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Evaluating the toxicity of biomass derived platform chemicals†

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Furans and their derivatives are well-known chemical building blocks common in plant biomass, and are abundantly used in food, medicines and industrial processes. As a bio-renewable resource, obtainable from the abundant and inexpensive lignocellulosic biomass, there is a growing interest in their study, physico-chemical characterization and application. Due to their biological origin, there is a presumption of low toxicity and high biodegradability for these compounds, which makes them "green" solvents, and thus potential substitutes of the classical organic solvents or oil derived commodities. Surprisingly, their ecotoxicity is poorly characterized. The few studies dealing with the toxicity of furans, namely towards animals, have presented contradictory results. In this work, the toxicity of eighteen furans and their derivatives was evaluated by the Microtox toxicity assay, using the marine bacterium *Vibrio fischeri*. Different levels of toxicity were observed among the furan derivatives investigated. The results obtained suggest that it might not be adequate to consider furans and their derivatives as "green" solvents as, in general, furans are more toxic than the classical solvents. Nevertheless, more data and studies across more trophic levels are necessary to fully understand the effects of furans on the environment as well as their biodegradability.

Introduction

Furans are common in nature and in the environment, furfural being the most widespread furan.[1] Furan and its derivatives, namely furfural and furfuryl alcohol, are abundantly used in food, medicines and in various industrial processes.[2,3] The commercial production of furans is mainly performed from lignocellulosic plant materials by acid hydrolysis reactions (hemicelluloses are hydrolysed and sugars are dehydrated to furans).[1] Other forms were already described regarding the production of furans, namely in paper mills and during the hot treatment of municipal wastes.[1] Meanwhile, there has been growing interest in furans, since these compounds are being considered as platform chemicals, *i.e.* chemical building blocks with numerous applications (*e.g.* adhesives, biofuels, lubricants and solvents) with relevance to the chemical industry.[4] They represent an interesting biorenewable resource as an abundant, inexpensive, and non-edible lignocellulosic biomass, which may be easily converted to furans.[5] These compounds may be a good renewable alternative to fossil fuels[6] and the classic petrochemical solvents,[4] since biomass is a renewable resource and may contribute to minimize CO_2 emissions,[5] along with a presumably lower toxicity and higher biodegradability.[4] Furans can only replace organic solvents if they can meet the requirements of the REACH legislation (regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals), nowadays mandatory for the commercialization of chemical products.[7] This legislation demands a high level of environmental and human health protection from the possible risks caused by a substance.[7] For this purpose ecotoxicity tests are required. Although furans and their derivatives have many interesting properties, their ecotoxicity is poorly studied[8] and have been often denied in the definition of their "greenness". Among their properties, low volatility must be emphasized,[8–10] minimizing atmospheric pollution. Nevertheless, they present a wide range of water solubility[6,11–13] and thus, they should be considered as a possible threat to aquatic environments.

The Microtox toxicity test is a simple, fast, cost-effective, sensitive, widely used and accepted method for toxicity assessments.[14] It is based on the bioluminescence response of the marine bacteria *Vibrio fischeri* (standard Microtox liquid-phase assays, here abbreviated as the Microtox test). The use of a test with marine bacteria, having in mind that the sea is the final destination of all aquatic pollution,[15] seems adequate for this

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first systematic approach to the ecotoxicity of furans and their derivatives.

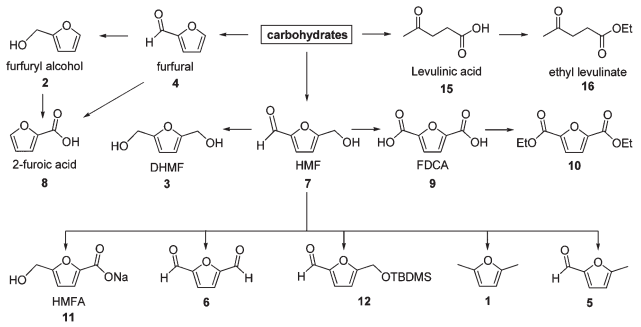
Of all furans, derivatives and other common molecules, only six had been studied with the Microtox test, namely furfural, furfuryl alcohol, levulinic acid, ethyl levulinate, *tert*-butylmethyl ether and butyl levulinate (the last one is not herein studied).[8,16–18] In these studies, their toxicity was generally reported to be very low, together with a high biodegradability.[8] These support their use as green substitutes of traditional chemicals.[10] However, there are also studies revealing the toxicity of many of these furanic compounds in animals and humans.[2,3,18] Furan itself, the parent compound of this family, is a potent liver toxicant and carcinogen in rats and mice. This fact, along with its ubiquity in the environment, leads to the classification of furan as a possible human carcinogen.[3] It is known that furans from diet have potential hepatic and renal toxicity to humans.[19] Many xenobiotics containing a furan ring are toxic and/or carcinogenic. Small structural differences affect the toxicity, as well as the presence of competing metabolic pathways and detoxification mechanisms.[3] The toxicity of the furan ring-containing compounds requires oxidation of the furan ring and it is influenced by three main factors: the involvement of other metabolic pathways, rapid detoxification of the reactive intermediate and the reactivity of the furan ring-oxidized metabolite (which will influence the cellular targets and, consequently, the overall toxicity of the compound).[3]

