Mixture toxicity predictions to address groundwater contamination

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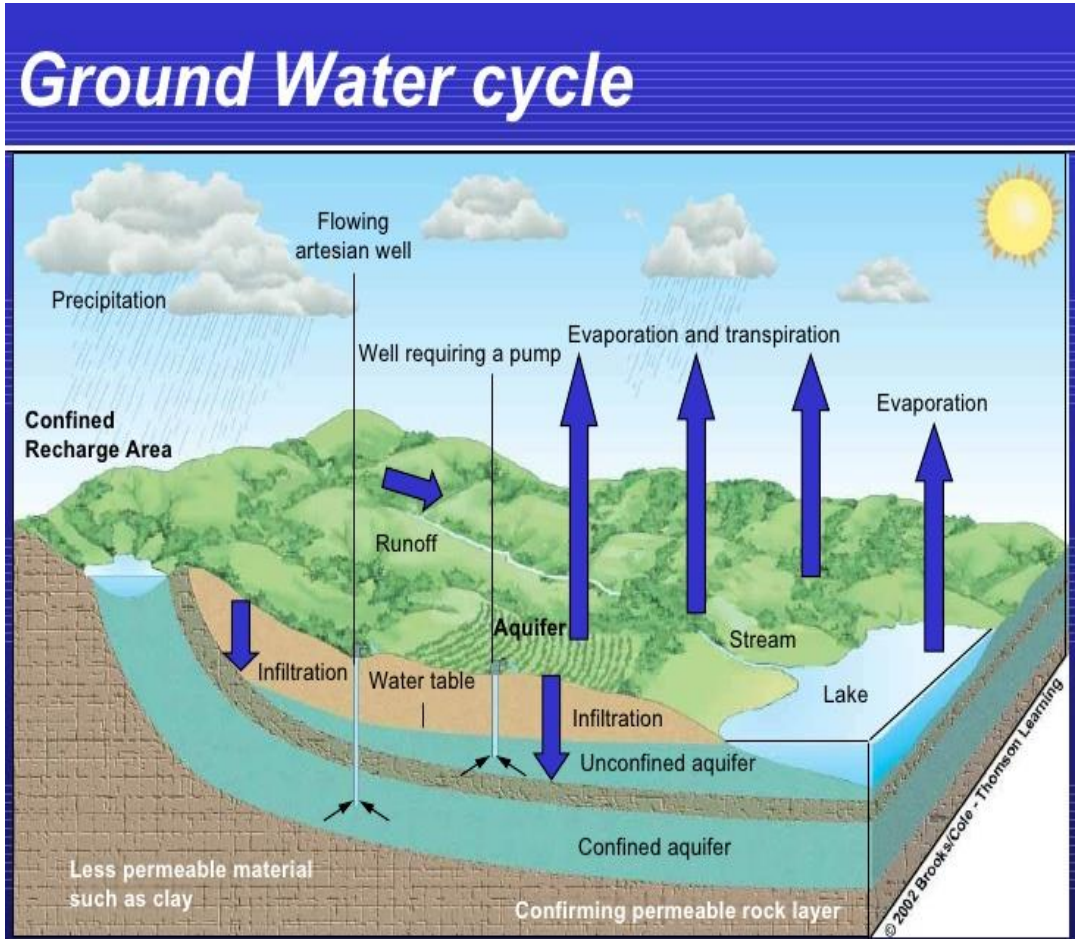
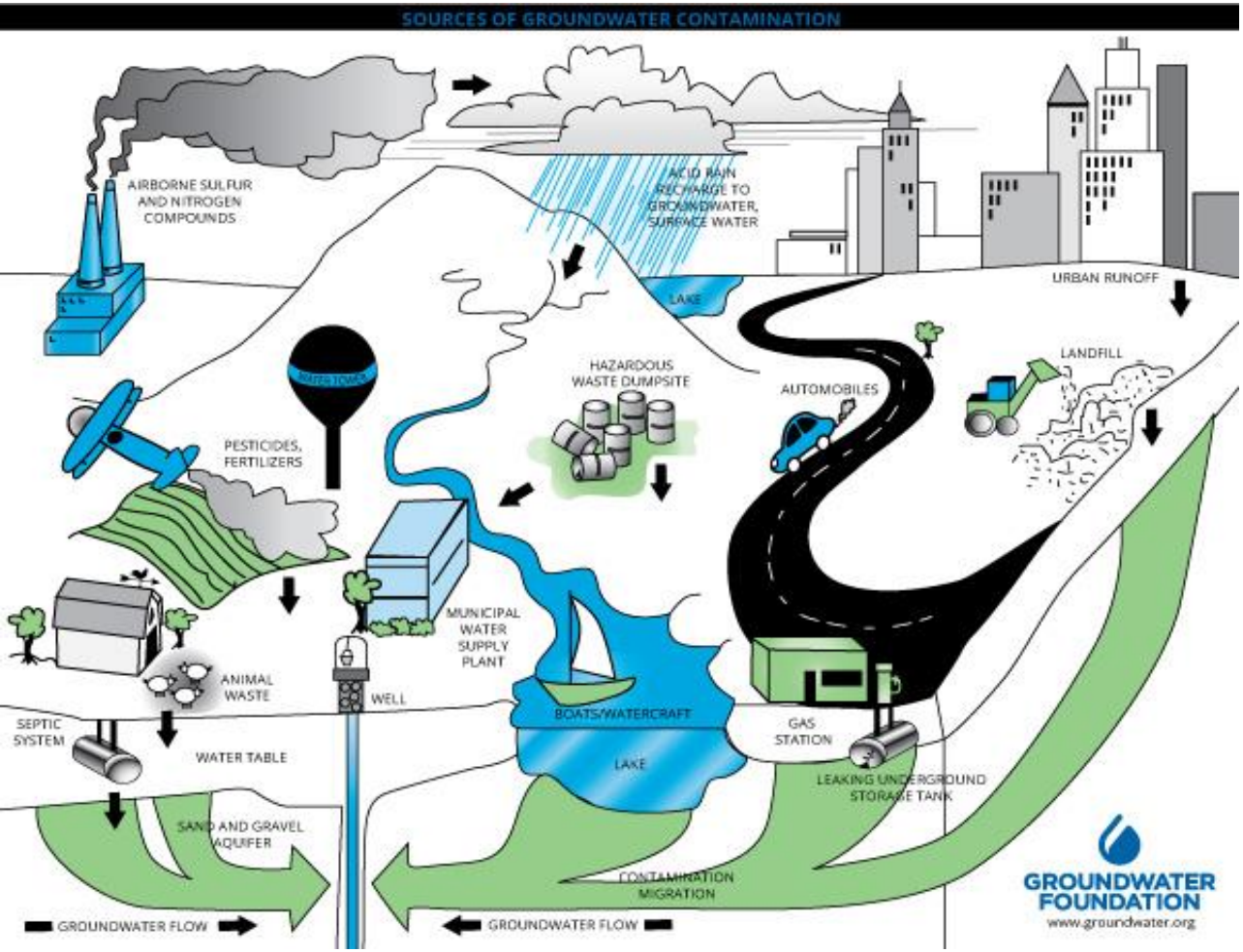


