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SUPPLEMENTARY MATERIAL

Environmental concerns about the effects of effluents from wastewater treatment plants in tourist areas of the Alps: toxicity in aquatic microorganisms

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Keywords: Pharmaceuticals, mixture toxicity, concentration addition, combination index, mountain tourism, Alpine convention.

Tab. S1. Aliquots (%) of the treated effluent (a) and concentrations (Conc, mg L^{-1}) of the pharmaceutical active compounds (b) acetaminophen (paracetamol), (c) clarithromycin, (d) naproxen, and (e) of the multicomponent mixture (mixMEC) tested on *A fischeri*.

a		b		c		d		e	
Sample	**Conc (%)**	**Sample**	**Conc**	**Sample**	**Conc**	**Sample**	**Conc**	**Sample**	**Conc**
Control	0	Control	0	Control	0	Control	0	Control	0
treated effluent	0.39	**paracetamol**	39.02	**clarithromycin**	1.56	**naproxen**	0.36	**mixMEC**	0.0002
	0.78		58.55		2.34		0.72		0.0006
	1.56		87.80		3.51		1.19		0.0019
	3.13		131.70		5.27		1.44		0.0058
	3.9		197.6		7.9		1.8		0.0174
	5.9		296.3		11.9		2.7		0.0215
	8.8		444.5		17.8		2.9		0.0523
	12.5		666.5		26.7		4.0		0.0645
	13.2		1000		40		6		0.157
	19.8		2000		60		6		0.194
	25.0						9.0		0.471
	29.6						11.5		0.581
	44.4						13.6		1.412
	50.0						20.3		1.743
	66.7						23		2.615
	100.0						45		5.230
							90		7.9
									15.7
									23.5
									47.1
									141.2

Tab. S2. Relevant physical and chemical properties of the tested pharmaceutical compounds. Information on the other PhACs (amoxicillin, carbamazepine, diclofenac, and ibuprofen) in the mixMEC mixture are reported in Di Nica *et al.* (2017a).

PhACs class	PhACs	CAS n°	Water solubility ($mg\ L^{-1}$)	pKa	Log D_{OW}
Antibiotics	Clarithromycin	81103-11-9	0.33	8.99[a]	3.16[b]
Anti-inflammatory	Naproxen	22204-53-1	15.9	4.18[a]	0.09[a]
Analgesics	Acetaminophen (paracetamol)	103-90-2	14000	9.63[a]	0.34[a]

CAS, Chemical Abstracts Service; pKa=dissociation constant; DOW, octanol–water distribution constant; a, Avdeef (2003).

Tab. S3. IC_{50} values on *Aliivibrio fischeri* predicted by QSAR equations and the obtained toxicity ratios for narcotic compounds (TR1) and polar narcotic compounds (TR2).

	IC_{50} narc. ($mg\ L^{-1}$)	TR1	IC_{50} polar narc. ($mg\ L^{-1}$)	TR2
Acetaminophen	29502	58.52	51.85	0.1
Naproxen	77200	589.04	105.4	0.8

Tab. S4. Combination Index (CI) values obtained for the tested mixMEC on *A. fischeri*.

Fa	CI Value
0.05	0.615
0.10	0.808
0.15	0.960
0.20	1.096
0.25	1.223
0.30	1.347
0.35	1.472
0.40	1.600
0.45	1.734
0.50	1.878
0.55	2.034
0.60	2.207
0.65	2.406

References

Avdeef A, 2003. Absorption and drug development: Solubility, permeability, and charge state. John Wiley & Sons, Inc.

Di Nica V, Villa S, Finizio A, 2017a. Toxicity of individual pharmaceuticals and their mixtures to *Aliivibrio fischeri*: experimental results for single compounds and considerations of their mechanisms of action and potential acute effects on aquatic organisms. Environ. Toxicol. Chem. 36:807-814.