A number of studies deal with the toxicity of some furans and contradictory results are frequently reported. To our knowledge, there are no records of toxicity assessment for some of the compounds herein studied, namely furan-2-carbaldehyde or furfural (**4**), furan-2,5-di-carboxylic acid (**9**), diethyl furan-2,5-dicarboxylate (**10**), 5-(((*tert*-butyldimethylsilyl)oxy) methyl)furan-2-carbaldehyde (**12**), and terephthalaldehyde (**13**). For most of the remaining compounds, some reports about toxicity exist for different target organisms. Although not mutagenic by the Ames test,[20] 2,5-dimethylfuran (**1**) was found to induce chromosomal damage in cultured murine cells[20] and sister chromatid exchanges in human lymphocytes *in vitro*.[21] Also, it is neurotoxic to *Drosophila melanogaster*, causing locomotory impairment, partly due to the production of reactive oxygen species.[22] However, no impact on gene expression was found when human lung alveolar epithelial cells were exposed to 2,5-dimethylfuran (**1**).[23] A moderate level of aquatic toxicity of this compound, together with bioaccumulation and persistence, had been predicted.[20]

Levulinic acid (the common name of 4-oxopentanoic acid or **15**) is an additive in cigarettes and, in this sense, its possible toxic effects were previously discussed[24] but were not studied until recently: it inhibited cell growth and ethanol production of *Saccharomyces cerevisiae*[25] and Lomba and collaborators analysed its toxicity using the Microtox test.[8] One of the most studied furans, in terms of toxicity, is 5-hydroxymethylfurfural (HMF) (**7**). When *in vitro*, it induced sister chromatid exchanges in human lymphocytes.[21] It may induce genotoxic and mutagenic effects in bacterial and human cells and promote colon and liver cancer in rats and mice.[26] Also, it is cytotoxic, irritating to the eyes, upper respiratory tract, skin and mucous membranes.[27] Curiously, several studies confirm the toxic effect of 5-hydroxymethyl-furfural (**7**) on bees[26] and many countries strictly limit its content in honey, beer and glucose injections.[28] Despite the controversial results on its toxicity, it does not seem to pose a serious health risk.[28] In the Ames test, **7** was found to be not mutagenic or weakly mutagenic.[29] Moreover, in *in vivo* experiments with mice, a single oral dose of 100 mg kg^{-1} was efficacious in prolonging the survival time under severe hypoxic conditions.[30] 5-Methylfurfural (**5**) has been reported to directly interact with cellular DNA without metabolic activation in Chinese hamster ovary cells.[31,32] It is even considered a potential anti-tumour agent.[33] The production of ethyl levulinate, whose common name is ethyl-4-oxopentanoate (**16**), is expected to impact the environmental and human health, probably more than diesel production.[34] Recently, Lomba *et al.*[8] analysed its toxicity using the Microtox test. Furfural had also been found to directly interact with cellular DNA without metabolic activation in Chinese hamster ovary cells[32] and gave a positive result in the Ames test.[35] Furfuryl alcohol has cytotoxic potential[19] and it induced sister chromatid exchange in mouse bone marrow.[36] In 1997, Irwin *et al.* exposed mice to furfuryl alcohol also known as furan-2-yl-methanol (**2**) vapour and some toxicity was detected, especially for nasal passages.[37] More recently, it was reported to be highly toxic in laboratory animals, with evidence of carcinogenic activity in rats. Additionally, exposure may have an impact on immunologic responses.[38] It is also associated with kidney neoplasm and adenoma, lung neoplasm, liver injury and cirrhosis.[20] With such toxicity studies in the past, the furans' family of compounds must be extensively studied before its use can be generalized, as has been recently suggested.[39]

Concerning simpler life forms, the toxicity of furans is expressed in fermenting organisms by slower growth, slower ethanol production and inhibition of certain enzymes.[40] Besides levulinic acid (**15**),[25] furfural (**4**) and 5-(hydroxymethyl) furan-2-carbaldehyde (HMF) (**7**) are toxic to different fermenting organisms, including *Saccharomyces cerevisiae*,[29,40] with furfural causing a more severe phenotype.[29,41] Reduction to the less toxic alcohols, like furfural reduction to furfuryl alcohol, reduces toxicity; this is achieved by several enzymes, namely reductases that have been studied in yeast and bacterial cells.[40] Despite being considered "green",[42] most of these compounds present some toxicity to most organisms studied. Given the contradictory results and generalized paucity of toxicological data, the ecotoxicity of furans deserves further investigation.[42]

To the best of our knowledge, this is the first systematic ecotoxicity study on different compounds of the furans' family and their derivatives, and the first record ever on the toxicity of some of its members. As a first approach, a comparative study on the toxicity of different compounds towards the marine bacterium *Vibrio fischeri* was carried out. For comparison purposes, the study was extended to some non-furan compounds



Scheme 1 Furanic compounds (**1–12**), levulinic acid (**15**) and ethyl levulinate (**16**) used in this study with potential interest as biomass derived platform chemicals.

such as terephthalaldehyde (**13**), levulinic acid (**15**), ethyl levulinate (**16**), hexane-1,6-diol (**17**), *tert*-butylmethyl ether (**18**) and terephthalic acid (**18**) because those compounds are already used for different purposes. The set of compounds tested in this work comprises 12 furanic compounds and two others derived or synthesised from carbohydrates namely levulinic acid (**4**) and ethyl levulinate (**16**) (Scheme 1). The possible mechanisms of toxicity and, above all, the seemingly "green" character of these lesser-known compounds will be discussed. Perspectives for future work on the ecotoxicity of furans will also be addressed.

