Universität Bremen*

Hazard Assessment of Liquid Organic Hydrogen Carriers (LOHCs) in Terrestrial Environment

– evaluation of ecotoxicological effects and adsorption behavior –

Doctoral Thesis

for the attainment of the academic degree of

Doktor der Naturwissenschaften

- Dr. rer. nat. -

Submitted to the Department of Biology and Chemistry

at the University of Bremen

08th November, 2017

presented by

Ya-Qi Zhang

Title: Hazard Assessment of Liquid Organic Hydrogen Carriers (LOHCs) in Terrestrial Environment

– evaluation of ecotoxicological effects and adsorption behavior

by

Ya-Qi Zhang

Thesis reviewers:

Dr. habil. Stefan Stolte

(Sustainable Chemistry, University of Bremen)

Prof. Dr. Juliane Filser

(General and Theoretical Ecology, University of Bremen)

Date and place of the colloquium:

08th December, 2017, at

Center for Environmental Research and Sustainable Technology (UFT), University of Bremen

Period of work: from December 2012 to December 2017.

at the Faculty 2 (FB2) Biology and Chemistry,

Centre for Environmental Research and Sustainable Technology (UFT),

Department Sustainable Chemistry,

University of Bremen, Germany.

Table of Content

I Table of Abbreviations and Symbols

Abbrev./Symb.	*Implication*
%	percent
°C	degree Celsius
w/w	% weight per weight
a	annum
A. globiformis	*Arthrobacter globiformis*
bpt	boiling point
BT	benzyltoluene
CEC	cation exchange capacity
CGH2	compressed gaseous hydrogen
COSMO	Conductor Like Screening Model for Realistic Solvation
d	days
D. magna	*Daphnia magna*
DBT	dibenzyltoluene
DCM	dichloromethane
DIN	Deutsches Institut für Normung (German Institute for Standardization)
DMSA	dimercaptosuccinate
DOE	Department of Energy, US
dw	dry weight
e.g.	exempli gratia
EC_{10}	concentration for 10% of maximal effect
EC_{50}	concentration for 50% of maximal effect
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EHA	environmental hazard assessment
EJ	exajoule
EPA	Environmental Protection Agency
EPI	Estimation Programs Interface Suite

(Continued)

Abbrev./Symb.	***Implication***
et al.	et and alii
etc.	et cetera
F. candida	*Folsomia candida*
FCEVs	fuel cell electric vehicles
Fe	iron
GC/MS	gas chromatography-mass spectrometry
GHG	greenhouse gases
GHS	Globally Harmonized System
h	hours
hetero-PAHs	heterocyclic polycyclic aromatic hydrocarbons
HPLC	high performance liquid chromatography
i.e	id est
IONP	iron oxide nanoparticles
ISO	International Organization for Standardization
JRC	Joint Research Centre
K_d	soil-water partition coefficient
kg	kilogram
K_{oc}	organic carbon-water partition coefficient
K_{ow}	octanol-water partition coefficient
KWh	kilowatt hour
L	liters
L. minor	*Lemna minor*
LC_{10}	concentration causes the death of 10% of a group of test animals
LC_{50}	concentration causes the death of 50% (one half) of a group of test animals
Log D	ionization corrected octanol-water partition coefficient
LOHC	liquid organic hydrogen carriers
mL	milliliters

Abbrev./Symb.	*Implication*
mM	millimolar
MW	molecular weight
NEC	N-ethylcarbazole
N-PAHs	nitrogen-substituted polycyclic aromatic hydrocarbons
OC	organic carbon
OECD	Organization for Economic Co-operation and Development
PAHs	polycyclic aromatic hydrocarbons
PBT	persistent, bioaccumulative and toxic
PECs	predicted environmental concentrations
pK_a	acid dissociation constant
PNECs	predicted no-effect concentrations
PHV	photovoltaic
PVC	polyvinylchlorid
QSAR	quantitative structure–activity relationship
R. subcapitata	*Raphidocelis subcapitata*
RCR	risk characterization ratio
RE	renewable energy
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SOM	soil organic matter
S_w	water solubility
TWh	terawatt hours
V. fischeri	*Vibrio fischeri*
vPvBs	very persistent and very bioaccumulative
WHC	water holding capacity
wt%	mass fraction
µg	microgram
µm	micrometer

II List of Figures

III List of Tables

IV Abstract

Liquid Organic Hydrogen Carriers (LOHCs) are part of a novel energy system that can efficiently and relatively safely store and transport hydrogen, which is a clean, high energy density fuel. LOHCs have high hydrogen holding capacities and are superior to most current energy sources, such as fossil fuels, because they have the potential to reduce CO_2 emission and have advantages in operational and handling safety; they are also adaptable to renewable energy. Therefore, massive integration and circulation of hundreds of thousands of tons of LOHCs in the market are anticipated in the near future. However, LOHC chemicals will likely be released into the environment during the process of producing and circulating the large forecasted volumes of these compounds. In addition to the interest in developing LOHC systems to improve the technological performance, increasing attention has been focused on the behavior, fate and toxicity of LOHCs in the environment.

When LOHCs enter the environment, the behavior and subsequent toxic effects of LOHCs on organisms are critical metrics used to evaluate and predict the environmental hazards of LOHCs. However, limited data are available to perform such comprehensive predictions. To help fill this gap, this thesis was conducted to characterize the potential adsorption and mobility behavior of different LOHC candidates in soils. Specifically, the organic carbon-water partition coefficient (K_{oc}), soil-water partition coefficient (K_d) and leaching capacity of LOHCs were investigated via instrumental analyses and software predictions. These outcomes were correlated to the physicochemical properties of the compounds to determine their potential adsorption mechanisms. In general, the K_{oc} values were correlated to hydrophobicity in the following order: indoles < quinaldines < carbazole derivatives < benzyltoluenes < dibenzyltoluene. When ionizable LOHC structures were investigated, the K_d and leaching capacity (with quinaldines as examples) revealed adsorption governed by ionic interactions. In such a case, ionization corrected octanol-water partition coefficient (log D) was found a propriate indicator for the prediction of adsorption.

Ecotoxicity tests for acute and chronic toxicity were conducted in soil (quinaldines) and aquatic (quinaldines and carbazole derivatives) test scenarios. Data were compiled and integrated with the adsorption and mobility of LOHCs in soils to interpret the extent of the exposure, bioavailability and mode of toxic action. The toxicity of LOHCs appeared to be dominated by hydrophobicity. With the deduction of toxicity classification and predicted no-effect concentrations (PNECs) as well as the evaluations for the behavior and fate, a proactive assessment of the potential environmental hazards of these chemicals was ultimately conducted in the context of realistic environmental conditions and potential application quantities.

A comparative analysis showed that LOHCs presented fewer potential environmental hazards than traditional energy systems (e.g., gasoline, diesel, and oil) and analogous compound structures, such as typical N-PAHs (nitrogen-substituted

polycyclic aromatic hydrocarbon, which share a similar structure with certain LOHCs). Although LOHCs seem not appear to present the same hazards as these equivalent energy sources, further studies regarding several key considerations are recommended for a better understanding of the potential environmental hazards of LOHCs. The careful application of LOHCs under appropriate monitors is suggested because of the likelihood that these compounds will be used in large application volumes in the future.

V *Zusammenfassung*

Flüssige organische Wasserstoffträger (LOHCs) sind Teil eines neuartigen Energiesystems, das molekularen Wasserstoff, als sauberen Brennstoff mit hoher Energiedichte, effizient und verhältnismäßig sicher speichern und transportieren kann. LOHCs haben eine hohe Wasserstoffspeicherkapazität und sind den meisten gegenwärtigen Energiequellen, wie zum Beispiel fossilen Brennstoffen, überlegen, weil sie das Potenzial haben, den CO_2-Ausstoß zu reduzieren und Vorteile bei der Betriebs- und Handhabungssicherheit bieten; sie sind auch an erneuerbare Energien anpassbar. Vor diesem Hintergrund ist zu erwarten, dass LOHC in naher Zunkunft großtechnisch verwendet werden und in hohen Quantitäten Anwendung finden. Damit verbunden ist allerdings auch die erhöhte Wahrscheinlichkeit, dass LOHC über Produktion, Transport oder Anwendung in die Umwelt freigesetzt werden. Neben dem Interesse LOHC-Systeme für die technologische Anwendung zu optimieren, wird daher vermehrt gefordert den Fokus auch auf die Untersuchung des Umweltverhaltens zu legen.

Das Wissen zum Umweltverhalten und zur Ökotoxikologie von LOHC ist bis heute sehr begrenzt, wodurch eine verlässliche Abschätzung von Umweltrisiken, die mit der Verwendung von LOHC potenziell einhergehen, nicht umfänglich möglich ist. Daher wurde diese Arbeit mit dem Ziel durchgeführt, Wissenslücken in diesem Bereich aufzufüllen. Dazu wurden das Adsorption- und Transportverhalten im Boden über die Ermittlung von Adsorptionskoeffizienten (K_{oc}) und Verteilungskoeffizienten (K_d) für unterschiedliche LOHCs bestimmt. Diese Ergebnisse wurden in Beziehung zu den physikochemischen Eigenschaften der Verbindungen gesetzt, um die möglichen Adsorptionsmechanismen zu bestimmen. Überwiegend korrelierten die K_{oc}-Werte mit der Hydrophobizität der Substanzen in der folgenden Reihenfolge: Indol < Chinaldin < Carbazol-Verbindungen < Benzyltoluole < Dibenzyltoluol. Bei ionogenen LOHC Verbindungen offenbarten die K_d -Werte (z.B. bei den Chinaldin-Verbindungen), dass ionische Wechselwirkungen das Adsorptionsverhalten wesentlich bestimmte. In einem solchen Fall wurde der ionisationskorrigierte Oktanol-Wasser-Verteilungskoeffizient (log D) als geeigneter Indikator für die Vorhersage der Adsorption gefunden.

Ökotoxikologische Tests zur Untersuchung der akuten und chronischen Toxizität wurden im Boden (Chinaldin-Verbindungen) und in aquatischen Tests (Chinaldin- und Carbazol-Verbindungen) durchgeführt. Die Daten wurden zusammengestellt und mit den Adsorptions- und Transporteigenschaften im Boden verglichen, um die Exposition, Bioverfügbarkeit und die Art des toxischen Effekts abzuschätzen. Die Toxizität der LOHCs wurde vor allem durch die Hydrophobizität dieser Verbindungen bestimmt. Über die Daten zur Toxizität und zum Verbleib in der Umwelt wurde schlussendlich eine erste Abschätzung der Umweltgefährlichkeit dieser Chemikalien im Kontext von realistischen Umweltbedingungen und Umweltkonzentrationen durchgeführt.

Eine erste vergleichende Analyse deutet darauf, dass LOHCs weniger umweltgefährdend sind als traditionelle Energiesysteme (z.B. Benzin, Diesel oder Öl) und andere N-PAHs (Stickstoff-substituierte polyzyklische aromatische Kohlenwasserstoffe, strukturell ähnlich zu den LOHCs). Für eine abschließende Bewertung sollten allerdings weitere Studien durchgeführt werden, um ein besseres Verständnis zu deren Umweltgefährdungspotenzialen zu erhalten. Eine gewissenhafte Anwendung unter sorgfältiger Testung und gegebenenfalls Regulation der LOHCs durch geeignete Vorschriften ist empfohlen, weil in der Zukunft wachsende Anwendungsmengen zu erwarten sind.

VI Structure of the Thesis

This thesis consists of five main chapters (**Figure I**): (i) Introduction, (ii) Materials and Methodology, (iii) Publications and Manuscripts, (iv) Summarized Discussion, and (v) Conclusions and Perspectives.

The "Introduction" chapter (i) provides an overview of the current landscape for conventional energy, renewable energy, hydrogen technology, and liquid organic hydrogen carrier (LOHC) systems. Available data on these different systems and techniques are compared to highlight their benefits and disadvantages with regard to their technological performance, application status, balance between production and demand of energy, and economic and environmental impacts. Environmental concerns and hazards related to the utilization of LOHCs, particularly the behavior, fate, and toxic effects of LOHCs in the environment, are emphasized in accordance with international criteria for environmental management. The research gaps and objectives as well as the hypotheses of this study are provided at the end of this chapter.

In the second chapter, "Materials & Methodology" (ii), basic information on the soil and aquatic test organisms are provided with a focus on "what are they?" and "why are they appropriate as the model organisms for the ecotoxicological tests in this thesis?" The experimental methods and setups used in this study are subsequently described as illustrative supplements to those provided in the manuscripts/publications. These methods include adsorption batch equilibrium experiments, soil column leaching tests, extraction procedures, and soil ecotoxicological tests.

In the third chapter "Publications and Manuscripts" (iii), the results obtained from laboratory work targeting the study objectives are presented in the format of articles/manuscripts for publication. The format of the text, figures, and tables are adjusted to the layout of this thesis. These manuscripts are described as follows:

a) Subchapter 3.1: A comprehensive review of the evolution and development of LOHC systems, including their technical and economic merits and deficiencies. Preliminary data on the ecotoxicity and biodegradation of LOHCs are provided to highlight the necessity of more detailed studies on the environmental hazards of these chemicals.

b) Subchapter 3.2: LOHC behavior is characterized with respect to its adsorption, mobility and leaching capacity in soil via software supported estimations, experimental analysis, and modeling. The relationships among adsorption, mobility, and bioavailability of LOHCs are discussed through assessments of the environmental hazards of LOHCs.

c) Subchapter 3.3: Ecotoxicity investigations of the quinaldine-based LOHC system are performed on typical soil-dwelling organisms, with classification of the toxicity and extrapolations of the predicted no-effect concentrations (PNECs) for

a proactive environmental hazard assessment of the LOHCs.

d) Subchapter 3.4: Ecotoxicity of the quinaldine- and carbazole derivative-based LOHC systems is assessed in aquatic scenarios and through a test battery that involves typical aquatic organisms. Toxicity classifications and the environmental fate of LOHCs (regarding biodegradation and persistence) are provided.

In the "Summarized Discussion" (iv), the key findings in terms of the potential behavior and toxicity of LOHCs are discussed with consideration for the physicochemical properties of the chemicals and environmental factors. A preliminary environmental hazard assessment is conducted in the context of possible application volumes of LOHCs and realistic conditions in the environment. By comparing the environmental impacts between different LOHCs and between LOHCs and conventional energy systems, LOHC candidates that are most suitable for meeting green energy goals are presented.

In the last chapter, "Conclusions and Perspectives" (v), the outcomes of the work in this thesis are provided. Perspectives and suggestions for future studies that can help improve our understanding of the impacts of LOHC technology in the environment are presented.

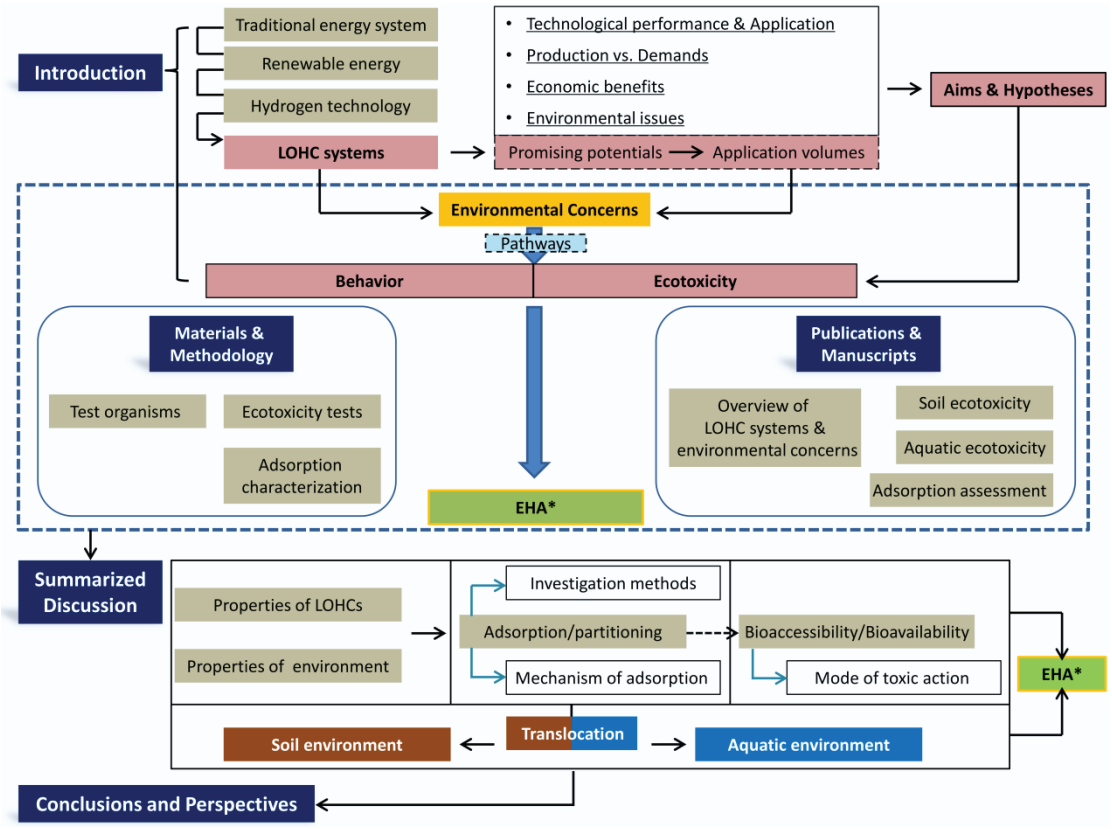


Figure I. Structure of the thesis showing the main chapters. *EHA: environmental hazard assessment.

Chapter-I Introduction

1. Introduction

1.1. Traditional energy supply system

Fossil fuels, including crude oil, coal and gas, have been the main sources for the global energy supply for many decades. Currently, approximately one billion vehicles are operating on roads worldwide (Eberle, Müller and von Helmolt, 2012), and they consume more than 95% of the fuel produced from fossil sources (Eberle, Müller and von Helmolt, 2012). Rapid economic development and population growth together with enhanced life styles, especially in recent decades, has resulted in increased energy demand. The total global energy demand was 548 EJ (1 exajoule = 10^{18} J) in 2016 (Jones and Warner, 2016). By 2050, the use of world energy is forecast to increase to 800 EJ or more (Moriarty and Honnery, 2012). In 2100, this level is expected to be as high as 1146 EJ (Jones and Warner, 2016). The consumption levels of oil, gas, and coal in 2015 were 36%, 27% and 23% of global energy, respectively (Abas, Kalair and Khan, 2015). It has been estimated by the World Energy Outlook that the consumption of oil and gas will increase from 36 million barrels per day in 2006 (IEA, 2007) to 81 million barrels per day by 2035 (IEA, 2010). The consumption of coal will rise by 73% from 2005 to 2030 (IEA, 2007).

