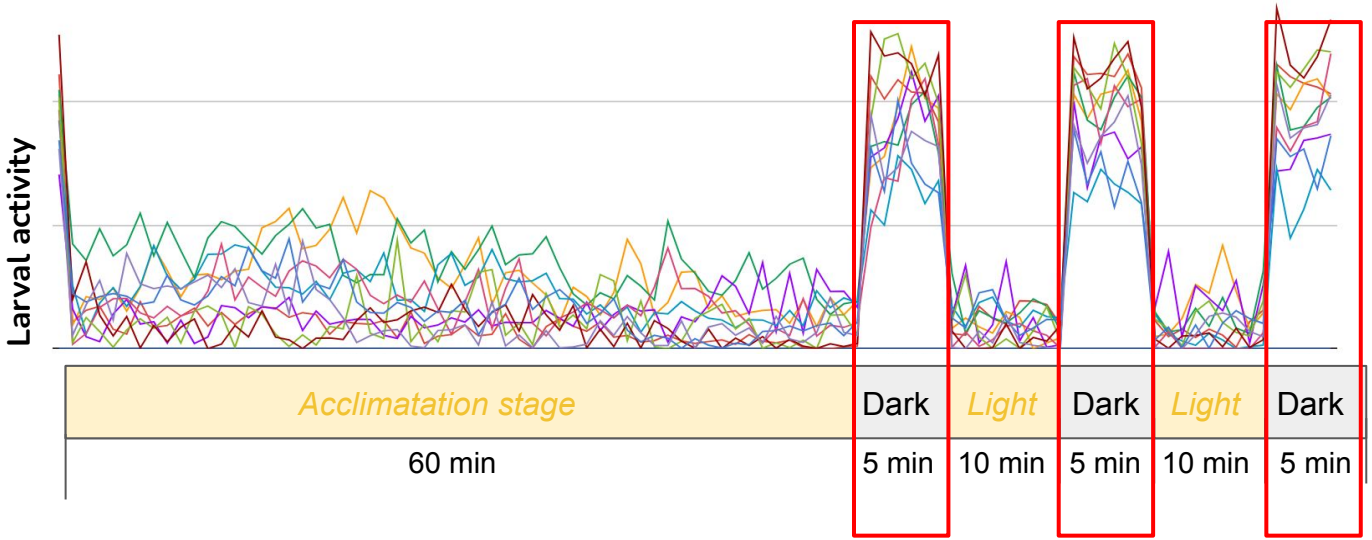
Poisson zèbre

Danio rerio

0 - 6 dpf

- Anomalies
- $O_2^{\bullet}$ Stress oxydant ***DCFDA***
- Comportement

Zebrabox : Appareil permettant la quantification de l'activité locomotrice des larves de poisson zèbre [1]