Experimental

Test chemicals

The compounds used, whose molecular structures are provided in Table 1, were 2,5-dimethylfuran (**1**), furan-2-yl-methanol or furfuryl alcohol (**2**), furan-2,5-diyldimethanol or DHMF (**3**), furan-2-carbaldehyde or furfural (**4**), 5-methylfuran-2-carbaldehyde or 5-methylfurfural (**5**), furan-2,5-dicarbaldehyde (**6**), 5-(hydroxymethyl)furan-2-carbaldehyde (**7**), 2-furoic acid (**8**), furan-2,5-dicarboxylic acid or FDCA (**9**), diethyl-furan-2,5-dicarboxylate (**10**), sodium 5-(hydroxymethyl)furan-2-carboxylate or HMFA (**11**), 5-(((*tert*-butyldimethylsilyl)oxy)methyl) furan-2-carbaldehyde (**12**), terephthalaldehyde (**13**), terephthalic acid (**14**), 4-oxopentanoic acid or levulinic acid (**15**), ethyl-4-oxopentanoate or ethyl levulinate (**16**), hexane-1,6-diol (**17**), and *tert*-butylmethyl ether or MTBE (**18**).

Terephthalaldehyde (**13**), 2,5-dimethylfuran (**1**), 5-methylfuran-2-carbaldehyde or 5-methylfurfural (**5**), 4-oxopentanoic acid or levulinic acid (**15**), ethyl-4-oxopentanoate or ethyl levulinate (**16**), 2-furoic acid (**8**), hexane-1,6-diol (**17**), *tert*-butylmethyl ether or MTBE (**18**), and terephthalic acid (**14**) were obtained from commercial sources and used without further purification. Furan-2-yl-methanol or furfuryl alcohol (**2**) and furan-2-carbaldehyde or furfural (**4**) were obtained from Aldrich and distilled. 5-(((*tert*-Butyldimethylsilyl)oxy)methyl) furan-2-carbaldehyde (**12**); furan-2,5-dicarboxylic acid or FDCA (**9**); furan-2,5-dicarbaldehyde (**6**); 5-(hydroxymethyl)furan-2-carbaldehyde (HMF) (**7**); furan-2,5-diyldimethanol or DHMF (**3**); and sodium 5-(hydroxymethyl)furan-2-carboxylate or HMFA (**11**) were prepared following the same procedures recently reported.[43] Diethyl-furan-2,5-dicarboxylate (**10**) was prepared by esterification of furan-2,5-dicarboxylic acid (**9**) in ethanol following a procedure reported.[44] For specific details of commercial sources, preparation, purification and purity see the ESI (Table S1†). These samples were characterized according to the water content, determined by Karl Fischer titration using a Metrohm 831 KF coulometric titrator. Some exceptions were dried with a nitrogen flow[6] due to practical impossibilities to inject the samples in the Karl Fischer equipment (these samples were considered pure, meaning without any measurable water content). Then, the mass concentration of each compound (**1–18**) was corrected for the water content of the bulk compound used to prepare the stock solution, thus guaranteeing the accuracy of the estimated EC_{50} values.

Microtox assay

The standard Microtox toxicity test[45,46] liquid-phase assay was used to evaluate the inhibition of the marine bacteria *Vibrio fischeri* bioluminescence following the exposure to each compound at 15 °C. Briefly, the test was performed by measuring the bacteria luminescence variation when exposed to nine different concentrations of each tested compound, with successive dilutions by a factor of 2 (from 0.32 to 81.9 percent), in which 100 percent of the compound corresponds to a known concentration (this concentration varies with the compound tested) of a stock solution, in which 0 percent corresponds to the control (without each compound **1–18** present).

After 5, 15, and 30 minutes of exposure to the compound, the light output of the luminescent bacteria was measured and compared with the light output of a blank control. The toxicity was evaluated, a 50 percent reduction in luminescence, corresponding to the EC_{50}, and the respective 95% confidence intervals were computed using the Microtox Omni™ Software version 4.3.0.1.[47] Additional statistical analysis, namely the respective 95% confidence intervals, which were estimated for each compound by non-linear regression, using the least-squares method to fit the data to the logistic equation, was performed by the STATISTICA software, version 8.0.

Results and discussion

The set of compounds tested in this work comprises 12 furanic compounds and six others derived or synthesised from furanic-based chemical structures. These are identified and ordered considering their chemical structure represented in Table 1, in accordance with the increase of their oxidation character. The toxicity results for 5, 15 and 30 minutes of exposure are presented in Table 2. The analysis of these data will be carried out considering the EC_{50} values after 30 minutes of exposure as the standard measurement of toxicity, except otherwise stated. The toxicity experimental data defined and further analysed in this work is defined in mg L^{-1}

Table 1 Chemical representation, code name, and molar mass (g mol^{-1}) of the compounds studied in this work

