

# Green Chemistry

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## Green Chemistry

## ARTICLE

## Ecotoxicity studies of glycerol ethers in *Vibrio fischeri*: checking the environmental impact of glycerol-derived solvents

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The toxicities of a series of glycerol mono-, di-, and trialkyl ethers against *Vibrio fischeri* bacteria have been determined. A systematic study has been carried out and the possible structure-toxicity relationships have been discussed using different QSAR models. Inhibition of bioluminescence after 30 minutes of exposure shows relatively low toxicity for many of the glycerol derived chemicals studied. Results indicate, that, as a general rule, the ecotoxicity increases as the length and number of substituents do. However, if the size of the molecule increases, an extra substituent at position 2, makes the toxicity lower than that of the corresponding analogues.

### Introduction

The search for new solvents, coming from new sources and/or able to provide special features (often known as neoteric solvents), is a field of growing interest, especially in connection with the possibility of using renewable raw materials to produce harmless solvents, more respectful with the environment than those derived from petroleum (the so-called green solvents).<sup>1</sup> In this context, biomass-derived chemicals have attracted a great deal of attention in the last years, in connection with the development of the biorefinery concept. Agricultural and some industrial activities are able to generate huge amounts of raw materials, capable of being used to produce commodity and fine chemicals.<sup>2</sup> In this sense, glycerol is one of the platform molecules that has received much attention in recent years.<sup>3–5</sup> Glycerol appears as a concomitant product in the production of biodiesel, amounting ca. 10% weight of the total output. At present, the world production of glycerol coming from vegetable oil transformations surpasses 2 million metric tons, so it constitutes a valuable starting point to obtain bio-based chemicals, useful as, for instance, solvents.<sup>6–9</sup>

Our research group<sup>10–14</sup> and others<sup>15–18</sup> have described the synthesis and application as solvents of glycerol ethers. These kinds of solvents have the additional advantage of being much more chemically inert than other glycerol-derived solvents,

such as esters, acetals or carbonates. Some of these glycerol ethers, namely those bearing fluoroalkyl chains, exhibited especial physical-chemical features, in some way similar to those displayed by some IL: high polarity, low vapour pressure at room temperature, immiscibility both with hydrocarbons and with water. The most prominent example of these compounds is 1,3-bis(2,2,2-trifluoroethoxy)propan-2-ol, which can be efficiently prepared from trifluoroethanol and epichlorohydrin (a commodity chemical currently produced from glycerol using the Solvay procedure).<sup>12</sup> Other authors have also recognized the special character of these new fluorinated solvents.<sup>19</sup>

Apart from this particular case, glycerol ethers have a high potential for chemical diversity, given that mono-, di, and trialkylation, either symmetrical or unsymmetrical at positions 1 and 3, leads to 1,2-diols, alcohols and trialkyl ethers. These possibilities allow tuning important solvent properties, such as polarity, hydrogen-bonding ability, hydrophobicity or viscosity. Some QSPR studies have been conducted to develop models able to predict some key physical-chemical properties of glycerol ethers, prior to their synthesis.<sup>20</sup>

Concerning the greenness of glycerol-derived solvents, not only their renewable origin must be taken into account, but also other aspects of their life cycle, including their fate and the consequences on the environment. Being solvents coming from glycerol and short chain alkyl alcohols, which can in turn be obtained from biomass to a large extent, low toxicity of glycerol ethers is generally taken for granted. The pertinent question arises, however, as to whether this family of solvents can be considered environmentally benign or not, given the lack of systematic experimental evidences on its toxicity and ecotoxicity.

In this paper, we present our first results on the ecotoxicity of a series of glycerol-derived ethers. In this case, we have studied the inhibition of the bioluminescence of *Vibrio fischeri* (*V. fischeri*) bacteria. This bioindicator has been frequently

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† Electronic Supplementary Information (ESI) available: Tables with the molecular descriptors used and additional QSAR analyses. Analyses of the predictive ability of the QSAR equations. Experimental details of the synthesis of the compounds tested. See DOI: 10.1039/x0xx00000x

used,<sup>21,22</sup> since it is a well-known organism and operating protocols are standardized. Inhibition bioluminescence tests offer robust, easy handling and cost-effective responses.<sup>23</sup> Finally, this study provides some clues on the Structure-Toxicity relationships found in the studied solvent set (Fig. 1). This solvent set, though not quite large, have been chosen to cover a wide range of structural and physical-chemical features, such as number of free hydroxyl groups, length and ramification of the alkyl chains, hydrophobicity, solubility in water, and presence of fluorinated groups, among others.

