Comparative ecotoxicity study of glycerol-biobased solvents

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Environmental context. The search for alternative solvents to prevent environmental damage is one of the main interests in ‘green’ sciences. Five of these new substances from biodiesel production were evaluated to assess their negative environmental effects. The results obtained showed that three of these chemicals may be harmless for short exposure in aquatic biomodels. Although more tests are required, this family of compounds promises to be safe and useful for industrial purposes.

Abstract. Glycerol-biobased ethers have a high potential as solvents owing to their chemical inertness and diversity, which allows modulation of their properties, such as polarity, hydrophobicity or viscosity, depending on the specific needs in each case. Despite their renewable source, the environmental compatibility of these solvents needs to be checked. The acute ecotoxicity of five glycerol-derived solvents (3-ethoxy-1,2-propanediol, 1,3-diethoxy-2-propanol, 3-butoxy-1,2-propanediol, 1,3-dibutoxy-2-propanol and 1,2,3-tributoxypropane) was evaluated in a systematic study using several bioindicators covering the trophic chain (the crustacean Daphnia magna, the fish Danio rerio and the green alga Chlamydomonas reinhardtii). These results were compared with the previously studied bioindicator Vibrio fischeri. According to the hypothesis of the present work, the toxicity of these solvents increased as a function of their lipophilicity, which is related to the increase in the number and length of the alkyl chains in the basic structure; accordingly, the least toxic compound for all the aquatic organisms was 3-ethoxy-1,2-propanediol and the most toxic solvent was 1,2,3-tributoxypropane, except in the case of D. rerio and V. fischeri, with 1,3-dibutoxy-2-propanol the most toxic chemical. Potential damage caused by eventual emissions, was evaluated using the Environmental Health and Safety Approach, a methodology used for detecting risks related to the environment and the human health. Using available physicochemical and toxicity data, each chemical compound receives a score for the categories health, safety and environment. The best candidates considered as least dangerous for a short exposure time according to the studied biomodels are 3-ethoxy-1,2-propanediol, 3-butoxy-1,2-propanediol and 1,3-diethoxy-2-propanol.

Additional keywords: ecotoxicology, chemical toxicology.

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Introduction

Organic solvents are one of the main sources of anthropogenic volatile organic compounds (VOCs). At present, most organic solvents come from petroleum and are used in huge amounts for industrial and household applications, so they constitute an important concern for air and water pollution because of their toxic and ecotoxic effects. Harmful VOCs are not acutely toxic in most cases, but they often have long-term effects on health and the environment, and also are not considered renewable media. A more sustainable chemistry requires new solvents, coming from new sources, able to provide the concrete features needed for the application in which they will be used but, above all, that are more respectful to the environment than those derived from petroleum. In this context, chemicals derived from renewable sources (such as biomass) are attracting a great deal of interest of late.

Current agricultural and industrial activities generate huge amounts of raw materials, which can be employed to produce useful chemicals (either commodities or fine chemicals). One of the most promising platform molecules that has received much attention in the last few years is glycerol (1,2,3-propanetriol). Although in the past it was also produced from fossil sources, nowadays glycerol is a byproduct in the production of biodiesel (~10% by weight of total production) and also in the oleochemical industry. In recent years, the world production of glycerol from vegetable oil transformations has surpassed 2 million metric tonnes, which may pose a problem if the surpluses have to be disposed of. However, glycerol constitutes
Acute ecotoxicological study of glycerol derivatives

Glycerol ethers have a high potential for chemical diversity, given that mono-, di and trialkylation, either symmetrical or asymmetrical at positions 1 and 3, leads to 1,2-diols, alcohols and trialkyl ethers respectively. These possibilities allow modifications in the structure of the molecules depending on the solvent properties needed, such as polarity, hydrophobicity or viscosity.[8–9] With respect to the harmlessness of these bio-based solvents, the low risk of being dangerous for the environment for glycerol ethers is generally taken for granted.[5,10] The question arises, however, as to whether solvents coming from glycerol can be considered environmentally benign or not, given the lack of systematic experimental evidence on their toxicity and ecotoxicity. Our group recently published the EC50 (effective concentration at 40% of the individuals exposed to the chemical) acute values of a series of glycerol-derived ethers in a typical bioindicator, the bacteria Vibrio fischeri[11] as a first approximation for the toxic effect of these chemicals. Only those ethers with long-chain substituents were found to be slightly toxic for this bioindicator. Further, we also published the EC50 values for short exposure of a fluorinated glycerol derivative and a commercially available ionic liquid, comparing these data to the octanol/water partition coefficient (log P) as an estimate of their overall lipophilicity, i.e. something that modulates their behaviour in relevant biological processes such as permeability through biological membranes and hepatic clearance.[17]

