16 octobre 2020

- Café des sciences -

Ecotoxicological study of an emerging pharmaceutical pollutant: the Pyridinium of Furosemide (PoF)

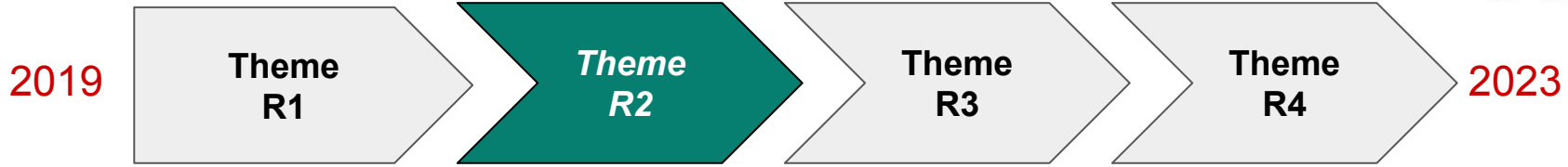
Fidji SANDRÉ (PhD student) ;
Christophe MORIN (Pr.) ;
Laure GARRIGUE-ANTAR (Pr.)

Laboratoire Eau Environnement et Systèmes Urbains
École des Ponts ParisTech
UPEC UNIVERSITÉ PARIS-EST CRÉTEIL
OPUR

OPUR *"Observatoire des Polluants Urbains"*

→ Improve knowledge about the production and transfer of pollutants in urban waters

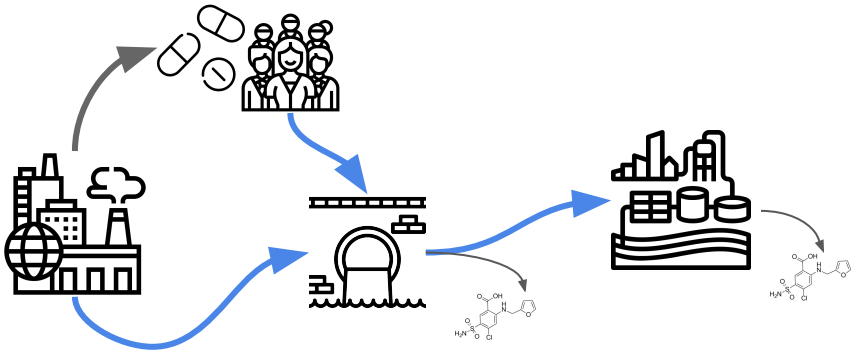
Phase 5 :



→ **Theme R2** : Diagnosis and optimization of wastewater systems regarding pollutants and micropollutants

→ **Action R2.6** : New methods for the characterization of micropollutants : Analysis by qualitative screening and ecotoxicology

Ecotoxicological study of an emerging pharmaceutical pollutant: the Pyridinium of Furosemide (PoF)



- Incomplete elimination or uncontrolled releases of pharmaceuticals compounds
- Systematic detection of some compounds in water (Diclofenac, Paracetamol, Ibuprofene,..

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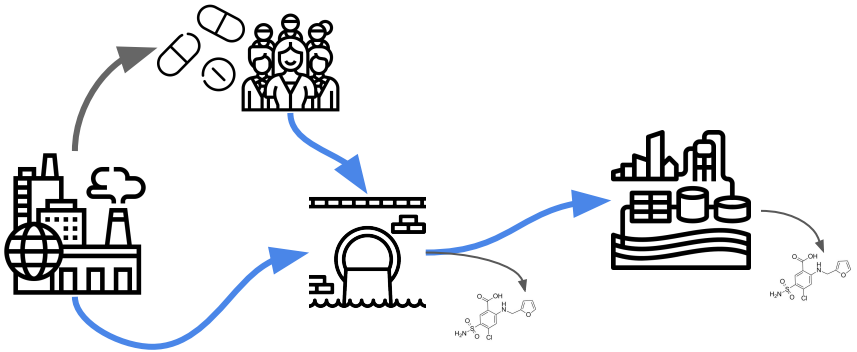

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...*furosemide*,..)

Maximum concentration found in water	
River	**1 230 ng.L^{-1}** (Cantwell et al. 2018)
Influent WWTP	**15 320 ng.L^{-1}** (Papageorgiou et al. 2016)
Effluent WWTP	**11 000 ng.L^{-1}** (Rozman et al. 2017)
Influent Hospital	**392 000 ng.L^{-1}** (Thomas & Langford 2010)
Effluent Hospital	**12 000 ng.L^{-1}** (Wahlberg et al. 2011)
Effluent pharmaceutical facilities	**1 200 000 ng.L^{-1}** (Kleywegt et al. 2019)

Ecotoxicological study of an emerging pharmaceutical pollutant: the Pyridinium of Furosemide (PoF)

Furosemide

- Widely used diuretic since 1965
- Occur at concentrations higher than the predicted environmental concentration (PEC)
  PEC > 100 ng.L^{-1}

- Incomplete elimination or uncontrolled releases of pharmaceuticals compounds
- Systematic detection of some compounds in water (Diclofenac, paracetamol, ibuprofene,..