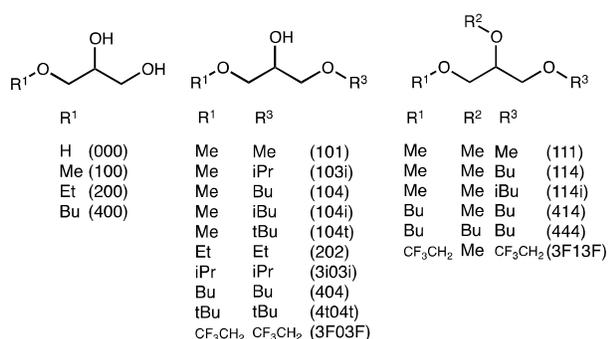


Fig. 1 Structures and codes of the 20 glycerol derivatives included in this study.

## Results and Discussion

EC<sub>50</sub> values obtained in the biotests using *V. fischeri*, with their respective adjustable parameters and standard deviations are gathered in Table 1. Furthermore, results are graphically represented in Fig. 2–9.<sup>†</sup>

Table 1 Experimental EC<sub>50</sub> values for *V. fischeri* obtained for studied solvents with their adjustable parameters for Eq. 1 and standard deviations.

Solvent code	EC <sub>50</sub> (mg·L <sup>-1</sup> )	a	b	SD
000	108,421	0.01544	0.09568	4.791
100	21,052	0.01698	0.05195	3.587
200	4,240	0.01333	0.05534	3.910
400	941	0.00873	0.02610	1.839
101	13,702	0.01795	0.10270	3.798
111	969	0.01655	0.10070	4.003
202	1,215	0.02520	0.07998	4.082
404	11	0.00777	0.01893	1.831
114	453	0.01967	0.13460	5.365
104	464	0.02099	0.06516	3.468
414	58	0.01066	0.03351	2.937
444	473	0.01796	0.04461	3.564
103i	2,188	0.01553	0.10940	3.764
104i	142	0.01280	0.03896	2.876
3i03i	1,064	0.00980	0.03596	2.523
114i	258	0.02083	0.05875	3.905
104t	189	0.03629	0.03283	3.391
404t	16	0.02154	0.04298	3.544
3F03F	1,597	0.01006	0.05688	2.375
3F13F	4,033	0.02849	0.06963	3.845

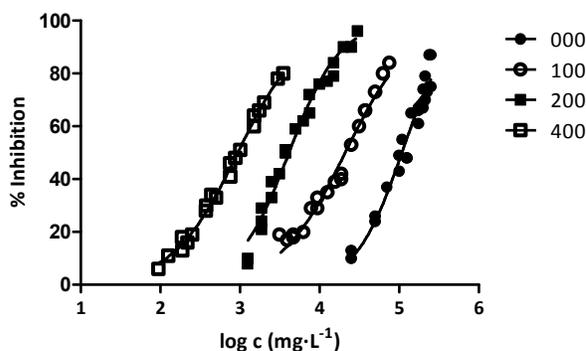


Fig. 2 Dose-response curves for studied solvents for *V. fischeri*: ● 000, ○ 100, ■ 200, □ 400.

As far as we know, there is no previous experimental ecotoxicological information of any of the studied glycerol derivatives. Regarding glycerol itself (000), there are no experimental data of ecotoxicity in *V. fischeri*. However, previous studies indicate that glycerol is of low toxicity towards microorganisms; in a 16 hour test with *Pseudomonas putida* no inhibition of bacterial growth was found at concentrations between 100 and 10,000 mg·L<sup>-1</sup><sup>24,25</sup> and similar results are found for other microorganisms such as *Entosiphon sulcatum* or *Clostridium sp.*<sup>26,27</sup> This information supports the conclusion that, overall, glycerol is of low toxicity to microorganisms. In this study, EC<sub>50</sub> of glycerol for *V. fischeri* is higher than 100,000 mg·L<sup>-1</sup>, indicating the low toxicity of this chemical for this bioindicator.

In view of the results, ecotoxicity of the studied solvents is higher than that of glycerol in all the cases. However, taking into account the results obtained for the bioindicator *V. fischeri*, only few solvents can be considered hazardous for the environment. According to Passino and Smith classification,<sup>28</sup> just 404, 404t and 414 are slightly harmful, while 111, 104, 114, 444, 104i, 114i and 104t can be considered practically harmless. The rest of the studied solvents (100, 200, 400, 101, 202, 103i, 3i03i, 3F03F, 3F13F) are clearly harmless for the environment.

Although the action mechanism is still unidentified, it is known that bacterial bioluminescence reactions are coupled to the electron transport system in cellular respiration. Consequently, bioluminescence reactions are indicative of cellular metabolism; *i.e.*, lower bioluminescence implies decreased cellular respiration. Thus, the presence of the studied solvents in the culture medium affects to a greater or lesser extent, the cellular respiration by the modification of both lipid and protein biosynthetic pathways and therefore, alters the bioluminescence emission.<sup>29</sup>

In general, the alteration of the bioluminescence emission and thus, the ecotoxicity, increases as the length of the radical does. This trend can be easily observed in Fig. 2, where we show the results obtained for the series 000-100-200-400 and ecotoxicity for *V. fischeri* increases substantially with the length of the substituent at position 1.