ViewPoint Behavior Technology

PRAMMICS

[1] Protocole Sandré et al. 2022

Toxicité sur le poisson

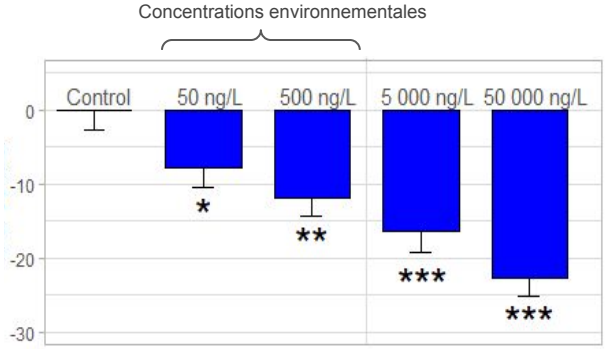
Poisson zèbre

Danio rerio

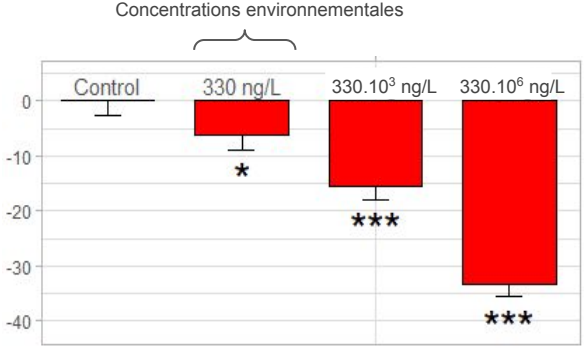
0 - 6 dpf

- Anomalies
- $O_2^{\bullet}$ Stress oxydant ***DCFDA***
- Comportement

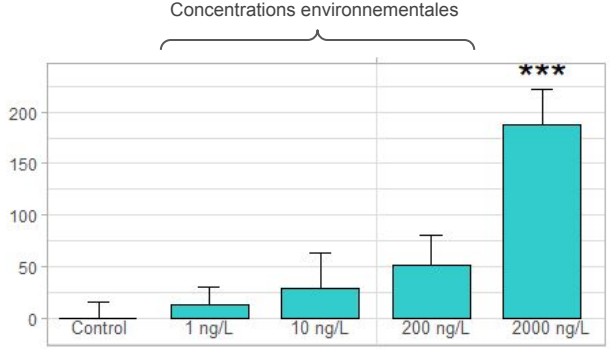
Furosemide



Pyridinium du Furosemide



Saluamine



→ FUR et PYR causent une inhibition significative de l'activité larvaire à des concentrations environnementales

Significant difference from control : * (p-value<0.05) ** (p-value<0.01) *** (p-value<0.001), kruskal-multiple comparison

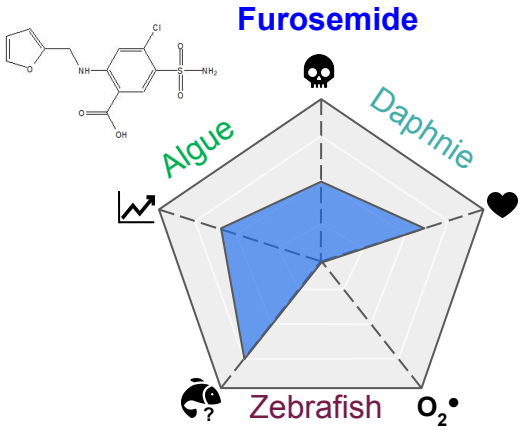
Messages clés

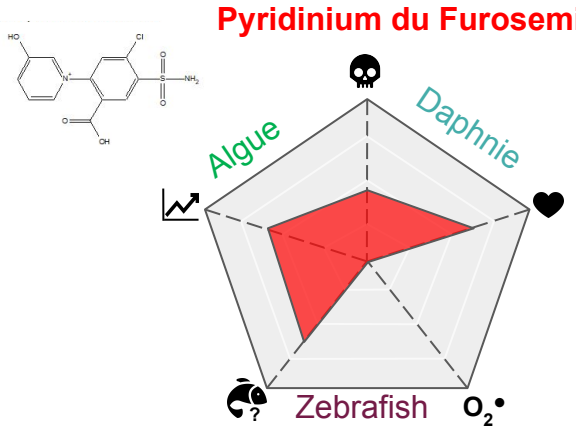
→ Première évidence de SAL et PYR dans les eaux de surface (50 - 250 $ng.L^{-1}$)

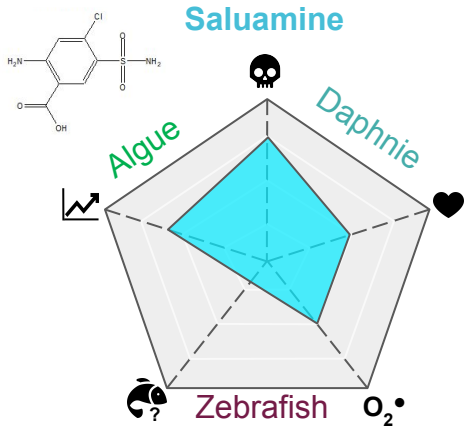
Messages clés

→ Première évidence de SAL et PYR dans les eaux de surface (50 - 250 $ng.L^{-1}$)

→ Impacts significatifs sur les trois organismes à concentrations fortes et environnementales