### Ecotoxicity studies

Chlamydomonas reinhardtii culturing and exposure to solvents

The unicellular algae C. reinhardtii CC125 in exponential phase were used for the experiments. Algae were growing for 72 h in an incubator at 25 °C, on an orbital shaker at 90 rpm under a continuous illumination of 130 µE PAR m⁻² s⁻¹ (micro-Einstein photosynthetically active radiation per second and square metre), given by four fluorescent tubes (Blau Aquaristic T5HO, 39 W 10000 K⁻¹, Blau Aquaristic, Barcelona, Spain). Talaqual culture medium was prepared as described in Szivák et al.[11] using CuCl₂·2H₂O and ZnCl₂ instead of the corresponding sulfates.

<table>
<thead>
<tr>
<th>Solvent code</th>
<th>Chemical name</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Molecular structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>3-Ethoxy-1,2-propanediol</td>
<td>Ethyl</td>
<td>–</td>
<td>–</td>
<td><img src="image" alt="Molecular structure" /></td>
</tr>
<tr>
<td>202</td>
<td>1,3-Diethoxy-2-propanol</td>
<td>Ethyl</td>
<td>–</td>
<td>Ethyl</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>3-Butoxy-1,2-propanediol</td>
<td>Butyl</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>404</td>
<td>1,3-Dibutoxy-2-propanol</td>
<td>Butyl</td>
<td>–</td>
<td>Butyl</td>
<td></td>
</tr>
<tr>
<td>444</td>
<td>1,2,3-Tributoxypropane</td>
<td>Butyl</td>
<td>Butyl</td>
<td>Butyl</td>
<td></td>
</tr>
</tbody>
</table>
Different concentrations of the studied solvents were tested in order to obtain dose–response curves, with two replicates (flasks) for each one. The exposure medium was prepared by adding the appropriate amount of solvents into Millipore-filtered water buffered with 10 mM MOPS (3-N-morpholinopropanesulfonic acid) adjusted to pH 7.5, and then adding the algae. In the case of [2.0.0], concentrations ranged between 5000 and 90 000 mg L$^{-1}$, for [2.0.2] between 1250 and 50 000 mg L$^{-1}$, for [4.0.0] between 500 and 10 000 mg L$^{-1}$, for [4.0.4] between 20 and 2000 mg L$^{-1}$, and for [4.4.4] between 50 and 500 mg L$^{-1}$. Negative controls (two replicates consisting of water with 10 mM MOPS, 7.5 pH, with the same algae concentration) were also tested, and the dose–response curve measurements were repeated at least 3 times. The 72-h-old algae were centrifuged (10 min, 1275 g, ambient temperature) and the concentrate was used to obtain an optical density (OD$_{680}$) of 0.3, equivalent to 1 150 000 cells mL$^{-1}$.