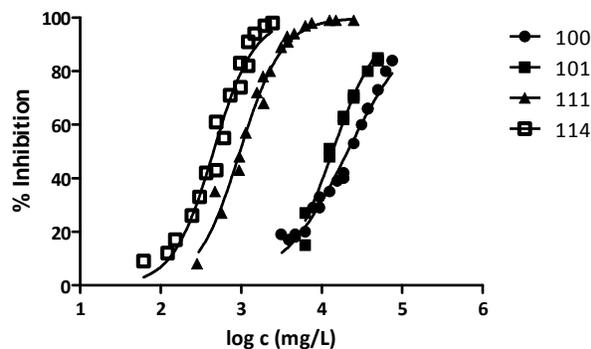


Fig. 3 Dose-response curves for studied solvents for *V. fischeri*: ● 100, ■ 101, ▲ 111, □ 114.

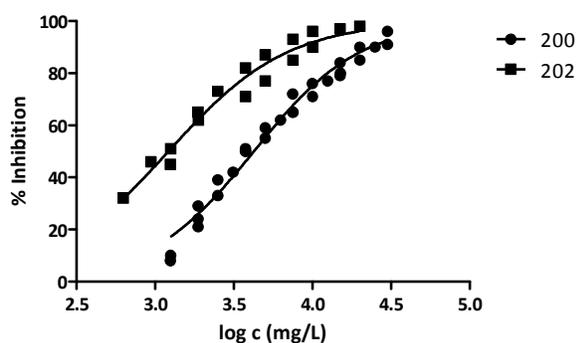


Fig. 4 Dose-response curves for studied solvents for *V. fischeri*: ● 200, ■ 202.

The presence of a substituent at position 3, makes toxicity for *V. fischeri* higher comparing with the corresponding analogous solvent. This is the case of the pairs 100-101, 200-202 and 400-404, whose behaviour is illustrated in Fig. 3, 4 and 5 respectively.

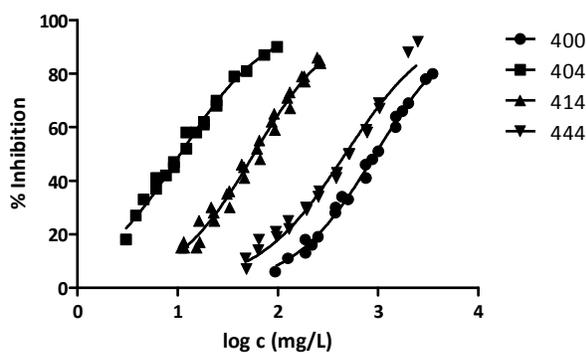


Fig. 5 Dose-response curves for studied solvents for *V. fischeri*: ● 400, ■ 404, ▲ 414, ▼ 444.

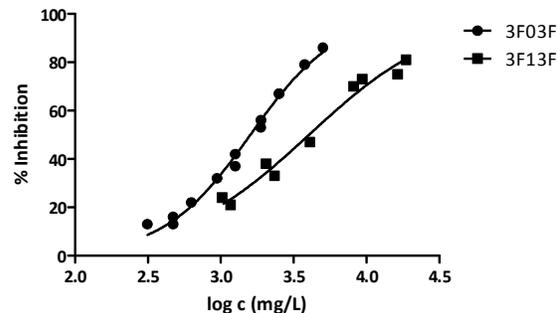


Fig. 6 Dose-response curves for studied solvents for *V. fischeri*: ● 3F03F, ■ 3F13F.

Fig. 3 shows the representation of the 100-101-111-114 series. Results indicate that toxicity increases with the length of the alkyl chain and also with the number of substituents, independently of the position of the radical. However, if the size of the molecule increases, an extra substituent at position 2, makes the toxicity lower than the corresponding analogues. Fig. 5, 6 and 8 exemplify this behaviour. For example, toxicity for 404 is *ca.* 5 times higher than 414, while for 3F03F is *ca.* 2.5 times higher than 3F13F.<sup>‡</sup> It is worth mentioning, that in the case of the pairs 104-114 and 104t-114t when molecular size is between that of 101-111 and 404-414, the ecotoxicological behaviour is quite similar and the extra radical at position 2 seems to affect only slightly the toxicity (Fig. 7 and 8).