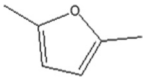
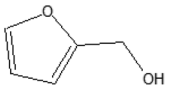
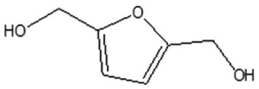
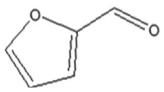
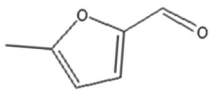
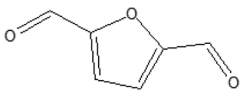
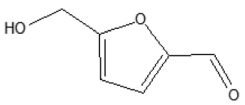
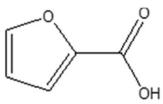
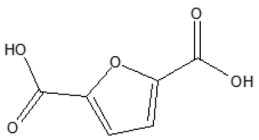
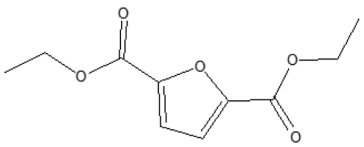
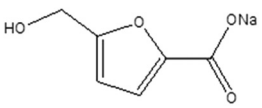
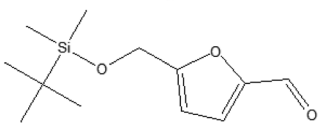
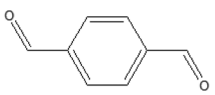
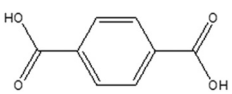
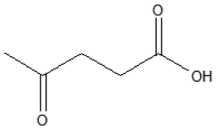
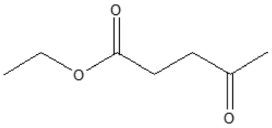
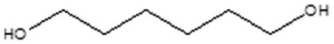
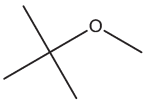
Chemical compound (common name)	Code name	Chemical structure	M (g mol^{-1})
2,5-Dimethylfuran	**1**		96
Furan-2-yl-methanol (furfuryl alcohol)	**2**		98
Furan-2,5-diyldimethanol (DHMF)	**3**		128
Furan-2-carbaldehyde (furfural)	**4**		96
5-Methylfuran-2-carbaldehyde (5-methylfurfural)	**5**		110
Furan-2,5-dicarbaldehyde	**6**		124
5-(Hydroxy-methyl)furan-2-carbaldehyde (5-hydroxymethyl-furfural)	**7**		126
2-Furoic acid	**8**		112
Furan-2,5-di-carboxylic acid (dehydromucic acid, FDCA)	**9**		156
Diethyl furan-2,5-dicarboxylate	**10**		212
Sodium 5-(hydroxymethyl)furan-2-carboxylate (HMFA)	**11**		164
5-(((*tert*-Butyl-dimethylsilyl)-oxy)methyl)-furan-2-carbaldehyde	**12**		240
Terephtaldehyde	**13**		134
Terephthalic acid	**14**		166
4-Oxopentanoic acid (levulinic acid)	**15**		116

Table 1 *(Contd.)*

Chemical compound (common name)	Code name	Chemical structure	M (g mol^{-1})
Ethyl 4-oxopentanoate (ethyl levulinate)	**16**		144
Hexane-1,6-diol	**17**		118
tert-Butylmethyl ether (MTBE)	**18**		88

units. However, the same parameter is also presented in units of mM (Table S2 in ESI†). Nevertheless, weight per volume has been adopted as a standard unit in the field of biology, which is followed by different authors for distinct solvents/toxicants.[48–50] Moreover, the mass units are analysed in this work to make the toxicological classification of these compounds easier considering the categories adopted by the European Commission,[51] which follow the same mass units. Meanwhile, the table with EC data in mM units is also presented in this work in the ESI (Table S2†).

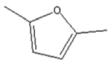
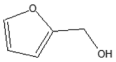
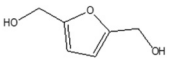
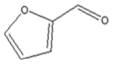
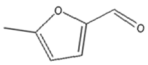
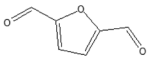
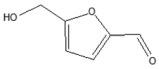
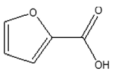
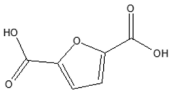
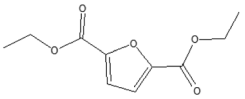
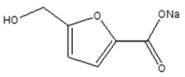
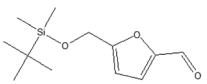
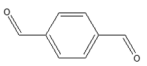
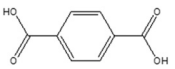
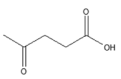
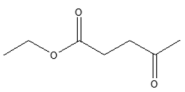
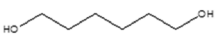
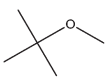
The exposure time did not have a significant influence on the toxicity, the exceptions being: (i) furan-2,5-dicarbaldehyde (**6**) and furfural (**4**); in both cases, the toxicity nearly doubled within 5 to 30 minutes of exposure and (ii) ethyl levulinate (**16**) in which the toxicity (mg L^{-1}) diminished to about 2 and 3 times within 5 to 15 and 5 to 30 minutes of exposure, respectively. The same trend is verified for the toxicity data analysed in terms of mM units. In most cases (9 out of 18), the toxicity [Tables 2 (mg L^{-1}) and S2† (mM)] increased with the exposure time, namely 5-methylfurfural; furan-2,5-dicarbaldehyde; 5-hydroxymethyl-furfural; diethyl furan-2,5-dicarboxylate; 5-(((*tert*-butyl-dimethylsilyl)-oxy)methyl)-furan-2-carbaldehyde; levulinic acid; ethyl levulinate; hexane-1,6-diol; *tert*-butylmethyl ether (**5–7**, **10**, **12**, **15–18**), which may indicate the presence of different mechanisms of toxicity to the bacteria in the studied compounds. To facilitate the interpretation and analysis of the various set of results, considering the main characteristics of the compounds under study, the results of toxicity for 30 minutes of exposure time are presented in Fig. 1 (mg L^{-1}). The compounds were depicted in Fig. 1, considering their decreasing order of toxicity: **12** > **13** > **9** > **8** > **18** > **6** > **1** > **15** > **10** > **2** > **5** > **4** > **3** > **7** > **16** > **11** > **17** > **14** (without toxicity). Fig. S1† shows the same toxicity results in mM units for the same compounds studied in this work. In this graphical representation, the result of **14** is not depicted, due to the observed lack of toxicity of this compound until its maximum of solubility in water. Since this derivative is devoid of toxicity it will be no longer analysed. Meanwhile, almost the same tendency is verified in Fig. S1 (ESI†) in which the EC parameter for 30 minutes is described in mM units. In fact, some exceptions are evidenced, namely those describing the switch between the compounds furan-2-yl-methanol (**2**) and 5-methylfuran-2-carbaldehyde (**5**); furan-2,5-dicarbaldehyde (**6**) and *tert*-butylmethyl ether (**18**) and those describing the similarity between the toxicity results in mM found for the compounds sodium 5-(hydroxymethyl)furan-2-carboxylate (**11**) and hexane-1,6-diol (**17**). Also for the common solvents some exchanges were obtained, namely for acetic acid and acetaldehyde.