The world is currently facing a conflict between continuously growing energy demand and gradually shrinking original resources of fossil fuels. Shafiee and Topal established a model to predict the time depletion of the reserve of fossil fuels by considering the influence of the rate of production or consumption (Shafiee and Topal, 2009). It turned out that the potential depletion time for crude oil, coal and gas would be in 35, 107 and 37 years, respectively (Shafiee and Topal, 2009). In addition, dramatic escalation of the consumption of fossil fuels is accompanied by enormous public attention to environmental pollutions (Moriarty and Honnery, 2012). Emission of greenhouse gases (GHG), which is closely associated with the utilization of fossil fuels in construction, electricity generation and automobile transport, leads to global warming and air pollution. The Intergovernmental Panel on Climate Change in 2013 predicted a total emission of 6,180 gigatons of CO_2 between 2012 and 2100; Covert et al. claimed that the total carbon emissions could even be as much as 12,744 to 17,407 gigatons after 2100 (Covert, Greenstone and Knittel, 2016). In parallel with the resource depletion and environmental pollution comes economic instability as a result of price fluctuation of fossil fuels[†]. Reserves of fossil fuels will shrink when prices are too low and expand when prices increase (Covert,

[†] The oil price was $ 50.64, $ 109 and $ 55.52 per barrel in 2005, 2012 and 2017 (19th Oct., 2017), respectively. OPEC: http://www.opec.org/opec_web/en/data_graphs/40.htm.

Greenstone and Knittel, 2016).

The combined problems mentioned above hinder fossil fuels from being “ideal” energy sources in the future, especially when minimization of environmental pollution has gained more and more attention nowadays. In this context, enhanced efforts have been shifted to research and development in the field of renewable and green energy resources.

1.2. Renewable energy resources

Renewable energy (RE), also called green energy or sustainable energy, is defined as “energy obtained from natural and persistent flows of energy occurring in the immediate environment” (Twidell and Weir, 1986). The definition of RE matches very well with the concept of “sustainable development” – a concept proposed in 1987 (in the seminal report of the World Commission on Environment and Development). It has also been a core guiding principle for related policies in the 21st century to maintain and improve the ecological processes upon which life relies (Twidell and Weir, 1986).

RE resources consisting of solar, wind, geothermal, hydro, and biomass are easily accessible worldwide because the natural sources are enormously abundant in nature (see Appendix A.1). RE is therefore expected to be able to contribute up to 50% of the gross energy demand after the mid-21st century (Akella, Saini and Sharma, 2009). Moreover, the average of CO_2 emission on a global basis could be reduced to 349 g kWh^{-1} by the share of RE sources – a 40% reduction compared to the level in 1990 (Pablo-Romero *et al.*, 2016) (Appendix A.1).

Nevertheless, RE resources in some cases are less competitive than the traditional energy supply systems. The energy production is intermittent due to the dependency of the process on geographical and weather conditions, which are usually of only limited predictability (Evans, Strezov and Evans, 2009) (Kalogirou, 2009). This leads to case by case mismatches of the energy production to the consumption/demand (Moriarty and Honnery, 2012). Energy supplies over spatiotemporal scales cannot be continuously guaranteed by RE resources (Evans, Strezov and Evans, 2009). Specific examples regarding the intermittence of energy production by RE are given in Appendix A.1. In the economic aspect, the inherent instability of the production of RE energy will lead to fluctuation of stock prices on the energy markets (Eberle, Müller and von Helmolt, 2012). As a consequence, a complete integration of RE sources into current energy supply systems is impeded, although from the aspects of abundance and environmental sustainability, they have become more desirable than fossil fuels. In addition, utilization of RE in automobiles is still not sufficiently advanced because of the lack of matched high-performance energy storage systems (Liu *et al.*, 2010).

Ambitious goals exist for the increase of the share of RE sources worldwide in order to reduce the dependency on fossil fuels. The European Union has announced

a goal to increase the share of RE resources in total energy consumption to 20% and to 30–40% in electricity production by 2020 (IEA, 2010). In Germany, the goal is to achieve a 50% share in electricity production by 2030 (Teichmann *et al.*, 2012). Moreover, an enormous share of energy should be accompanied by equivalent energy storage facilities. The total storage demand in Germany by 2020 is estimated to be up to 40 TWh, whereas the current total storage capacity is limited to approximately 0.04 TWh, which is due to the low storage density of the current technology where energy is stored solely mechanically (Teichmann *et al.*, 2012).

To compensate for energy shortages over the long term and to bridge the energy over-rich and over-lean periods, establishing RE equivalents with improved storage and distribution efficiency is required. These are also very important issues for the stability of the global energy systems (Papp *et al.*, 2014). Energy storage units developed for medium- and long-term application will become crucial components (Müller, Geng and Arlt, 2013) to overcome the unmatched energy production and demand.

1.3. Hydrogen as energy vector and challenges

To buffer the problems discussed above, hydrogen seems a good option. Hydrogen is the most abundant element, accounting for approximately 15 mol% of the Earth's surface (Møller *et al.*, 2017). Molecular hydrogen can be produced by using renewable resources as sources via water electrolysis (i.e., $H_2O \rightarrow H_2 + ½O_2$) (Mazloomi and Gomes, 2012) to realize the conversion and storage of excess energies (Geburtig *et al.*, 2016). Subsequently, power can be generated through re-oxidation of the energy-loaded hydrogen to water (Müller, Geng and Arlt, 2013). To store excess energy produced from RE resources in such a way is considered attractive for the future energy supply on a large scale regardless of geographical limits (Ibrahim, Ilinca and Perron, 2008) (Andrews and Shabani, 2012).

Hydrogen has excellent gravimetric energy density of 120 MJ kg^{-1} (i.e., 33.3 kWh kg^{-1}) (Preuster, Papp and Wasserscheid, 2017) – three times that of petroleum (Zhu and Xu, 2015). Hydrogen is thus a more powerful vector enabling long-term energy storage over days (Crotogino and Hamelmann, 2007). Furthermore, energy-loaded hydrogen can be employed not only in stationary devices but also in automobile applications (Eberle, Müller and von Helmolt, 2012) (Papp *et al.*, 2014) without emission of CO_2 as the byproduct (zero-emission) (Preuster, Papp and Wasserscheid, 2017). However, the low volumetric storage density of hydrogen (3 Wh L^{-1} (Preuster, Papp and Wasserscheid, 2017)) makes its practical use problematic. One liter of hydrogen contains only 10.8 kJ of energy under normal conditions (Markiewicz, Zhang, *et al.*, 2015). Applying very high pressures (200–700 bar, "Compressed Gaseous Hydrogen" or "CGH2") or keeping it in a liquid state under an extremely low temperature at (-253 °C, "Liquid or Cryogenic Hydrogen") (Teichmann, Arlt and Wasserscheid, 2012b) (Tietze, Luhr and Stolten, 2016) helps increase the volumetric storage density; however, this occurs at the expense of a decline in the overall

gravimetric storage density (5.6 MJ L^{-1}, even lower than that of gasoline 32.0 MJ L^{-1} (Lemmon, Huber and McLinden, 2013)), increased weight of tanks (Müller, Geng and Arlt, 2013), and large investment costs for the build-up of specialized and dedicated infrastructure (Eberle, Müller and von Helmolt, 2012) (Teichmann, Arlt and Wasserscheid, 2012b). Moreover, handling elemental hydrogen *per se* is a big challenge with respect to safety issues. Hydrogen is easily combustible, with the flammability limits in air spanning broad concentration ranges (4.0–75 vol%) (Häussinger, Lohmüller and Watson, 2000). Gaseous hydrogen has the fastest diffusion speed in air (approximately 20 m s^{-1} at room temperature), meaning quick dispersion if a leak occurs (Møller *et al.*, 2017). The use of cryogenic hydrogen also faces the loss of hydrogen from evaporation (von Wild *et al.*, 2010).

Overall, it is complex and costly to store and transport large amounts of elemental hydrogen by using the current technology. Therefore, various storage systems have been studied and proposed in the past few years to overcome the problems. Extensive research, including physisorption of hydrogen to condensed materials (Liu *et al.*, 2010), chemical storage through chemical bonds in metal hydride (e.g., aluminum borohydride) (Møller *et al.*, 2016), ammonia boranes (NH_3BH_3) (Zhu and Xu, 2015), methane (Dufour *et al.*, 2009) (Saxena, Kumar and Drozd, 2011), methanol (CH_3OH) (Nielsen *et al.*, 2013) and formic acid (HCOOH) (Grasemann and Laurenczy, 2012) etc.. These technologies, however, are subject to major drawbacks such as low hydrogen storage capacity (Papp *et al.*, 2014), high temperatures for hydrogen release (Pukazhselvan, Kumar and Singh, 2012) (Møller *et al.*, 2017), liberation of undesirable gaseous by-products (Dufour *et al.*, 2009) (Müller, Geng and Arlt, 2013), high cost (Dalebrook *et al.*, 2013), deterioration of the materials (Zhu and Xu, 2015) and irreversible conversion (Pukazhselvan, Kumar and Singh, 2012) (Müller, Geng and Arlt, 2013).

1.4. Liquid organic hydrogen carriers

Liquid organic hydrogen carriers (LOHCs) are expected to be a promising technology to buffer the problems in hydrogen-based energy storage and delivery. LOHCs were proposed in recent years and have been considered suitable energy carriers to gradually substitute fossil fuels. **Figure 1** summarizes the amount of research that has been published thus far on LOHCs.

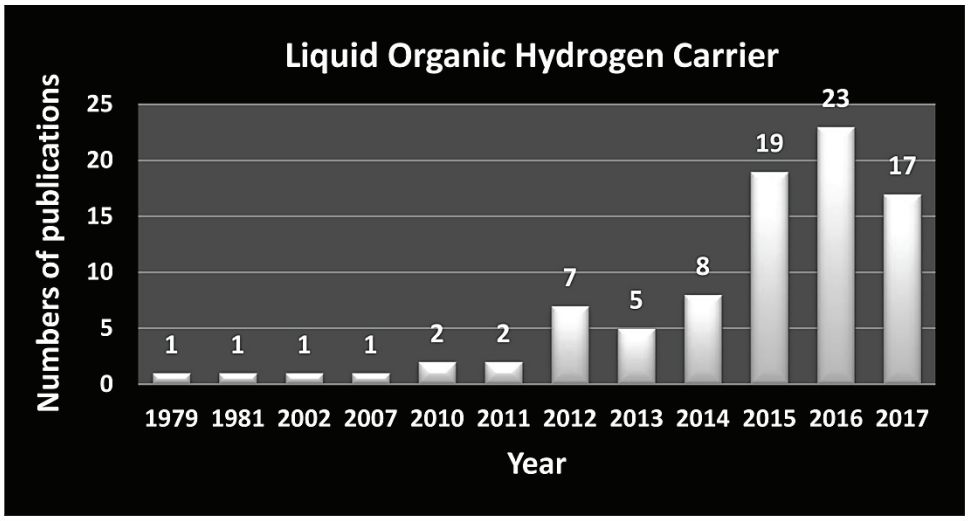


Figure 1. Numbers of publications (in total 88) regarding LOHCs since 1979 (to 23th October, 2017). Data collection was based on the SciFinder Scholar database‡ using the search terms "liquid organic carrier" and "hydrogen".

LOHCs are organic liquids existing in pairs as hydrogen-lean and hydrogen-rich forms. Reversible processes and transformation between the paired LOHC chemicals are possible by catalytic hydrogenation/dehydrogenation cycles under ambient conditions (**Figure 2**) (Teichmann *et al.*, 2011) (Preuster, Papp and Wasserscheid, 2017). Excess energy produced from RE in times of high loads can be used to produce hydrogen by water electrolysis or steam reforming. Energy in the form of hydrogen can thus be stored in the hydrogen-lean form of LOHCs via hydrogenation (Teichmann *et al.*, 2011) with comparatively high energy densities (usually 5–8 wt% H_2) (Kariya, Fukuoka and Ichikawa, 2002) (Hodoshima *et al.*, 2005) (Eberle, Felderhoff and Schüth, 2009) (Eblagon *et al.*, 2010) (Teichmann *et al.*, 2011). Storage for extended periods without significant energy losses (Teichmann *et al.*, 2011) or great decreases in the storage density could be achieved because hydrogen diffusion or boil-off (which occur if higher pressures and cryogenic temperatures are applied) seem less likely (Papp *et al.*, 2014). As a consequence, effective transportation over longer periods and distances to the spots where energy is demanded could be realized (Teichmann *et al.*, 2011) (Preuster, Papp and Wasserscheid, 2017). Furthermore, the process of hydrogen storage does not release other substances (e.g., GHG such as CO and CO_2) to the atmosphere (Eblagon *et al.*, 2010) (Preuster, Papp and Wasserscheid, 2017). Pure hydrogen is released through dehydrogenation (Preuster, Papp and Wasserscheid, 2017) and can be effectively used as energy sources in fuel cells or internal combustion engines (Papp *et al.*, 2014), electricity transmission (Teichmann, Arlt and Wasserscheid, 2012b) and residential heating (Teichmann *et al.*, 2012). Hydrogen-lean compounds are recycled by returning back to the energy sources to be reloaded with H_2. LOHCs *per se* are not consumed

‡ Chemical Abstracts Service (CAS), American Chemical Society.

(Teichmann *et al.*, 2011) in the reaction and are expected to be used repeatedly in further hydrogenation/dehydrogenation cycles (Teichmann *et al.*, 2012) (Teichmann, Arlt and Wasserscheid, 2012b). In this way, storage of large quantities of energy is possible to meet short- and long-term energy needs (Teichmann, Arlt and Wasserscheid, 2012b). The improved flexibility and dynamics in the energy storage by LOHCs are expected to compensate for the fluctuation of energy demand, thereby facilitating the decoupling of energy production and usage (Fikrt *et al.*, 2017). The mismatch between the energy demand and supply might be balanced to a considerable extent (Teichmann *et al.*, 2011).

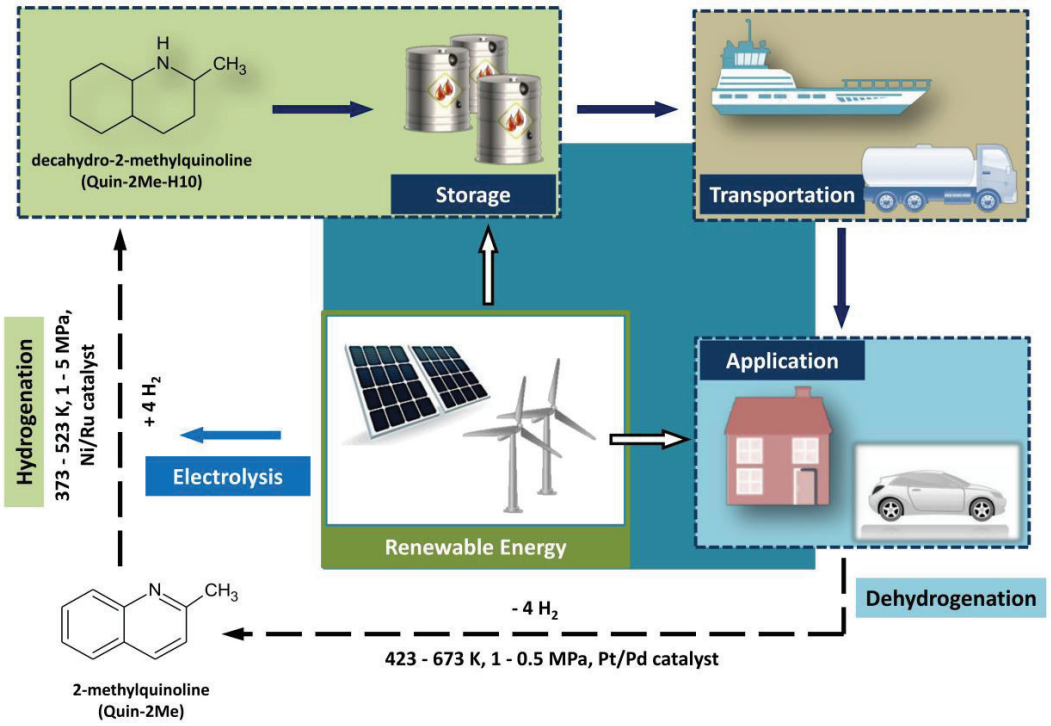


Figure 2. Schematic diagram describing the cyclic dehydrogenation/hydrogenation of LOHCs. Hydrogen (H_2) can be generated from RE such as solar and wind power (light blue arrow). Hydrogen-rich form – product of hydrogenation process (dashed arrow), can be safely generated, stored and transported (navy blue arrows); energy in the form of H_2 can be effectively released and applied in stationary devices and automobile applications via dehydrogenation (dashed arrow); the hydrogen-lean form will return and be reloaded with H_2 thus return to the cycle. Reaction conditions of hydrogenation/dehydrogenation (Markiewicz, Zhang, *et al.*, 2015) are given. In such a process, energy utilization is more stable and effective than use of RE directly (white arrows).

Another attractive aspect of LOHC systems is the compatibility of the technology with the existing infrastructure. Current facilities used in storage and transport (e.g., fueling stations and pipelines) or onboard applications (such as ships and trucks) (Papp *et al.*, 2014) (Preuster, Papp and Wasserscheid, 2017), which have been

employed for common fuels such as gasoline and diesel, are considered feasible to LOHCs because of the similarities of the chemicals to traditional fuels (Teichmann, Arlt and Wasserscheid, 2012b). The potential on-board application of LOHCs in vehicles via combustion under comparatively mild conditions is also expected to be safer. Furthermore, compatibility with the current infrastructure (Teichmann *et al.*, 2011) is also very important for economic efficiency as the transition of the current energy system to the new technology could possibly be realized with less costs. The preservation of at least parts of the current network infrastructure also enables step-wise and steady transition of the energy systems from fossil fuels to sustainable sources (Teichmann *et al.*, 2011), which is expected to facilitate the massive adoption of the technology (Teichmann *et al.*, 2012).