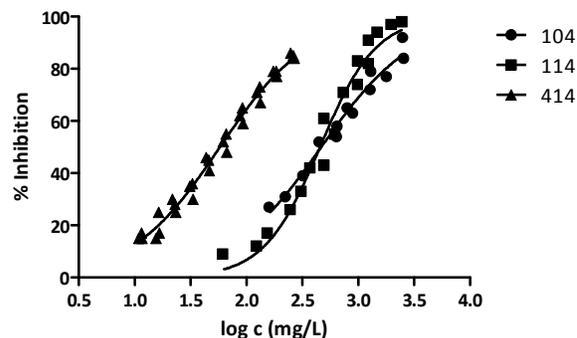


Fig. 7 Dose-response curves for studied solvents for *V. fischeri*: ● 104, ■ 114, ▲ 414.

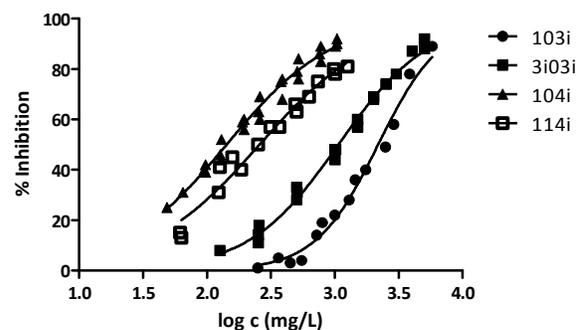


Fig. 8 Dose-response curves for studied solvents for *V. fischeri*: ● 103i, ■ 3i03i, ▲ 104i, □ 114i.

Finally, to analyse the effect of the structure of the substituents, we have studied derivatives with branched radicals. The obtained results are graphically represented in Fig. 8 and 9. Once again, the higher the size of radicals, the higher the ecotoxicity, both in the case of *iso*- or *tert*-substituents.

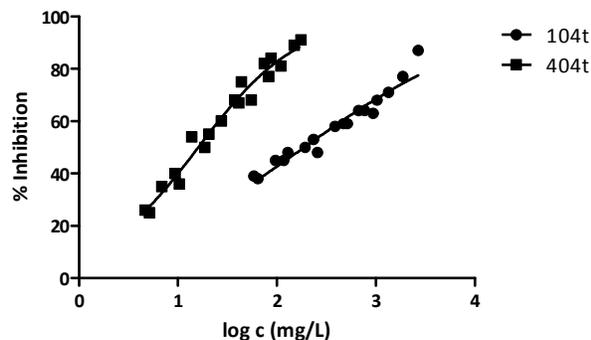


Fig. 9 Dose-response curves for studied solvents for *V. fischeri*: ● 104t, ■ 404t.

In Table 2, *V. fischeri* ecotoxicity data of a selection of some conventional solvents and ionic liquids (ILs) are shown for comparative purposes.<sup>30–33</sup> Ecotoxicity of most of the studied glycerol derivatives is smaller than that of some traditional solvents, such as toluene or phenol. Smaller compounds (100, 200 or 101) show values of  $EC_{50}$  similar to those of low molecular weight alcohols (methanol, ethanol or propan-2-ol) or acetone, known for being innocuous for *V. fischeri*. Only glycerol derivatives with large substituents (404, 414 and 404t) are as ecotoxic as *o*-xylene or phenol, which are solvents traditionally known for being toxic for the environment.

Table 2. Several  $EC_{50}$  values for different traditional solvents in *V. fischeri* bioassay during 30 minutes of exposure.

Molecular Solvents	$EC_{50}$ (mg·L <sup>-1</sup> )
Methanol	101,068 <sup>1</sup>
Propan-2-ol	35,383 <sup>2</sup>
Ethanol	23,089 <sup>2</sup>
Acetonitrile	21,172 <sup>2</sup>
Acetone	19,311 <sup>1</sup>
Dichloromethane	2,532 <sup>1</sup>
Chloroform	1,199 <sup>1</sup>
Ethylene glycol	621 <sup>1</sup>
Benzene	108. <sup>1</sup>
Toluene	32 <sup>1</sup>
Phenol	31 <sup>1</sup>
<i>o</i> -Xylene	9 <sup>1</sup>
Ionic Liquids	
1-Methyl-3-propylimidazolium tetrafluoroborate	1850 <sup>3</sup>
1-Pentyl-3-ethylimidazolium tetrafluoroborate	350 <sup>4</sup>
1-Butyl-3-ethylimidazolium tetrafluoroborate	151 <sup>3</sup>
1-Butylpyridinium dicyanamide	98 <sup>4</sup>
1-Heptyl-3-methylimidazolium tetrafluoroborate	74 <sup>3</sup>
1-Ethyl-3-hexylimidazolium tetrafluoroborate	38 <sup>3</sup>
1-Methyl-3-octylimidazolium tetrafluoroborate	7 <sup>3</sup>
1-Hexyl-3-methylpyridinium bromide	2 <sup>4</sup>

Regarding the comparison with ILs, it is interesting to note that ionic liquids have been catalogued as “green solvents” in several occasions,<sup>34,35</sup> although the toxicity of a number of them is quite high to *V. fischeri* and many other bioindicators.<sup>36</sup> Our solvents are generally much eco-friendly from the ecotoxicity point of view than the selected ILs, independently of the cation (Table 2); only in the case of ILs with small substituents toxicity is comparable to the vast majority of the studied glycerol-derived solvents.