Only the recent work of Lomba *et al.*[8] and Roslev *et al.*[18] reported the toxicity of three of the compounds herein studied (levulinc acid – **15** and ethyl levulinate – **16**)[8] and *tert*-butylmethyl ether (**18**)[18] under the same time conditions (30 minutes) and for the same microorganism. According to their results a moderate level of toxicity would be expected for these compounds. This was only verified in our results for levulinic acid (**15**). Fig. 1 shows not only the tendency of toxicity by comparing the results of all derivatives, but also their classification in terms of toxicity.

The compounds represented with the red bars are considered as moderately toxic, those represented in orange are practically harmless and the compound depicted in green should be considered as non-toxic or harmless.[52] However, the interpretation of the results presented in Fig. 1 in mass units (the numerical data is reported in Table 2) and in Fig. S1† in mM units (numerical data found in Table S2†) is not straightforward. This analysis hereafter presented does not seem to suggest any clear tendency with the chemical features studied, *i.e.* the effect of the functional (carbonyl, hydroxyl, carboxyl, ether and ester) groups of each compound on its toxicity. However, looking more carefully for this set of compounds, five chemicals draw attention due to their structural similarity regarding the presence of at least one CH_2OH functional group. These are furan-2-yl-methanol (**2**), furan-2,5-diyldimethanol (**3**), 5-(hydroxy-methyl)furan-2-carbaldehyde (**7**), sodium 5-(hydroxymethyl)furan-2-carboxylate (**11**) and hexane-1,6-diol (**17**). These are all considered as "practically harmless" or even "harmless", which means that the alcohol functional group is less toxic. Comparing compounds (**6**) and (**7**) it is possible to conclude that the replacement of an aldehyde by one hydroxyl group decreases significantly the toxicity of these chemicals.

In an attempt to better understand what may be the principal driven force(s) behind the results assessed in this work,

Table 2 Median effective concentration (EC_{50}) values, in mg L^{-1}, and respective confidence intervals (c. i.) at 95%, obtained with *Vibrio fischeri* (Microtox) after 5, 15 and 30 minutes of exposure to different furans and derivatives

Code name	Chemical structure	EC_{50} at 5 min (mg L^{-1}) (95% c. i.)	EC_{50} at 15 min (mg L^{-1}) (95% c. i.)	EC_{50} at 30 min (mg L^{-1}) (95% c. i.)
1		33.5 (18.8–48.1)	24.3 (14.8–33.8)	23.4 (16.6–30.2)
2		167 (110–225)	131 (92.2–169)	101 (75.7–127)
3		314 (261–368)	306 (266–345)	290 (247–333)
4		339 (255–424)	255 (188–321)	188 (142–235)
5		85.6 (73.8–97.5)	94.7 (83.4–106)	107 (94.1–120)
6		39.2 (26.7–51.7)	21.3 (15.1–27.6)	22.8 (12.5–33.1)
7		407 (334–480)	385 (321–448)	389 (285–492)
8		15.6 (14.8–16.5)	15.4 (14.3–16.4)	14.9 (14.2–15.6)
9		10.4 (9.30–11.5)	9.66 (8.53–10.8)	9.57 (8.31–10.8)
10		77.2 (60.4–94.0)	85.4 (67.2–104)	92.7 (78.8–107)
11		1331 (1020–1642)	1086 (821–1350)	983 (760–1206)
12		3.64 (3.35–3.94)	4.10 (3.96–4.24)	5.44 (4.89–5.99)
13		8.41 (6.73–10.1)	7.67 (6.80–8.54)	6.66 (5.99–7.32)
14		n.d. Not toxic	n.d. Not toxic	n.d. Not toxic
15		26.6 (25.5–27.7)	27.8 (26.5–29.0)	28.4 (27.2–29.5)
16		253 (206–300)	471 (312–629)	694 (416–972)
17		900 (635–1165)	1103 (733–1472)	1188 (711–1666)
18		20.6 (14.7–26.4)	20.8 (13.1–28.4)	21.1 (9.8–32.3)

the 1-octanol–water partition coefficients (K_{ow}) for all the compounds were estimated using ChemSpider (accessed on February 25th, 2016), with the exception of 5-(((*tert*-butyldimethylsilyl)-oxy)methyl)-furan-2-carbaldehyde (**12**), due to the unavailability of K_{ow} value and **14** (since this compound is devoid of toxicity). This parameter is useful for the environmental risk assessment of chemicals, since the partition coefficients of 1-octanol–water systems display similarities to the partition of compounds between water and the biological membranes of common microorganisms. 1-Octanol has an amphiphilic nature, similar to a generalized lipid phase in terms of their dielectric properties.[53] Correlations between the