As toxicity endpoint, the photochemical quantum yield, Y (i.e. the efficiency in transforming light energy into biochemical energy by photosynthesis) was used, and measured using a Mini-PAM fluorometer (from Walz, Effeltrich, Germany). The settings used were a low-frequency measuring light (ML) level of 0.15 μmol m$^{-2}$ s$^{-1}$, SP (saturating pulse) of 1577 μmol m$^{-2}$ s$^{-1}$, and 0.8-s pulses. Fluorescence parameters were measured on 2-mL algal suspension after 1 h of exposure. After 30 s of acclimatisation to measuring light conditions (measured on 2-mL algal suspension after 1 h of exposure. After 72 h of exposure to the chemicals to determine lethal concentration (for EC 50 and LC 50) values and the dose–response curves, positive controls with K$_2$Cr$_2$O$_7$ and negative controls were also tested. Concentrations tested were prepared using culturing medium as solvent. The ranges for [2.0.0], [2.0.2], [4.0.0], [4.0.4] and [4.4.4] were 550–15 000, 1000–3000, 1000–4000, 50–500 and 0.5–75 mg L$^{-1}$ respectively. The pH of the dilutions was measured and adjusted to between 7 and 7.5 before exposure. A total of 20 newborn daphnids (aged <24 h) were exposed to the test compounds in complete darkness for 24 h at 20 °C per concentration and compound. The crustaceans were separated into four groups of five organisms, four replicates per concentration exposure. The test was repeated at least three times. Immobilisation of the organisms was measured through observation. Crustaceans that were not able to swim for 15 s after gentle agitation were considered immobilised.

**Vibrio fischeri culturing and exposure to solvents**

Test conditions and the operating protocol of the *V. fischeri* acute toxicity experiments were carried out in accordance with the UNE-EN-ISO 11348–3 protocol (Spanish Association for Standardization, International Organization for Standardisation).[19] A description of the complete experimental procedure can be found elsewhere.[12] The lyophilised *V. fischeri* (strain NRRL-B-11177) used for the tests were purchased from Macherey-Nagel (ref. no. 945006). A 2% NaCl stock solution was used to prepare the dilutions of each of the studied solvents, adjusting the pH to 7–7.5 using either 0.1 M HCl or 0.1 M NaOH solutions in 2% NaCl. For [2.0.0], concentrations ranged between 625 and 20 000 mg L$^{-1}$, for [2.0.2] between 475 and 10 000 mg L$^{-1}$, for [4.0.0] between 100 and 3500 mg L$^{-1}$, for [4.0.4] between 3 and 100 mg L$^{-1}$, and for [4.4.4] between 50 and 2500 mg L$^{-1}$. Positive controls were zinc sulfate (2.2 mg L$^{-1}$) and phenol (42.5 mg L$^{-1}$). Negative controls were culturing medium (Biofix® Lumi medium for freeze-dried luminescent bacteria by DIN EN USO 11348–3, Macherey-Nagel, Düren, Germany). Two replicates for each control were tested.

The time exposure of bacteria to the solvents was 30 min at constant temperature (15 °C). Luminescence measurements were obtained with a Biofix® Lumi-10 luminometer (Macherey-Nagel) in acute mode (Biotox B). The test was repeated at least twice.

**Daphnia magna culturing and exposure to solvents**

This test was performed according the guidelines in the Organisation for Economic Co-operation and Development (OECD) 202 Test conditions.[22,23] Once again, a complete and detailed description of the experimental protocols can be found elsewhere.[12] The *D. magna* ephippia used were purchased from Vidrafor (Toxkit, Daphktik F Magna, ref. DM090812, Barcelona, Spain) and stored at 4 °C until their use. The tests were carried out with a new batch each time. The preparation of the eggs consisted in their incubation with culturing medium prepared according to the specifications of the supplier for 72 h at 22 ± 2 °C with 6000 lx in a Toxkit CH-0120D-AC/DC incubator (supplied by Ecotest, Valencia, Spain) and fed with *Spirulina* algae 2 h prior to starting the experiment. Positive controls with K$_2$Cr$_2$O$_7$ and negative controls were also tested.

Concentrations tested were prepared using culturing medium as solvent. The ranges for [2.0.0], [2.0.2], [4.0.0], [4.0.4] and [4.4.4] were 550–15 000, 1000–3000, 1000–4000, 50–500 and 0.5–75 mg L$^{-1}$ respectively. The pH of the dilutions was measured and adjusted to between 7 and 7.5 before exposure. A total of 20 newborn daphnids (aged <24 h) were exposed to the test compounds in complete darkness for 24 h at 20 °C per concentration and compound. The crustaceans were separated into four groups of five organisms, four replicates per concentration exposure. The test was repeated at least three times. Immobilisation of the organisms was measured through observation. Crustaceans that were not able to swim for 15 s after gentle agitation were considered immobilised.