1.4.1. Development of LOHC systems

The first report on liquid organic carriers for hydrogen in energy storage can be traced back to 1979 when the energy was envisaged for on-board fuel cells in the form of hydrogen by dehydrogenation of liquid carriers consisting of methylcyclohexane and toluene (Taube and Taube, 1979). Related studies followed on the potential application of these carriers in vehicles (Taube *et al.*, 1983), and subsequently, the potential use of this carrier system in a prototype truck was proposed (Taube *et al.*, 1985). The possibility of seasonal energy storage by these carriers were also evaluated (Grünenfelder and Schucan, 1989). Since then several potential chemicals considered as potential LOHCs were investigated in more detail than in earlier studies, including methylcyclohexane/toluene (Scherer, Newson and Wokaun, 1999) (Schildhauer, Newson and Uller, 2001) (Pradhan *et al.*, 2011) (Geburtig *et al.*, 2016), cycloalkane/benzene (Kariya, Fukuoka and Ichikawa, 2003) (Biniwale *et al.*, 2008), and decalin/naphthalene (Hodoshima *et al.*, 2003) (Biniwale *et al.*, 2008).

These LOHC systems usually have the hydrogen holding capacities of 5–8 wt%; however, they have low boiling points and need high temperatures for dehydrogenation (e.g., ≥ 300 °C) (Biniwale *et al.*, 2008). The practical uses are therefore constrained in view of efficiency and safety considerations (Crabtree, 2008) (Zhu and Xu, 2015) (Preuster, Papp and Wasserscheid, 2017). Reduction of the dehydrogenation enthalpy is necessary for effective thermodynamic utilization of hydrogen storage (Zhu and Xu, 2015). Furthermore, lower temperature is desirable for maintaining reactions in a condensed liquid state thus to minimize side reactions (Zhu and Xu, 2015) (e.g., dealkylation (Yang *et al.*, 2014)). In particular, for the application in automobiles milder temperatures are more appropriate for the optimal operation of fuel cells (Dong *et al.*, 2015).

Incorporation of heteroatoms (e.g., nitrogen) into the rings of LOHC structures as atoms or ring substituents has been reported to facilitate the dehydrogenation by decreasing the enthalpy of dehydrogenation (Pez *et al.*, 2006) (Clot, Eisenstein and Crabtree, 2007) (Crabtree, 2008). The temperature required for hydrogen release

could consequently be efficiently reduced to 100–200 °C (Yamaguchi *et al.*, 2009) (Dean, Davis and Jessop, 2011). In addition, the presence of nitrogen atoms minimizes the interaction of the alkyl chains of LOHCs with catalyst surface, thus relieving the deactivation of the catalysts in the cyclic reactions (Zhu and Xu, 2015). This strategy was first proposed by Pez *et al.* in 2006 at Air Products (Air Products And Chemicals, Inc.) with the patents from Pez et al. (Pez *et al.*, 2006) (Pez *et al.*, 2008). These findings rendered nitrogen-substituted heterocycles in many cases as viable and promising candidates of LOHCs and stimulated fundamental screening of a number of compounds of this kind (Crabtree, 2017).

1.4.2. Promising LOHC candidates

A variety of potential compounds has been investigated to date, typically such as nitrogen-substituted heterocyclics, e.g., indoles (Cui *et al.*, 2008) (Dean, Davis and Jessop, 2011) (Dong *et al.*, 2015), quinaldines (Yamaguchi *et al.*, 2009) (Hu *et al.*, 2015) (Manas *et al.*, 2015), and carbazole derivatives such as N-ethylcarbazole (Pez *et al.*, 2006) (Eblagon *et al.*, 2010) (Teichmann, Arlt and Wasserscheid, 2012b) (Papp *et al.*, 2014) (Preuster, Papp and Wasserscheid, 2017). Other LOHC candidates such as benzyltoluene (BT) and dibenzyltoluene (DBT) (Müller *et al.*, 2015) (Geburtig *et al.*, 2016) (Fikrt *et al.*, 2017) (Preuster, Papp and Wasserscheid, 2017) have also been proposed. These candidates have high boiling points and usually have hydrogen storage capacities between 1 and 7 wt% H_2. The alkyl chains present such as in carbazole derivative-based LOHCs lower the melting point (Stark *et al.*, 2016) and in turn facilitate reversible hydrogenation (M. Yang *et al.*, 2013). For example, the melting point decreases from 247 °C for carbazole to 68 °C for N-ethylcarbazole (NEC) (Zhu and Xu, 2015) and even to as low as 48 °C for N-propylcarbazole (Stark *et al.*, 2016) though with a small loss in the hydrogen capacity from 5.8 wt% (Yang *et al.*, 2014) (Preuster, Papp and Wasserscheid, 2017) to 5.4 wt% (Zhu and Xu, 2015). Therefore, a further extension of the lengths of the substituted alkyl chains is usually attempted. Recently, for instance, a new indole-based LOHC, N-ethylindole with an ethyl group, was investigated due to the low melting point (-17.8 °C) and milder temperature for dehydrogenation (190 °C) (Dong *et al.*, 2015).

However, there are challenges for the application of the LOHC candidates. First, the H_2-lean form of NEC has a melting point of 69.1 °C, meaning that it will remain solid at ambient conditions (Teichmann, Arlt and Wasserscheid, 2012b). This property complicates the handling and use of the LOHC for instance in tanks for automobiles where additional requirements are needed (Markiewicz, Zhang, *et al.*, 2015). Moreover, LOHCs may undergo side reactions under the conditions for dehydrogenation, which could lead to the deterioration of the chemicals. In addition, high loading catalysts are required for the reactions in quinaldine-based LOHCs (Hu *et al.*, 2015); more suitable catalysts (in terms of efficiency promotion and low cost) are still needed for the hydrogenation/dehydrogenation to improve the long-term stability (Amende *et al.*, 2014). Dibenzyltoluene, a rather new and promising

candidate that was proposed in recent years, consists of different isomers in the initial material; a mixture of complex components will be generated in the hydrogenation reaction of this potential LOHC (Müller *et al.*, 2015). In addition, the presence of partially hydrogenated forms in mixtures with H_2-rich forms would decrease the energy capacity of LOHCs (Crabtree, 2017).

1.4.3. Potential applications and environmental concerns

Notwithstanding the needs for further improvement in the technological performance, LOHCs are considered promising in many areas of application. A very interesting prospect for LOHC application is in stationary facilities, such as on-grid applications (Preuster, Papp and Wasserscheid, 2017) in residential buildings and industrial sites (Teichmann *et al.*, 2012) (Fikrt *et al.*, 2017). LOHCs can be loaded with hydrogen at peak energy times (from roof-top photovoltaic (PHV) systems and power grids) and released via dehydrogenation when there is a shortage in energy supply and/or prices are high (Teichmann *et al.*, 2012). Utilization of this technology can be expanded to the community scale (Teichmann *et al.*, 2012) or isolated sites such as deserts or islands (Preuster, Papp and Wasserscheid, 2017).

Another potential of application that makes LOHCs even more interesting is the possibility of integration of the technology into automobiles (Teichmann, Arlt and Wasserscheid, 2012a). LOHCs are prospected to meet the target proposed by the U.S. Department of Energy (DOE) for the development of hydrogen storage materials to increase the capacities of on-board hydrogen storage systems to 7.5 wt% (0.07 kg H_2 L^{-1})[§]. Moreover, LOHCs are considered compatible with current infrastructure (e.g., gasoline fueling stations and tanks) for energy storage and distribution in automobiles to power a combustion engine or a fuel cell (Teichmann *et al.*, 2011). Moreover, long-distance travel can be envisaged with zero-emission (Teichmann *et al.*, 2012). A car powered by a Direct-LOHC-fuel cell integrated with an 80-kg LOHC tank could support a maximum driving range of approximately 700 km (Preuster, Papp and Wasserscheid, 2017). One hundred liters of *N*-ethylcarbazole are required for 500 km of driving (Teichmann *et al.*, 2011). A project “Reversible Liquid Carriers for an Integrated Production, Storage & Delivery of Hydrogen,“ funded by the U.S. DOE, has been proposed by the BMW Group Research and Technology together with the Air Products, United Technologies Research Center and Pacific Northwest National Lab to design and evaluate a reactor prototype based on LOHCs with a hydrogen production rate of 1 g H_2 min^{-1} (von Wild *et al.*, 2010).

In addition, an efficient share of RE with lower costs in logistics, both domestic and global, is expected to be realized by using LOHCs. Exports of RE from Northern Africa (solar) and Iceland (hydroelectric, geothermal and wind power) (Teichmann, Arlt and Wasserscheid, 2012b) to Germany in hydrogen-loaded LOHCs by established logistics such as ships and trucks would be possible, thus reducing costs and energy consumption during the transport (Preuster, Papp and Wasserscheid, 2017).

[§] https://energy.gov/eere/fuelcells/hydrogen-storage-current-technology.

Transport of the electricity produced by wind plants primarily installed on- and off-shore in northern Germany to the industrial centers in the south and west could also be achieved without enhanced construction of grids (Teichmann, Arlt and Wasserscheid, 2012b). Wind farms of small to medium sizes could produce approximately 200 tons of loaded LOHCs per day (Teichmann, Arlt and Wasserscheid, 2012b). A LOHCs demonstration on the logistics was established very recently (Preuster, Papp and Wasserscheid, 2017); a total storage of 1,000 L loaded perhydrodibenzyltoluene (H18-DBT) (using solar power as the energy source for the production of H_2 at Erlangen, Germany) was delivered to Stuttgart, Germany via road transport as an energy source for the local electric vehicles. In addition, a series of LOHC-based products for the storage and release of energy generated from RE sources have been developed by H_2-INDUSTRIES for electricity supplies[**].

Driven by these appealing potentials, penetration of LOHCs into the market once they are successfully implemented is very likely with huge quantities on a wide scale (Markiewicz, Zhang, *et al.*, 2015). It is therefore reasonable to anticipate that LOHCs would become high production volume (HPV) chemicals, which are defined by the Organization for Economic Co-operation and Development (OECD) as “chemicals which are produced or imported at levels greater than 1,000 tons per year in at least one member country/region” (OECD, 2009a). Potential hazards of HPV chemicals to the environment and the humans are subject to strict regulations and supervisions such as OECD HPV chemical program (OECD, 2009a) and REACH (a European Union regulation concerning Registration, Evaluation, Authorisation & restriction of Chemicals). The development of LOHC systems is still at an early stage, and various issues in addition to technological performances and economics need to be addressed (Teichmann *et al.*, 2011). Handling safety and environmental impacts while using these chemicals are definitely among the “crucial concern issues” and must be taken into account before the massive circulation of LOHCs in the market. The environmental hazards of LOHC chemicals in detail are very necessary for selecting the most “ideal” LOHC system (Teichmann *et al.*, 2011) (Markiewicz, Zhang, *et al.*, 2015) in view of environmental sustainability that is required for the development of future energy systems. The ecotoxicity profiles, i.e., whether or not and to what magnitude the LOHCs present potential adverse effects on the environment, are definitely required to ensure environmental safety (Teichmann *et al.*, 2011) (Markiewicz, Zhang, *et al.*, 2015). Moreover, the behavioral properties of these compounds in the environment are important for understanding the bioavailability and fate, which are closely related to the evaluation and prediction of the potential risks.

1.5. Environmental hazard assessment

1.5.1. Basic criteria

[**] H_2-INDUSTRIES: http://www.h2-industries.com/products/.

The goal of environmental hazard assessment (EHA) is to understand the harm caused by the utilization of chemicals in order to protect humans and the environment from any risk caused by these chemicals. To assess the possibility that a chemical can cause adverse effects, comprehensive data on physicochemical properties and (eco)toxicity are necessary. Moreover, the behavior and fate of the chemicals in the relevant environment (compartments) are often considered and connected to the toxic effects. Accordingly, EHA preferably involves the estimation of "to what magnitude" and "by what route" the target chemicals are influencing the environment. Data are consequently compiled to establish safety reports for substances manufactured or imported in quantities equal to or greater than 100 or 1,000 tons per year (European Chemicals Agency, 2011).

Briefly, EHA usually begins with data collection and gathering; data are then compared to the requirements that are necessary to conduct hazard evaluation on the basis of international regulations. Gaps in the data are identified to develop testing strategies for filling the gaps (European Chemicals Agency, 2011). The gaps may be insufficient information regarding physicochemical properties, ecotoxicity and behavior of the substances and the relevance of these characteristics to the hazard assessment in terms of production, utilization and logistics. The more the data (or the knowledge regarding the relationship between the data) are available, the "better" the assessment will be, although there will always be uncertainties due to unpredictability in natural processes. Data collection based on reliable evaluations will reduce the uncertainties and facilitate the assessment.

Data on ecotoxicity can be obtained in quantitative levels by conducting a series of ecotoxicological tests with individual organisms by including various endpoints over short or/and long terms. According to REACH, regarding extent of exposure, short-term toxicity to soil organisms needs to be considered for chemicals produced ≥ 100 t per year, and long-term toxicity is required for chemicals with production ≥ 1,000 t per year (European Chemicals Agency, 2011). Dose-response relationships between concentrations and observed effects are usually established simultaneously with the extrapolation of an effective/lethal concentration (ECx/LCx). The obtained EC_{50}/LC_{50} (concentration for 50% of maximal effect/concentration causing the death of 50% of a group of test animals) and EC_{10}/LC_{10} (concentration causing 10% maximal effect/10% of death) values are accordingly used for describing potential toxic effects. Moreover, these parameters are usually used as the bases for ecotoxicity classification of chemicals. Effects of the chemicals are identified and assigned to certain categories, e.g., toxic, harmful or not toxic (towards a specific compartment) to promote the management of the chemicals. Toxicity can be classified according to international classification criteria, such as GHS (Globally Harmonised System of Classification and Labelling of Chemicals), REACH proposed CLP [††] (European Chemicals Agency, 2015), Joint Research Centre (JRC)[‡‡]. However, it has been

[††] Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of Substances and Mixtures.

[‡‡] European Chemicals Agency-guided harmonized criteria for PBT/vPvB assessment in non-aquatic organisms at European level.

recognized that the highly diverse ecosystem is usually more sensitive to chemicals than individual organisms tested in laboratories where comparatively limited numbers of species are feasibly involved. Consequently, the results from ecotoxicological tests are usually not directly applied for EHA but are employed as a basis for the derivation of the hazard threshold – one of which is known as the predicted no-effect concentration (PNEC) (European Chemicals Agency, 2008). This is defined as the concentration of the substance below which adverse effects in the environment compartment of concern are unlikely to occur (European Chemicals Agency, 2008). PNECs are required for EHA for the Chemical Safety Assessment (CSA) of chemicals that are manufactured, imported and/or used in a level exceeding 10 tons per year (European Chemicals Agency, 2008).

If the outcome shows ecotoxic effects on individual organisms in certain environment compartment(s), and the toxicity regarding to hazard identification of the chemicals is classified as harmful, toxic, PBTs (persistent, bioaccumulative and toxic) or vPvBs (very persistent and very bioaccumulative), exposure assessment and risk characterization are often required and needed to be carried out quantitatively for further determination of the environmental impacts of the chemicals (European Chemicals Agency, 2011).

Release of chemicals to the environment can occur throughout the entire life cycle of the chemicals, i.e., it could occur during manufacturing, formulation and packing, as well as during logistics, use and disposal (European Chemicals Agency, 2016). In the aspect related to behavior, released chemicals will be subjected to transport and transformation after entering the environment by interacting with different environment compartments including air, water, soil, sediment and biota. Transport processes govern the spatial and temporal distribution of the chemicals in the environment (ECETOC, 1992). This process usually consists of advection and diffusion within specific compartments and partitioning between those compartments (ECETOC, 1993). Transfer by partitioning can be achieved by volatilization, adsorption, sedimentation, leaching, bioaccumulation (though passive or active uptake depending on bioavailability) and biomagnification (ECETOC, 1992) (ECETOC, 1993) (European Chemicals Agency, 2016). Transformation processes, on the other hand, determine the persistence of chemicals in the environment (ECETOC, 1992). Important reactions such as biological transformation (i.e., biodegradation and metabolism), hydrolysis, phototransformation, and speciation need to be considered (ECETOC, 1992) (ECETOC, 1993) (European Chemicals Agency, 2016) to understand the environmental fate (European Chemicals Agency, 2016) of the chemicals. A comprehensive characterization of the behavior can eventually be achieved as the consequence of the evaluation of transport and transformation processes (ECETOC, 1992).

Ultimately, by the combination of the aforementioned behavior characterizations along with the physicochemical properties of the chemicals, environmental parameters and possible release patterns, the quantity of the chemicals that will enter and be distributed within specific environmental compartments can be

evaluated (ECETOC, 1993). Estimates of the degree that organisms would be exposed and thus bioavailability of the chemicals will be possible. The results are usually integrated into the outcome of the ecotoxicological assessment in order to characterize the environmental impacts the compounds may pose.

1.5.2. State-of-the-art of LOHCs in terms of EHA

Since the development of LOHC technology is at an early stage, most studies available to date focus on the promotion of the technological performance and the handling in terms of hydrogenation/dehydrogenation processes. Comprehensive and reliable information of the potential effects and behavior of LOHCs targeting EHA is rarely available. Far more data are accessible on heterocyclic aromatic hydrocarbons (hetero-PAHs). Hetero-PAHs are polycyclic aromatic hydrocarbons (PAHs) in which one or more of the carbons in the aromatic ring are replaced by a nitrogen, sulfur, or oxygen atom. Therefore, hetero-PAHs usually present partial similarities in chemical structures and physicochemical properties to LOHCs. Due to N-substituted LOHCs constituting one dominant portion of LOHC systems, hetero-PAHs with carbons substituted by nitrogen (N-PAHs) are selected for a further elaboration of the current research.

N-PAHs in many studies usually show comparatively higher water solubility (Lopes and Furlong, 2001) (Feldmannová *et al.*, 2006) (Anyanwu and Semple, 2015a) and lower octanol-water partition coefficients (K_{ow}) (Lopes and Furlong, 2001) (Anyanwu and Semple, 2015a) compared to the parental PAHs. Consequently, they are prone to adsorb to soil particles less but show higher tendency to partition into the water phase (e.g., soil pore water). Thus, N-PAHs generally exhibit increased mobility in the environment compared to their homologous PAHs (Anyanwu and Semple, 2015a). Therefore, these chemicals seem more likely to contaminate groundwater and drinking water via leaching though soil pores (Schlanges *et al.*, 2008) (Anyanwu and Semple, 2015a). Moreover, for the (eco)toxicity, negative impacts of N-PAHs on both aquatic and soil organisms have been revealed in many studies (**Table 1**). Increased potential of water partitioning also results in greater bioavailability of the chemicals to many organisms (Eisentraeger *et al.*, 2008) (Kobetičová *et al.*, 2008) and accordingly leads to greater toxicity compared to their parental PAHs (Anyanwu and Semple, 2015a) (Anyanwu and Semple, 2015b). In addition, mutagenic/carcinogenic effects of these compounds have also been indicated (Yamada *et al.*, 2004) (Eisentraeger *et al.*, 2008).