- ✓ Freshwater availability and quality- key for human life.
- ✓ Groundwater is the world's most important source of freshwater.
- ✓ Ecosystem quality, energy and food security.
- ✓ 2 billion people: drinking water and irrigation for world's food supply.

Major sources of groundwater contamination



Natural resource endangered by several factors, including over-exploitation and contamination by anthropogenic activities.

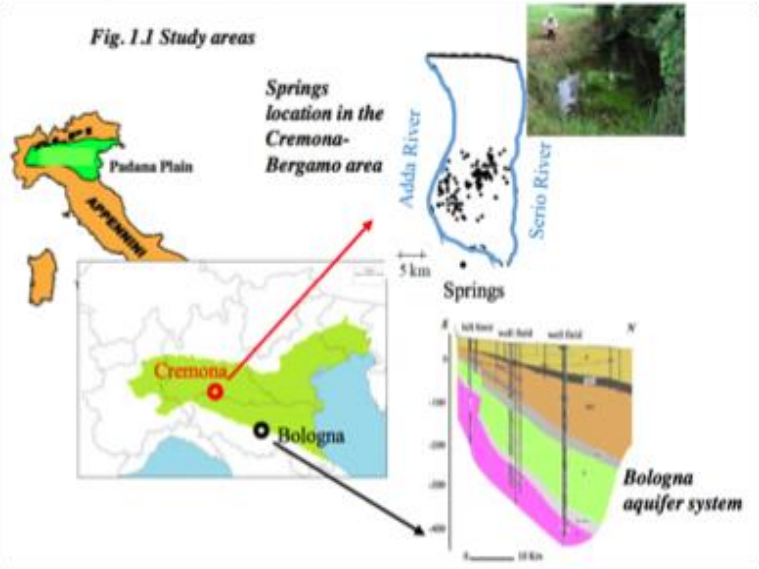
These elements severely affect the water-energy-food nexus, with critical environmental, sociological and economic consequences.

WE-NEED

WatEr NEEDs, Availability, Quality and Sustainability

WP4 Objective:

Quantitative assessment of potential deleterious effects to the environment of emergent contaminants (e.g. PPCPs, nanoparticles) and risk assessment of any implications related to potential hazards of groundwater pollution in ecosystems



Cremona Aquifer:
Main supply for agricultural usage and key environmental driver

Bologna Aquifer:
80% of water used for public consumption and industrial use

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To request chemical analysis you should know what to request…

So, what about when you fail on one crucial compound??

What about those below the chemical detection limit??

Specific Objectives:

1. Assess the potential toxicity of groundwater samples
2. Infer potential increases in toxicity (synergism) due to multiple chemical exposure

Two freshwater model organisms

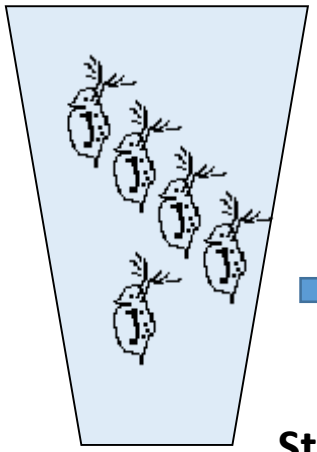
Daphnia magna

Danio rerio

Under controlled laboratory conditions, following ISO/OECD guidelines and procedures

Water Flea

Daphnia magna



24h

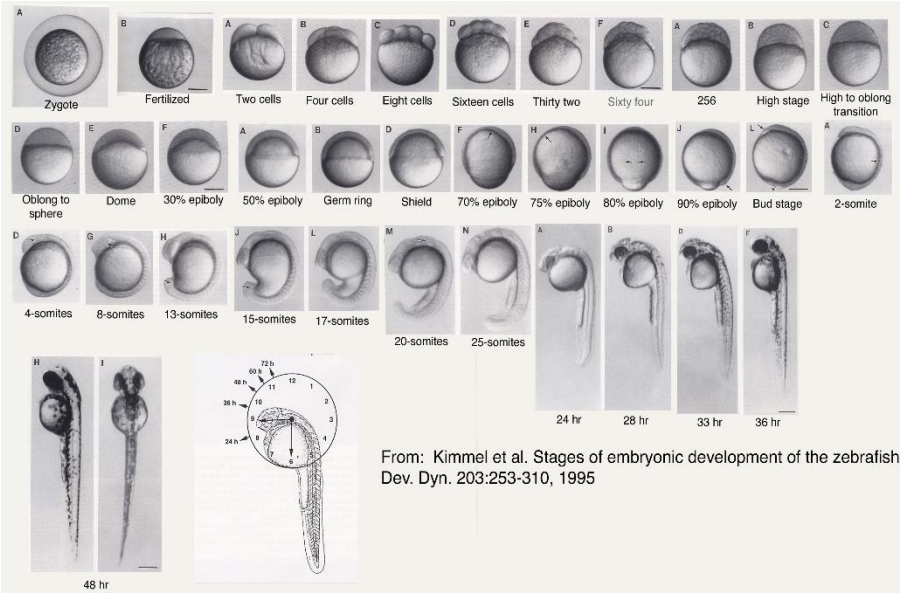
48h

Stirring for 10 sec…
Wait for 10 sec…
Count how many are still swimming or moving

Zebra fish

Danio rerio

https://owlcation.com/stem/The-why-and-how-of-breeding-zebrafish-for-research



From: Kimmel et al. Stages of embryonic development of the zebrafish Dev. Dyn. 203:253-310, 1995