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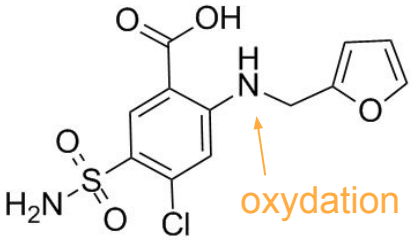
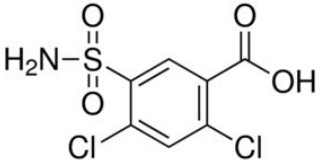
Ecotoxicological study of an emerging pharmaceutical pollutant: the Pyridinium of Furosemide (PoF)

Furosemide



(1)

Saluamine



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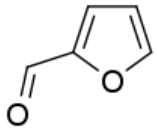
Furfural



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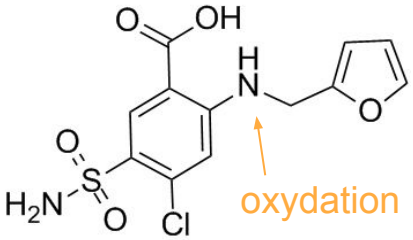
Furosemide



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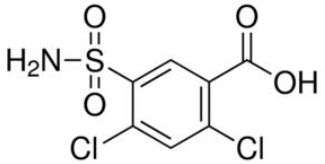

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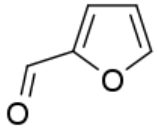

- Both known as a Furosemide metabolite since 1987 (Andreasen et al. 1981)
- Furfural : occurs naturally, not very toxic, rapidly biodegradable (Hoydonckx et al. 2007)
- Saluamide : highly irritating (European chemical agency) more toxic than furosemide (Olvera-vargas et al. 2016) genotoxic, cytotoxic & E.D. (Al-Omar et al. 2009)

Ecotoxicological study of an emerging pharmaceutical pollutant:
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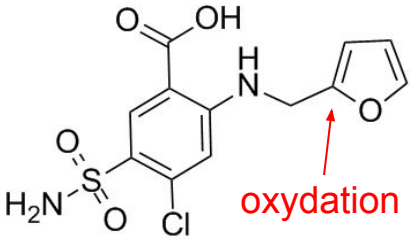
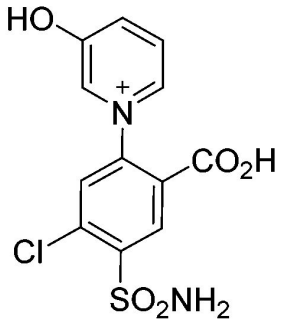
Furosemide



(2)

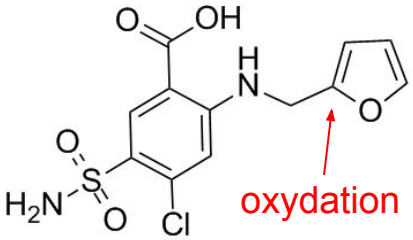


Pyridinium of Furosemide (PoF)

- Produced by electrochemistry (Laurencé et al. 2011)

Ecotoxicological study of an emerging pharmaceutical pollutant: the Pyridinium of Furosemide (PoF)

Furosemide



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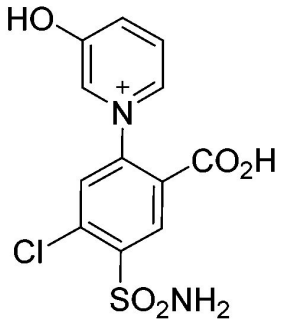

Pyridinium of Furosemide (PoF)

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Cunninghamella echinulata

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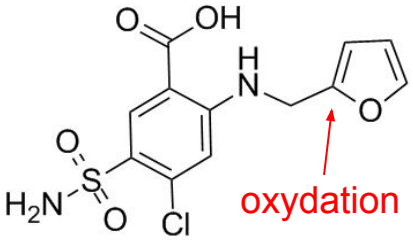

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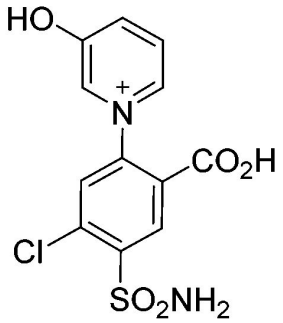

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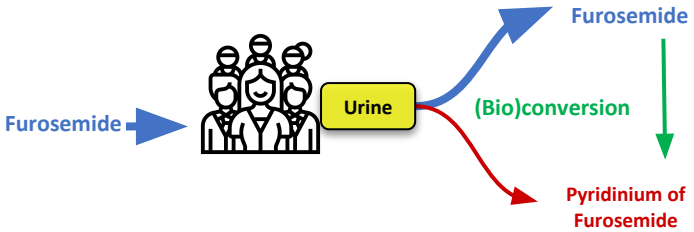

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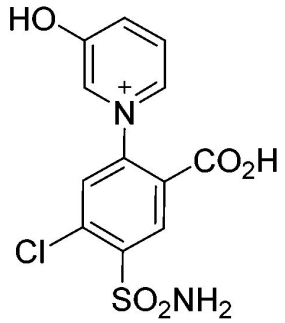

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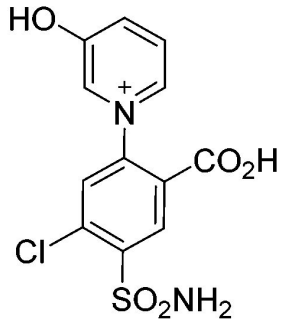
→ Is it toxic ?

