

# Ecotoxicity of EAN and Doped EAN Ionic Liquids <sup>†</sup>

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**Abstract:** The ecotoxicity of a nitrate-based ionic liquid (IL) and saturated mixtures with four nitrate salts towards the bioluminescent bacteria *Vibrio fischeri*, were tested in this work, using the Microtox<sup>®</sup> standard toxicity test. The selected IL was Ethylammonium Nitrate (EAN) and the nitrate salts were Lithium Nitrate ( $\text{LiNO}_3$ ), Calcium Nitrate ( $\text{Ca}(\text{NO}_3)_2$ ), Magnesium Nitrate ( $\text{Mg}(\text{NO}_3)_2$ ), and Aluminum Nitrate ( $\text{Al}(\text{NO}_3)_3$ ). The effective concentration ( $\text{EC}_{50}$ ) of these mixtures was determined over three standard periods of time, namely 5, 15, and 30 min. Results of  $\text{EC}_{50}$  for pure EAN at 15 min are in relatively good concordance with the literature. To the best of our knowledge, no ecotoxicity studies have been performed for doped EAN. Similar results have been found for pure EAN and for EAN doped with  $\text{LiNO}_3$  and  $\text{Mg}(\text{NO}_3)_2$ , whose values indicated low toxicity. Nevertheless, the addition of  $\text{Ca}(\text{NO}_3)_2$  and  $\text{Al}(\text{NO}_3)_3$  cause an increase in the ecotoxicity of EAN, especially for the IL doped with  $\text{Al}(\text{NO}_3)_3$ , which present values associated to highly toxic compounds, comparable even with benzene or atrazine.

**Keywords:** ecotoxicity; ionic liquids; *Vibrio fischeri*; microtox; doped

## 1. Introduction

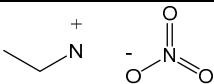
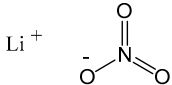
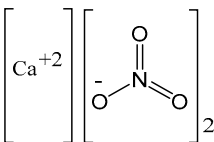
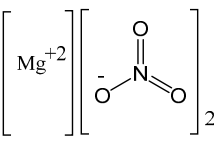
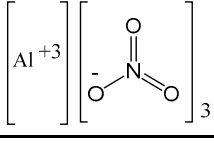
Ionic Liquids (ILs) are generically regarded as environmentally “harmless” and thus, accepted as “non-toxic” [1]. However, owing to the limitless possibilities in their design, their ecotoxicity is still poorly known. Due to their ionic character, almost all ILs are soluble in water [2], which can represent an environmental problem. Different studies showed that ILs present some toxicity [3,4], with their environmental impact strongly dependent on the cation and anion used to synthesize them [5,6]. Under the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation [7], an accurate knowledge on the toxicity of a substance is required before their application and/or commercialization. Focusing on the use of ILs as: Electrolyte for chemical devices, base lubricants or lubricant additives, or even as potential working fluids for absorption heat pumps, a spill of IL can occur, and it is necessary to know their possible environmental effects. Although, thus far, toxicity of ILs have been evaluated through different models, it is extremely important to study the effects of their ecotoxicity across various trophic levels. In addition, the knowledge on the relation between the chemical structure and the toxic effect is essential for the future design of greener solvents. The ecotoxicity of pure and doped ethylammonium nitrate (EAN) with four different ionic salts has been analyzed using the standard organism *Vibrio fischeri*.

## 2. Materials and Methods

### 2.1. Chemicals

The main characteristics of selected Ionic Liquid (IL), EAN, are indicated in Table 1. To obtain doped EAN, lithium, calcium, magnesium, and aluminum nitrate salts were used.

**Table 1.** Chemical structure, identification number, molecular mass, and purity of EAN and nitrate salts.

Name Molecular Mass (g·mol <sup>-1</sup> )	Abbreviation CAS Number	Chemical Structure	Purity Provenance	Water Content
Ethylammonium Nitrate 108.0965	EAN 22113-86-6		>0.999 Iolitec	<100 ppm
Lithium Nitrate 68.946	LiNO <sub>3</sub> 7790-69-4		>0.999 Merck	<100 ppm
Calcium Nitrate Tetrahydrate 236.088	Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O 13477-34-4		>0.999 Merck	30 <sub>wt</sub> %
Magnesium Nitrate Hexahydrate 256.3	Mg(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O 13446-18-9		>0.999 Merck	42 <sub>wt</sub> %
Aluminium Nitrate Nonahydrate 374.996	Al(NO <sub>3</sub> ) <sub>3</sub> ·9H <sub>2</sub> O 7784-27-2		>0.999 Merck	43 <sub>wt</sub> %

The different solutions of salts were prepared by mixing both components with the help of an ultrasound bath and a magnetic stirrer during 24 to 48 h. Saturated solutions have been reached using the hydrated salts by increasing molality in intervals of 0.5 mol·kg<sup>-1</sup> until saturation point at room temperature.