However, it should be mentioned that the comparison made regarding the toxicity of glycerol-derived chemicals and some other solvents is limited to only one bioindicator, *V. fischeri*, in this case. Furthermore, to completely assess the greenness of the studied compounds, not only ecotoxicity data have to be taken into account; the analysis and evaluation of some other important properties such as bioavailability or biodegradability would change this first approach. Finally, the whole lifecycle of the product; production processes, uses and applications, removal rate in depuration processes, final fate and toxicity of degradation products are some parameters that should be also taken into account to determine definitively the environmental properties of the solvents.

#### Quantitative Structure-Activity Relationships

To carry out a deeper analysis of the quantitative relationships between the molecular structure and the measured ecotoxicity we use the same QSAR approaches previously applied to some physical-chemical properties of a larger family of glycerol-derived solvents.<sup>20</sup>

The first part of a QSAR study is to choose the quantitative description of the molecular structure. To this end, we used two different approaches: local structure descriptors and global structure descriptors. In the first ones, each descriptor indicates the presence or absence of a group of atoms at a given molecular position. As in our previous study, we used the DARC-PELCO approach,<sup>37</sup> following the definition shown in Fig. 10.

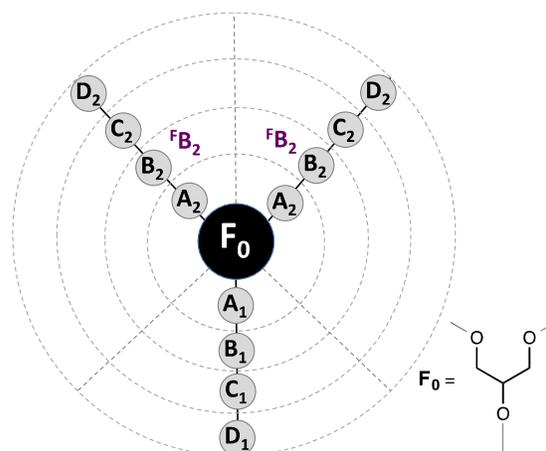


Fig. 10 DARC/PELCO scheme used to describe the molecular structures of the glycerol-derived solvents.

The DARC/PELCO method is particularly suitable for studying families of compounds with a common chemical substructure, and is based on the exhaustive generation of all possible substitution sites around the reference structure ( $F_0$ )—corresponding to the glycerol skeleton common to all ethers—and the evaluation of their contribution to the property. In this definition we have incorporated the symmetry of the glycerol derivatives used, by assuming that the contributions of groups occupying equivalent positions (*i.e.* those linked to carbons 1 and 3 of the glycerol moiety) will display the same influence on the property under study. Previous studies have demonstrated that this simplification does not alter the results of the regression analyses.<sup>20</sup>

The second approach to molecular codification is the use of topological parameters,<sup>38,39</sup> which are easily derived from the connectivity and adjacency matrixes of each compound. The number of connected components of a graph is a topological invariant that measures the number of structurally independent or disjoint subnetworks. These parameters are excellent descriptors of molecular size, shape and flexibility. They are global parameters in the sense that the whole molecular structure is condensed in a single number. The full list of DARC-PELCO and topological descriptors used in the QSAR analyses is available in the Supplementary Information.

The second part of the QSAR analysis consists in relating the dependent variable (ecotoxicity) with the independent ones (the molecular descriptors), through a quantitative model, derived in most cases from a least squares fit. In our case, we used Multiple Linear Regression (MLR) analysis, whose detailed description has already been done elsewhere.<sup>20</sup>