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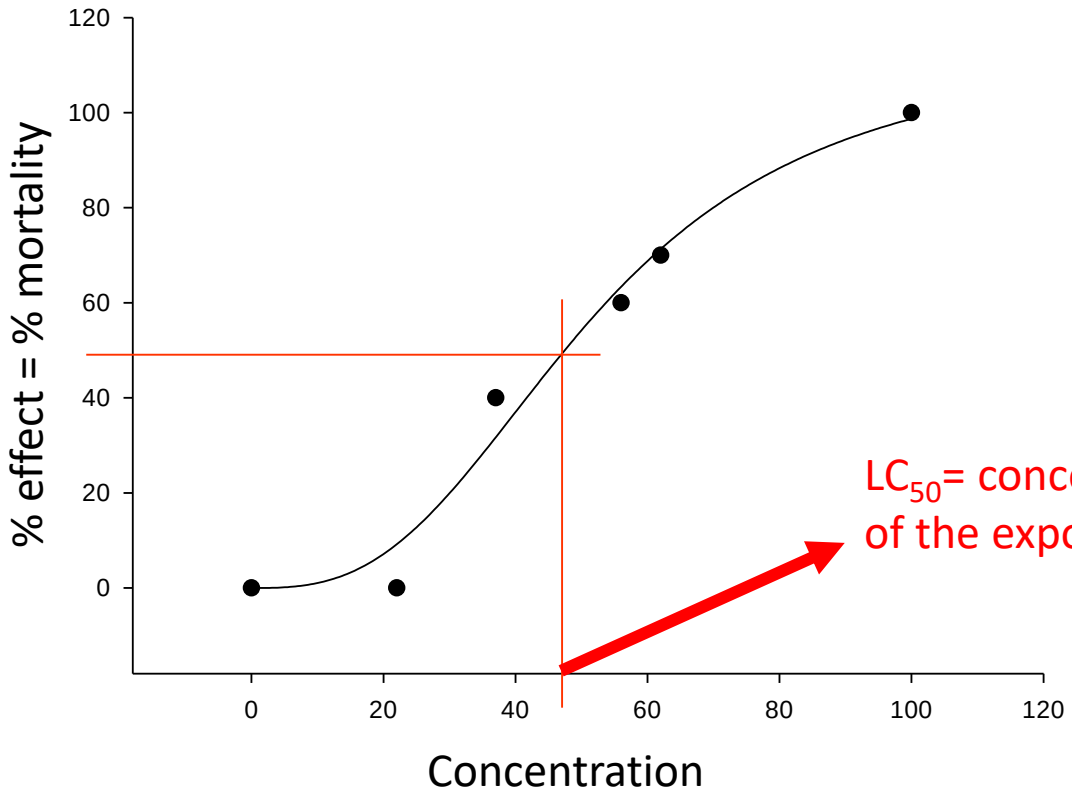
OUTPUT of EcoToxicity tests…



LC_{50}= concentration that kills 50% of the exposed population

Ground water characteristics

Synthetic groundwater composition provided by Partner 2: Department of Earth and Planetary Sciences, Weizmann Institute of Science, Israel

מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

	Bologna	Cremona
Composition	Concentration (mg/L)	Concentration (mg/L)
$CaCO_3$	475	158.3
$MgSO_4$	138	46.1
$Ca(HCO_3)_2$	673	224.2
NaCl	67	22.4
$NaNO_3$	34	11.3
Humic acid (sodium salt)	5	5
	µg/L	µg/L
tetrachloroethylene (PCE)	30	10.0
NaF	75	25
$(NH_4)OH$	100	33.3
H_3BO_3	800	266.7

Anthropogenic contaminants

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1st experimental setup:

… compare **anthropogenic contaminants toxicity** found in ground waters …
… **individually** …
… **different water types** …

Bologna and Cremona waters testing contaminants individually

48h-LC_{50} values (mg/L) with confidence intervals between brackets for *Daphnia magna* k6 exposed to different compounds for ASTM, Cremona and Bologna waters.

	ASTM		CREMONA		BOLOGNA
Boric acid	697.6 (568.6-792.4)	=	664.0 (626.8-705.6)	<	165.1 (132.4-199.1)
Ammonium hydroxide	91.9 (79.5-115.1)	>	105.0 (102.0-108.1)	>	323.5 (295.7-357.3)
NaF	540.2 (436.2-647.4)	=	513.4 (493.9-533.5)	>	594.0 (543.1-646.3)

96h-LC_{50} values (mg/L) with confidence intervals between brackets for *Danio rerio* exposed to different compounds for FSW, Cremona and Bologna waters.

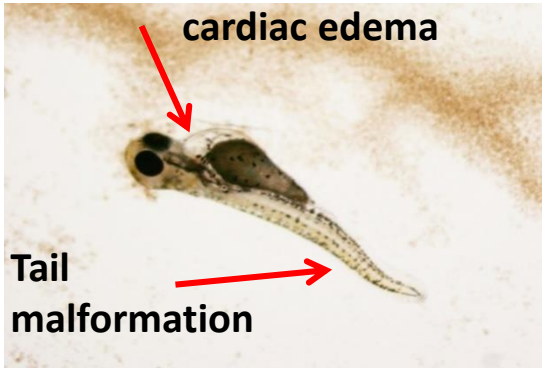
	FSW		CREMONA		BOLOGNA
Boric acid	1617.6 (1426.4-1876)	<	991.2 (863-1160.7)	<	401.5 (348.3-466.6)
Ammonium hydroxide	>20		94.2 (80.6-112.3)	=	122.2 (108.2-138.8)
NaF	1009.1 (848.6-1169.5)	<	664.1 (580.7-746.4)	>	993.8 (876.6-1171.6)

Danio rerio e.g. Sodium Fluoride (NaF)

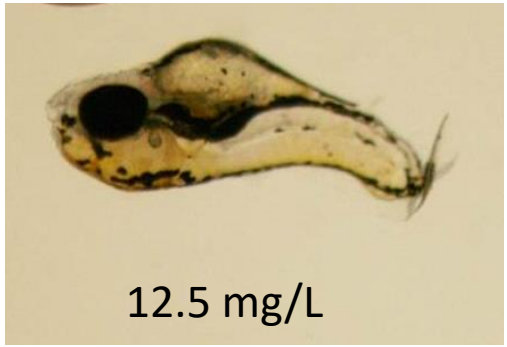
96 h larvae

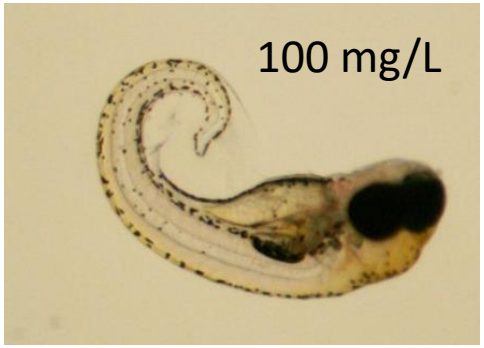
FSW (CTR)

