

# Environmental and Health Impact Assessment of Liquid Organic Hydrogen Carrier (LOHC) systems - Challenges and Preliminary Results

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## Materials and methods

### Liquid Organic Hydrogen Carriers

The aromatic forms of LOHC: MSH, Quin-2Me, NEC were purchased from Hydrogenious Technologies GmbH, Erlangen ([www.hydrogenious.net](http://www.hydrogenious.net)). The partially hydrogenated and fully hydrogenated forms, that is H<sub>18</sub>-MSH, H<sub>x</sub>-NEC, Quin-2Me-pH and Quin-2Me-H<sub>10</sub> were prepared by catalytic hydrogenation reaction using literature proceedings.<sup>25</sup>

### Acute immobilization assay with *Daphnia magna*

The 48-h acute immobilization test with the crustacean *Daphnia magna* was performed using the commercially available Daphtoxkit F (MicroBioTest Incorporation, Gent, Belgium) in accordance to ISO standard (ISO 6341). The *Daphnia* neonates were hatched from dormant ephippia at 20 °C under constant illumination. For each replicate 5 pre-fed animals, less than 24-h old, were placed in 10 mL of mineral medium (controls) or solution of test substances in mineral medium. The number of immobilized or dead organisms was checked after 24 and 48 h. The relative toxicity of the samples was expressed as percentage of not affected organisms compared to the controls. All substances were tested in three independent experiments (five concentrations, five replicates).

### Solubility in media

For the media/water solubility assessment, a so called generator column method was used according to OECD 105.<sup>1</sup> Shortly, the chemical of interest was dissolved in hexane and deposited on glass beads by evaporating the solvent. Beads were placed in generator column and medium or water was pumped through for at least 8 hours in constant temperature (20 °C). The solution was then extracted with hexane using phenanthrene as a surrogate standard. Concentration of target compound/surrogate was measured using GC/FID (HP 6890 series) with split-less injection of 1 µL. Column used was FS-supreme-5ms (length=30m, id=0.25mm, film thickness 0.5 µm) purchased from CS Chromatographie Service, Langerwehe, Germany. GC method parameters: inlet temperature 250 °C, oven program 40 °C hold 0.6 min, ramp 20 °C min<sup>-1</sup> to 280 °C, ramp 35 °C min<sup>-1</sup> to 320 °C hold 1 min; detector temperature 320 °C; column pressure 2 bar, column flow 3.7 mL min<sup>-1</sup>.

The WST-1 medium is used for assessment of cytotoxicity towards promyelocytic leukemia rat cell line IPC-81. It contains RPMI medium (with L-glutamine, without NaHCO<sub>3</sub>, supplemented with 1% penicillin–streptomycin and 1% glutamine, pH 7) with 10% horse serum. Here 1% (v/v) of DMSO was added as a co-solvent.

The *Lemna minor* medium is a Steinberg medium containing: 3.46 mM KNO<sub>3</sub>, 1.25 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.66 mM KH<sub>2</sub>PO<sub>4</sub>, 0.072 mM K<sub>2</sub>HPO<sub>4</sub>, 0.41 mM MgSO<sub>4</sub>, 1.94 µM H<sub>3</sub>BO<sub>3</sub>, 0.63 µM ZnSO<sub>4</sub>, 0.18 µM Na<sub>2</sub>MoO<sub>4</sub>, 0.91 µM MnCl<sub>2</sub>, 2.81 µM FeCl<sub>3</sub>, 4.03 µM EDTA; pH 5.5 ± 0.2. Test is performed in plastic six-well plates incubated in climate chamber with controlled temperature, humidity and light intensity and lasts 7 days. For solubility in *Lemna minor* medium a test solution of test compound at day 0 and after incubation for 7 days were extracted and measured as described above.

## GC/MS analysis of the isomeric mixture of dibenzyltoluenes

For the analysis of GC/MS spectrum of the isomeric mixture of dibenzyltoluenes, a HP series 6890N GC with HP 5973 MSD and a FS-supreme-5ms column (length=30m, id=0.25mm, film thickness 0.5  $\mu\text{m}$ ) from CS Chromatographie Service, Langerwehe, Germany were used. The GC method parameters were: inlet temperature 280 °C, split-less injection of 1  $\mu\text{L}$ , oven program: 100 °C hold 3 min, ramp 15 °C  $\text{min}^{-1}$  to 280 °C hold 5 min. The MSD was working in EI positive ion mode, using electron ionization energy of 70 eV. Spectrum was recorded in full scan mode. 50 mg  $\text{L}^{-1}$  solution of MSH in hexane was injected. The identity of target compound was confirmed by the presence of molecular ion  $m/z$  272.

## Ultimate biodegradation

Ultimate biodegradation was measured by manometric respirometry method according to OECD guideline 301F using automated OxiTop®, thermostatically controlled from WTW GmbH, Weilheim, Germany.<sup>2</sup> The activated sludge from the municipal wastewater treatment plant in Delmenhorst (Germany) was used as a source of inoculum. The flocs were allowed to settle and remaining supernatant was aerated for 5 days prior to use. Test lasted 28 days and was performed in standard OECD medium with nitrification inhibitor (allylthiourea). Target compounds were weighed directly to test vessels to yield BOD of 200 mg  $\text{O}_2 \text{L}^{-1}$ . Two replicates were run for each compound accompanied by two blanks and two positive controls (benzoic acid).

## Data analysis and image processing

Dose-response curve parameters and plots were obtained using drfit package (version 3.1.0) for R language and environment for statistical computing (<http://www.r-project.org>).<sup>3</sup> Marvin software was used for drawing, displaying and characterizing chemical structures, Marvin 6.3.1, 2014, available from ChemAxon (<http://www.chemaxon.com>).<sup>4</sup>

## Bibliography

1. OECD, *Guideline for testing of chemicals 105 - Water solubility*, 1995.
2. OECD, *Guideline for testing of chemicals 301 - Ready Biodegradability*, 1992.
3. R Core Team, 2014.
4. ChemAxon, 2014.