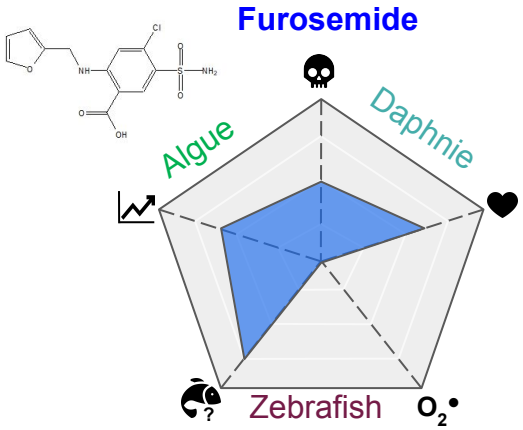


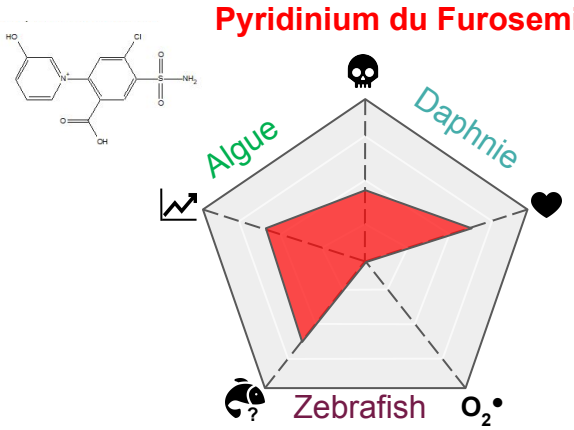
Messages clés

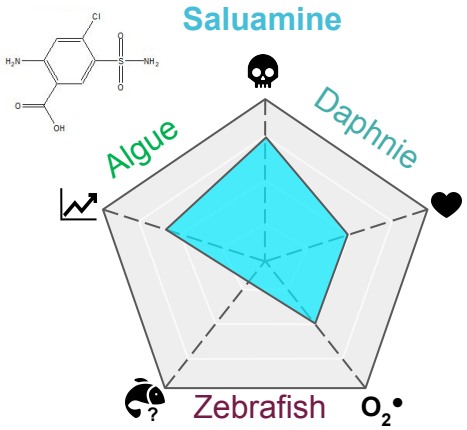
→ Première évidence de SAL et PYR dans les eaux de surface (50 - 250 ng.L^{-1})

→ Impacts significatifs sur les trois organismes à concentrations fortes et environnementales

→ Toxicité plus importante de SAL par rapport à FUR (bioactivation)







→ Mieux caractériser le risque des produits de transformation des pharmaceutiques sur l'environnement

Merci pour votre attention !

Christophe MORIN

Laure GARRIGUE-ANTAR

leesu
laboratoire eau environnement systemes urbains

UPEC
UNIVERSITÉ PARIS-EST CRÉTEIL

OPUR

PRAMMICS

ICMPE
Institut de Chimie et des Matériaux Paris-Est

OSU Efluve

This work is supported by the OPUR 5 program (phase 5: 2019-2023) piloted by Leesu

The Zebrabox® was financed by the Prammics platform (OSU-Efluve)

Thanks to Michael Rivard for the synthesis of the PYR

Fidji SANDRE is recipient of phD fellowship from the french ministry of research

sandrefidji@gmail.com

Furosemide is considered as a priority pollutant

	Zuccato 2004	Zuccato et. al 2005	Besse & Garric 2008	Munoz et al. 2008	De Voogt et al. 2009	Christensen et al. 2009	Roos et al. 2012	Dong et al. 2013	Kostich et al. 2014	Riva et al. 2014	Daouk et al. 2015	Guo et al. 2016	Mansour et al. 2016	Chinnaiyan et al. 2018	Li et al. 2019	Ahmed 2020
Production, sale or consumption (kg/year)					x			x		x		x	x	x		x
Removal in WWTP										x			x	x		
Persistance/ stability in freshwater	x	x		x	x		x				x					
Occurence in environment	x				x		x		x		x			x		
PEC			x						x							
Toxicity				x	x			x			x		x			
Ecotoxicity				x		x	x	x			x	x	x			
MEC/PNEC ratio or equivalent							x	x								
Other				x										x		
Bioconcentration factor (BCF)											x		x			
Excretion rate										x						

Synergic effect with combenefit

Di Veroli, G.Y., Fornari, C., Wang, D. et al. (4 more authors) (2016) *Combenefit: an interactive platform for the analysis and visualization of drug combinations.* Bioinformatics, 32 (18). pp. 2866-2868. ISSN 1367-4803