Cremona + NaF



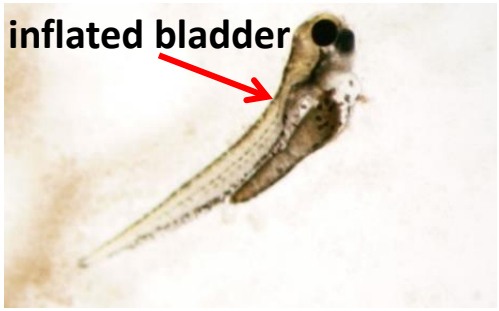
FSW + NaF





Tail malformations

Bologna + NaF



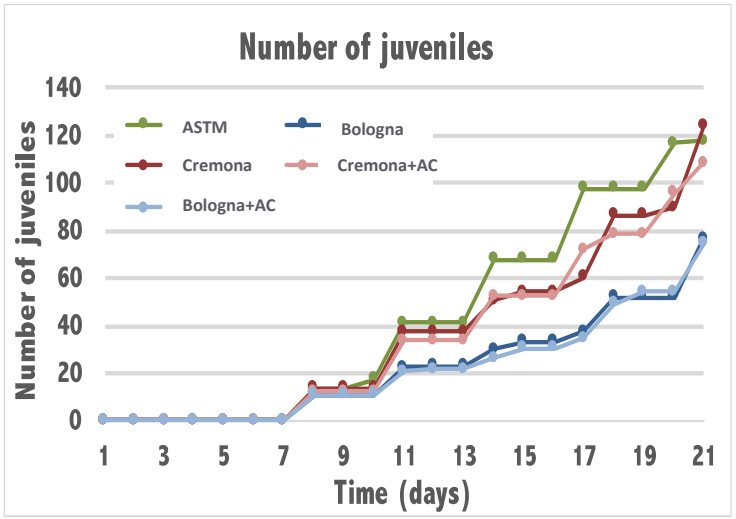
2nd experimental setup:

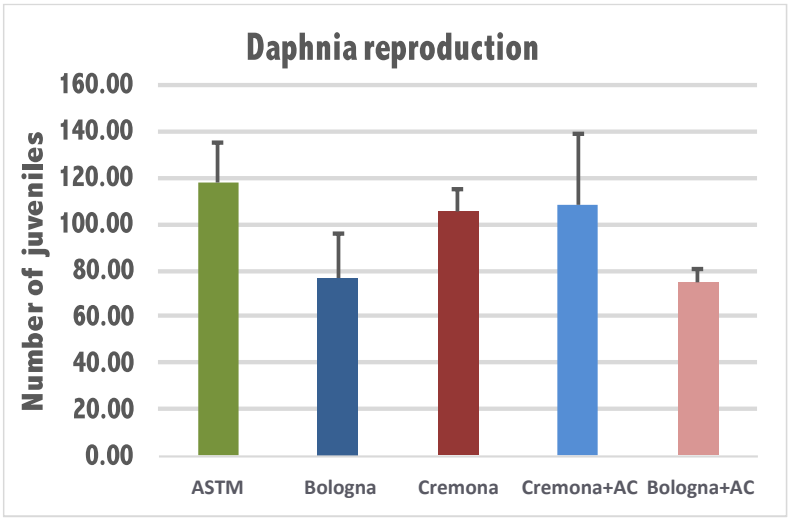
… compare **anthropogenic contaminants toxicity** found in ground waters …
… **mixtures** …
… **mimicking different ground waters**…

Survival data (24 and 48h) and reproduction for *Daphnia magna* k6 Cremona and Bologna waters with anthropogenic contaminants: - PCE, Boric acid, NaF and Ammonium hydroxide

Survival	ASTM	CREMONA	CREMONA + AC	BOLOGNA	BOLOGNA + AC
24h	100%	100%	100%	100%	100%
48h	100%	100%	100%	100%	100%

Reproduction data (21 days)





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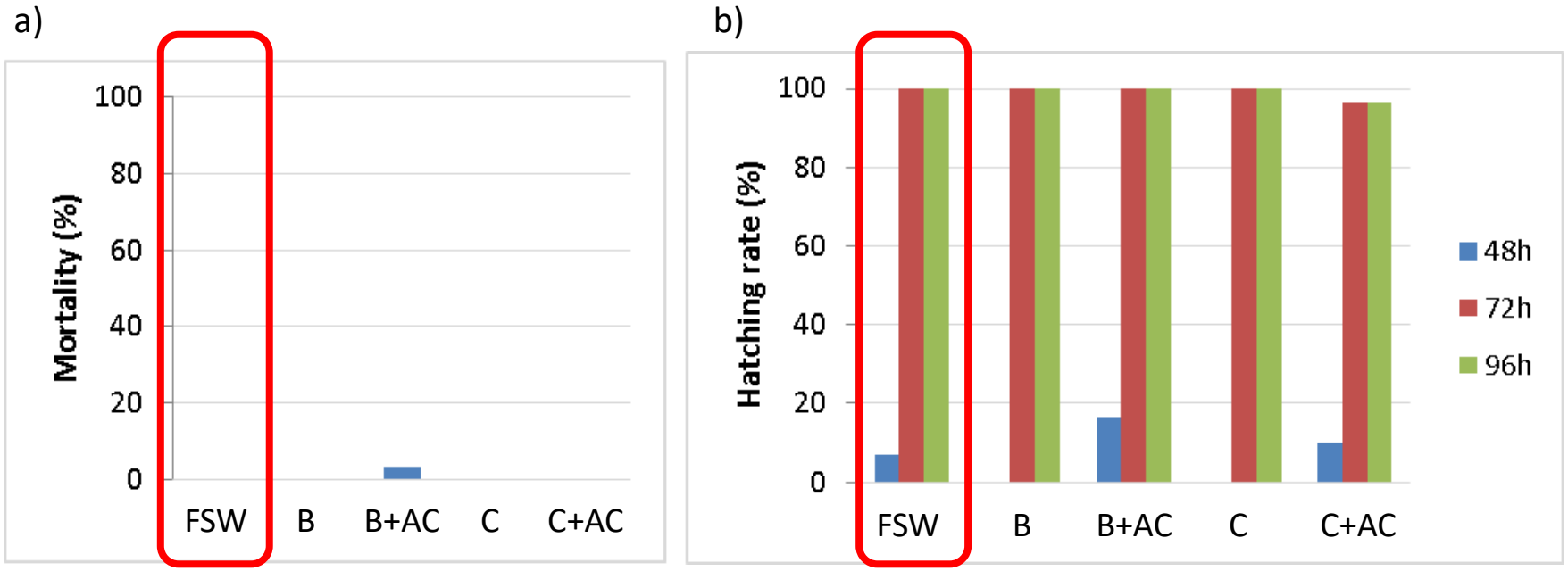
FET (Fish Embryo Acute Toxicity) test with zebrafish (*D. rerio*) after 96h