Table 1 summarizes several studies concerning the toxicity of typical N-PAHs towards different model organisms in aquatic and soil scenarios (a review specifically for the toxicity of (N-)PAHs towards the reproduction of soil invertibrates *Folsomia candida* with test duration of 28 days is given in **Table I in Appendix A.2**). The toxicity mostly can be assigned to the class for the aquatic organisms as “acute 1” ($EC_{50} \leq 1$ mg L^{-1}), “acute 2” ($1 < EC_{50} \leq 10$ mg L^{-1}), or “acute 3” ($10 < EC_{50} \leq 100$ mg L^{-1}) and for the soil organisms as “harmful” ($100 < EC_{50} \leq 1000$ mg kg^{-1} dw (dry weight) soil)

according to GHS (GHS, 2011) and JRC (Hartmann, Stefania and Sokull-Klüttgen, 2014), respectively.

Table 1. Review of the literature regarding the ecotoxicity of typical N-PAHs towards aquatic (Part A) and soil (Part B) organisms. "n.a." indicates data not available in the literature. Effective concentrations are all unified in mg L^{-1} or mg kg^{-1} dw soil.

N-PAHs	Log K_{ow}	Water solubility (25°C) [mg L^{-1}]	Organisms	Test medium / soil	Endpoint & Effective concentration in aquatic or soil scenario		Ref.
Part A ------ Aquatic scenario [mg L^{-1}]							
Quinoline	2.03	6110	*Desmodesmus subspicatus*	ISO 8692	Inhibition EC_{50} (72 h)	60.9	[a]
			Daphnia magna	ISO 6341	Immobilization EC_{50} (24 h)	14.7	[a]
			Salmonella typhimurium	ISO 16240	Mutagenicity	++*	[a]
	2.23	1711	*Chironomus riparius*	water	Lethality LC_{50} (96 h)	4.9	[d]
Indole	2.14	3560	*Daphnia magna*	ISO 6341	Immobilization EC_{50} (24 h)	1.3	[c]
4,7-Phenanthroline	2.40[a]	38.04[a]	*Daphnia magna*	ISO 6341	Immobilization EC_{50} (48 h)	16.99	[e]
1,7-Phenanthroline	2.51[b]	30.64[b]	*Daphnia magna*	ISO 6341	Immobilization EC_{50} (48 h)	17.8	[e]
1,10-Phenanthroline	2.51[b]	30.64[b]	*Daphnia magna*	ISO 6341	Immobilization EC_{50} (48 h)	1.05	[e]
6-Methylquinoline	2.57	631	*Desmodesmus subspicatus*	ISO 8692	Inhibition EC_{50} (72 h)	33.2	[a]
			Daphnia magna	ISO 6341	Immobilization EC_{50} (24 h)	8.6	[a]
			Salmonella typhimurium	ISO 16240	Mutagenicity	+*	[a]
Acridine	3.40	38.4	*Desmodesmus subspicatus*	ISO 8692	Inhibition EC_{50} (72 h)	2.1	[a]
			Daphnia magna	ISO 6341	Immobilization EC_{50} (24 h)	4.6	[a]
	n.a.	n.a.	*Daphnia magna*	ISO 6341	Immobilization EC_{50} (48 h)	2.4	[e]
	3.27	5.38	*Chironomus riparius*	water	Lethality LC_{50} (96 h)	0.07	[d]

N-PAHs	Log K_{ow}	Water solubility (25°C) [mg L^{-1}]	Organisms	Test medium / soil	Endpoint & Effective concentration in aquatic or soil scenario		Ref.
Part A ------ Aquatic scenario [mg L^{-1}]							
Benzo(h)-quinoline	3.43[b]	78.70[b]	*Daphnia magna*	ISO 6341	Immobilization EC_{50} (48 h)	3.48	[e]
	3.37	5.07	*Chironomus riparius*	water	Lethality LC_{50} (96 h)	0.59	[d]
Phenan-thridine	n.a.	n.a.	*Daphnia magna*	ISO 6341	Immobilization EC_{50} (48 h)	2.69	[e]
	3.44	4.6	*Chironomus riparius*	Water	Lethality LC_{50} (96 h)	0.61	[d]
Carbazole	3.72	1.8	*Daphnia magna*	ISO 6341	Immobilization EC_{50} (24 h)	3.4	[a]
	3.23[c]	3.27[c]	*Fundulus heteroclitus*	Buffer	EROD* activity inhibition IC_{50}	4.56	[f]
Phenazine	n.a.	n.a.	*Daphnia magna*	ISO 6341	Immobilization EC_{50} (48 h)	2.9	[e]
Part B ------ Soil scenario [mg kg^{-1} dw soil]							
1,10-Phenan-throline	1.78	2690	*Eisenia fetida*	OECD artif. soil*	Reproduction EC_{50} (28 d)	1033	[g]
			Enchytraeus crypticus	OECD artif. soil	Reproduction EC_{50} (28 d)	796	[g]
			Folsomia candida	OECD artif. soil	Reproduction EC_{50} (28 d)	928	[g]
	2.51	30.64	*Eisenia fetida*	UK agricul. soil*	Growth EC_{50} (30 d)	31.1	[b]
Quinoline	2.03	6110	*Eisenia fetida*	OECD artif. soil	Reproduction EC_{50} (28 d)	1948	[g]
			Enchytraeus crypticus	OECD artif. soil	Reproduction EC_{50} (28 d)	990	[g]
			Folsomia candida	OECD artif. soil	Reproduction EC_{50} (28 d)	230	[g]
			Folsomia candida	LUFA 2.2 soil*	Reproduction EC_{50} (28 d)	75.0	[d]
	2.23	1711	*Enchytraeus crypticus*	LUFA 2.2 soil	Reproduction EC_{50} (28 d)	285.4	[d]
4,7-Phenan-throline	2.4	38.04	*Eisenia fetida*	UK agricul. soil	Growth EC_{50} (30 d)	50.4	[b]

N-PAHs	Log K_{ow}	Water solubility (25°C) [mg L^{-1}]	Organisms	Test medium / soil	Endpoint & Effective concentration in aquatic or soil scenario		Ref.
Part B ------ Soil scenario [mg kg^{-1} dw soil]							
1,7-Phenanthroline	2.51	30.64	*Eisenia fetida*	UK agricul. soil*	Growth EC_{50} (30 d)	5.3	b
Phenazine	2.84	16.00	*Eisenia fetida*	OECD artif. soil	Reproduction EC_{50} (28 d)	649	g
			Enchytraeus crypticus	OECD artif. soil	Reproduction EC_{50} (28 d)	1073	g
			Folsomia candida	OECD artif. soil	Reproduction EC_{50} (28 d)	298	g
Acridine	3.27	n.a.	*Folsomia fimetaria*	Danish agricul. soil*	Reproduction EC_{50} (21 d)	460	h
			Folsomia fimetaria	Danish agricul. soil	Reproduction EC_{10} (21 d)	290	h
	3.40	38.40	*Eisenia fetida*	OECD artif. soil	Reproduction EC_{50} (28 d)	1460	g
			Enchytraeus crypticus	OECD artif. soil	Reproduction EC_{50} (28 d)	1412	g
			Folsomia candida	OECD artif. soil	Reproduction EC_{50} (28 d)	1212	g
Benzo[h]-quinoline	3.43	78.70	*Eisenia fetida*	UK agricul. soil	Growth EC_{50} (30 d)	11.4	b
Phenanthridine	3.44	4.61	*Folsomia candida*	LUFA 2.2 soil	Reproduction EC_{50} (28 d)	208	i
			Enchytraeus crypticus	LUFA 2.2 soil	Reproduction EC_{50} (28 d)	646	i
			Folsomia candida	LUFA 2.2 soil	Reproduction EC_{50} (28 d)	37.3	d
			Enchytraeus crypticus	LUFA 2.2 soil	Reproduction EC_{50} (28 d)	115.4	d
Carbazole	3.51	n.a.	*Folsomia fimetaria*	Danish agricul. soil	Reproduction EC_{50} (21 d)	35	h
			Folsomia fimetaria	Danish agricul. soil	Reproduction EC_{10} (21 d)	10	h

N-PAHs	Log K_{ow}	Water solubility (25°C) [mg L^{-1}]	Organisms	Test medium / soil	Endpoint & Effective concentration in aquatic or soil scenario		Ref.
Part B ------ Soil scenario [mg kg^{-1} dw soil]							
Carbazole	3.51	n.a.	*Eisenia veneta*	Danish agricul. soil	Growth EC_{50} (28 d)	54	[j]

[*] EROD: CYP1A (mono-oxygenase cytochrome P4501A)-mediated ethoxyresorufin-*O*-deethylase.

[*] +: Mutagenic potential. The mutagenesis marked with "++" is higher than that with "+".

[*] OECD artificial soil OC% : 4.68; UK agricultural soil OC% : 2.7; LUFA 2.2 soil OC%: 2.3 ± 0.2; Danish agricultural soil OC%: 1.6.

[a] (Eisentraeger *et al.*, 2008). [b] (Anyanwu and Semple, 2016). [c] Predicted by Estimation Programs Interface Suite (EPI)[‡]. [d] (Bleeker *et al.*, 2003). [e] (Feldmannová *et al.*, 2006). [f] (Wassenberg *et al.*, 2005). [g] (Kobetičová *et al.*, 2008). [h] (Sverdrup *et al.*, 2001). [i] (Droge *et al.*, 2006). [j] (Sverdrup *et al.*, 2002).

The findings regarding the behavior and (eco)toxicity of these chemicals indeed raise concerns with respect to the potential risks of the compounds of this kind (such as LOHCs). Although data on N-PAHs are available as mentioned above, simple extrapolation for the environmental hazards of LOHCs from these structurally partially similar compounds may lead to incomplete and incorrect conclusions. Even if data on the toxicity are obtainable for some of the chemicals that have more relevant structures to LOHCs (e.g., quinoline, indole and carbazole) (**Table 1**), they are still too limited to make any comprehensive assessment because these data were gathered in the following ways: i) by testing the compounds typically in the dehydrogenated forms, i.e., only one of the paired LOHCs, such as indole; ii) only involving the simpler (unsubstituted) homologues of LOHCs, such as carbazole and quinoline; and iii) by various tests established in different conditions (e.g., varied media, incubation times and endpoints), which impede the comparison of the toxic effects among these compounds.

As mentioned in section 1.4.3 about the applications of LOHCs, the production, utilization and transport of these chemicals in large quantities on a global scale are very likely. As possible HPV chemicals, LOHCs cause increased concerns for the environmental safety, particularly when accidental spills on site/road or leakages occur (Markiewicz, Zhang, *et al.*, 2015) (Crabtree, 2017). In this context, soil is usually considered a highly relevant exposure scenario of LOHCs; soil has been demonstrated in many studies as the major and ultimate sink of pollutants (Markiewicz, Jungnickel, *et al.*, 2015) such as organic contaminants e.g., tar, oil (Hentati *et al.*, 2013) and PAHs (Eom *et al.*, 2007) or N-PAHs (Stokes, Paton and Semple, 2005) (Anyanwu and Semple, 2015a). To establish a comprehensive EHA based on reliable data (see section 1.5.1),

[‡] EPI Suite: https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface.

the potential behavior of LOHCs after release needs to be considered both in terms of transportation and transformation (**Figure 3**). These processes control the extent of exposure of organisms to these compounds and thus the level of bioavailability and toxicity. Related aspects are listed below to briefly specify the processes that LOHCs could be subjected to after entering the environment:

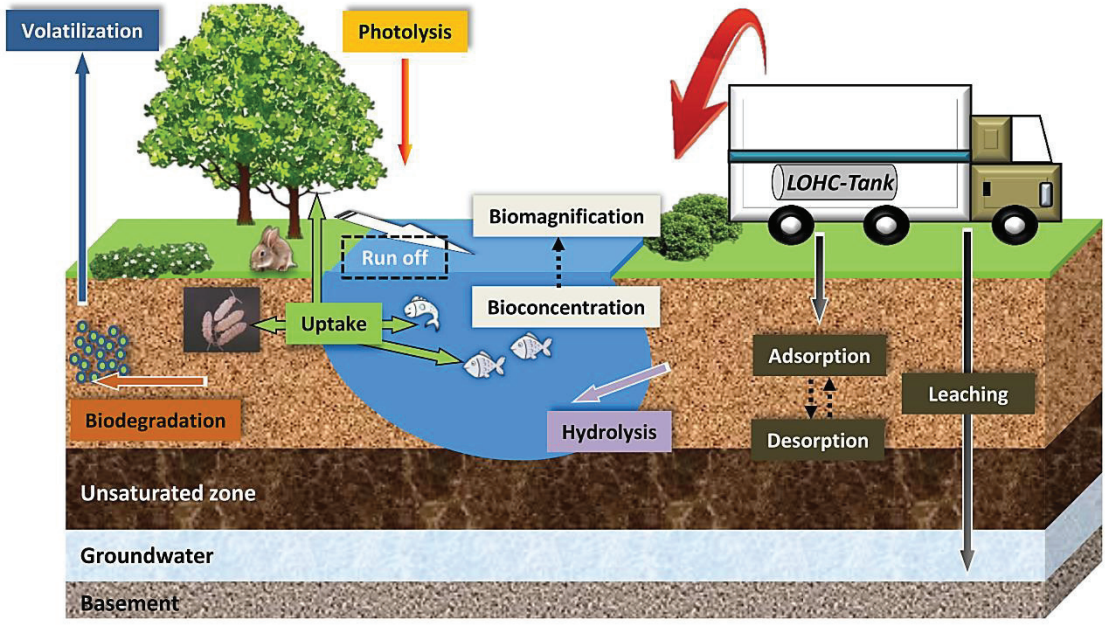


Figure 3. Schematic diagram of the typical transport and transformation processes of LOHCs in soil matrix networks. Relevant environmental compartments include air, water, soil and biota.

I. Transport of LOHCs could occur between air, water (e.g., pore water, groundwater and surface water), soil solids and biota.

(i) Transfer from soil/water surface to the air via volatilization (can be described by boiling point, Henry's Law constant and air-water partition coefficient).

(ii) Transfer between soil solids and water phases via adsorption (e.g., binding to soil particles and soil organic matters (SOM)), desorption and leaching. Physicochemical parameters helping to describe such processes include water solubility (S_w), octanol-water partition coefficient (K_{ow}, for neutral structures), ionization corrected octanol-water partition coefficient (log D, particularly for ionizable structures), organic carbon-water partition coefficient (K_{oc}), and soil-water partition coefficient (K_d) of the LOHCs.

(iii) Uptake by biota such as macrofauna (e.g., earthworm and ants), mesofauna (e.g., arthropods such as Collembola), microfauna (nematodes, small arthropods and protozoans) and soil bacteria with the consideration of bioaccessibility and bioavailability. This process could be realized through indirect uptake such as skin contact with soil and pore water (dominant route of exposure of arthropods and

nematodes), and/or the direct ingestion of soil particles (e.g., earthworms), pore water and organic biomass (ECETOC, 1990). Accumulation of these compounds would be achieved by bioconcentration and biomagnification (if transport in food chain and to higher tropic levels is involved).

II. Transformation could occur via two main processes (Schwarzenbach, Gschwend and Imboden, 2003) by which transformation products such as "degradation products" or "metabolites" are generated (European Chemicals Agency, 2016) (hazards could be altered due to changed stabilities and toxicities compared to the parental substances). These processes control the persistence of the chemical in the environment, which is treated as an important index for hazard evaluation:

(i) Abiotic processes, e.g., hydrolysis, redox reactions, and photochemical reactions (e.g., direct/indirect photolysis).

(ii) Biotic processes, i.e., biologically mediated reactions such as metabolism of arthropods and microbial biodegradation.

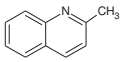
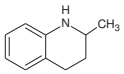
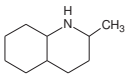
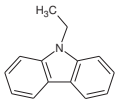
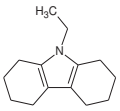
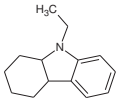
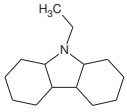
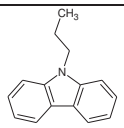
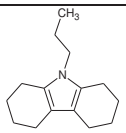
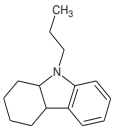
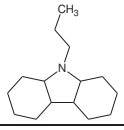
Unfortunately, few data is available regarding the aspects mentioned above for the behavior characterization of LOHCs. Studies on the exposure and fate exclusively of LOHC structures are sparse; information with respect to the potential behaviors in the environment such as partitioning and mobility is missing. A lack of data for the ecotoxicity and behavior of LOHCs hinders a comprehensive and reliable assessment of the environmental hazards of these chemicals.

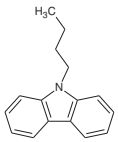
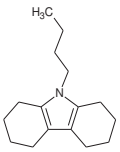
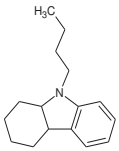
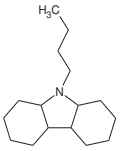
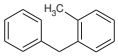
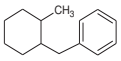
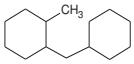
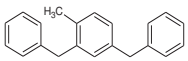
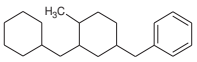
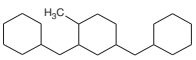
1.6. Study objectives and hypotheses

1.6.1. Study objectives

The overall objective of the thesis was to establish a proactive environmental hazard assessment of LOHC systems (**Table 2**). Specifically, the thesis sought to determine the potential behavior (i.e., adsorption properties, mobility and retention) and ecotoxicity of LOHCs in soil environment. Aquatic toxicity was also considered in view of soil-water interrelationship. Characterization of behavior was correlated to ecotoxicity to evaluate the bioavailability of chemicals and the effect of LOHCs on organisms and the environment.

Table 2. Basic physicochemical properties of the currently promising LOHCs. More information can be found in *Table 3.2.1 in subchapter 3.2*.