Ecotoxicological study of an emerging pharmaceutical pollutant: the Pyridinium of Furosemide (PoF)

(Laurencé et al. 2019)

- Death of dopaminergic neurons
- Alteration of the complex I of the mitochondrial respiratory chain
- Accumulation of alpha synuclein in neurons

Possible inducer of neurodegeneration
... at high concentration



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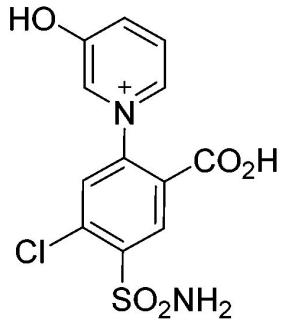

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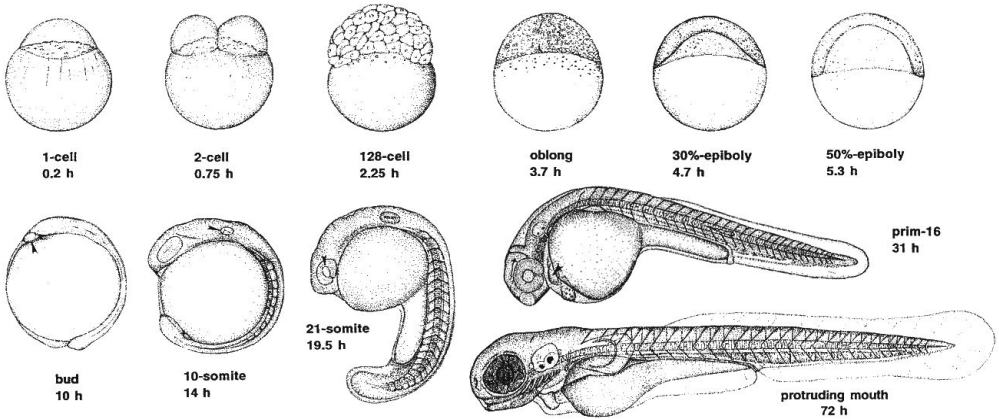
→ Is it toxic at environmental concentration?

~~→ Is it toxic ?~~

Experimental model : Danio *rerio*

- optically transparent eggs
  → easily observable and manipulable
- Integrative "whole animal" model
- Strong homology with man
  → 80% of genes in common
- Model for a large number of human pathologies
  (cancer, neurodegenerative diseases...)



(kimmel et al. 1995)

Hb9 - GFP strain
Tests at 5, 6 & 7 dpf

Organism

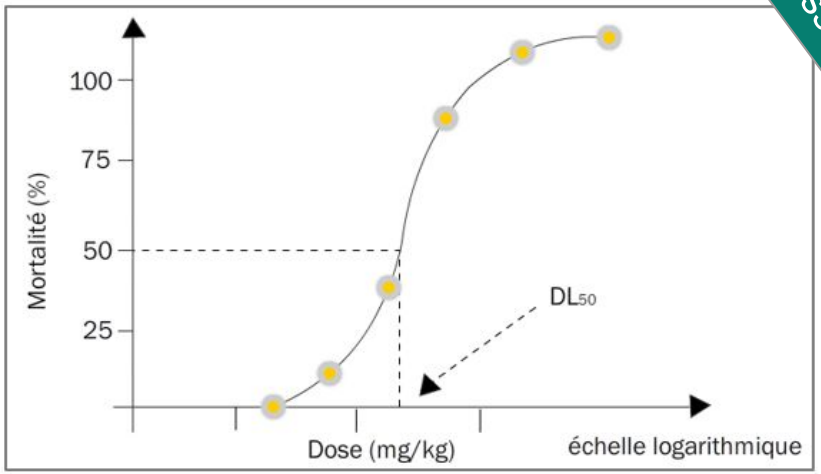
- **Letality (LC50 / LD50)**
- **Deformations / developmental abnormalities**
- **Cardiotoxicity**