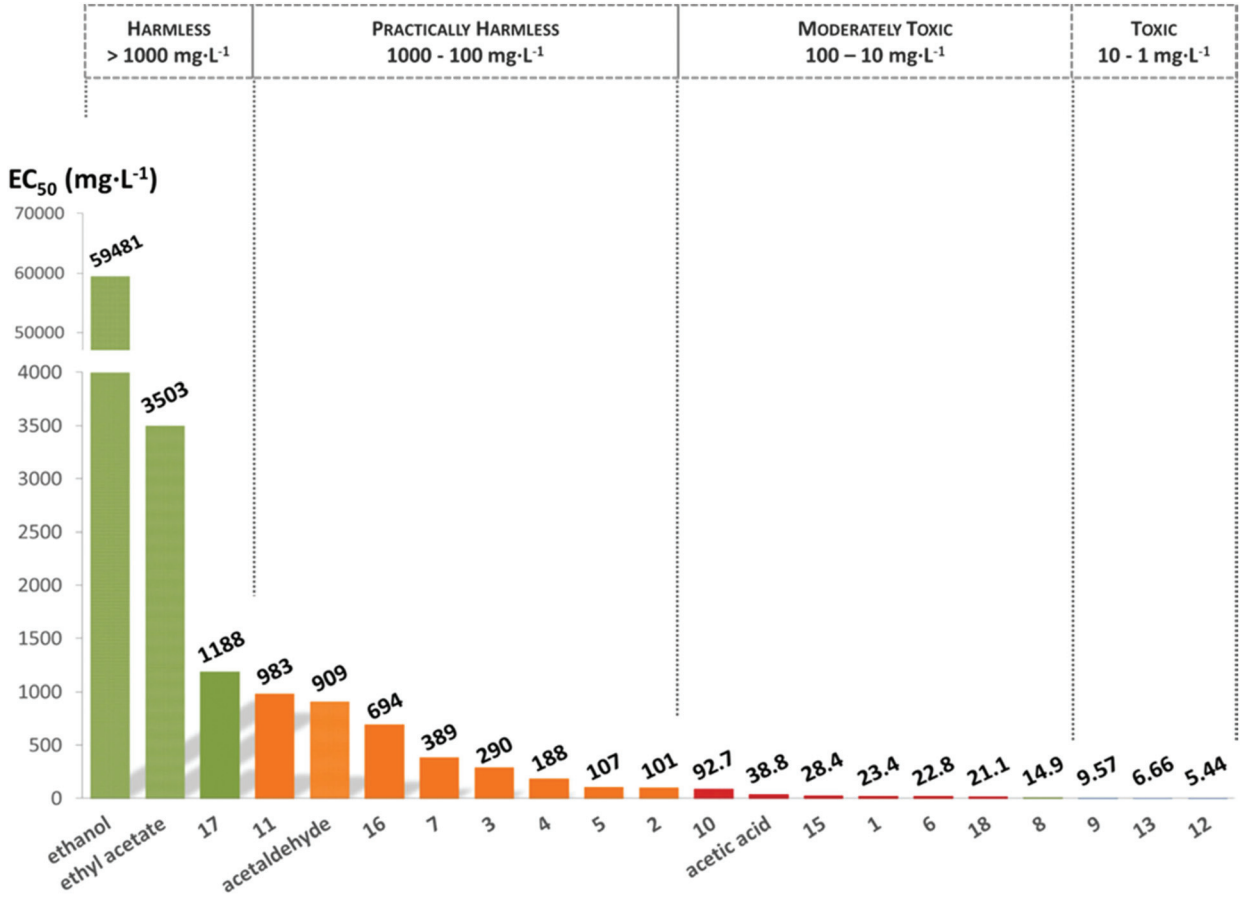


Fig. 1 Values of the median effective concentration (EC_{50}), in mg L^{-1}, obtained after 30 minutes of exposure of each compound tested in this work and the marine bacteria *Vibrio fischeri*, ordered by the level of toxicity: green bars represent the non-toxic/harmless compounds; orange bars represent the practically harmless compounds; and red bars represent the moderately toxic chemicals. The results for some organic solvents are also represented considering 15 minutes of exposure time.[60]

toxicity results and K_{ow} values have been successfully obtained for diverse classes of compounds/solvents,[14,54,55] due to the 1-octanol ability to mimic the behaviour of a lipid phase. The K_{ow} values indicate the higher or lower affinity of a solute for 1-octanol (more hydrophobic phase, representative of the fatty tissue on organisms) or water (directly related to the hydrophilicity of the solute). The results for the entire set of compounds analysed are shown in Fig. 2, except for the compound sodium 5-(hydroxymethyl)furan-2-carboxylate (**11**), due to the absence of its K_{ow} value. It seems that there is a general correlation between the EC_{50} data (in mg L^{-1}) measured and the estimated K_{ow} values. The results suggest that there is, in general, an increase of the toxicity with the compound hydrophobicity. The same tendency is verified when the results of the K_{ow} values are analysed against the toxicity experimental data in mM units, as depicted in Fig. S2.†

In other words, the higher the K_{ow} values the higher is the affinity of the target chemicals for the phospholipidic membrane of microorganisms represented by 1-octanol. However, the results depicted in Fig. 2 also show that the hydrophobic/hydrophilic nature of these compounds is not enough to explain all the results, since the correlation between the K_{ow} and EC_{50} values is not observed for all the compounds studied, in particular for furfuryl alcohol (**2**), furan-2,5-dicarbaldehyde (**6**), 2-furoic acid (**8**), furan-2,5-di-carboxylic acid (**9**) and 4-oxopentanoic acid (**15**).