LOHC candidates	Abbrev.	Formula	MW [g mol^{-1}]	Chemical structure	Log K_{oc} [a]	Log K_{oc} [b]
Quinaldines						
2-methyl-quinoline	**Quin-2Me**	$C_{10}H_9N$	143.2		2.25	2.18
Tetrahydro-2-methylquinoline	**Quin-2Me-pH**	$C_{10}H_{13}N$	147.2		2.52	2.48
Decahydro-2-methylquinoline	**Quin-2Me-H10***	$C_{10}H_{19}N$	153.3		2.52	2.53
Indoles						
Indole	**Indole**	C_8H_7N	117.2		1.86	2.21
Indoline	**Indoline**	C_8H_9N	119.2		2.02	1.94
Carbazole derivatives						
9-Ethyl-9H-carbazole	**Car-2**	$C_{14}H_{13}N$	195.3		3.34	3.36
9-Ethyl-octahydro-carbazole	**Car-2-pH***	$C_{14}H_{21}N$	203.3		5.08	3.26
9-Ethyl-hexahydro-carbazole		$C_{14}H_{19}N$	201.3		2.99	3.08
9-Ethyl-dodecahydrocarbazole	**Car-2-H12**	$C_{14}H_{25}N$	207.4		2.76	n.a.
9-Propyl-9H-carbazole	**Car-3**	$C_{15}H_{15}N$	209.3		3.53	3.61
9-Propyl-octahydro-carbazole	**Car-3-pH***	$C_{15}H_{23}N$	217.4		5.50	3.50
9-Propyl-hexahydro-carbazole		$C_{15}H_{21}N$	215.3		3.26	3.36
9-Propyl-dodecahydrocarbazole	**Car-3-H12**	$C_{15}H_{27}N$	221.4		3.03	n.a.

LOHCs candidates	Abbrev.	Formula	MW [g mol^{-1}]	Chemical structure	Log K_{oc} [a]	Log K_{oc} [b]
Carbazole derivatives						
9-Butyl-9H-carbazole	**Car-4**	$C_{16}H_{17}N$	223.3		3.80	3.89
9-Butyl-octahydro-carbazole	**Car-4-pH***	$C_{16}H_{25}N$	231.4		5.93	3.79
9-Butyl-hexahydro-carbazole		$C_{16}H_{23}N$	229.4		3.53	3.62
9-Butyl-dodecahydrocarbazole	**Car-4-H12**	$C_{16}H_{29}N$	235.4		3.30	n.a.
Benzyltoluenes						
Benzyltoluene	**MLH**	$C_{14}H_{14}$	182.3		3.96	3.34
Benzyltoluene 50% hydrogenated	**MLH-pH***	$C_{14}H_{20}$	188.3		4.96	3.65
Benzyltoluene 100% hydrogenated	**MLH-H12**	$C_{14}H_{26}$	194.4		5.88	n.a.
Dibenzyltoluenes						
Dibenzyltoluene	**MSH***	$C_{21}H_{20}$	272.4		5.72	4.52
Bis(cyclohexylmethyl)methyl-benzene	**MSH-pH**	$C_{21}H_{32}$	284.5		7.72	n.a.
Bis(cyclohexylmethyl)methyl-cyclohexane	**MSH-H18**	$C_{21}H_{38}$	290.5		8.64	n.a.

[a] Predicted by Estimation Programs Interface Suite (EPI).

[b] Calculated by Conductor like Screening Model for Realistic Solvation (COSMO-RS, see section *S3.2.2 in subchapter 3.2*)[‡] assuming chemicals marked with "*" were mixtures of "*cis*" and "*trans*" configurations.

[‡] COSMO-RS: http://www.cosmologic.de/theory/cosmo-rs.html.

The study objectives were achieved by investigating different parameters with different methods:

I. Characterization of the adsorption properties and mobility

(i) Carbon-water partition coefficient (K_{oc})

K_{oc} is an indicator that describes the affinity of organic contaminants for organic matter. The adsorption capacity of 13 LOHC structures to organic matter based on the K_{oc} value were estimated and analyzed by software and high performance liquid chromatography (HPLC) screening with the mobility predictions.

(ii) Soil-water partition coefficient (K_d)

K_d is often used to describe the partitioning of organic contaminants between the solid and water fractions in soil. The K_d values of LOHCs in standard soil were measured via adsorption batch equilibrium experiments, and adsorption isotherm modeling was performed using quinaldines (Quin-2Me, Quin-2Me-pH, and Quin-2Me-H10) as examples for cross-check.

(iii) Leaching capacity

The leaching capacity of LOHCs was evaluated in soil leaching columns using quinaldines (Quin-2Me, Quin-2Me-pH, and Quin-2Me-H10) as examples. Breakthrough curves were illustrated, and leaching-based K_d values were calculated to dynamically investigate the adsorption and mobility of these LOHCs. The possible contamination of groundwater by these chemicals was evaluated.

II. Ecotoxicity of LOHCs towards organisms

The toxicity of LOHCs was evaluated in soil and aquatic test scenarios by investigating the acute and chronic effects. The toxic effects were determined by considering the bioavailability of LOHCs for different test organisms in two test scenarios.

(i) Soil ecotoxicity

The toxicity of the quinaldines was investigated using the soil bacteria *Arthrobacter globiformis* (growth, 2 h) and Collembola *Folsomia candida* (survival and reproduction at 14 days and 28 days, respectively). The toxic effects were determined by considering the pore-water exposure of the organisms via the integration of adsorption properties and the performance of pore-water tests. The toxicity was classified, and the PNECs were extrapolated.

(ii) Aquatic ecotoxicity

The toxic effects of LOHCs (quinaldines and carbazole derivatives) were estimated in a test battery with the aquatic organisms *Vibrio fischeri*, *Raphidocelis subcapitata*, *Lemna minor,* and *Daphnia magna*. Toxicity was assigned to defined categories.

A preliminary assessment regarding the environmental hazard of LOHCs was then conducted in the soil and water phases based on the inherent relations observed in the two environmental scenarios as well as by considering the fate, realistic environmental conditions and application volumes of LOHCs.

1.6.2. Hypotheses

The hypotheses of this thesis mainly addressed the adsorption characteristics and ecotoxicological effects of LOHCs. The hypotheses were as follows.

I. Organic chemicals with a higher octanol-water partition coefficient (K_{ow}) and molecular weight or lower water solubility usually have stronger affinity for organic sorbents. Therefore, the K_{oc} was expected to increase in the order of indole, indoline, Quin-2Me, Quin-2Me-H10, and Quin-2Me-pH followed by the carbazole derivatives, MLHs and MSH.

II. The K_d is often considered to be closely related to the K_{oc} by correction with the total organic carbon content of soils. Moreover, the K_{ow} is often correlated with the K_{oc}. Therefore, adsorption in terms of the K_d values of the three components of the quinaldine-based LOHC system were expected to follow the order of octanol-water partitioning (log K_{ow}) with Quin-2Me (2.45) < Quin-2Me-pH (3.04) < Quin-2Me-H10 (3.25).

III. The leaching capacity describes the mobility of chemicals through soil layers, and a reverse correlation of the affinity of chemicals for soils was anticipated. Therefore, the leachability of the quinaldines was expected to be in the order of Quin-2Me-H10 < Quin-2Me-pH < Quin-2Me.

IV. Organic compounds with higher octanol-water partitioning are usually more toxic to organisms due to their greater hydrophobicity and lipophilicity. In soil, however, compounds with higher octanol-water partitioning potentials might also exhibit increased adsorption to the soil matrix, which can result in a reduced distribution of compounds in the water phase than the soil phase, thereby decreasing their bioavailability to the test soil organisms (when pore water exposure dominates). Therefore, Quin-2Me-H10 and Quin-2Me-pH, which both have higher K_{ow}, were expected to be less toxic to the two tested soil organisms than Quin-2Me.

V. In the aquatic exposure scenario where soil was absent, the toxicity of LOHCs was anticipated to follow the hydrophobicity; therefore, a higher octanol-water partitioning was expected to correlate with higher toxicity.

VI. Due to partitioning in the soil solids, the extent of exposure and thus bioavailability of the test LOHCs to the test organisms in soil were expected to be lower (when pore-water exposure dominates) than that in the aquatic scenario. Therefore, the soil exposure scenario presented in the thesis was expected be less sensitive than the aquatic exposure scenario.

Chapter-II Materials and Methodology

2. Materials and Methodology

Details on the culturing of the test organisms are available in *subchapters 3.3 and 3.4*; and the relevant physicochemical properties of the test LOHCs are described in *subchapters 3.2, 3.3 and 3.4*. The experiments presented in the study, including the ecotoxicity tests, adsorption batch equilibrium experiment, soil column leaching experiment and chemical extractions, were all subjected to pre-tests to establish and validate the methods before performing the experiments.

2.1. Test organisms

Ideal ecotoxicological studies on the effects of (a class of) chemicals would screen all chemicals in question toward all relevant organisms that are present in the scenario under consideration. However, such screening is usually an impossible task. Therefore, standard and representative organisms are usually selected for a preliminary hazard assessment of a group of new chemicals to generate new ecotoxicological data. Thus, this study was conducted to investigate the toxicity of LOHCs by evaluating typical biological endpoints. The organisms studied are common in the selected scenarios, thus enabling easy rearing and most of them are considered sensitive to the changes in the environment. Moreover, these same organisms have frequently been used for ecotoxicity tests in laboratories and tests that follow standard test guidelines (e.g., OECD, EPA, DIN and ISO guidelines), facilitating data comparison and sharing between studies.

2.1.1. Soil bacteria *Arthrobacter globiformis*

Arthrobacter globiformis are gram-positive microbes that are aerobic (anaerobic is also possible) and non-pathogenic (Dworkin *et al.*, 2006), and they are typical organisms found in natural soils and sediments (Dworkin *et al.*, 2006). Furthermore, *A. globiformis* is located at the bottom of the food chain and presents as an food source for organisms on higher trophic levels (Doelman *et al.*, 1984). Thus, they are considered important components of soil ecosystems. These bacteria are able to grow on a wide range of substrates through the aid of different enzymes, which offers a significant benefit for biological tests because alterations in enzyme activity can be employed as toxicity parameters and indicators for environmental hazards (Ahtiainen, 2002) (Neumann-Hensel and Melbye, 2006). Measuring and estimating total microbial enzyme activity is often complex. According to the German Standard Sediment Contact Test with *A. globiformis* (DIN, 2002), tests can be conducted based

on spectrophotometrical measurements by detecting a color change of the dye resazurin as it interacts with the bacterial enzyme dehydrogenase. Dehydrogenase in vital bacterial cells catalyzes blue resazurin to pink resorufin via chemical reduction (Thomsom, Liu and Kaiser, 1986). The production of resorufin is spectrophotometrically measured at an optical density of 620 nm to detect bacterial survival. This test allows for a fairly rapid and efficient screening with 2-h incubation; therefore, it is recommended for the assessment of chemicals in soils and remediated soils.

A microbial contact test with *A. globiformis* using changes in enzyme activities as index provides for the rapid screening of chemicals of environmental concern. Therefore, these bacteria are the optimal organism for proactive ecotoxicological evaluations of newly emerged chemicals for which limited ecotoxicological data are available.

Bacteria is usually considered primarily exposed through pore water (Peijnenburg *et al.*, 2012) (Kördel *et al.*, 2013). Consequently, the bonded fraction of organic compounds to soil solids is considered unavailable for uptake by the bacteria (Gevao *et al.*, 2001).

2.1.2. Soil invertebrate *Folsomia candida*

Collembola (springtails) are one of the most abundant soil arthropods. They present a reported density of several million individuals per m^2 with a species richness ranging in an ecosystem from 1–3 to 50–60 (Rusek, 1998). These organisms are an important component of soil mesofauna (Rusek, 1998) and are important decomposers of leaf litter and soil organic matter in the terrestrial ecosystems (Xu *et al.*, 2009). Collembola are particularly sensitive to changes in soil conditions (Santorufo *et al.*, 2012) (e Silva *et al.*, 2017); therefore, they are vulnerable to soil contamination. The un-pigmented species *Folsomia candida* is often used to study the environmental impact of various contaminants on soil, such as pesticides, heavy metals, fertilizers, polycyclic aromatic hydrocarbons (PAHs) and nanomaterials. Most studies have shown that *F. candida* is among the most sensitive springtails compared with e.g., *Sinella curviseta* (Bandow *et al.*, 2014), *Proisotoma minuta* (Cristhy *et al.*, 2016), and among the most sensitive taxon of soil invertebrates[*] to a majority of chemicals (**Table 1**). *Folsomia candida* has been selected as a standard organism by the OECD and ISO for ecotoxicological testing in soil under laboratory conditions.

Populations of *F. candida* consist exclusively of parthenogenetic females (Fountain and Hopkin, 2005). This mode of reproduction renders these animals easily rearable in laboratories in large numbers, thus promoting testing on the basis of reproduction (Crouau, Chenon and Gisclard, 1999). Compared with tests established for

[*] Examples: *Enchytraeus crypticus* (Droge *et al.*, 2006) (Kobetičová *et al.*, 2008) (e Silva *et al.*, 2017), *Eisenia fetida* (Fountain and Hopkin, 2005) (Kobetičová *et al.*, 2008), *Eisenia andrei* (Giesen and Van Gestel, 2013) (e Silva *et al.*, 2017), *Lumbricus rubellus* (Giesen and Van Gestel, 2013), and *Hypoaspis aculeifer* (Kamoun *et al.*, 2017).

monitoring endpoints, such as survival or mortality, changes in the reproduction of *F. candida* based on chronic (long-term) effects have been ranked as a highly sensitive and informative biological parameter by numerous studies (Crouau, Chenon and Gisclard, 1999) (Eom *et al.*, 2007) (Giordano *et al.*, 2010). Generally, instar individuals at 21 to 24 days are sexually mature and lay approximately 30 to 50 eggs per batch (**Figure 4 A**) (Fountain and Hopkin, 2005). Hatching usually requires 7 to 10 days (juveniles, **Figure 4 B**) at the optimal temperature of 21 °C (Fountain and Hopkin, 2005). Environmental stresses induced by substrate contamination can lead to reduced fecundity (Fountain and Hopkin, 2005) of these animals through various modes of toxic action. Moreover, these arthropods have been reported to be sensitive to oil spillage regardless of the amount applied, and extreme sensitivity was found in *F. candida* exposed to oily waste-contaminated soils (Juvonen *et al.*, 2000). By partially sharing the chemical structures and properties of oils and fuels, the toxicity of LOHCs was expected to be effectively evaluated using this species.

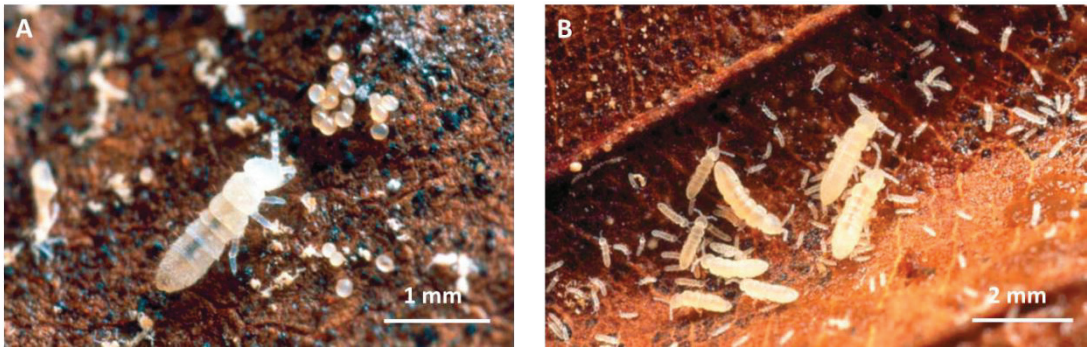


Figure 4. *Folsomia candida* in soil. Adapted from Fountain and Hopkin (Fountain and Hopkin, 2005). **A**: individual adult and eggs; and **B**: adults and juveniles.

Collembola *F. candida* is also defined as a semi soft-bodied organism (Kördel *et al.*, 2013) to distinguish it from other terrestrial species classified as soft-bodied organisms, such as nematodes and earthworms (Peijnenburg *et al.*, 2012) (Kördel *et al.*, 2013). For soft-bodied organisms, the exchange process with the external environment, such as the uptake of oxygen, water, and contaminants, is mostly governed via the skin. Collembola, however, have evolved special organs to mediate uptake processes and consumption via pore water, which represents the dominant exposure pathway (Van Gestel, 1997) (Peijnenburg *et al.*, 2012) (Kördel *et al.*, 2013) for this animal. Two main manners are often considered in such exposure pathway. One is via the ventral tube (VT, **Figure 5 A**), which is composed of a pair vesicles appended on the ventral side of the first abdominal segment (Fountain and Hopkin, 2005) participates in fluid exchange (Fountain and Hopkin, 2005) and pore water-mediated uptake (Peijnenburg *et al.*, 2012). The second pathway is via the cuticle (**Figure 5 B**), which is a hydrophobic lipid-contained waxy layer (composed of fatty acids, wax esters and terpenes envelopes (Nickerl *et al.*, 2014)) covering the

outer surface of the animal (Peijnenburg *et al.*, 2012) (Schmidt *et al.*, 2013) that controls the uptake of oxygen (Fountain and Hopkin, 2005) and water through absorption from wet or humid substrates (Peijnenburg *et al.*, 2012). In the case of exposure to organic contaminants, the cuticle mediates the contact of the organism to organic compounds (Domene, Alcañiz and Andrés, 2007) by providing sorption sites (Schmidt *et al.*, 2013). In such a context, it is reasonable and necessary to investigate the toxicity of LOHCs by considering the partitioning processes of these chemicals between soil and water phases to evaluate the effective exposure.

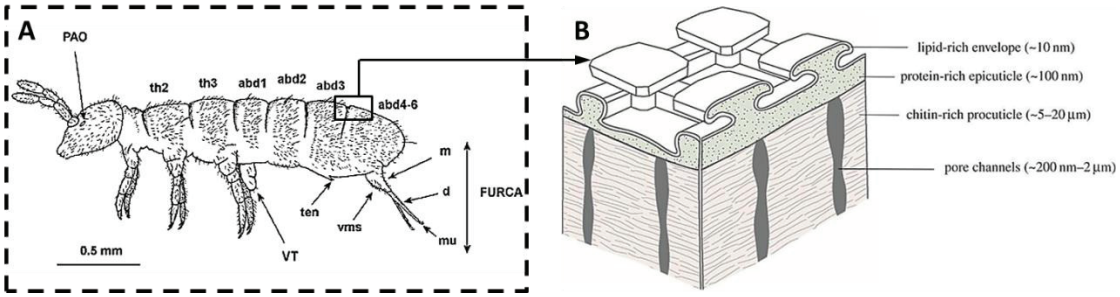


Figure 5. Schematic diagram showing the body structure of adult *F. candida*. **A** (Fountain and Hopkin, 2005): typical structures, including the post-antennal organ (PAO), thoracic segments (th), abdominal segments (abd), ventral manubrial setae (vms), and ventral tube (VT); and **B** (Nickerl *et al.*, 2014): model of the cuticle of Collembola, with multiple layers composed of lipids, proteins, chitins, and other materials.