Fish System Water (FSW), Cremona (C) and Bologna (B)



(AC- Anthropogenic contaminants)

3rd experimental setup:

… compare **anthropogenic contaminants toxicity** found in ground waters …
… **other possible ground water contaminants** …
… **Acetaminophen**
… **Triclosan**
… **PFOA**
… **PFOS**

Bologna and Cremona waters (total composition)

48h-LC_{50} values (mg/L) with confidence intervals between brackets for *Daphnia magna* k6 exposed to different compounds for ASTM, Cremona and Bologna waters.

	ASTM	CREMONA	BOLOGNA
Acetaminophen	3.39 (3.04-3.76)	3.11 (2.75-3.48)	5.7 (4.8-7)
Triclosan	0.98 (0.70-1.69)	0.95 (0.86-1.03)	2.23 (1.65-3.48)
PFOA	414.3 (374.9-453.6)	428.7 (363.7-507)	501.6 (442-582)
PFOS	21.1[1] (n.d.)	4.51 (3.74-5.28)	6.25 (5.88-6.62)

[1] Yang et al (2019), Sci Total Environ

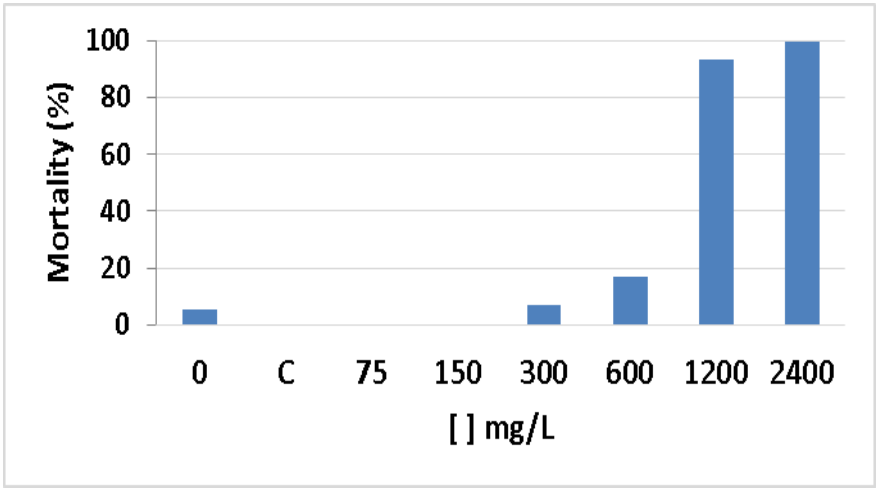
Bologna and Cremona waters (total composition)

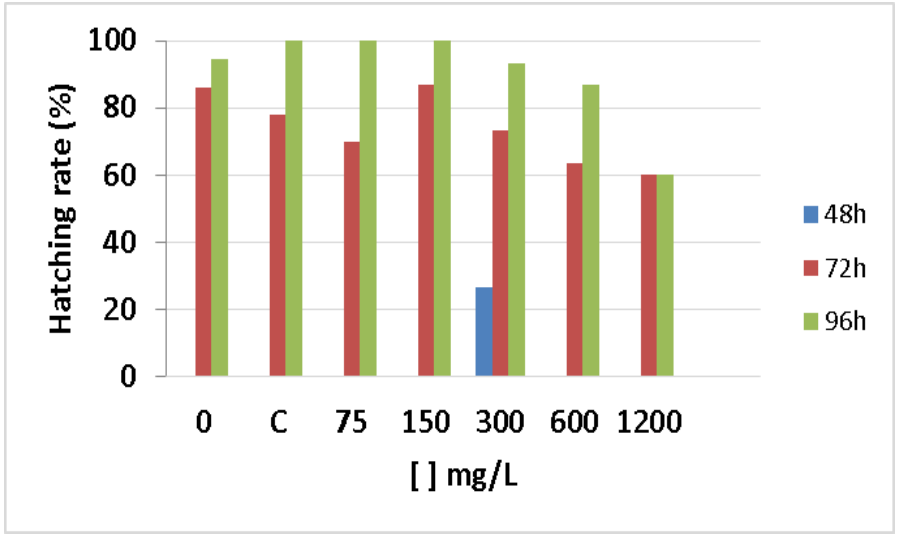
96h-LC_{50} values (mg/L) with confidence intervals between brackets for *Danio rerio* exposed to different compounds for FSW, Cremona and Bologna waters.

	FSW	CREMONA	BOLOGNA
Acetaminophen	1483.2 (n.d.)	736.7 (594.3-928.9)	634.6 (564.3-741.9)
Triclosan	0.42[1] (0.38-0.45)	0.80 (n.d.)	0.73 (n.d.)
PFOA	759[2] (643-875)	545.1 (483.7-611.7)	377.9 (343.9-413.7)
PFOS	3.04 (1.35-14.88)	6.34 (4.79-9.33)	2.88 (2.02-4.47)

[1] Oliveira et al (2009), Environ Sci Pollut Res
[2] Stengel et al (2018), Environ Sci Pollut Res

Danio rerio – e.g. Acetaminophen





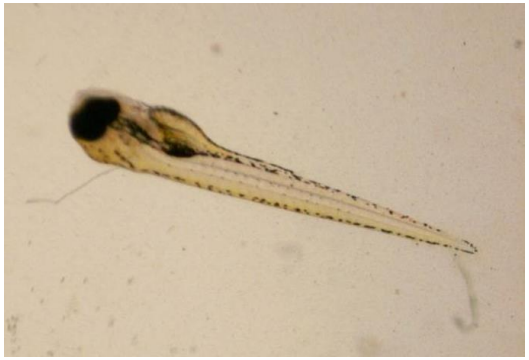
FET (Fish Embryo Acute Toxicity) test with zebrafish (*D. rerio*) in the Cremona water and exposed to Acetaminophen after 96h: a) cumulative mortality; b) hatching rate.