**Danio rerio culturing and exposure to solvents**

The fish embryo toxicity (FET) test in zebrafish was developed by ZFBiolabs and consists of an adaptation of FET approved as OECD 236.[24] Embryos of *D. rerio* were obtained by in vitro fertilisation and hatchlings were selected when percentage viability was over 80%.

A 0.25 % DMSO solution was used to dilute the compounds. DMSO was used to increase the permeability of the embryo chorion. The temperature was maintained between 24 and 26 °C, and oxygen saturation between 60 and 100%. The pH was adjusted to 6.5–8.5 if necessary. For [2.0.0], concentrations ranged between 2300 and 300 000 mg L$^{-1}$, for [2.0.2] between 78 and 10 000 mg L$^{-1}$, for [4.0.0] between 156 and 20 000 mg L$^{-1}$, for [4.0.4] between 1 and 312 mg L$^{-1}$, and for [4.4.4] between 78 and 10 000 mg L$^{-1}$. Positive controls contained 4-aminophenol (paracetamol) (4155 mg L$^{-1}$). Negative controls were also included. Twelve embryos were exposed to each concentration with a dilution factor of 2 applied to the concentrations above. When toxicity range was low, a dilution factor of 1.5 was used to repeat the protocol ([2.0.0], [2.0.2] and [4.0.0]).

Signs of lethality to embryos were observed after both 24 and 48 h of exposure to the chemicals to determine lethal concentration 50%, LC$_{50}$, using data from observation at 48 h. Assays were repeated at least twice.

**Statistics and graphical representation**

To obtain the half-maximal effective or lethal concentration (for EC$_{50}$ and LC$_{50}$) values and the dose–response curves, the following procedures were carried out: for *C. reinhardtii*, results were fitted using *R* and the *drc* package to a four-parameter logistic curve whereas the ‘compPAR’ function was used to perform comparison tests. The null hypothesis is that the ratio obtained dividing EC$_{50}$ values equals 1; if it significantly differs from 1, the null hypothesis is rejected because those values are significantly different ($P < 0.05$). Analogously, for the rest of the bioindicators, Tukey’s method...
for multiple comparisons was applied. In all cases, the results were significantly different.

Experimental data for *V. fischeri*, *D. magna* and *D. rerio* were fitted to Eqn 1 obtain the corresponding EC or LC50 values and standard deviations (s.d.) using the least-squares method:

\[ \%I = 100 \left( \frac{1}{1 + 10^{(a - \log c) b}} \right) \]  

where \( \%I \) denotes % bioluminescence inhibition for *V. fischeri*, % immobilisation for *D. magna* and % death for *D. rerio*, \( c \) is concentration (in mg L\(^{-1}\)) in all cases and \( a \) and \( b \) are adjustable parameters.

### Results and discussion

#### Ecotoxicity results

All the glycerol ethers studied showed concentration-dependent toxicity to the organisms tested after acute exposure. Figs 1–3 show the experimental dose–response results obtained for *C. reinhardtii*, *D. magna* and *D. rerio*, and Table 2 shows their corresponding EC or LC50 and standard deviation (s.d.) values. For comparison, the corresponding EC50 and log \( P \) values from previous work for the studied compounds in *V. fischeri* are gathered in Table 3 and Fig. 4\[11\]

The acute EC50 values of 1,2,3-propanetriol (glycerol) in the environment are well established in the literature, including for