2.1.3. Aquatic organisms

Organisms used in the aquatic toxicity tests were bioluminescent bacteria *Vibrio fischeri*, fresh water green algae *Raphidocelis subcapitata*, water plants *Lemna minor*, and water fleas *Daphnia magna*. Standard test procedures were used to investigate the effects of LOHCs on the growth of *V. fischeri, R. subcapitata*, and *L. minor* and the mobilization of *D. magna*. These organisms are typically used in laboratory tests of aquatic toxicities. Briefly, the gram-negative bacteria *V. fischeri* are normally found in seawater and freshwater. A short-term bioluminescence inhibition assay (30 min) with the bacteria provides a fast and low-cost screen for toxicity. The algae *R. subcapitata* (**Figure 6 A**), which was formally known as *Pseudokirchneriella subcapitata*, commonly occurs in freshwater. This species has been broadly used in ecotoxicological tests for the effects of chemicals on growth (72 h) and is usually highly sensitive when exposed to herbicides (Ma *et al.*, 2006). *L. minor* (**Figure 6 B**) is a freshwater floating plant that often serves as a biological monitor for toxic substances and domestic/industrial effluents (Radić *et al.*, 2011) based on the inhibitory effects of these substances on their growth (7 days). Unlike algae, *Lemna* represents characteristic of a higher plant. *D. magna* (**Figure 6 C**) is widespread in freshwater and plays an important role in aquatic ecosystems. These animals are widely used as a standard organism for testing the toxicity of chemicals (e.g., towards

the mobilization of the animals for 48 h) and display a high sensitivity to contaminants. These typical organisms were used in an aquatic test scenario to test the toxicity of LOHCs and establish a battery test with a tiered characterization.

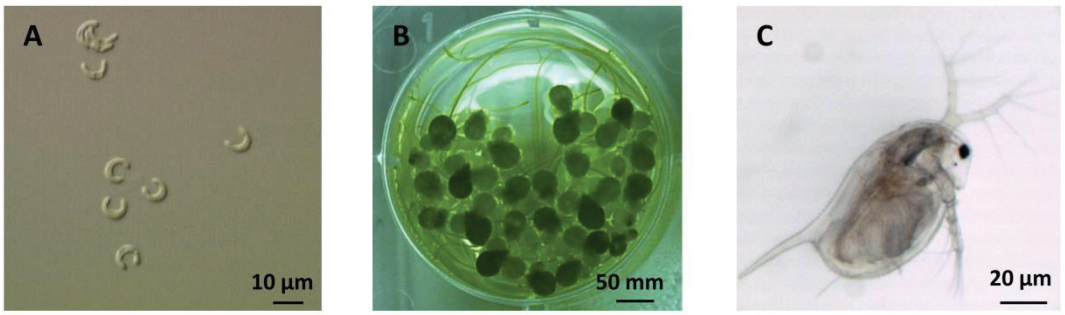


Figure 6. Aquatic organisms. Microscopic or visual observation of **A:** algae *Raphidocelis subcapitata* (magnification × 400); **B:** water plant *Lemna minor*; and **C:** water flea *Daphnia magna* (magnification × 16). Adapted from Zhang et al. (Zhang *et al.*, 2016).

2.2. Adsorption batch equilibrium experiment

The adsorption isotherms were investigated and adsorption modeling of the quinaldines were performed in three main successive steps: i) preliminary tests to identify the appropriate soil/water ratio and adsorption equilibrium time; ii) establishment of adsorption isotherms; and iii) adsorption modeling. Samples were prepared according to OECD guideline 106 (OECD, 2000). Two independent tests performed in triplicate were conducted for both the preliminary and final adsorption isotherm experiment. In each test, additional samples consisting of one control (10 mL mixed solution containing the test substance and 0.01 M $CaCl_2$) and one blank (without the test substance but a mixture of a specific amount of soil and 10 mL 0.01 M $CaCl_2$ solution) were prepared to evaluate the possible loss that could occur from adsorption to the test vessels and operations. The test substrate was pure artificial soil equilibrated with 9 mL 0.01 M $CaCl_2$ for 24 h before it was spiked with the test substances. Detailed descriptions of steps ii) and iii) are included in *subchapter 3.2*, Details of the preliminary tests (i.e., step i)) are given here.

Preliminary tests were performed to determine the soil/liquid ratio and equilibrium time. The soil/liquid ratios were first estimated from the empirical correlation between the ratio and K_d value by considering a preferable ultimate adsorption of 50–80%, which was determined by the relationship established between the two parameters at various percentages of adsorption as provided in OECD guideline 106 (OECD, 2000). The K_d values were calculated from the estimated K_{oc} by using the empirical equation (Eq. (1)):

$$K_{oc} = \frac{K_d}{f_{oc}} \qquad (1)$$

where f_{oc} is 0.0121 and represents the fraction of organic carbon in the artificial soil I (pH ($CaCl_2$) = 5.41, organic carbon 1.21%, sand 76.7%, silt 17.2%, clay 6.1%), and the K_{oc} of each quinaldine was predicted by the Estimation Programs Interface (EPI) Suite™ v4.1. Three soil/liquid ratios, 1:2, 1:1 and 2:1, were selected as the starting ratios, and the adsorption equilibrium tests were conducted over a period of 48 h. The ratio of 2:1 was then selected for the adsorption kinetics to determine the adsorption equilibrium time. In the kinetic test, a specific amount of quinaldine was applied to achieve the estimated original concentration in the aqueous phase for Quin-2Me, Quin-2Me-pH and Quin-2Me-H10 of 200, 50 and 750 mg L^{-1}, respectively. The test substances in the aqueous solution were recovered and analyzed using the parallel method (OECD, 2000), and the samples were collected sequentially at 2, 4, 24, 48 and 72 h.

An adsorption plateau was not observed for Quin-2Me and Quin-2Me-H10 at the test ratio within the test period. Instead, increased adsorption was found. Moreover, the adsorption of Quin-2Me-pH was so strong that the concentration in the aqueous phase was too low to be instrumentally quantified. Therefore, the test procedure was subsequently modified. A soil/liquid ratio of 1:10 and a prolonged equilibrium time of 6 days were finally used for the ultimate adsorption isotherm test. **Figure 7** shows the procedure of the adsorption batch equilibrium experiment that was ultimately performed to determine the adsorption isotherms and to establish the modeling. Additional details of this procedure can be found in *subchapter 3.2*.

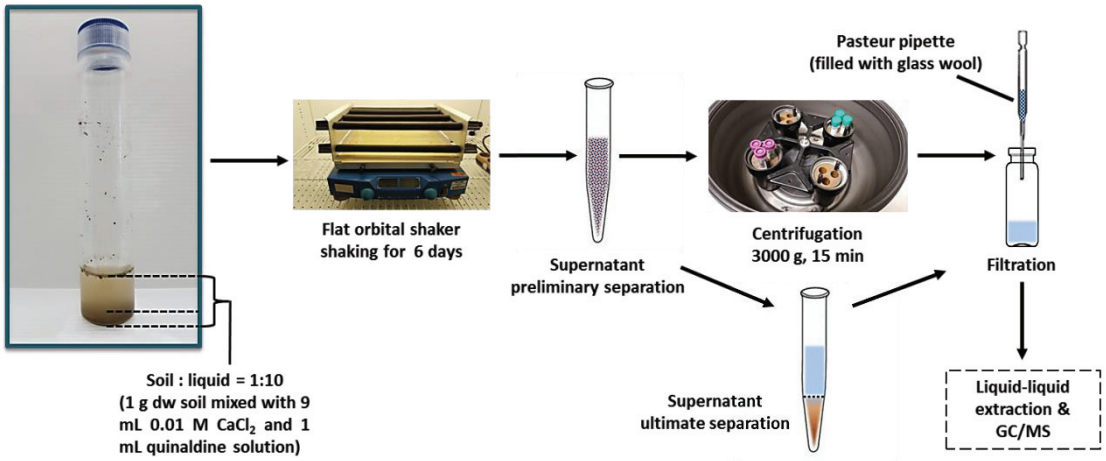


Figure 7. Illustration of the test procedure of the adsorption batch equilibrium experiment. GC/MS: gas chromatography-mass spectrometry.

2.3. Soil column leaching

The soil column leaching experiment was performed following OECD guideline 312 (OECD, 2004b). A more detailed description of this experiment is provided in *subchapter 3.2*. Here, details of the column design and setup are provided (**Figure 8**).

According to the guideline, four carbon steel columns (3.8 cm inner diameter × 35 cm height) with PVC (polyvinylchloride) caps at both ends connected with a steel capillary as the inlet and outlet were constructed. Each PVC cap had an inserted section (the part plugged into the column) of 3.8 cm × 1.15 cm. The inlet and outlet capillaries were filled with glass wool to avoid blockage from soil. Artificial soil II (pH ($CaCl_2$) = 5.33, with organic carbon 0.80% of the same texture as the soil I) was packed stepwise in small portions with a spoon and pressed with a plunger to obtain a packing that was as uniform as possible. Soil was packed until the space between the soil surface and the upper end of the column was approximately 6.5 cm. After equilibrating by artificial rain (0.01 M $CaCl_2$) from the bottom to the top for 1.5–2 h and a drainage period of 2.5 h, a soil vector (20 g) containing the test substances and atrazine (reference substance) was added to the top. This soil vector was prepared one day before by spiked with the test substance prepared in acetone. Atrazine was added to the soil vector directly on the day of packing. The ultimate packed soil was 510 ± 5 g dw in each column, and the ultimate concentration of quinaldine and atrazine in the soil was 100 and 20 mg kg^{-1} dw soil, respectively. The porosity of the packed soil for each column in each test was calculated to evaluate the pore volumes (PV). Finally, the soil surface was covered with a filter paper to obtain an even distribution of artificial rain. Artificial rain was supplied from the top to bottom in drops by a peristaltic pump at a flow rate of 0.108 mL min^{-1}.

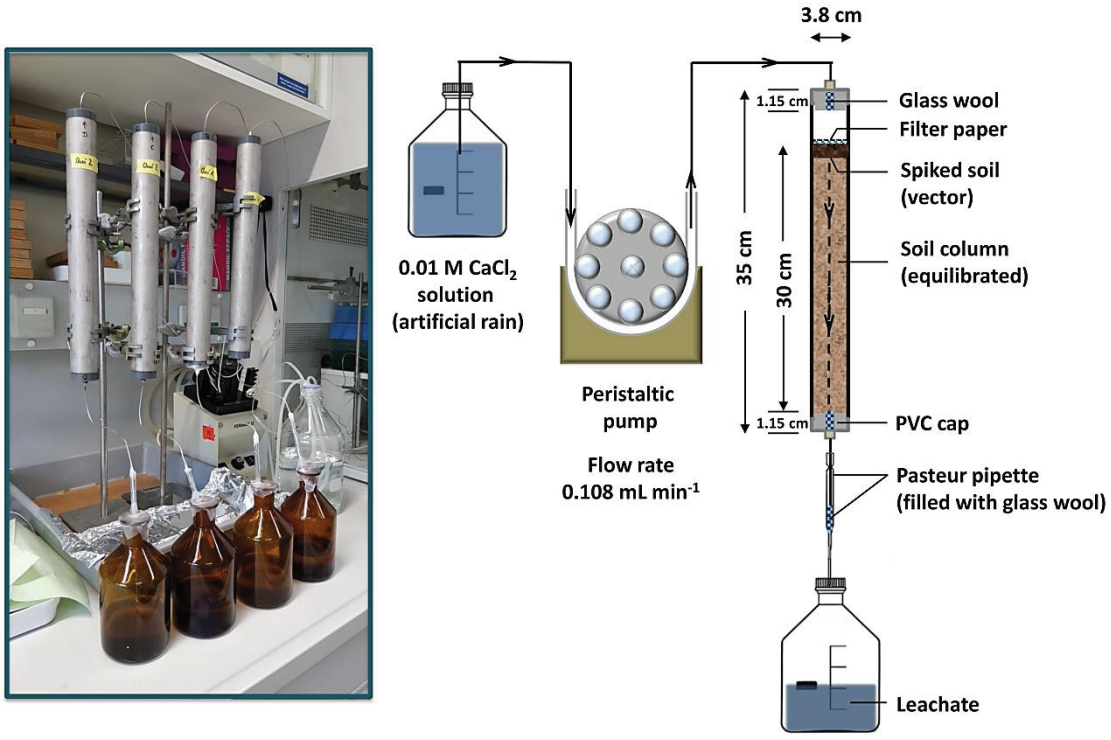


Figure 8. Schematic diagram of the design of the soil column leaching system. The setup consists of the inflows and outflows through a soil column by the aid of a peristaltic pump.

A preliminary test was performed for each quinaldine to estimate the potential leaching time. In the final test, leachates were collected after every 24 h (at the early and mid-stage of each test run) or 48 h (at the later stage) for a period of 172, 648 and 720 days for Quin-2Me-H10, Quin-2Me, and Quin-2Me-pH, respectively. The leachate was first passed through a filter (Pasteur pipette filled with glass wool) and then extracted in duplicate. The content of the test substance was determined by gas chromatography-mass spectrometry (GC/MS).

2.4. Ecotoxicity tests

The soil toxicity of the quinaldine-based LOHC system was investigated with *A. globiformis* and *F. candida* in two test scenarios (without and with soil). More details on these test procedures are available in *subchapter 3.3*. Descriptions of the standard test procedures for aquatic toxicity are available in *subchapter 3.4*. Here, in this subchapter, supplementary information on the soil test procedures with schematic illustrations is provided.

2.4.1 Contact test with *A. globiformis*

Tests were performed based on DIN 38412 L48 (DIN, 2002) and Engelke et al. (Engelke *et al.*, 2014). In the test scenario with soil (**Figure 9**), for each quinaldine, test plates with triplicates for each concentration were prepared. To each well, 0.6 (± 0.02) g of quinaldine spiked soil and 600 µL of sterilized water were added followed by 500 µL of bacterial suspensions ("+" plate). Another set of plates were similarly prepared in parallel, and the bacterial suspensions were substituted by the addition of 500 µL growth medium DMS-B ("-" plate). The "-" plates were used to detect the direct reduction of the dye resazurin by the soil and/or test substance (Engelke *et al.*, 2014). Each test also contained paired plates ("+", "-") of negative controls (uncontaminated soil) and positive controls (benzalkonium chloride of 1000 mg kg^{-1} dw soil). In the test without soil (i.e., pore-water test), the test substrate was replaced by 500 µL of quinaldine solution prepared in sterilized water. Sterilized water was used as the negative control. Paired plates were prepared for all treatments. After the bacteria were inoculated on a shaker (150 min^{-1} at 30 °C) for 2 h and further incubation for 40 min by reacting with resazurin (45 mg L^{-1}), the supernatants were separated by centrifugation (3,000 g for 5 min at 20 °C) and transferred to 96-well plates and analyzed via spectrometry to determine the cell density.

Dehydrogenase activity, which indicates the survival of the bacteria, was determined by the decreased optical density of resazurin that was reduced to resorufin. The inhibition from each concentration was calculated by Eq (2) according to DIN 38412 L48 (DIN, 2002), and survival measurements were then obtained.

$$\text{Inhibition } [\%] = 100 - \left[\frac{(OD_{QB} - OD_Q)}{(OD_{NB} - OD_N)} \times 100\right] \quad (2)$$

where:

OD_{QB}: optical density of the sample with quinaldine but without bacteria,
OD_Q: optical density of the sample with quinaldine and with bacteria,
OD_{NB}: optical density of the negative control without bacteria,
OD_N: optical density of the negative control and with bacteria.

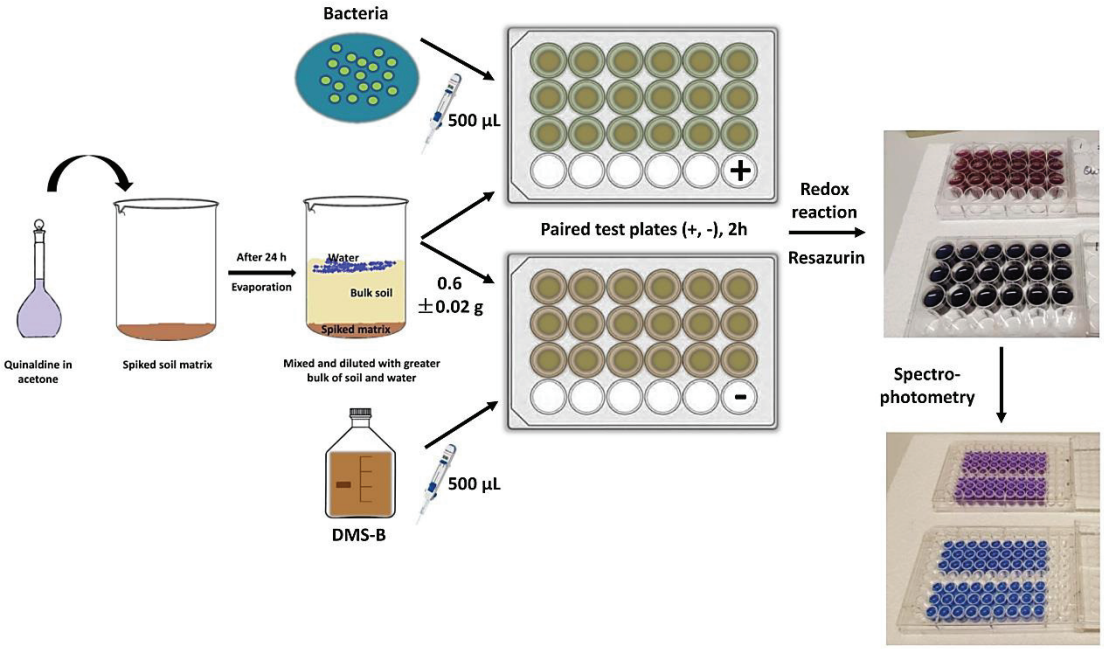


Figure 9. Illustration of the contact test with bacteria *A. globiformis* (2 h) in the soil. The test procedure included soil spiking, preparation of the paired test plate, and spectrophotometric measurements. The pore-water exposure test (without soil) was similarly designed with the test soil replaced by the test solution.