Danio rerio – e.g. Acetaminophen

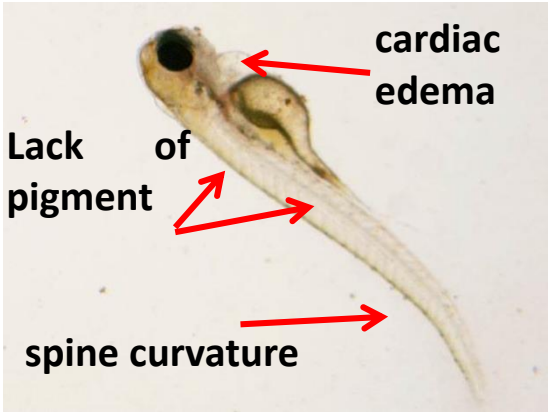
96 h larvae

FSW

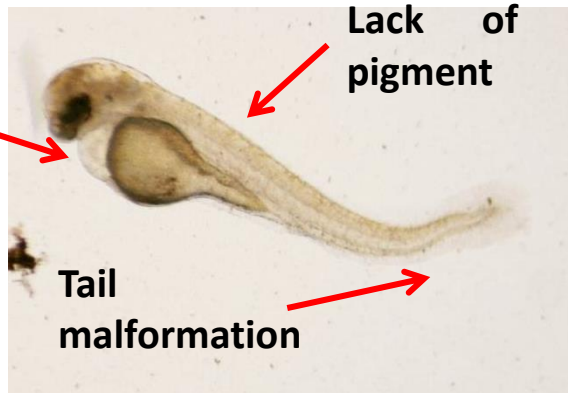
CREMONA



CREMONA – 600 mg/L



CREMONA – 1200 mg/L



4th experimental setup:

… compare **anthropogenic contaminants toxicity** found in ground waters …
… **looking at mixtures**…

Binary mixtures

Component-based approach

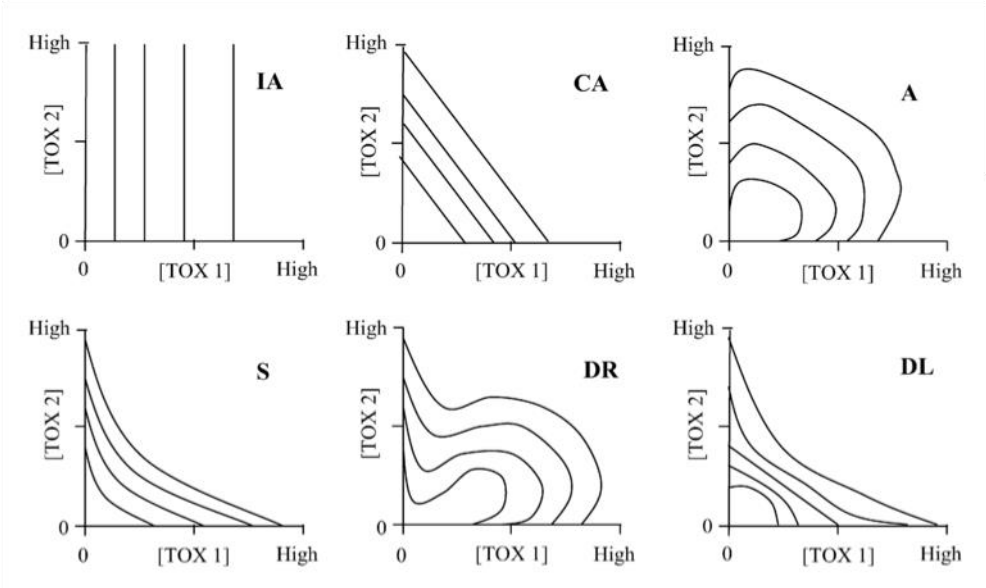
Identification of interaction between chemicals

Concentration Addition (CA)

Reference model
Widely accepted by risk assessors
Conservative model
Assuming no interaction

Nested framework - MIXTOX

Start with reference model
Add extra parameters to assess possible deviations

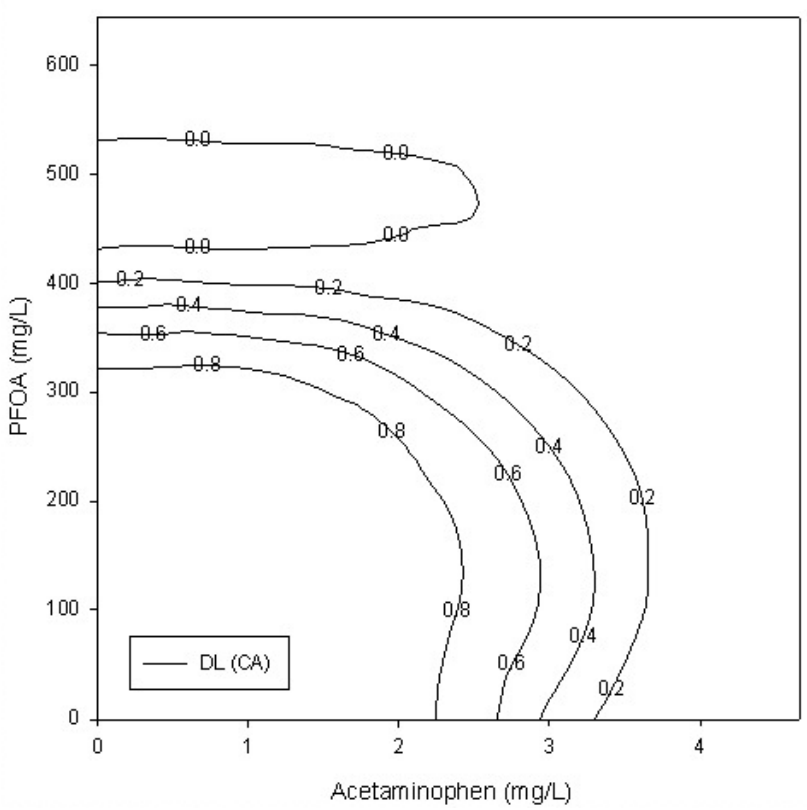


Binary mixtures – e.g. ACET – PFOA

Summary of the analysis of the effect of ACET and PFOA in Cremona water on the mortality of *D. magna*.

