Ecotoxicity of binary mixtures of cholinium-based ionic liquids and salts to microalgae

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Supplementary material

Figure S1: Schematic representation of the experimental design used to assess the effect of binary mixtures of ILs and salts. For each ratio, 5 concentrations were tested.

Table S1: Results of the chemical analysis of the tested ions, given as the ratio between measured concentrations and nominal concentrations (average ± SD).

Table S2: ECx values (x = 50%, 20% and 10%) of each tested chemical (i.e., cholinium bicarbonate, benzyldimethyl(2-hydroxyethyl)ammonium chloride, cholinium bitartrate and cholinium dihydrogencitrate, potassium phosphate tribasic and sodium citrate dihydrate) to the microalgae *R. subcapitata* after 96 h of exposure, considering both yield and growth rate as endpoints.

Table S3: Statistical and estimated parameters for the fitting of the experimental data to the CA and IA models (baseline models) and their deviation functions (S/A: synergism/antagonism; DR: dose-ratio dependence; DL: dose-level dependence). The models best describing the toxicity of each mixture are highlighted in grey. Statistically significant values are highlighted in bold.

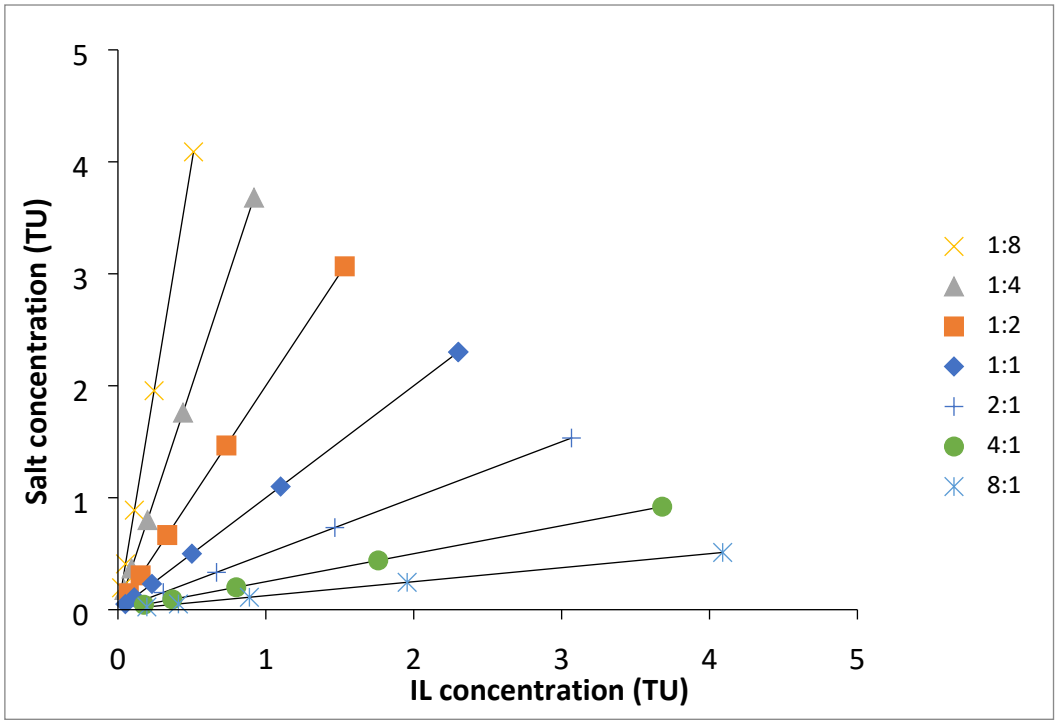

Figure S1: Schematic representation of the experimental design used to assess the effect of binary mixtures of ILs and salts. For each ratio, 5 concentrations were tested.

Table S1: Results of the chemical analysis of the tested ions, given as the ratio between measured concentrations and nominal concentrations (average ± SD).