Phenotype

→ **Multi-scale testing panel**

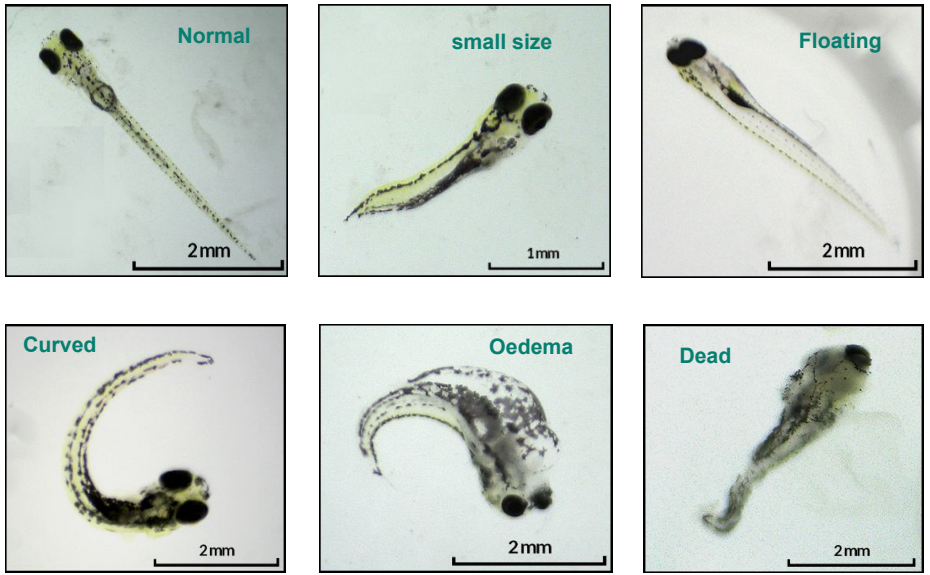
Letality (LC50 / LD50) :

In progress



→ Development of OECD standardized tests (OECD 236)

Deformations / developmental abnormalities :

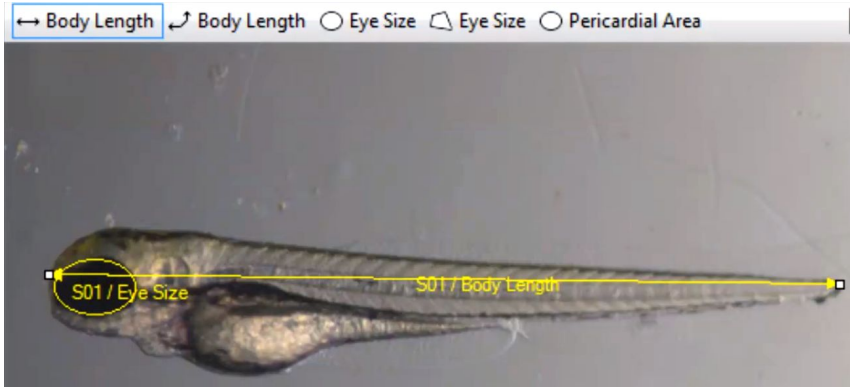


Photographies of 6 or 7 dpf larvae with malformations

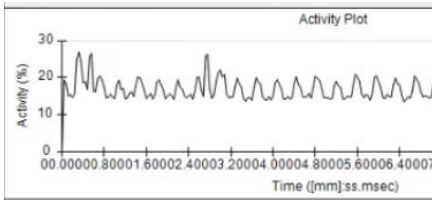
Deformations / developmental abnormalities (2) :

In progress

→ Measurement of body length, eye size and pericardial area

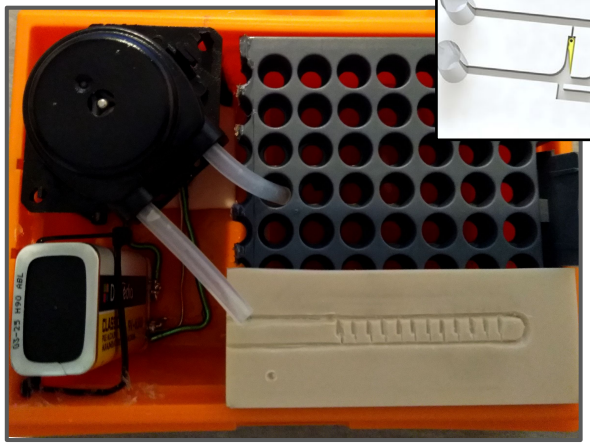


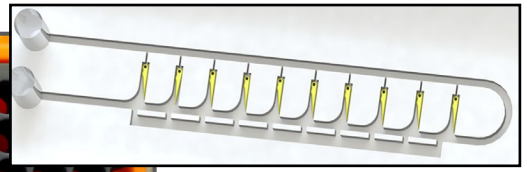
Cardiotoxicity :



In progress

→ Monitoring of cardiac activity using a millifluidic device





Fuad et al. 2016
Fuad et al. 2017

Organism

Sub-organism

→ **Multi-scale testing panel**

- Letality (LC50 / LD50)
- Deformations / developmental abnormalities
- Cardiotoxicity