	Concentration Addition			
	Reference	**S/A**	**DR**	**DL**
max	0.92	0.85	0.85	**0.83**
β_{ACET}	1.65	5.86	5.86	**10.7**
β_{PFOA}	479.9	486.2	486.2	**419.8**
$EC_{50\ ACET}$	4.03	2.80	2.8	**2.92**
$EC_{50\ PFOA}$	424.9	334.4	334.4	**323.8**
a	-	2.25	2.25	**0.66**
b	-	-	0.01	**-1.44**
SS	198.4	149.67	149.67	**144.6**
R^2	0.60	0.70	0.70	**0.71**
$p(\chi^2)$	-	< 0.0001	0.97	**0.02**

Concentration–response data (isoboles) of survival after 48 hours of exposure to acetaminophen and PFOA showing a dose-level dependent response.

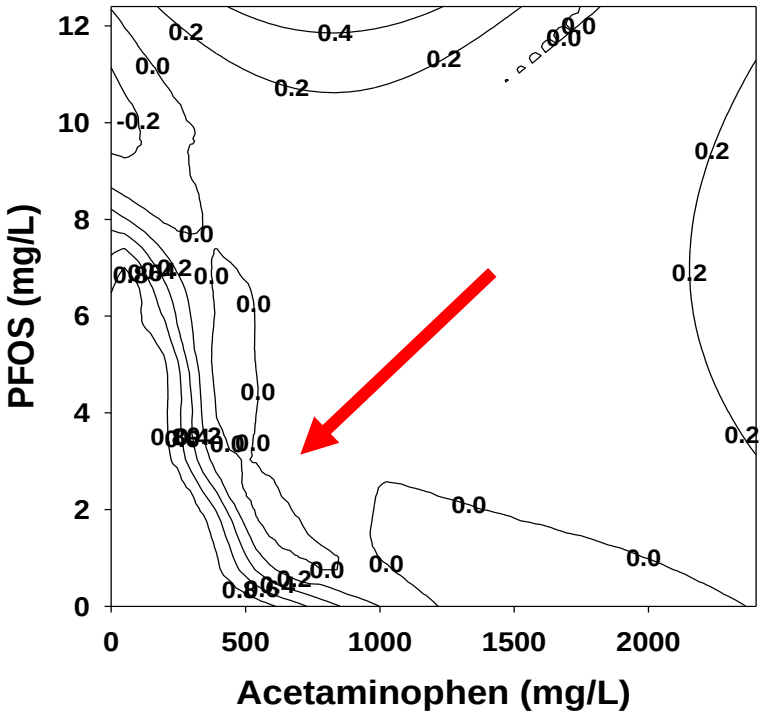


Binary mixtures – e.g. Acetaminophen – PFOS

Summary of the analysis of the effect of ACET and PFOS in Cremona water on the mortality of *D. rerio*.

		Deviations from CA model		
	CA	S/A	DR	DL
R^2	0.86	0.86	0.89	0.87
SS	43.7	43.7	35.7	41.49
p(F-test)	< 0.05	-	-	-
$p(\chi^2)$	-	> 0.05	< 0.05	< 0.05
max	0.88	0.88	0.86	0.87
a	-	0.04	1.06	-2.17
b	-	-	-2.94	1

Concentration–response data (isoboles) of survival after 96 hours of exposure to acetaminophen and PFOS showing a synergistic response at low dose levels.



Ternary mixtures – e.g. ACET– TCS – PFOA

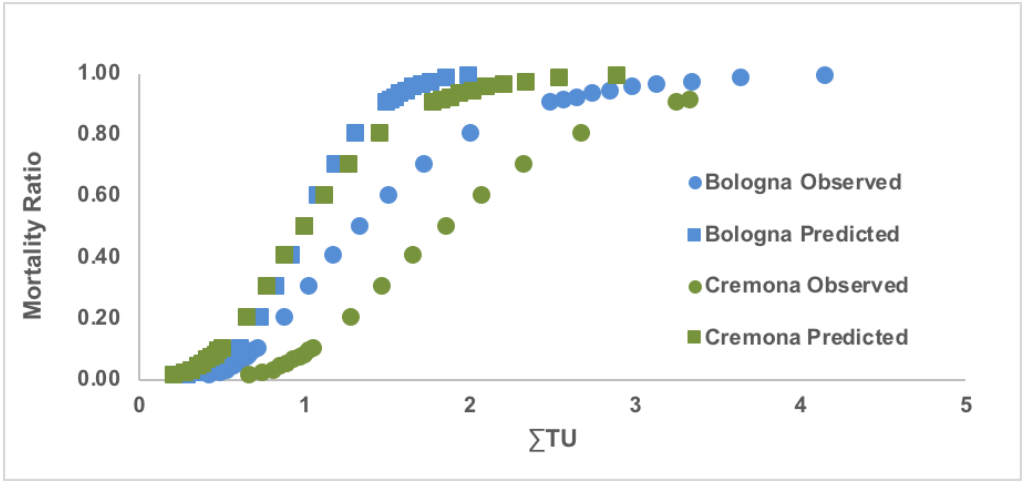
Experimental design for acute toxicity experiments in *D. magna* testing three-component mixtures containing ACET, TCS and PFOA. Fixed-ratio design based on TU-based approach.

Cremona Mixture	ACET TU	TCS TU	PFOA TU	∑ TU
M1	0.0078	0.0078	0.0078	0.02
M2	0.0156	0.0156	0.0156	0.05
M3	0.03125	0.03125	0.03125	0.09
M4	0.0625	0.0625	0.0625	0.19
M5	0.125	0.125	0.125	0.38
M6	0.25	0.25	0.25	0.75
M7	0.375	0.375	0.375	1.13
M8	0.5	0.5	0.5	1.50
M9	1	1	1	3.00
M10	2	2	2	6.00

CA-predicted vs observed acute toxicity three-compound mixture *Daphnia magna*

Performed for synthetic groundwaters

- **Higher toxicity (increased mortality) observed in the Bologna water when compared to Cremona water caused by the ternary mixtures → Differences in the chemical composition of the two synthetic groundwaters**
- **CA model → conservative towards Cremona and Bologna ternary mixture**



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Risk characterization

$$RQ = \frac{PEC_i}{PNEC_i}$$

Stepwise approach for calculation of an "ecosystem risk quotient":