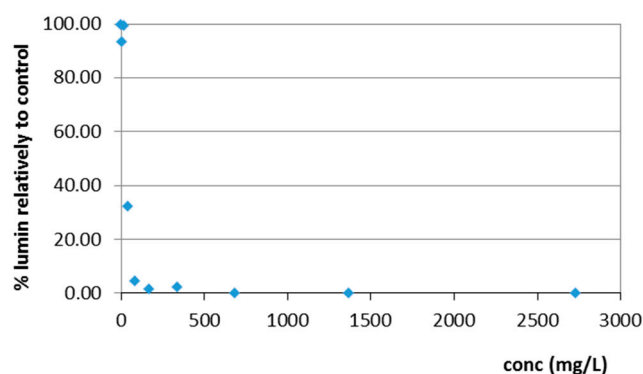
### 2.2. Experimental Section

Microtox® Toxicity Test [8] was used to assess the toxicity of the tested chemical compounds through the inhibition of the luminescence of the marine bacteria *Vibrio fischeri*. Exposure to a toxic substance caused a disruption of the respiratory process of the bacteria, and the light production was directly proportional to the metabolic activity of the bacterial population. This test was performed using a range of diluted aqueous solutions (from 0 to 81.9%) of each tested compound (100% corresponds to a known concentration of a stock solution). After 5, 15, and 30 min of exposure to the IL, the light output of the luminescent bacteria was measured and compared with the light output of a blank control sample (without the presence of the IL). These data were used to estimate the concentrations that promote 50%, 20%, and 10% of luminescence inhibition (corresponding to the effective concentration, EC<sub>50</sub>, EC<sub>20</sub>, and EC<sub>10</sub>, respectively) and the corresponding 95% confidence intervals through a non-linear regression, using the least-squares method to fit the data to the logistic equation.

## 3. Results and Discussion

Figure 1 shows the typical behavior of *Vibrio fischeri* bacteria when it is exposed to a toxic, in this case % of luminescence relative to control versus concentration, of EAN + Al 2m. Furthermore, the

effective concentration values ( $EC_{10}$ ,  $EC_{20}$ , and  $EC_{50}$ ) of the different compounds have been published in Table 2.



**Figure 1.** Effective concentration ( $EC_{50}$ ) at 30 mins for ethylammonium nitrate (EAN) + Al 2m.

**Table 2.** Mean effective concentration values ( $EC_{10}$ ,  $EC_{20}$ ,  $EC_{50}$ ) in  $mg\ L^{-1}$  and the respective 95% confidence intervals (CI), obtained after 5, 10, and 30 min of exposure of the marine bacteria *Vibrio fischeri*.

IL	Time/min	$EC_{50}$ (Lower Limit; Upper Limit)/ $mg\ L^{-1}$	$EC_{20}$ (Lower Limit; Upper Limit)/ $mg\ L^{-1}$	$EC_{10}$ (lower Limit; Upper Limit)/ $mg\ L^{-1}$
EAN	5	12,582 (8186; 16977)	4314 (1548; 7081)	2304 (248; 4361)
	15	10,665 (6650; 14680)	3236 (951; 5522)	1609 (56; 3163)
	30	9711 (6561; 12860)	3012 (1264; 4761)	1517 (332; 2703)
EAN + Li 2m	5	13,911 (12469; 15232)	8892 (7412; 10373)	6842 (5316; 8368)
	15	11,210 (9613; 12808)	7495 (5603; 9386)	5920 (3841; 8000)
	30	9706 (7233; 12179)	6145 (3301; 8988)	4701 (1744; 7658)
EAN + Ca 1m	5	7354 (5672; 9036)	3939 (2427; 5450)	2732 (1304; 4159)
	15	6064 (4343; 7784)	2858 (1475; 4240)	1938 (632; 3046)
	30	4502 (3067; 5937)	1804 (800; 2808)	1059 (263; 1849)
EAN + Mg 2m	5	12,724 (10834; 14615)	7471 (5520; 9421)	5469 (3551; 7387)
	15	13,384 (12522; 14247)	8933 (7887; 9979)	7049 (5925; 8174)
	30	14,266 (13128; 15403)	10,222 (8450; 11994)	8409 (6357; 10461)
EAN + Al 2m	5	45 (32; 58)	15 (8; 22)	8 (3; 13)
	15	37 (33; 41)	23 (18; 27)	17 (12; 21)
	30	32 (29; 36)	19 (15; 23)	14 (10; 18)

Results found in Table 2 demonstrate the well-known effect of time in the toxicity results, which normally describes the lowest EC values for the highest time of exposure. Montalbán et al. [9] have found lower values than ours for pure EAN in the effective concentration for  $EC_{50}$  at 15 min of 2256  $mg\ L^{-1}$ , this value is quite different than ours, although both of them can be considered as non-toxic according to GHS (Globally Harmonized System of Classification and Labeling of Chemicals) [10]. Similar results have been found for the solutions of lithium and magnesium (non-toxic), as well as for calcium solution, although in this case it was a bit more toxic. On the other hand, the aluminum solution can be categorized as Acute III, which is comparable with atrazine and benzene [11].

#### 4. Conclusions

The main conclusion of this work is that ethylammonium nitrate and three of the studied salts solutions are non-toxic, although when the studied solution is saturated with aluminum, it is highly toxic.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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