<table>
<thead>
<tr>
<th>Solvent code</th>
<th>C. reinhardtii EC50 (mg L(^{-1}))</th>
<th>D. magna EC50 (mg L(^{-1}))</th>
<th>D. rerio LC50 (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>[2.0.0]</td>
<td>52,811</td>
<td>6458</td>
<td>36,700</td>
</tr>
<tr>
<td>[2.0.2]</td>
<td>8613</td>
<td>1819</td>
<td>2800</td>
</tr>
<tr>
<td>[4.0.0]</td>
<td>4445</td>
<td>2332</td>
<td>4300</td>
</tr>
<tr>
<td>[4.0.4]</td>
<td>631</td>
<td>248</td>
<td>17</td>
</tr>
<tr>
<td>[4.4.4]</td>
<td>64</td>
<td>13.7</td>
<td>2700</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solvent code</th>
<th><em>V. fischeri</em> EC50 (mg L(^{-1}))</th>
<th>Log ( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>[2.0.0]</td>
<td>4240</td>
<td>-0.63</td>
</tr>
<tr>
<td>[2.0.2]</td>
<td>1215</td>
<td>0.07</td>
</tr>
<tr>
<td>[4.0.0]</td>
<td>941</td>
<td>0.28</td>
</tr>
<tr>
<td>[4.0.4]</td>
<td>11</td>
<td>1.88</td>
</tr>
<tr>
<td>[4.4.4]</td>
<td>473</td>
<td>3.48</td>
</tr>
</tbody>
</table>
Thus, glycerol can be considered clearly harmless in the aquatic environment, according to the classification of Passino and Smith, a logarithmic hazard ranking for aquatic biomodels that allows classification of soluble chemical substances in acute exposure. Comparing with our results, the EC₅₀ value of the original compound is higher than the studied glycerol derivatives. As far as we know, there are few studies on the toxicities of this group of compounds. Only Sutter et al. evaluated one of these chemicals, 1,2,3-trimethoxypropane, in several toxicity studies, including in algae, crustaceans and fish.

As a general trend and according to the hypothesis of the present work, the toxicity of these solvents increases as the lipophilic character does (Fig. 5), which is also related to the presence and the increase in length of the alkyl chains attached to the hydroxyl groups in the glycerol molecule. Mainly for *D. magna* and *C. reinhardtii*, there was a correlation between log *P* and log EC₅₀ values. Organic compounds with no toxically active functional groups have an action mechanism called ‘narcosis’. This mechanism involves non-specific non-covalent interactions of the organic molecule with the lipophilic cell membrane of the biomodel. The final result of this interaction is the reversibly altered structure and function of the membrane, causing the toxic effects. However, results indicate that *D. rerio* and *V. fischeri* did not show a direct relationship between log *P* and log EC₅₀.

From our results, the highest EC or LC₅₀ for all the aquatic organisms was shown by [2.0.0]. The most toxic solvent for *V. fischeri* and *D. rerio* was [4.0.4], whereas [4.4.4] was the most toxic chemical for *D. magna* and *C. reinhardtii*. In line with the Passino and Smith classification, only [4.0.4], in the case of *V. fischeri* and *D. rerio*, and [4.4.4], for *D. magna* and *C. reinhardtii*, can be considered as slightly harmful for these bioindicators, because their EC or LC₅₀ values are below 100 mg L⁻¹. [4.0.0] and [4.4.4] can be considered practically harmless for *V. fischeri*, having an EC₅₀ value between 100 and 1000 mg L⁻¹, and the rest of the studied solvents, displaying EC or LC₅₀ values over 1000 mg L⁻¹, are clearly harmless for the aquatic environment in the same classification ([2.0.0] and [2.0.2]) (Fig. 6).

Fig. 5. Plots of log EC₅₀ (logarithm of the lethal concentration), 50%, *v*. log *P*, logarithm of the octanol/water partition coefficient *P* for the organisms tested.

Fig. 6. Graph of the harmfulness of the five solvents studied for the organisms tested, according to the Passino and Smith classification.
Focussing on the structure of the studied compounds, it is remarkable that for *D. magna* and *D. rerio*, the effect on toxicity of two ethyl substituents in positions 1 and 3 of the glycerol derivative is higher than the presence of only one butyl substituent. Furthermore, we detected another anomaly in the trend; when position 2 is substituted with the biggest substituent (butyl), toxicity does not increase in all cases: for *D. rerio*, toxicity clearly decreases. This result has been previously observed for the bioindicator *V. fischeri*. In that case, when the molecular size of the substituent in position 2 was methyl, the extra radical at this position seemed to only slightly affect the toxicity. Here, in the case of *D. rerio*, this effect is particularly clear: the toxicity of [4.0.4] is ~160 times higher than that of [4.4.4].

Effectively, the size of the substituent in position 2 is a key factor in toxicity, depending on the bioindicator studied, and tailoring opportunities arise from this fact.