2.4.2 Ecotoxicity test with *F. candida*

The inhibitory effect of the quinaldines on the reproduction and survival of Collembola was assessed in the test scenario with soil (**Figure 10**) (following OECD guideline 232 (OECD, 2009b) and a miniaturized method (Filser, Wiegmann and Schröder, 2014)) and without soil (pore-water exposure scenario (Houx *et al.*, 1996)) (**Figure 11**). These procedures are described in detail in *subchapter 3.3*. The experimental design and procedure in each of the test scenarios are illustrated here as auxiliary instructions.

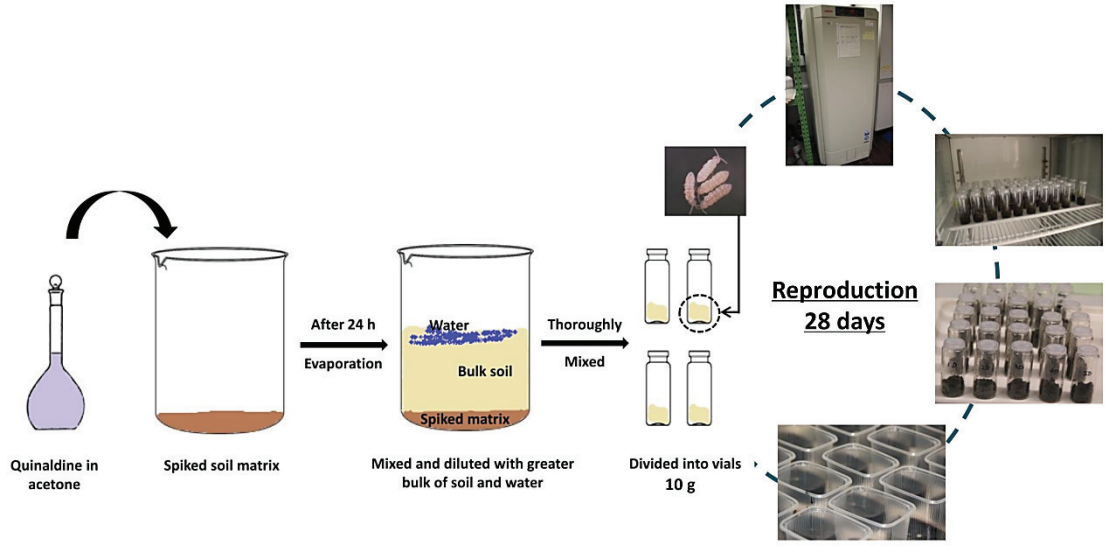


Figure 10. Illustration of the ecotoxicological test of the quinaldines on the reproduction (28 days) of *F. candida* in test soil. The test procedure included soil spiking, exposure of the Collembola, condition-controlled reproduction, and Collembola extraction.

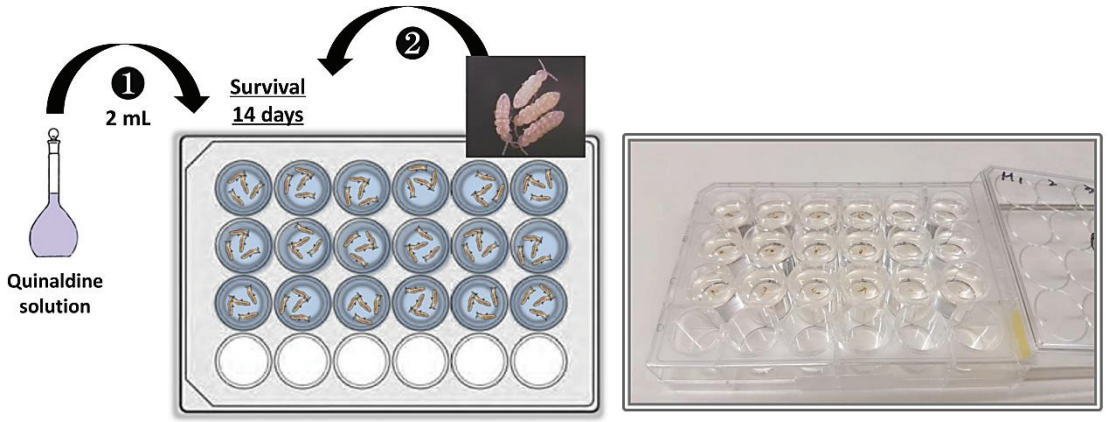


Figure 11. Illustration of the ecotoxicological test of the quinaldines on the survival (14 days) of *F. candida* in pore-water exposure (scenario without soil). The test procedure included the preparation of test solutions and exposure of Collembola adults.

2.4.3 Aquatic toxicity test

The quinaldine- and carbazole-based LOHC systems were investigated for aquatic toxicity with the luminescent bacteria *V. fischeri* (growth, 30 min), fresh water algae *R. subcapitata* (growth, 72 h), water plants *L. minor* (growth rate, 7 days) and water fleas *D. magna* (immobility, 48 h). Detailed procedures are described in *subchapter 3.4*.

2.5. Extraction methods

Two extraction procedures were applied in this thesis for the samples that originated from liquids and soils. A detailed description of the GC/MS analysis of all the extracts is available in *subchapters 3.2 and 3.3*.

2.5.1 Liquid-liquid extraction system

A liquid-liquid extraction system was used to extract samples with a liquid origin. In this thesis, the system was applied to i) extract samples collected from the liquid phase that were separated in the adsorption batch equilibrium experiment, ii) extract leachates collected in the soil column leaching test, and iii) determine the concentration of samples obtained from ecotoxicological tests performed in the exposure scenario without soil. Details of the experimental steps are available in *subchapters 3.2 and 3.3*. A schematic of the setup is presented in **Figure 12**.

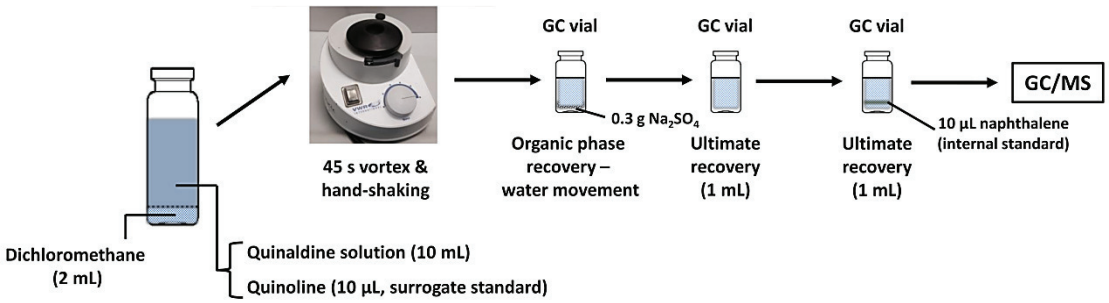


Figure 12. Schematic diagram of the liquid-liquid extraction system. The extraction procedure consists of the preparation of a biphasic solution, separation of the organic phase, water removal, and internal standard (naphthalene) spiking. Prepared extracts were measured by GC/MS analysis.

2.5.2 Soil extraction

Soil samples were extracted by double-extraction using an ultrasonic bath in ice water. This setup was mainly based on EPA guideline 3550c (U. S. Environmental Protection Agency, 2007). The samples obtained from the ecotoxicological tests in soil were extracted using this procedure for concentration determination. Quality controls (test substance directly diluted in dichloromethane (DCM)) and blanks (pure soil mixed with DCM) were tested in parallel. Detailed descriptions of these procedures can be found in *subchapter 3.3*. **Figure 13** illustrates the extraction setup to provide a better understanding of the procedure.

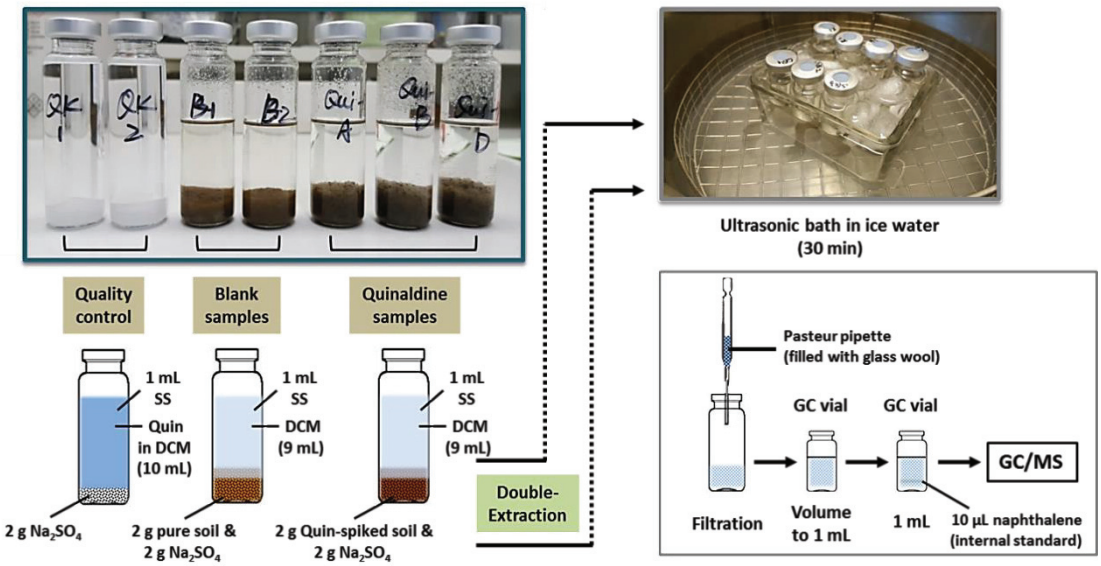


Figure 13. Double-extraction by ultrasonic bath in ice water for the soil samples. The samples included the quality control, blank control and ecotoxicological test samples. Each sample was extracted twice in dichloromethane (DCM) followed by filtration, internal standard (naphthalene) spiking, and GC/MS analysis (shown in rectangle). SS: surrogate standard (quinoline).

Chapter-III Publications and Manuscripts

3. Publications and Manuscripts

3.1. Manuscript 1

Published: Markiewicz, M., **Zhang, Y. Q.**, Bösmann, A., Brückner, N., Thöming, J., Wasserscheid, P. and Stolte, S. (2015) '**Environmental and health impact assessment of Liquid Organic Hydrogen Carrier (LOHC) systems – challenges and preliminary results**', *Energy Environ. Sci.*, 8(3), pp. 1035–1045. doi: 10.1039/C4EE03528C.

Impact factor: 29.5 (2016 Journal Citation Reports® (Clarivate Analytics, June 2017))

Page: 40–64

Contributions of Zhang, Y. Q.:

- Support in developing the research questions and experimental planning;
- Support in literature review; and
- Support in writing of the manuscript and (peer review) revisions (regarding structure, outline, interpretation of results and deriving conclusions).

The rest of the manuscript was prepared by the co-authors.

Environmental and health impact assessment of Liquid Organic Hydrogen Carrier (LOHC) systems – challenges and preliminary results‡

Marta Markiewicz[a]*, Ya-Qi Zhang[a], Andreas Bösmann[b], Nicole Brückner[b], Jorg Thöming[a], Peter Wasserscheid[b], and Stefan Stolte[a, c]

[a] UFT – Centre for Environmental Research and Technology, University of Bremen, Leobener Straße, D-28359 Bremen, Germany.

[b] Institute of Chemical Reaction Engineering, Friedrich Alexander University of Erlangen Nürnberg, Egerlandstraße 3, 91058 Erlangen, Germany.

[c] Department of Environmental Analysis, Faculty of Chemistry, University of Gdańsk, ul. Sobieskiego 18, 80-952 Gdańsk, Poland.

Abstract

Liquid Organic Hydrogen Carrier (LOHC) systems offer a very attractive way to store and transport hydrogen, a technical feature that is highly desirable to link unsteady energy production from renewables with the vision of a sustainable, CO_2-free, hydrogen-based energy system. LOHCs can be charged and discharged with considerable amounts of hydrogen in cyclic, catalytic hydrogenation and dehydrogenation processes. As their physico-chemical properties are very similar to diesel, today's infrastructure for liquid fuels can be used for their handling thus greatly facilitating the step-wise transition from today's fossil system to a CO_2 emission free energy supply for both, stationary and mobile applications. However, for a broader application of these liquids it is mandatory to study in addition to their technical performance also their potential impact on the environment and human health. This paper presents the first account on the toxicological profile of some potential LOHC structures. Moreover, it documents the importance of an early integration of hazard assessment in technology development and reveals for the specific case of LOHC structures the need for additional research in order to overcome some challenges in the hazard assessment for these liquids.

Broader context

Due to increasing environmental awareness, many countries try to optimize their economies for a low-carbon growth turning towards renewable energy sources.

Nevertheless, to fully exploit these sources fundamental change in our energy supplies is needed. Hydrogen is considered a main player in future energy systems, especially for mobile applications but its storage poses a technological challenge. Liquid Organic Hydrogen Carrier (LOHC) systems offer a very attractive way to store and transport hydrogen that links unsteady energy production from renewables with the vision of a sustainable, CO_2-free, hydrogen-based energy system. LOHCs can be charged and discharged with considerable amounts of hydrogen in cyclic, catalytic hydrogenation and dehydrogenation processes. As their physico-chemical properties are very similar to those of diesel, today's infrastructure for liquid fuels can be used for their handling thus greatly facilitating the step-wise transition from today's fossil system to a CO_2 emission free energy supply for both, stationary and mobile applications. However, for a broader application of these liquids it is mandatory to study in addition to their technical performance also their potential impact on the environment and human health.

Introduction

Modern societies depend on steady and reliable supply of energy.[1,2] Due to the increasing environmental awareness, many countries try to optimize their economies for a low-carbon growth, i.e., a growth that happens without a major increase in CO_2 emissions, e.g., without burning additional fossil fuels. Currently, with its *Roadmap 2050*, the European Union has set new long-term goals in energy policy including an 80% reduction of domestic CO_2 emissions.[3] To comply with these goals a fundamental change in our energy system is needed.

The amount of energy that can be harvested from renewable sources, such as sun, wind and hydropower is extremely high. It can satisfy the global energy demand over hundred times with the enormous benefit of being inexhaustible.[4,5] Energy from these renewable sources has many socio-economic advantages over fossil fuel or nuclear based energy: (a) zero or very low variable costs of generation; (b) lower environmental impact since there are almost no emissions and no waste production associated with the power generation; (c) applicability for decentralized power generation.[4]

However, some major technical challenges remain to be addressed before the full transition to a renewable-based energy system can take place successfully:

- The production of most renewable energies is geographically limited, dependent on unforeseeable weather conditions and intermittent; even though in virtually every location on the globe some kind of sustainable energy can be produced it usually does not meet the spatiotemporal demand;[5,6]
- Energy systems with a high share of energy from wind or sun are characterised by periods in time when overproduction of energy from renewable resources causes very low or negative stock energy prices;[7–9]

- Renewable energies are currently mostly used to power stationary consumers to which they are transported via the electric grid; their use in mobile applications, though extensively researched and certainly very relevant,[10–12] is still far less advanced.

Hydrogen is considered as a main player in future energy systems, especially for mobile applications as it is a clean fuel of very high gravimetric energy density (120 MJ kg^{-1}). Its gravimetric energy density is three times higher than that of gasoline and any other liquid fuel.[4,13] Furthermore, hydrogen powered cars using a fuel cell have efficiencies of energy conversion of 50–60%, much higher than today's cars using fossil fuels and an internal combustion engine where maximum efficiencies of about 25% are reported.[8,14,15] In order to use hydrogen as a fuel for vehicles an on-board storage system is required that contains suitable amounts of hydrogen. Note that a medium size vehicle needs between 0.8 and 1 kg H_2 per 100 km. The hydrogen storage system should be light, compact and safe. Moreover, the system should allow a dynamic hydrogen release on demand and a fast H_2 filling of the storage system without the need for specific or new infrastructure.

Given these very tough requirements it is important to state that the use of hydrogen in a future energy systems is by far not restricted to its use in cars and trucks. Many other mobile (e.g., forklifts), transportable (e.g., portable electronics), and stationary applications (stand-alone energy systems, back-up systems) are discussed and attract high commercial interest. Apart from using hydrogen in fuel cells there remains the option to burn hydrogen in a combustion engine. Using lean-hydrogen mixtures at not too high temperatures (to disfavour formation of NO_x) may also lead to more sustainable energy processes at somewhat lower investment cost and higher technical robustness.

While the gravimetric energy storage density of hydrogen is excellent its volumetric storage density suffers from the very low H_2 density. Under ambient conditions one liter of gaseous hydrogen contains only 10.8 kJ of energy. Even under very high pressures (70 MPa H_2, called “Compressed Gaseous Hydrogen” or CGH2) or in its liquid state which requires temperatures below 20 K (called “Liquid Hydrogen” or LH2) the volumetric energy density of hydrogen is low. Liquid hydrogen has a density of 71.2 kg m^{-3} resulting in an energy storage capacity under these very challenging conditions of 8.3 MJ L^{-1} which is by a factor of four lower than the volumetric storage density of typical fuels under ambient conditions. Note that the compression of hydrogen and especially the cooling of hydrogen are energy intensive and costly operations and that hydrogen losses by diffusion (high pressure storage) or by boil-off (cryogenic hydrogen storage) may lead to relevant hydrogen/energy losses. Under current technologically feasible conditions CGH2 storage uses around 15% of stored energy to achieve 70 MPa compression and LH2 as much as 30% for liquefaction (based on lower heating value of H_2 of 120 MJ kg^{-1}).[16] Other disadvantages result from time consuming loading and unloading procedures (mainly for LH2) and the need for specific infrastructures.[17]

So far several technical options have been proposed to store and transport hydrogen in a more economic and efficient manner. These include for example physical sorption on high surface area materials (e.g., nanostructured materials like active carbon) or chemical adsorption to solids leading to solid hydride materials.[12,14,18] When using hydrides there is always a trade-off between storage capacity and hydrogen desorption temperature, however, recent development especially regarding doped alanates brought these materials closer to fulfilling current hydrogen technology requirements.[19,20] However, these solutions have a number of severe drawbacks with respect to their practicability: apart from limited hydrogen carrying capacity and time consuming loading/unloading procedures with the significant heat production/heat demand, the handling of solids is impractical for the storage and transport of larger amounts of energy.