These exceptions are based on three acids (**8**, **9** and **15**), one alcohol (**2**) and one aldehyde (**6**), as highlighted in Fig. 2. The exceptional behaviour of the acids may be assigned to their low pH, which increases their toxicity towards the microorganisms. This hypothesis was proved to be correct for compounds **8** and **15**, for which the toxicity towards the marine bacteria was re-measured at a controlled pH of around 7. Their final results after neutralization are EC_{50} [for **8** EC_{50} (mg L^{-1}) = 515.9 (377.8; 704.6) or EC_{50} (mM) = 4.61 (3.37; 6.29) and for **15** EC_{50} (mg L^{-1}) = 2592 (2078; 3234) or EC_{50} (mM) = 22.35 (17.92; 27.88)].

For **9**, its toxicity after neutralization was not possible to be measured due to its low solubility in water at this pH. The only exceptions for which we do not have an explanation are those for the alcohol and the aldehyde.

The results for 5-(((*tert*-butyl-dimethylsilyl)-oxy)methyl)-furan-2-carbaldehyde (**12**), terephthalaldehyde (**13**), furan-2,5-

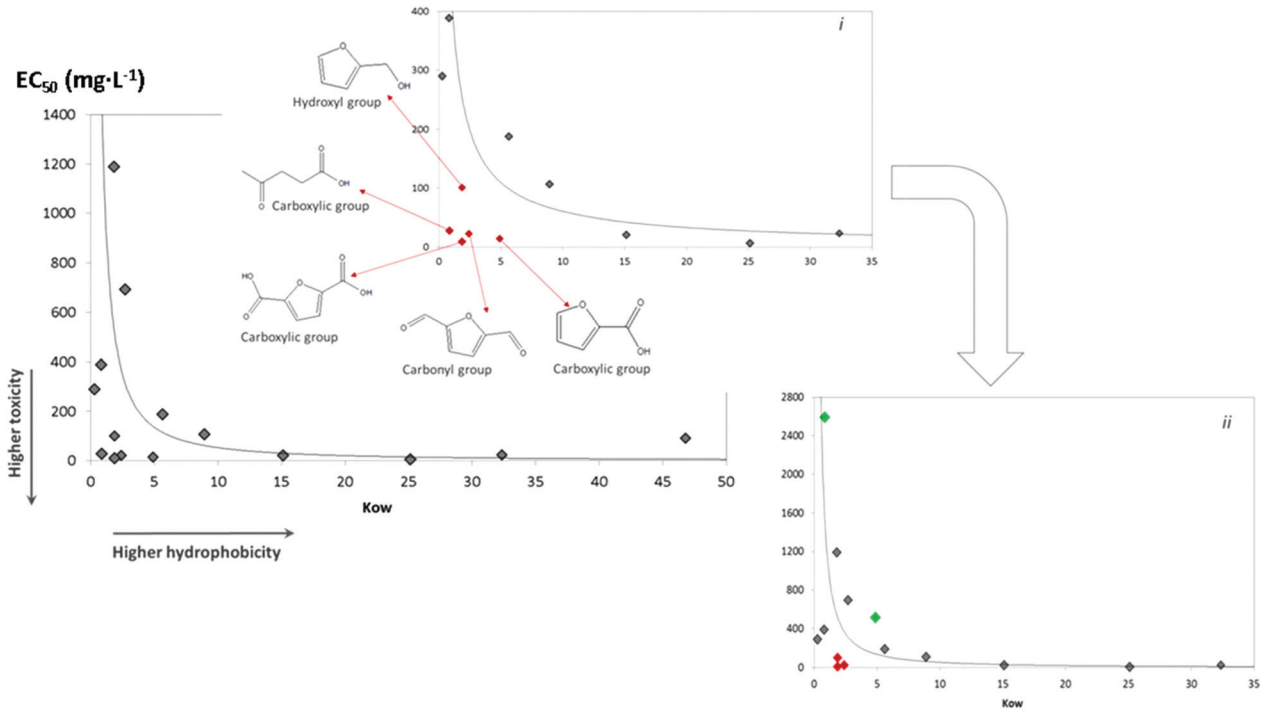


Fig. 2 Correlation between the values of the median effective concentration (EC_{50}), in mg L^{-1}, obtained after 30 minutes of exposure between the compounds under study and the marine bacteria *Vibrio fischeri* and the octanol–water partition coefficient (K_{ow}). The insets are representing the compounds with an exceptional behavior to the relation (red diamonds) and the respective chemical structures identified: before neutralization (i) and after neutralization (ii). The reader should note that the green diamonds are representing the compounds with exceptional behavior which after neutralization are following the tendency between the toxicity and the octanol–water partition coefficient.