Algal toxicity followed the expected trend for the studied chemicals: [2.0.0] is the least toxic chemical in acute exposure, followed by [2.0.2], [4.0.0], [4.0.4] and [4.4.4] (Fig. 1). A good correlation between log EC_{50} and log *P* was observed (correlation coefficient *r* = 0.988) and the values of EC_{50} decreased as the length and number of alkyl chains in the glycerol series increased (Fig. 5). Toxicity in the algae is measured as the decrease of the yield of Photosystem II, Y (II), indicating that these compounds can affect electron flow in photosynthesis. Previous studies have demonstrated that this effect is found in several chemicals and herbicides, like atrazine. The inhibited electron transfer in Y (II) results in oxidative stress, photooxidation of chlorophyll and cell necrosis. Another reason for the toxicity of these compounds to the algae can be explained by damage to the photosynthetic membranes, due to their lipophilicity. However, log *P* is considered an estimate of a compound’s overall lipophilicity, i.e. something that modulates its behaviour in relevant biological processes such solubility, permeability through biological membranes or hepatic clearance. In the present case, according to the linear relationship found between log *P* and log EC_{50}, this type of glycerol derivative could be harmful for algae if the log *P* value is higher than 3.

In the *D. magna* bioassay, the toxicity ranking of the studied chemicals decreased as follows: [2.0.0], followed by [2.0.2], [4.0.0], [4.0.4] and [4.4.4] (Fig. 2). In this case, a good correlation between lipophilic character and toxicity was also observed (correlation coefficient *r* = 0.994, Fig. 5). According to the linear relationship between log *P* and log EC_{50}, a glycerol derivative with a log *P* value higher than 3 may become a serious threat for both crustacean and algae. This is because compounds with higher log *P* values present lower EC_{50} concentrations (see Fig. 5) in the environmental range of concentrations found in natural systems.

Results in *D. rerio* (Fig. 3) showed that the most toxic compound was [4.0.4] in acute exposure, according the LC_{50} values. As in the previous biomodels, the least toxic was [2.0.0], followed by [4.0.0], [2.0.2] and [4.4.4]. There were significant differences among all these values. Because of the inversion of the toxicities of [4.0.4] and [4.4.4] LC_{50} values did not correlate well with lipophilic character. Again, the LC_{50} experimental values of [2.0.2] were lower than those of [4.0.0], as was already found in *D. magna*. The acute ecotoxicity of [4.0.4] was higher than that of [4.4.4], as was previously found in *V. fischeri*. The reason for this apparent lack of coherence with the trend in toxicity may be explained by the complexity of the biological processes of the tested organism (fish) compared with algae or cladocerans. Analogously, log *P* values between 1 and 2 (and [4.0.4] has a value of 1.88 whereas [4.4.4]'s is 3.48) are often considered optimal to achieve a compromise between permeability and first-pass clearance in vertebrates.

### Environmental, health and safety approach

Furthermore, in order to estimate the potential damage to the local and regional environment caused by eventual emissions from the chemical processes in which these new solvents could be involved, we have used the Envirnoment, Health and Safety Approach (EHSA), together with further information that has been gathered (Tables 4 and 5) and analysed.

To check hazards associated with the mobility of the solvent during its handling and use, two properties were selected at room temperature: volatility and boiling point. These physicochemical characteristics provide information about the probability of generating new phases and release to the atmosphere. Although associated hazards for both properties are linked to the temperature and pressure of the particular process, we can provide several conclusions. The higher the vapour pressure, the higher the environmental risk: in this case, values were quite low (below 0 on a scale from 0 to 1, 1 being the highest risk indicator), and none of them can be considered as potential hazards according to the methodology. However, boiling points were very high and thus, in most of the cases, when using these solvents at room temperature, the probability of generating new vapour phases is quite low owing to the index value below or very close to 0 on the scale for all the compounds. Therefore, the risk of overpressure in experimental devices is also low as well as the probability of the substances escaping from the system in case of equipment failure.