Ion	Parent compound	Nr of samples	Measured concentration/ nominal concentration
Bicarbonate	[Chol][Bic]	51	0.71 ± 0.04
Bitartrate	[Chol][Bit]	20	1.00 ± 0.12
Cholinium	[Chol][DHCit]; [Chol][Bic]; [Chol][Bit]	17	1.10 ± 0.41
Chloride	[BzChol]Cl	52	1.03 ± 0.04
Citrate	[Chol][DHCit]	48	0.94 ± 0.07
Phosphate	K_3PO_4	74	1.12 ± 0.09
Potassium	K_3PO_4	50	0.99 ± 0.07
Citrate	$NaCit.2H_2O$	51	1.12 ± 0.03
Sodium	$NaCit.2H_2O$	43	0.90 ± 0.05

Table S2: EC_x values (x = 50%, 20% and 10%) of each tested chemical (i.e., cholinium bicarbonate, benzyldimethyl(2-hydroxyethyl)ammonium chloride, cholinium bitartrate and cholinium dihydrogencitrate, potassium phosphate tribasic and sodium citrate dihydrate) to the microalgae *R. subcapitata* after 96 h of exposure, considering both yield and growth rate as endpoints. The values are presented as mean (SD). Data are expressed in $mg.L^{-1}$ and were obtained from the single exposures in the mixture toxicity tests; n refers to the number of tests performed and used to estimate EC values that were then averaged.

Chemical	**Endpoint**	**96 h-EC_{50}**	**96 h-EC_{20}**	**96 h-EC_{10}**	**n**
[Chol][Bic]	Yield	310.5 (75.4)	154.7 (64.8)	106.2 (59.5)	7
	Growth rate	1110.8 (235.0)	407.7 (83.5)	222 (41.5)	7
[BzChol]Cl	Yield	766.3 (197.7)	564.8 (119.8)	429.7 (113.2)	7
	Growth rate	1802 (288.0)	1067 (326.5)	841.7 (334.5)	7
[Chol][Bit]	Yield	110.8 (30.9)	82.8 (32.6)	70.4 (31.5)	8
	GR	183.4 (20.2)	130.9 (18.3)	107.9 (19.5)	8
[Chol][DHCit]	Yield	85.2 (15.0)	58.9 (20.1)	48 (20.9)	7
	Growth rate	174.8 (24.9)	100.2 (15.6)	73.2 (17.4)	7
K_3PO_4	Yield	583.0 (111.8)	354.9 (103.8)	267.7 (95.9)	14
	Growth rate	1237.7 (133.5)	783.7 (124.5)	600.7 (132.1)	14
$NaCit.2H_2O$	Yield	372.2 (149.4)	215.4 (133.0)	143.6 (83.0)	14
	Growth rate	1220.9 (359.6)	434.2 (163.2)	276.5 (133.4)	14

Table S3: Statistical summary regarding the fitting of the experimental data to the CA and IA models (baseline models) and their deviation functions (S/A: synergism/antagonism; DR: dose-ratio dependence; DL: dose-level dependence). The models best describing the toxicity of each mixture are highlighted in grey. Statistically significant values are highlighted in bold.

		CA				IA			
		baseline	S/A	DR	DL	baseline	S/A	DR	DL
[Chol][Bic] x $NaCit.2H_2O$	r^2 (n = 95)	0.899	0.902	0.907	0.903	0.890	0.901	0.905	0.902
	SS	52364	50923	47927	50388	55308	49543	47563	49166
	p (F-test or χ^2)	**6.87×10^{-44}**	vs CA: 0.104	**vs CA: 0.0149; vs S/A: 0.0164**	vs CA: 0.1608; vs S/A: 0.3163	**3.33×10^{-42}**	**vs IA: 0.00122**	**vs IA: 0.0008; vs S/A: 0.049**	**vs IA: 0.0037**; vs S/A: 0.3944
[BzChol]Cl x $NaCit.2H_2O$	r^2 (n = 86)	0.759	0.762	0.762	0.774	0.757	0.758	0.759	0.769
	SS	761332	753880	753517	716508	747217	743524	740986	710562
	p (F-test or χ^2)	**2.81×10^{-24}**	vs CA: 0.358	vs CA: 0.6417; vs S/A: 0.8386	vs CA: 0.0736; vs **S/A: 0.0365**	**4.31×10^{-24}**	vs CA: 0.514	vs CA: 0.6976; vs S/A: 0.5876	vs CA: 0.115; **vs S/A: 0.0483**
[Chol][Bit] x $NaCit.2H_2O$	r^2 (n = 89)	0.767	0.794	0.808	0.796	0.785	0.788	0.799	0.789
	SS	136653	120854	112682	119602	123250	121266	115345	121144
	p (F-test or χ^2)	**9.48×10^{-26}**	**vs CA: 0.001**	**vs CA: 0.0002; vs S/A: 0.0125**	**vs CA: 0.0027**; vs S/A: 0.3358	**3.19×10^{-27}**	vs IA: 0.229	vs IA: 0.0524; **vs S/A: 0.0348**	vs IA: 0.464; vs S/A: 0.764
[Chol][DHCit] x $NaCit.2H_2O$	r^2 (n = 64)	0.669	0.693	0.694	0.694	0.663	0.685	0.686	0.698
	SS	100034	92797	92514	92685	101470	94763	94549	90753
	p (F-test or χ^2)	**8.090×10^{-14}**	**vs CA: 0.027**	vs CA: 0.0789; vs S/A: 0.6559	vs CA: 0.0838; vs S/A: 0.7797	**1.471×10^{-13}**	**vs CA: 0.035**	vs CA: 0.1007 vs S/A: 0.7016	vs CA: 0.0266; vs S/A: 0.0937