We started by applying the MLR analysis to the DARC-PELCO model. Given that not all the molecular descriptors have to be statistically relevant to the final equation, we used a stepwise procedure for variable selection. The final regression equation derived was the following:

$$\log EC_{50} = 4.828(\pm 0.320) + 1.064(\pm 0.460)\square B_1 - 0.614(\pm 0.192)\square A_2 - 0.310(\pm 0.082)\square B_2 - 0.685(\pm 0.123)\square C_2 \quad (1)$$

$$N = 20, R = 0.93, R_{CV} = 0.83, \sigma(y) = 0.418$$

$$F = 22.6 (F_{(4,15, 0.05)} = 3.1)$$

As can be seen, the model fits fairly well the experimental data of ecotoxicity (86% of the experimental variance is explained by the model, as indicated by the  $R^2$  determination coefficient), with a ratio observations/adjustable parameters = 5, which is quite reasonable. The standard error of the model (0.42) is also close to the experimental value (0.54). However, to have a better knowledge of the true predictive ability of this equation, a cross-validation procedure was applied. In this procedure, the toxicity value of each solvent is predicted by the regression equation derived without using the experimental data of that solvent, so that it becomes a pure

prediction. In the case of eq. 1, the toxicity of solvent 444 could not be predicted by this procedure, as the  $B_1$  coefficient cannot be calculated without this toxicity value. Using the remaining 19 solvents a  $R_{CV} = 0.83$  was obtained, still quite reasonable for a biological response. Figure 11 plots the experimental values vs. those calculated with the MLR models.

From the QSAR point of view, a positive sign of the regression coefficient in eq. 1 means that the presence of an atom in the corresponding position leads to a decrease in toxicity, whereas the converse is true for negative coefficients. Thus, enlargement of the substituents at positions 1 and 3 results in a progressive increase of toxicity, which is easier to see by looking at the standardized regression coefficients for  $A_2$ ,  $B_2$  and  $C_2$  ( $-0.342$ ,  $-0.399$  and  $-0.600$ , respectively). On the other hand, substitution at the central oxygen atom tends to decrease the toxicity of these compounds, although in a less clear manner (standardized coefficient for  $B_1 = 0.241$ ).

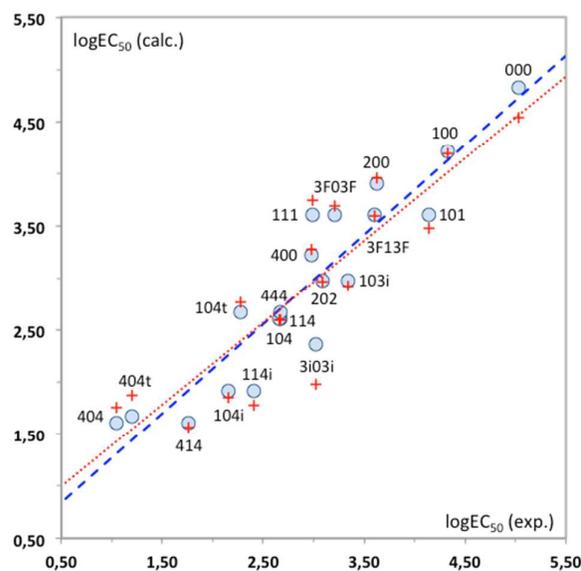


Fig. 11 Plots of predicted vs. experimental values of  $\log EC_{50}$  as calculated through MLR analysis using the DARC/PELCO model. Circles represent the predictions made with the plain regression equation and crosses are the cross-validated predictions. Dashed and dotted lines represent the least squares fit between both sets of data, respectively.

The application of the stepwise MLR procedure to the same data set, using the topological descriptors as structural variables led to the following equation:

$$\log EC_{50} = -7.715(\pm 2.710) - 1.455(\pm 0.200)\square \chi_2^v + 4.779(\pm 1.023)\square Bal^x + 0.553(\pm 0.180)\square HBD_{count} \quad (2)$$

$$N = 20, R = 0.91, R_{CV} = 0.85, \sigma(y) = 0.436$$

$$F = 27.0 (F_{(3,16, 0.05)} = 3.2)$$

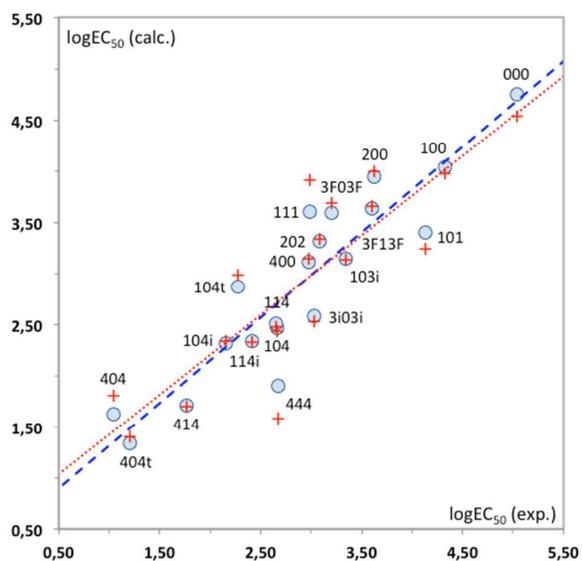


Fig. 12 Plots of predicted vs. experimental values of  $\log EC_{50}$  as calculated through MLR analysis using the topological descriptors. Circles represent the predictions made with the plain regression equation and crosses are the cross-validated predictions. Dashed and dotted lines represent the least squares fit between both sets of data, respectively.