dicarboxylic acid (**9**), furan-2,5-dicarbaldehyde (**6**), diethyl furan-2,5-dicarboxylate (**10**), 2-furoic acid (**8**), hexane-1,6-diol (**17**), furan-2,5-diylmethanol (**3**), and sodium 5-(hydroxymethyl)furan-2-carboxylate (**11**) are the first records ever on the ecotoxicity of these compounds. Furfural (**4**) and terephthalic acid (**14**) toxicity had already been studied but never using the Microtox test. Something similar was performed by McDonald *et al.*;[16] vacuum kiln condensate, including furfural in its composition, had a 3.3% Microtox toxicity after 15 minutes of exposure (no additional details were presented).[16] In the same work, there are references to the EC_{50} values, 11 and 32 mg L^{-1}, for a 14 days' test with guppies and a 96 hours' test with fat head minnows, respectively.[16] Despite the different tests used, these values are much lower than our result (188 mg L^{-1}), suggesting different mechanisms of toxicity for organisms with different degrees of complexity, which was often observed for other chemicals.[56] Another work stated that furfural is more toxic than 5-hydroxymethyl-furfural (HMF) (**7**) for yeast,[57,58] something also supported from the presented results [389 mg L^{-1} for 5-hydroxymethyl-furfural (**7**)]. 5-Methylfurfural (**5**) toxicity had been studied in Chinese hamster ovary cells and it was found to directly interact with cellular DNA,[32] an effect that increases with its concentration and the time of reaction (in our work, toxicity decreases with the time of exposure). Additionally, it is considered a potential anti-tumour agent.[33] To our knowledge, 5-methylfurfural was studied here for the first time with a non-animal model organism. 5-Hydroxymethyl-furfural (**7**) was found to be toxic to yeast[40,41,57] and genotoxic and mutagenic to bacterial, murine and human cells.[59] It was never studied with the Microtox test before. The 2,5-dimethylfuran (**1**) is five times more toxic than furfuryl alcohol, which means that it is a potent cytotoxic agent as found for cultured rat Schwann cells.[22] Only two previous studies used the Microtox assays for three of the compounds herein studied: levulinic acid (**15**) and ethyl levulinate (**16**),[8] and *tert*-butylmethyl ether (**18**).[18]

The EC_{50} values obtained after 30 minutes of exposure, were 5687 ± 1325 mg L^{-1}, 182 ± 5 mg L^{-1} and 10.9 (9.57; 13.24) mg L^{-1}, respectively.[8,18] Our results are comparable for ethyl levulinate [694 (416; 972) mg L^{-1}] and *tert*-butylmethyl ether [21.1 (9.8; 32.3) mg L^{-1}], and for levulinic acid tested after neutralization [2593 (2078; 3234) mg L^{-1}], a much higher value than the results obtained for it without neutralization [28.4 (27.2; 29.5) mg L^{-1}]. Accordingly, they stated that ethyl levulinate was more toxic than levulinic acid,[8] which is contrary to our findings in this work.

Comparing the furan toxicities with some organic solvents, using EC_{50} values from the Microtox tests, it is observed that some of the furans tested in this work are more toxic than ethanol and ethyl acetate, for example. Some examples of the toxicity of classic organic solvents are also presented in Fig. 1, those also being described for the bacteria *Vibrio fischeri* considering 15 minutes of exposure time.[60] Meanwhile, these results are comparable to ours, since the authors state that no

significant differences on the results were observed for different times of exposure, namely the 30 minutes of contact.

In spite of all these compounds being furans or closely related chemicals, their EC_{50} values are quite dissimilar. The difference can be of more than 3 orders of magnitude, from 5.44 to 1188 mg L^{-1}. This means that these compounds can range from toxic (1 to 10 mg L^{-1}) to moderately toxic (10 to 100 mg L^{-1}), practically harmless (100 to 1000 mg L^{-1}) and harmless (higher than 1000 mg L^{-1}), according to the hazard ranking adapted from Passino & Smith.[52]

All things considered, it seems that the hydrophobicity/hydrophilicity of the compounds is the main driven force for the general toxicity of these building block chemicals towards *Vibrio fischeri*, with a few exceptions. To gain a deep understanding of even more specific effects, including the mechanisms of toxicity acting, more studies are necessary, with other test organisms and at different levels of the food chain. For example, if these compounds are internalized by bacteria, they can be bio-augmented throughout the food chain, a risk to be taken into consideration. Regarding the environmental relevance of these results, and having in mind that this is just the first approach to this yet poorly studied group of chemicals in what concerns their ecotoxicity, it seems that much care is necessary before considering these compounds as "green" or "benign" building block compounds, since some of them exhibit a considerable level of toxicity to *Vibrio fischeri* and can be more toxic than classic organic solvents. Before their extensive industrial use, and before reaching the market, if ever, new tests are necessary and probably, new methods to improve their efficiency and simultaneously decrease their toxicity.

Although these biomass-derived compounds might be an alternative to classic fuels and solvents, based on the ecotoxicology data here reported their environmentally friendly character seems to be questionable.

Conclusions

The obtained results suggest that it might not be correct to consider furans and derivatives as devoid of toxicity and thus their "green solvent" status derived from their biomass origin is questionable. There is a general tendency for many of these compounds to show a higher toxicity level than classical petrochemical solvents. Very different levels of toxicity are found among closely related compounds, which may indicate different mechanisms of toxicity but in any case, with two exceptions, the main driving force for the toxicity seems to be the compounds' hydrophobicity, and thus their penetration into the cell. All things considered, furans and their derivatives must be cautiously adopted as alternatives to classic petrochemical solvents and more ecotoxicological tests are necessary to understand the effects of these compounds on the environment. Moreover, the evaluation of their sustainability cannot be based solely on their production from renewable resources but must be completed by biodegradability studies and a life cycle analysis for these compounds.

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