		CA				IA			
		baseline	S/A	DR	DL	baseline	S/A	DR	DL
[Chol][Bic] x K_3PO_4	r^2 (n = 95)	0.786	0.788	0.799	0.788	0.778	0.779	0.789	0.781
	SS	88599.0	88148.3	83571.4	87893.1	88278.9	87780.4	83881.3	87221.0
	p (F-test or χ^2)	**2.45×10^{-29}**	vs CA: 0.486	vs CA: 0.0624; **vs S/A: 0.0244**	vs CA: 0.6849; vs S/A: 0.600	**1.36×10^{-28}**	vs IA: 0.463	vs IA: 0.088; **vs S/A: 0.0377**	vs IA: 0.564; vs S/A: 0.436
[BzChol]Cl x K_3PO_4	r^2 (n = 91)	0.857	0.858	0.875	0.862	0.854	0.855	0.872	0.858
	SS	72341	71793	63473	69758	71163	70560	62316	69297
	p (F-test or χ^2)	**1.65×10^{-35}**	vs CA: 0.406	**vs CA: 0.0026; vs S/A: 0.0008**	vs CA: 0.1913; vs S/A: 0.1058	**4.55×10^{-35}**	vs IA: 0.3788	**vs IA: 0.0024; vs S/A: 0.0008**	vs IA: 0.298; vs S/A: 0.200
[Chol][Bit] x K_3PO_4	r^2 (n = 85)	0.588	0.689	0.691	0.693	0.573	0.637	0.639	0.637
	SS	216379	163193	162465	161179	220595	187549	186527	187546
	p (F-test or χ^2)	**9.54×10^{-15}**	**9.74×10^{-7}**	**vs CA: 5.14×10^{-6}**; vs S/A: 0.538	**vs CA: 3.66×10^{-6}**; vs S/A: 0.304	**3.77×10^{-14}**	**vs IA: 2.04×10^{-4}**	**vs IA: 8.01×10^{-4}**; vs S/A: 0.4954	**vs IA: 1.01×10^{-3}**; vs S/A: 0.9683
[Chol][DHCit] x K_3PO_4	r^2 (n=50)	0.759	0.816	0.859	0.871	0.774	0.852	0.860	0.847
	SS	199692.5	151851.8	116346.1	106973.1	186553.9	122132.3	115680.2	126747.9
	p (F-test or χ^2)	**2.337×10^{-13}**	**vs CA: 2.152×10^{-4}**	**vs CA: 1.364×10^{-6}; vs S/A: 2.630×10^{-4}**	**vs CA: 1.670×10^{-7}; vs S/A: 2.848×10^{-5}**	**5.154×10^{-14}**	**vs CA: 4.179×10^{-6}**	**vs CA: 6.477×10^{-6}**; vs S/A: 0.0995	**vs CA: 6.359×10^{-5}**; vs S/A: "ND"

r^2 is the coefficient of determination; n is the number of data points used to build the models; SS is the sum of squares (a measure of variability); p (χ^2 or F-test) indicates the outcome of the likelihood ratio test (for comparing the adjustment of different models) or the outcome of the F-test (for the baseline model; tests the null hypothesis that the experimental data do not follow the respective baseline model); Y max is control response (yield, cells.mL^{-1}); a, b are parameters in the deviation functions.