Again, a fairly good fit is obtained (84% of the total experimental variance explained), which is mostly kept in the cross-validation analysis ( $R_{CV} = 0.85$ ). Figure 12 plots the experimental values vs. those calculated with these MLR models. As can be seen, 444 displays the highest deviation in the predicted toxicity, a fact undoubtedly related to being the sole structure studied with a long alkyl chain at position 2.

The interpretation of results is not straightforward in this case, due to the global character of the topological descriptors. The positive coefficient of the hydrogen bond donor count reflects the fact that the more free hydroxyl groups has the solvent the lower its toxicity. The other two topological indices are mostly related to the molecular size, with some corrections to atom electronegativities and valence, which mainly affect to the fluorinated compounds. Looking at the standardized coefficients for  $Bal^{IX}$  and  $\chi_2^V$  (1.157 and  $-1.513$ , respectively) it can be seen that molecular size increase roughly results in an increase of toxicity, too.

We finally tested a classical approach in QSAR studies, *i.e.*, the correlation between the measured toxicity and the hydrophobicity of the compounds, as expressed by  $\log P$ , the logarithm of the octanol/water partition coefficient.<sup>40,41</sup> Figure 13 plots both sets of values. As can be seen, there are clear deviations from a linear behaviour in three solvents, namely 444 and the two fluorinated solvents. This indicates that hydrophobicity alone cannot account for the variations in toxicity obtained. In fact, it has also been shown that there is not always a direct correlation between lipophilicity and the adsorption, biodegradation rates and therefore, toxicity; the persistence of the substance appears to be a key factor when correlating the bioavailability of the substance and the biological effect.<sup>42–45</sup> Leaving aside these three solvents, the remaining seventeen show a good linear response:

$$\log EC_{50} = 3.243(\pm 0.112) - 0.992(\pm 0.110) \square \log P \quad (3)$$

$$N = 17, R = 0.92, R_{CV} = 0.88, \sigma(y) = 0.429$$

$$F = 81.9 (F_{(1,16, 0,05)} = 4.5)$$

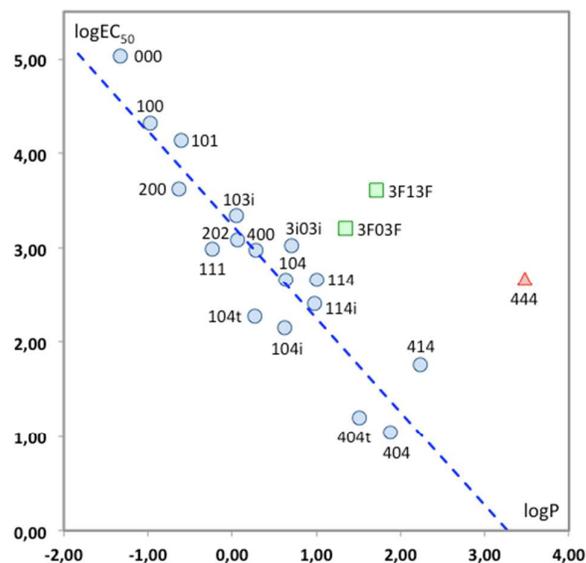


Fig. 13 Plot of  $\log EC_{50}$  vs.  $\log P$ . Only the solvents represented with circles have been used to obtain the least-squares fitting line.

In any case, it is always dangerous to use this kind of correlation to drive conclusions about causality, because of the cross-correlations that exist among different molecular properties. For instance, in the case of  $\log P$  in a rather homogeneous molecular set as the considered in this work, there are strong correlations of this property with other molecular properties mainly related with molecular size, such as molar volume (0.984) or solvent accessible surface (0.977). It is clear that a deep knowledge of the mechanisms responsible for the toxicity measured in *V. fischeri*, as well as of the bioavailability of the aqueous solutions of the glycerol derivatives would be necessary before assigning a clear causality in the SAR analyses. Studies in this direction, as well as the enlargement of the ecotoxicity studies to other bioindicators are currently in progress in our groups and will be reported in due course.

Concerning the predictive ability of the QSAR equations presented in this work in real-world situations, we have tried to provide some clues in this direction by synthesizing and testing a new compound, namely that coded as 114t (1-*tert*-butoxy-2,3-dimethoxypropane). First results obtained in the ecotoxicity measurements of this compound indicate a value of  $1,735 \pm 3.64 \text{ mg} \cdot \text{L}^{-1}$ , which fits well in the equations derived with the 20 original solvents. Furthermore, if the new solvent is included in the QSAR analyses, the derived equations show little difference with the original ones, indicating their robustness against the inclusion of new data. The

corresponding plots and equations can be found in the Supplementary Information.