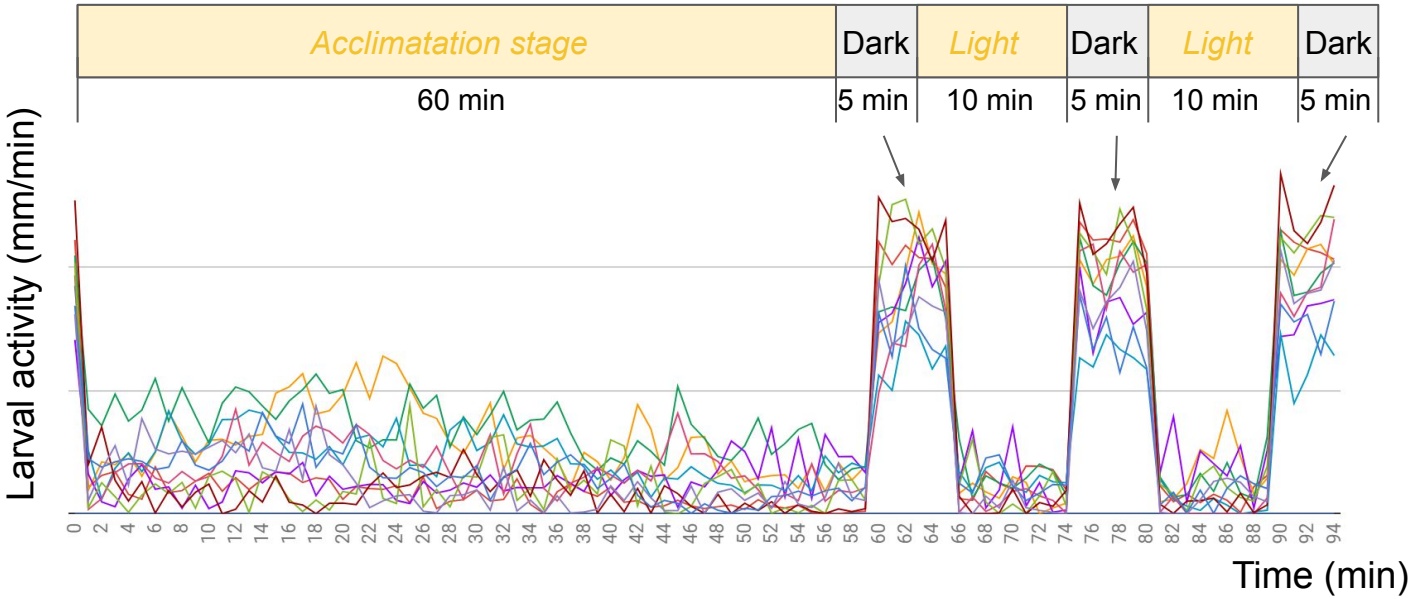
Phenotype

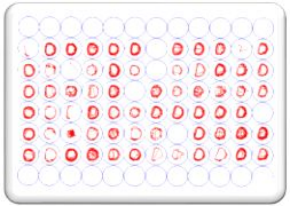
- **Dark/light transition test**
- **Escape test**

Comportemental effect

Dark/light transition test

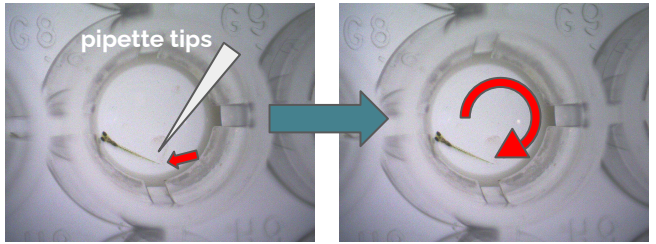
- Zebrabox : Automated analysis chamber for the quantification of the locomotor activity of zebrafish larvae
- Adapted protocol from Peng et al. 2016