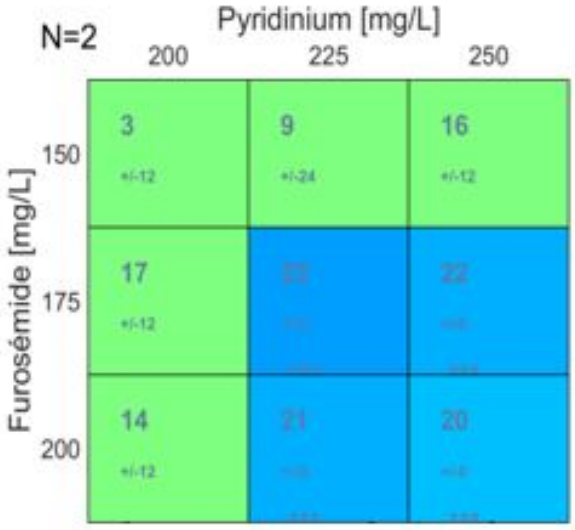


Figure 6a : Mélange PYR-FUR

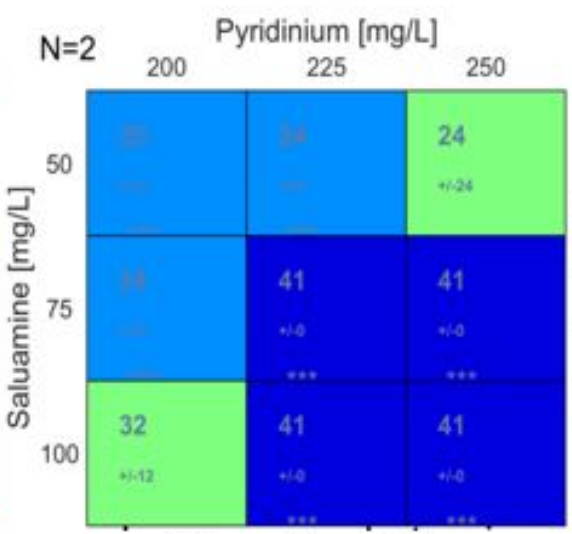


Figure 6b : Mélange PYR-SAL

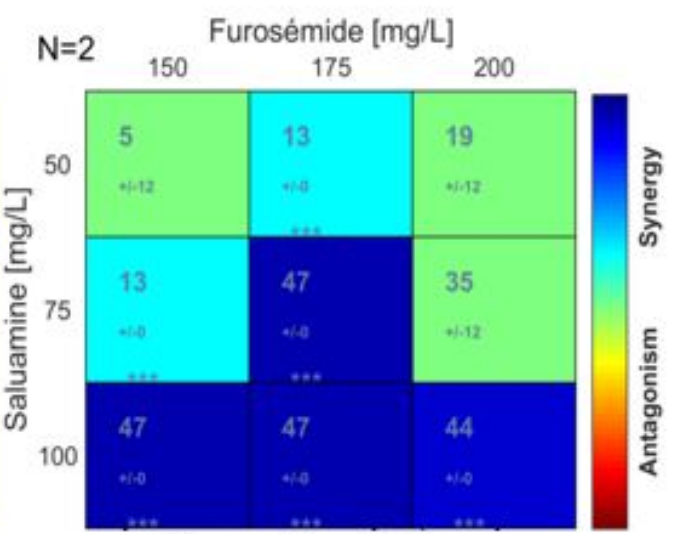


Figure 6c : Mélange FUR-SAL

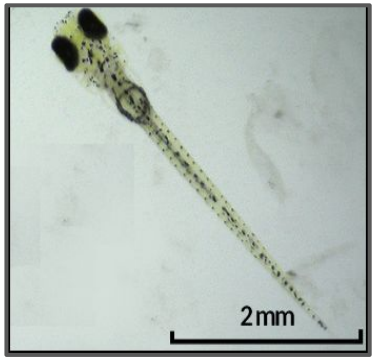
→ *acute toxicity on Daphnia magna*

Toxicity on zebrafish larvae

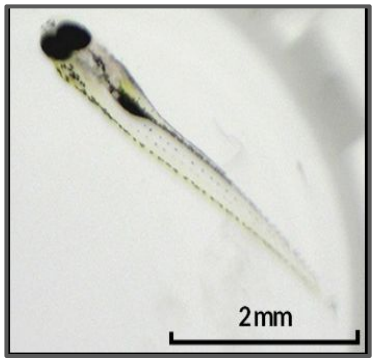
Zebrafish

Danio rerio

Photographies of 6 dpf larvae with malformations



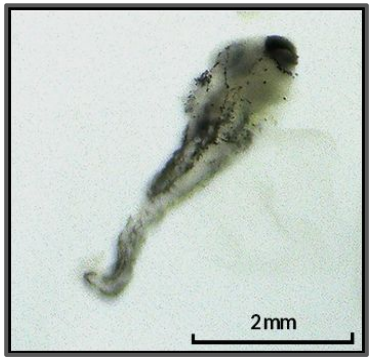
Normal



Floating

Curved

Oedema



Dead

very rare at environmental concentrations

→ No significant severe deformations at environmental concentrations