## Experimental

### Synthesis of the glycerol derivatives

Monoalkylated glycerol derivatives were obtained by ring opening of glycidol using the appropriate alcohol and catalytic amounts of potassium hydroxide. Non-symmetric dialkylated glycerol derivatives were obtained by reaction of glycidol ethers with the appropriate alkoxide. Symmetric dialkylated glycerol derivatives were obtained by reaction of epichlorohydrin with the appropriate potassium alkoxide. Finally, trialkylated glycerol derivatives were obtained from the corresponding dialkylated derivatives, by alkylation of the central free hydroxyl group. Full experimental details on the synthetic procedures are available as Supplementary Information.

### Ecotoxicity experiments

The evaluation of the ecotoxicity was based on the determination of the inhibition of luminescence of *V. fischeri* bacteria. The experiments were carried out in accordance with the test conditions and the operating protocol of the *V. fischeri* acute toxicity test (UNE-EN-ISO 11348-3 2007).<sup>‡</sup> Details of the experiments can be found elsewhere.<sup>46</sup>

Trend analysis and quantitative structure–activity relationship (QSAR) models were applied with the software QSAR Toolbox 2.3 to determine the solvents concentrations to be tested. Once the range of EC<sub>50</sub> was bounded, at least 10 dilutions for each of the studied solvents were prepared using 2% NaCl as stock solution. The pH of the solutions was adjusted to 7–7.5. Additionally, positive controls (phenol 42.5 mg·L<sup>-1</sup> and zinc sulphate, 2.2 mg·L<sup>-1</sup>) and negative controls were also tested.<sup>47</sup> The lyophilized *V. fischeri* (strain NRRL-B-11177) used were provided by Macharey-Nagel (ref. 945 006). Bacteria were exposed to the toxicants for 30 min at 15 °C.

Luminescence measurements were taken with a Biofix<sup>®</sup> Lumi-10 luminometer (Macharey-Nagel) using Biotox B mode (for acute measurements).

Obtained results have been fitted using the least squares method using Eq. 4 to obtain the corresponding EC<sub>50</sub> values and standard deviations (SD):

$$\%I = 100 / (1 + 10^{(a - \log c)^b}) \quad (4)$$

where %I denotes % bioluminescence inhibition, c concentration (in mg·L<sup>-1</sup>) and a and b are adjustable parameters.

## Conclusions

We have described for the first time the ecotoxicity of a series of glycerol alkyl ethers solvents (including glycerol itself), using the bacteria *Vibrio fischeri* as bioindicator. Although all the glycerol-derived structures display higher ecotoxicities than glycerol, half of them can be considered harmless for environment on the basis of this bioindicator, and only three are clearly harmful following the Passino–Smith classification. These results support the often assumed greenness of these bio-based solvents, as far as potential environmental damage is concerned, although more ecotoxicity studies representing different trophic levels, are necessary to have a whole view on this topic.

A comparison of the ecotoxicity towards *V. fischeri* between several traditional solvents and glycerol ethers indicates that EC<sub>50</sub> of some of our solvents is similar to low molecular weight alcohols, traditionally known as no harmful for this bacteria. Furthermore, in general terms, ecotoxicity of ionic liquids seems to be higher than glycerol derived solvents, especially when length of substituents increases.

The QSAR studies point to a dependence of the toxicity on alkyl chain size, manifest in the case of substitution at 1 and 3 positions of glycerol, but less clear in the case of substitution at the central 2 position. An enlargement of the solvent set seems necessary to establish with certainty the latter effect.

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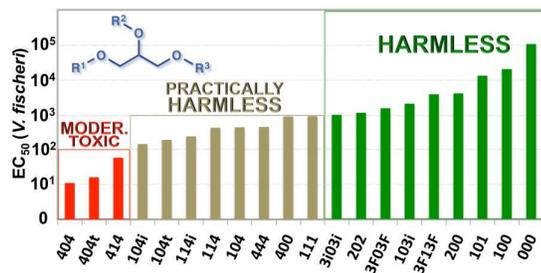
## Notes and references

‡ Concentration units in the UNE-EN-ISO 11348-3 2007 acute toxicity test used are expressed in mg·L<sup>-1</sup>. Given the different molar masses (M) of the solvents studied, this could be an issue when comparing relative toxicities. Of course, in the case of compounds with similar M, these differences tend to be negligible. However, for the sake of completeness, we have repeated all the QSAR analyses with EC<sub>50</sub> toxicities expressed in mM units, without noticeable changes in the conclusions reached. The results of these analyses are available as Supplementary Information.

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## Table of contents entry



Most glycerol-derived ethers display low or null ecotoxicity against *Vibrio fischeri* biomarker, supporting the low environmental impact of this biobased solvent family.