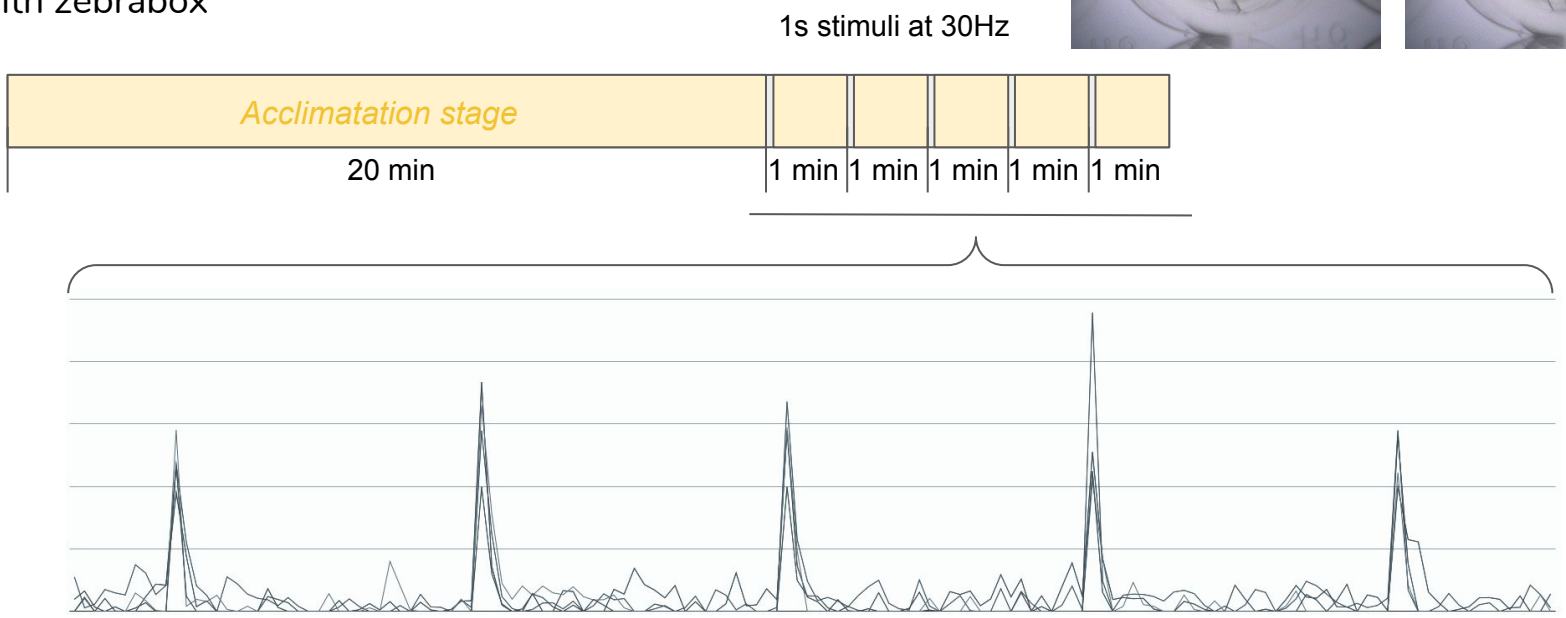




Escape test

- With manual contact
- With zebrabox





Organism

Sub-organism

Molecular

→ **Multi-scale testing panel**

- Letality (LC50 / LD50)
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- Cardiotoxicity

Phenotype

- Dark/light transition test
- Escape test

Comportemental effect

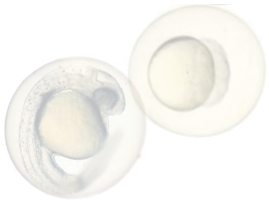
- **Identification of biomarker genes**
- **Identification of indicator proteins**

OMICs perspective

- **Genotoxicity**
- **Endocrine disruption**
- **Impact on mitochondria**

Methods still in discussion

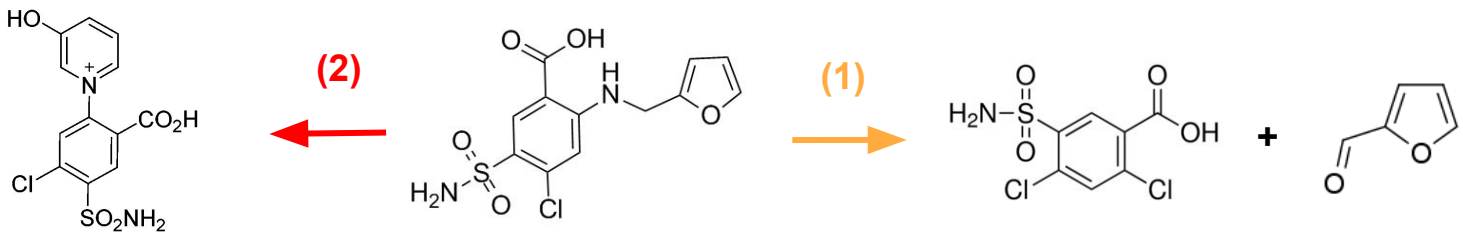
Thank you for your attention



Approximation of environmental concentrations

- 60-90% of Furosemide remain unchanged or conjugated → ~25% is transformed

Pyridinium of Furosemide **Furosemide** **Saluamine** **Furfural**

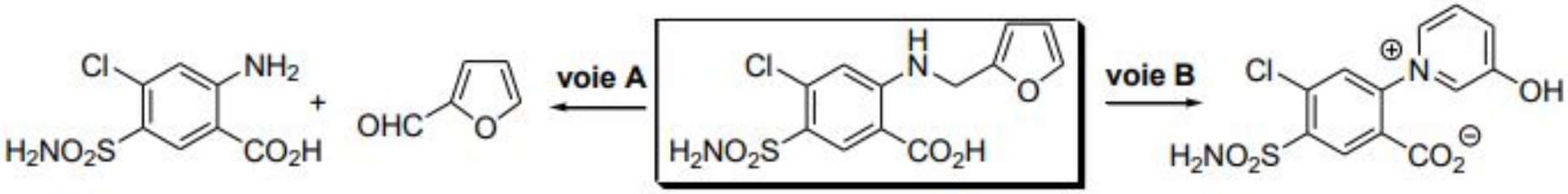


- Among the 25%, there is two possibilities : (1) is the major way, maximum 99%
- (2) is 1% of the 25% estimated, we consider 1000 ng/L as medium value of furosemide in step effluent
- 2,5ng/L minimum estimated, then successive dilution/2

Autres métabolites du Furosémide

oxydation du groupe amine

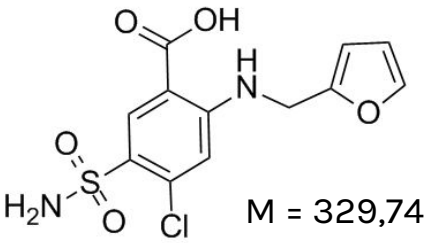
oxydation du cycle furane



Saluamine **Furfural** **Furosémide** **Pyridinium du Furosémide**

Laurencé C., et al., 2011.