Hazard related to fire or explosions were analysed using the flashpoint. Once again, the process temperature will determine potential risk in this case. However, to get a general idea of the fire or explosion risk associated with the studied solvents, we
can compare the raw flash-point values at a process temperature of 25 °C. According to EHSA, all of these solvents could be dangerous, and the highest risk of fire or explosion is found for [2.0.2] (0.76 on a scale from 0 to 1), whereas [2.0.0] and [4.0.4] (0.56 and 0.555 respectively) show the lowest risk in comparison. It is important, however, to put these values into context, because common organic solvents structurally related to glycerol alkyl ethers have, in general, much lower flash point values and indices: diethyl ether (−45 °C, 1.35), ethanol (13 °C, 1.06), 1-butanol (37 °C, 0.94), methyl tert-butyl ether (−28 °C, 1.265), diglyme (67 °C, 0.81). Only ethylene glycol shows a similar value (111 °C, 0.57).[38]

To assess human acute toxicity, we selected the oral LC50. High values estimated for this property indicated that the risk associated with toxicity was quite low, below 0.0001 on an index scale from 0 to 1 for all the compounds. However, environmental toxicity was assessed using the mean value obtained in all available aquatic acute toxicity experiments. In the present case, we used our own experimental values to evaluate this environmental parameter. Mean values above the threshold of 1000 mg L−1 (a 0 value on the index scale) are considered very low risk. Thus, only [4.4.4] (0.023) and [4.0.4] (0.161) showed environmental risk and, therefore, specific waste purification processes could be considered.

Another important environmental property is the ease with which the substances are degraded once they are in the environment. Potential for degradation has been assessed with ready biodegradability. It should be mentioned that this estimation depends strongly on the conditions of degradation, particularly in waste treatment plants. In this case, only [4.4.4] may be persistent in the environment, according to the predictions of the Biowin model.[34,37]

Finally, the potential of these chemicals to accumulate through the soil or the chain food was analysed. The property selected to check this risk is the log of the bioconcentration factor (log BCF). BCF is defined as the ratio of the concentration of a chemical in an organism to the concentration in the surrounding environment at steady state. It is a valuable indicator of the bioaccumulation potential of a substance, and hence has become an essential environmental measure required for regulatory purposes. The log BCF values used in the present work were calculated using the model of Meylan et al.[39] included in the Estimation Program Interface (EPI) suite programs.[34] Basically, this model employs different equations relating the experimental BCF values from a large database to calculated log P values, taking into account the ionic or non-ionic nature of the solutes, as well as their classification on the log P scale and other correction factors. In this case, only [4.4.4] (0.11 on an index scale from 0 to 1) showed a certain risk. Its calculated log BCF is 2.22, which is above the threshold of the Chemical Safety Assessment (2.00) (https://chesar.echa.europa.eu/es/, accessed 13 May 2016), but still below the threshold established by the REACH Regulation for Bioaccumulative Substances (3.30).[40]

To sum up, comparison of the EC50 values of five glycerol alkyl ethers in different aquatic bioindicators, along with V. fischeri [11] as well as the application of EHSA, allows some interesting conclusions to be reached. First of all, the glycerol ethers bearing either the shortest alkyl chains ([2.0.0], [2.0.2]) or just one long chain ([4.0.0]) can be classified as harmless for the environment in acute exposure, according to the Passino and Smith classification.[20] Those ethers bearing two or more butyl substituents ([4.0.4] and [4.4.4]) raise more concern, because they display moderate toxicity for two out of four bioindicators for the same classification. These results cannot be directly connected to the lipophilicity of these compounds in the case of V. fischeri [11] and D. rerio. For C. reinhardtii and D. magna, a strong linear relationship was found between the log P and EC50 values, and an approximate estimation of their degree of toxicity according to their lipophilicity is reported. In general, values of log P higher than 3 in these types of chemicals could lead to harmful environmental effects (because of their lower EC50).

None of the glycerol ethers studied seem to be of concern regarding human toxicity, and only [4.4.4] has several negative indices, such as higher persistence in the environment, moderate toxicity for some aquatic bioindicators, and quite low flash point (but still much higher than those of common organic solvents).

In the light of the results described in the present work, the best candidates for solvent substitution appear to be [2.0.0], [2.0.2] and [4.0.0]. Further studies are being conducted to improve the quality of the information about the overall greenness of these bio-based solvents.

Conflict of interest
The authors declare no conflicts of interest.

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