1. PEC/PNEC ratios of all mixture components

$$RQ_{PEC/PNEC} = \sum_{i=1}^{n} \frac{PEC_i}{PNEC_i} = \sum_{i=1}^{n} \frac{PEC_i}{\min(EC50_{daphnids}, EC50_{fish}) \times \left({}^{1}/_{AF_i}\right)}$$

2. Sum of Toxic Units (STU) calculated for each trophic level

$$RQ_{STU} = \max(STU_{daphnids}, STU_{fish}) \times AF$$

$$= \max\left(\sum_{i=1}^{n} \frac{PEC_i}{EC50_{i,daphnids}}, \sum_{i=1}^{n} \frac{PEC_i}{EC50_{i,fish}}\right) \times AF$$

Backhaus & Faust, 2012, *Environmental Science & Technology*, 46:2564–2573

Risk characterization

Environmental risk characterization of emerging contaminant mixtures - a comparison of standard protocols and groundwater adapted protocols.

PEC values based on maximum concentrations found for groundwater in literature

	PEC	ASTM / FSW		Cremona		Bologna	
		LC_{50} *D. magna*	LC_{50} *D. rerio*	LC_{50} *D. magna*	LC_{50} *D. rerio*	LC_{50} *D. magna*	LC_{50} *D. rerio*
	(mg / L)	(mg / L)		(mg / L)		(mg / L)	
Acetaminophen	0.12 [1]	3.388	---	3.105	736.69	5.741	643.61
Triclosan	0.0021 [1]	0.977	---	0.948	0.796	2.233	0.731
PFOS	0.000039 [2]	537.866	---	428.713	545.06	501.621	377.89
PFOA	0.000135 [2]	67.2	---	4.51	62.45	5.01	---
$RQ_{PEC/PNEC}$		**37.46829067**		**41.16886816**		**23.6652838**	
RQ_{STU}		**37.46829067**		**40.78707209**		**21.82496176**	

[1] Lapworth DJ et al, 2012, *Environmental Pollution*, 163: 287-303

[2] Loos R et al, 2010, Water Research 44: 4115-4126

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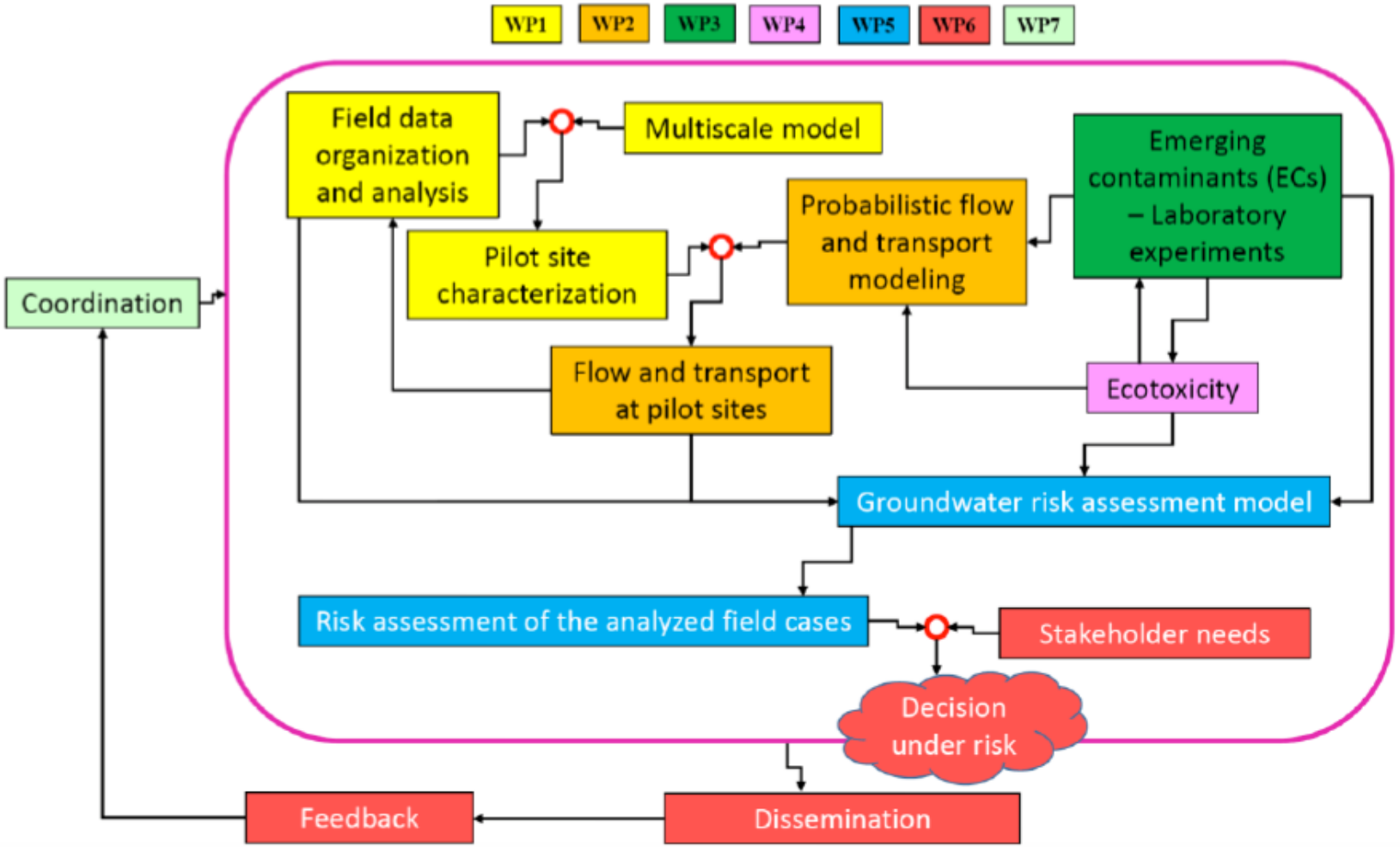
WP1
WP2
WP3
WP4
WP5
WP6
WP7
Field data organization and analysis
Multiscale model
Emerging contaminants (ECs) – Laboratory experiments
Probabilistic flow and transport modeling
Pilot site characterization
Coordination
Flow and transport at pilot sites
Ecotoxicity
Groundwater risk assessment model
Risk assessment of the analyzed field cases
Stakeholder needs
Decision under risk
Feedback
Dissemination

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