Modélisation du Furosémide et du PoF



M = 329,74

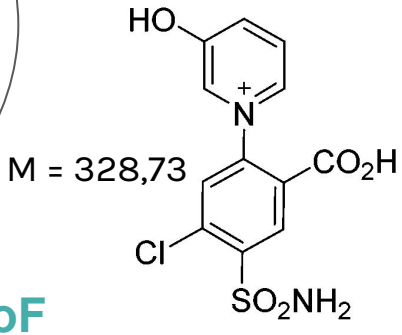
Probabilité de liaison aux récepteurs

Forte — Faible

Antagoniste récepteurs Androgènes

récepteurs Glucocorticoïdes

récepteurs α hormones Thyroïdiennes



M = 328,73

Furosémide

AR : -7.4	AR an.: -7.2	ER α: -7.7	TR α: -7.9
ER α an.: -7.1	ER β: -7.6	ER β an.: -7.3	RXR α: -7.5
GR: -7.7	GR an.: -6.4	LXR α: -8.1	PPAR γ: -7.5
LXR β: -8.1	PPAR α: -7.2	PPAR β: -7.6	TR β: -7.5

PoF

AR : -6.8	AR an.: -6.7	ER α: -7.7	TR α: -7.7
ER α an.: -7.8	ER β: -7.8	ER β an.: -7.7	RXR α: -7.4
GR: -8.7	GR an.: -6.6	LXR α: -8.3	PPAR γ: -6.2
LXR β: -8.0	PPAR α: -7.4	PPAR β: -7.6	TR β: -7.8

Microorganismes

Laurencé C., et al., 2014.

Aspergillus candidus

Cunninghamella echinulata

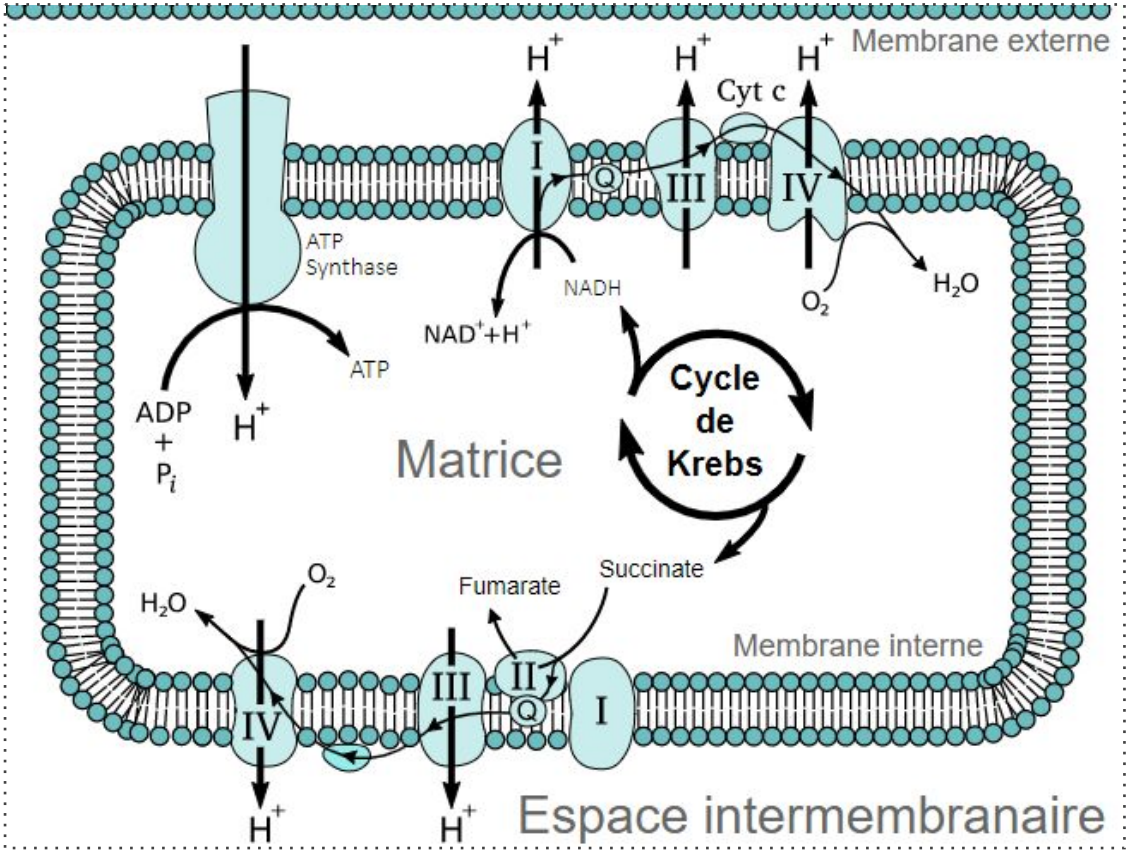
Agrobacterium tumefaciens

Arthrobacter ureafaciens

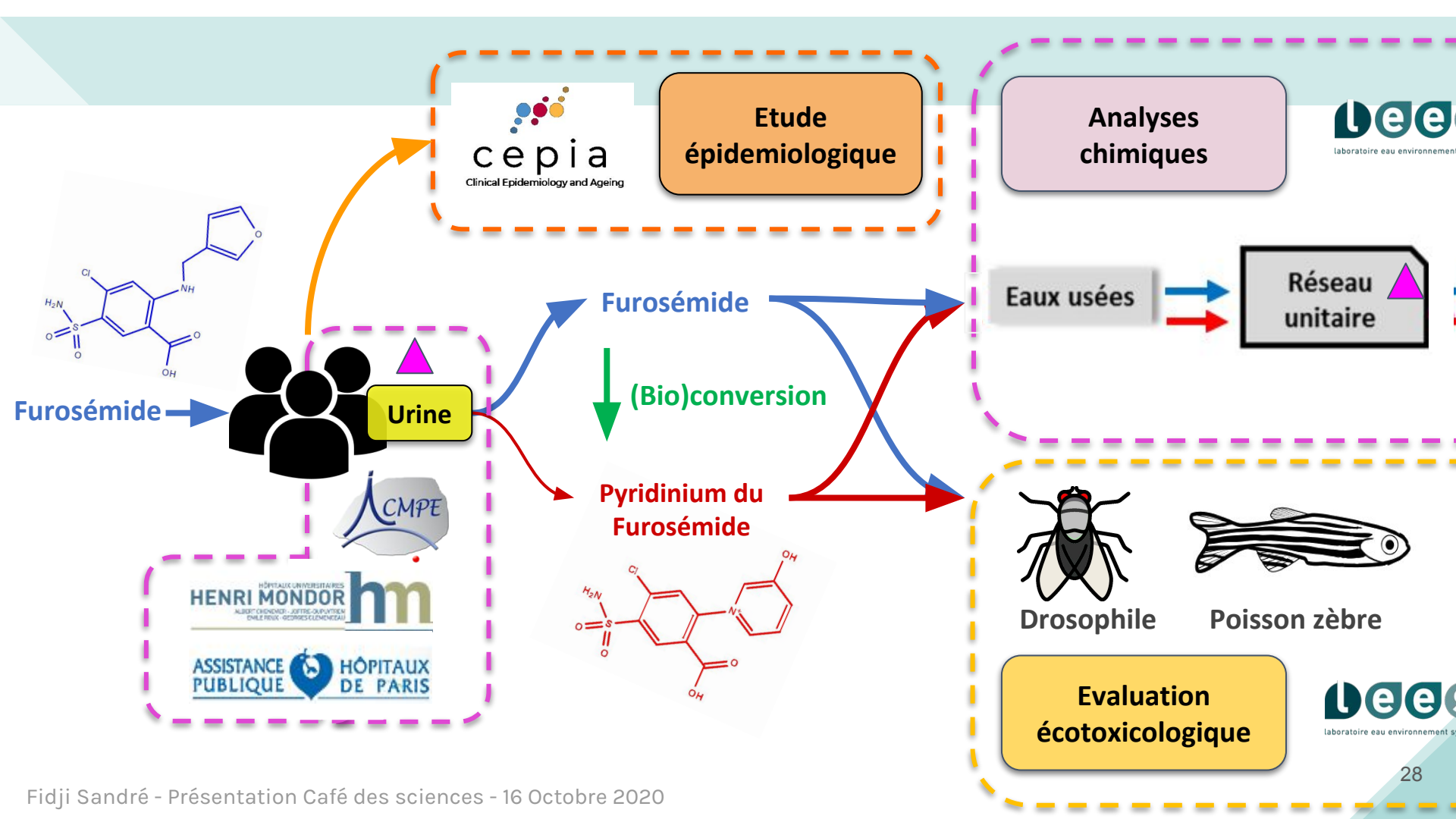
Métabolisation furosémide → PoF (Champignons)

Métabolisation furosémide → saluamine (Bactéries)

Chaîne respiratoire mitochondriale



Chaîne de transport d'électrons conduisant à la production d'ATP cellulaire


cepia
Clinical Epidemiology and Ageing
Etude épidemiologique
Analyses chimiques
laboratoire eau environnement
Furosémide
Urine
Furosémide
(Bio)conversion
Pyridinium du Furosémide
Eaux usées
Réseau unitaire
CMPE
HENRI MONDOR
ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS
Drosophile
Poisson zèbre
Evaluation écotoxicologique