The catalytic hydrogenation of hydrogen-lean molecules offers another option to store and transport energy in the form of hydrogen. Nitrogen can be hydrogenated to ammonia,[21] CO_2 can be hydrognated to either formic acid,[22] methane,[23] methanol,[24] or Fischer–Tropsch products.[25] However, all these options have one thing in common, they use gaseous substances as energy-lean molecules and as a consequence they require isolation of CO_2 or N_2 from air or exhaust gas streams in appropriate quality and quantity for the hydrogen storage process and they release mixtures of hydrogen and the hydrogen-lean gas during dehydrogenation reaction instead of pure hydrogen.

This important drawback is circumvented if organic liquids of low vapour pressure are used as hydrogen-lean compounds, a concept for which the name “Liquid Organic Hydrogen Carrier (LOHC)” has been coined. Starting from pioneering work in the 1990s, later patents by Pez, Scott, Copper and Cheng from Air Products[26,27] and continuous intensive research in the last couple of years,[28] LOHC systems have developed to a very promising technology for hydrogen storage and transport. LOHC systems are formed by pairs of organic compounds, the hydrogen-lean one being typically an aromatic or heteroaromatic compound, the other hydrogen-rich one being typically an alicyclic or heterocyclic compound.[26,29–32] LOHCs are loaded with hydrogen in analogy to large scale catalytic hydrogenation reactions of the chemical industry. Typical reaction conditions for the exothermic LOHC hydrogenation are hydrogen pressures of 1 to 5 MPa and temperatures of 373 to 523 K. Typical hydrogenation catalysts are Ni- or Ru on oxide supports applied in slurry phase tank reactors or trickle bed hydrogenation units.[33,34] Typical reaction conditions for the endothermic LOHC dehydrogenation are hydrogen pressures of 1 to 0.5 MPa and temperatures of 423 to 673 K. Typical dehydrogenation catalysts are Pt or Pd on oxide supports applied in slurry phase tanks or tubular reactors.[35,36]

In the context of the storage and transport of renewable energy equivalents, excess renewable electric energy is converted into high pressure hydrogen by electrolysis (typical H_2 pressures of electrolysers are 1 to 5 MPa) and the latter is used directly to hydrogenate the hydrogen-lean form of the LOHC. The hydrogen-charged LOHC can be regarded as a liquid transport form of hydrogen that can be handled in today's infrastructure for liquid fuels (pipelines, oil tanker and petrol stations).[37] Thus,

LOHCs enable long-time energy storage under ambient temperature and pressure conditions without significant losses. On energy or hydrogen demand, the hydrogen-rich LOHC molecule is heated to the dehydrogenation temperature and allowed to be in contact with the dehydrogenation catalyst. Further heat is added to the reactor to deal with the endothermic nature of the dehydrogenation reaction. The hydrogen-lean form of the LOHC-system is isolated by simple condensation from the dehydrogenation reactor together with very pure hydrogen. The hydrogen-lean LOHC compound is stored for its next charging cycle or transported to a place with cheap and available regenerative energy. Figure 3.1.1 illustrates the storage and transport of renewable energy equivalents using LOHC systems.

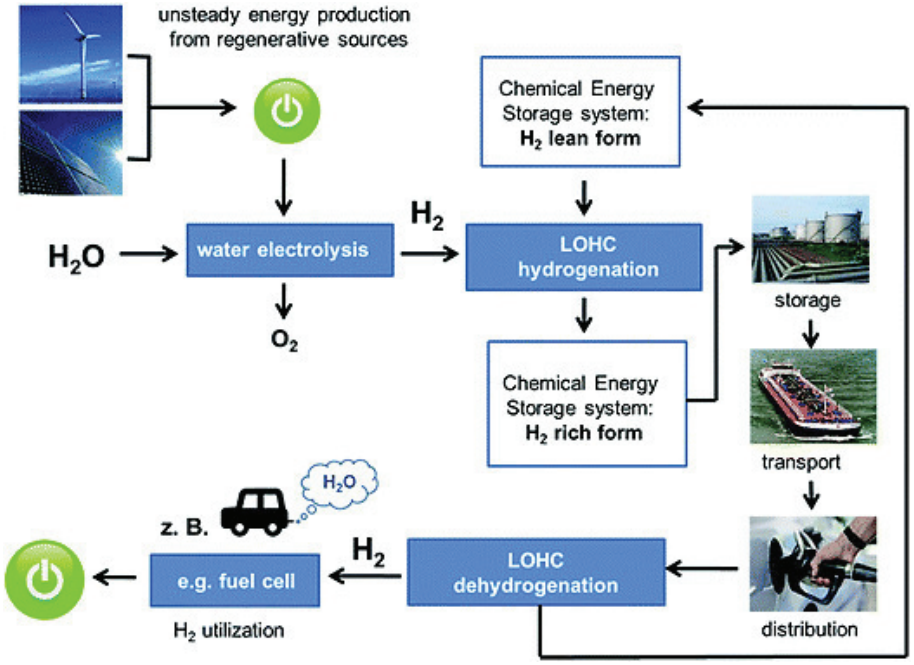


Figure 3.1.1. Schematic view on storage and transport of regenerative energy equivalents using Liquid Organic Hydrogen Carrier (LOHC) systems (reproduced with permission from ref. 38, Copyright American Chemical Society).

Initially one or two six-membered ring compounds like benzene, toluene, naphthalene, biphenyl and their corresponding hydrogenated equivalents cyclohexane, methylcyclohexane, decaline (dodecahydronaphthalene), and bicyclohexyl were suggested as LOHC systems (Figure 3.1.2).[39–41] These compounds have storage capacities between 6 and 7 wt% H_2 and can be hydrogenated under relatively mild conditions.[31] However, some of these compounds are too toxic (e.g., benzene) or too volatile (benzene/cyclohexane, toluene/methylcyclohexane) to be of greater practical relevance, at least so far.[26,40,42] More recently, thiophene, quinaldine and carbazole derivatives were also recommended as LOHC systems as the presence of a hetero atom reduces the heat of hydrogenation/dehydrogenation and thus allows for

dehydrogenation at milder temperatures.[43,44] In particular, *N*-ethylcarbazole (NEC)/perhydro-*N*-ethylcarbazole (H_{12}-NEC) has found a lot of interest as a LOHC system due to its relatively high H_2 storage capacity (5.8 wt% H_2) and its good dehydrogenation characteristics at 453–533 K (ambient pressure, heterogeneous Pd- or Pt catalyst).[26,30,37] The hydrogen-lean form, NEC is a solid with a melting point of 341 K. This is much lower than the melting point of carbazole (mp. = 518 K) but still not ideal. The solid nature of the fully dehydrogenated molecules complicates the technical use of this LOHC system as either the tank has to be heated to 343 K or the dehydrogenation degree has to be limited to ca. 90% for the mixture of fully and partially dehydrogenated substances to remain liquid. However, this reduces the effective hydrogen capacity to about 5.2 wt%. There is also a risk for NEC dealkylation at temperatures above 533 K. While these conditions are above the normal dehydrogenation conditions they still limit operating the hydrogen release reaction at very high temperature levels and thus much faster which would allow us to use smaller reactors.[38]

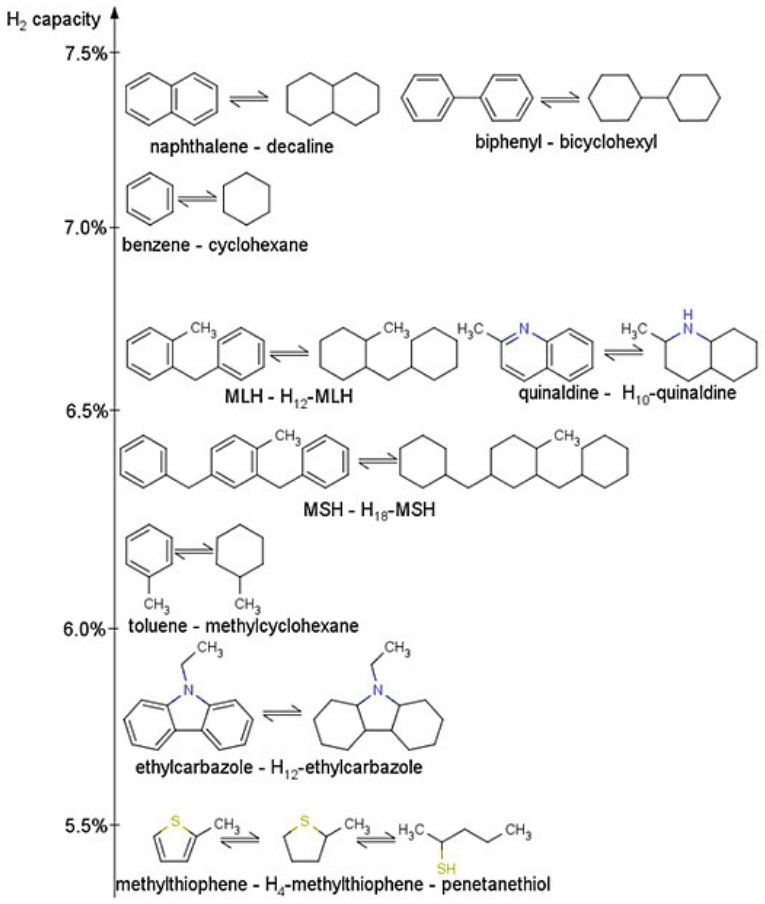


Figure 3.1.2. Examples of LOHCs systems (H_2-lean and H_2-rich forms) and their gravimetric hydrogen carrying capacity.

Recently isomeric mixtures of perhydro-benzyltoluene and perhydro-dibenzyltoluene were also proposed as LOHCs.[38] The hydrogen-lean form of these LOHC systems, benzyltoluene and dibenzyltoluene, are readily available and technically applied as heat transfer oils in the form of their isomeric mixtures. Typical trade names of these substances are Marlotherm LH (MLH, i.e., mixture of benzyltoluenes) or Marlotherm SH (MSH, i.e., mixture of dibenzyltoluenes). Dehydrogenation of the respective hydrogen-charged mixtures, H_{12}-MLH and H_{18}-MSH requires higher temperatures than for the dehydrogenation of H_{12}-NEC (553–633 K for H_{18}-MSH *vs.* 453–533 K for H_{12}-NEC). However, the MSH/H_{18}-MSH system offers low melting points of all relevant mixtures and species (< 243 K), high hydrogen capacity (6.2 wt% H_2), excellent technical availability, and a huge amount of available data concerning thermal stability and heat transfer properties.[38]

All the named compounds can undoubtedly store hydrogen, however, taking into account all possible structural variations of LOHC molecules, the enormous dimension of potential LOHC applications in the energy system and the cost related to implementation of the LOHC technology, industry can only afford to develop a very limited number of the most promising LOHC candidates to a full commercial scale. Therefore intense investigations to limit the set of potential structures to the most promising candidates are in progress.

Apart from technological and economic aspects safety and environmental criteria must also be taken into account for the selection of the most promising LOHC system. The hazard assessment should be performed at the early research and development stages – moving from "end of pipe" solutions (addressing environmental problems after they have manifested themselves) to proactive environmental protection – in order to anticipate and assess the hazards that each involved chemical might pose. This approach might open the chance to focus research and development efforts on such LOHC systems with reduced hazard potential and higher intrinsic safety. Regardless of which of the hundreds of possible LOHC structures will finally make it to the market, it will be handled, processed, stored and transported in vast quantities. An average stationary storage application will need to process 1 kg LOHC material for ca. 7.2 MJ of thermal energy stored in the form of its releasable hydrogen. For potential future mobile applications of the technology a car would need to dehydrogenate between 12 and 20 litres of LOHC material per 100 km driving range. Shall these technologies penetrate markets in an extended manner, LOHC chemicals will become high production volume chemicals (HPVC) that are globally used by the public with potential release into the biosphere, for example, during fuelling, via leakages or in accidental spills. In recent decades the extensive production, use and release of man-made chemicals have resulted in serious environmental problems and have raised public awareness of the hazards arising from chemical substances and technologies in general. Therefore especially HPVC are subjected to strict health/environmental regulations such as the European Union Regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). Generally, the public opinion and acceptance is of highest importance

when implementing new technologies – as the controversy concerning risk of hydraulic fracturing currently shows.[45]

Therefore this paper aims to demonstrate an approach for the early integration of hazard assessment into the development of the LOHC technology. First data give indications of possible hazards for some potential LOHC structures. More important we want to address the need for research and the challenges that have to be faced when assessing the environmental impact of LOHC systems. Hereby, we hope to encourage further research in this important field which will help to facilitate the LOHC selection on technological, economic and environmental grounds to provide the base for a broad public acceptance of this highly promising hydrogen storage and transport technology with the potential to contribute to a CO_2-free energy system.

Short introduction to risk assessment

In order to protect human health and the environment, chemicals produced or imported into European Union in quantities higher than 1 ton per year have to be subjected to REACH. REACH, depending on the production volume, requires varying extents of information on identity, physicochemical properties, mammalian toxicity, ecotoxicity, environmental fate (including biotic and abiotic degradation), manufacturing and applications which are used for the assessment of risk associated with chemicals. In risk assessment there are two important building blocks: hazard and release/exposure. In simple words risk assessment is based on identifying harmful effects that can be exerted by the chemical substance (hazard) and assessing the likelihood of these effects to occur based on predicted release (exposure). Figure 3.1.3 shows a simplified scheme of assessment of chemicals.

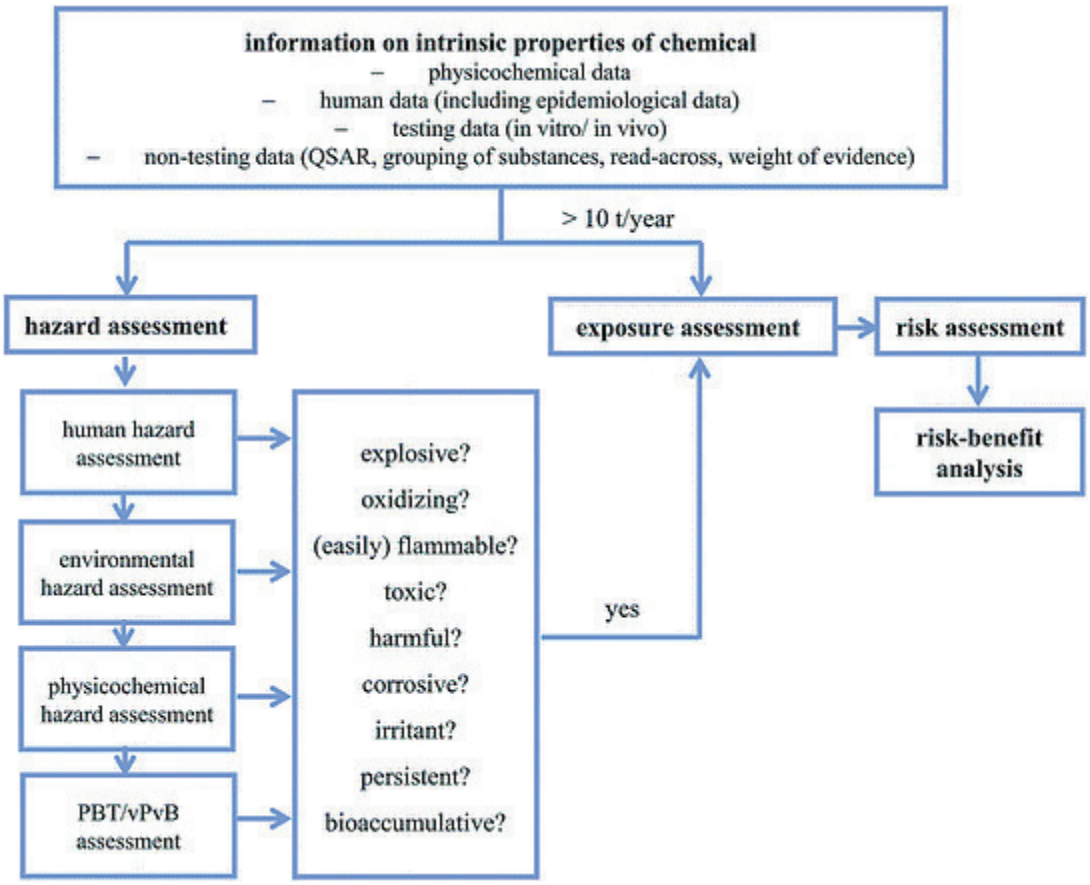


Figure 3.1.3. Simplified flowchart for chemical's risk assessment.

For a chemical to be recognized as environmentally safe it should be biodegradable, non-toxic and non-accumulative so that it can be assured that whenever it is released it will breakdown quickly to non-harmful products and will not persist in any of the environmental compartments including living organisms. In order to make that declaration possible it has to be made sure, at the very least, that the substance does not fulfil Persistent, Bioaccumaulative, Toxic/very Persistent, very Bioaccumaulative, Toxic (PBT/vPvBT) or Carcinogenic Mutagenic Toxic for Reproduction (CMR) criteria and does not act as an endocrine disrupter as those are the substances of particular concern.

When assessing the risk associated with chemicals we are most often interested in effects on humans and on the environment. Since it is not possible to test the effect of the chemicals in question directly on humans as well as under every relevant environmental condition some kind of model or approximation has to be made. The more distant this model is from the subject of interest the more uncertainty it carries. Therefore defining the risks associated with chemicals is always affected by uncertainties resulting mainly from the difficulty in extrapolating from model test results to real subjects. Note that the assessment of known pollutants, like the “dirty dozen” (twelve chemicals recognised in 2001 by United Nations Stockholm

Convention as persistent organic pollutants of particular concern) is easier due to the fact that historical or epidemiological data for those compounds exist together with a multitude of model tests conducted under different conditions. The risk assessment of "new chemicals", that were or are not regularly detected in the environment is challenging. No historical data and often limited model test data are available and the assessment has to be started from scratch by e.g., numerical models (Quantitative Structure-Activity Relationship QSAR) which – though very useful – result in a greater degree of uncertainty. The results obtained by QSARs can act as indicators or guidelines but have to be verified.

Specific challenges when assessing risks associated with LOHC systems

The aim of performing e.g., the ecotoxicological test with model organisms is to reduce the uncertainty factor in risk assessment by indicating hazards, possible modes of actions and organisms which might be particularly prone to the action of the chemical under investigation.

Even though some potential LOHC chemicals suggested in the literature are relatively common organic compounds, the amount of data that are needed for their risk/fate assessment is scarce. There is a strong need not only for basic ecotoxicological parameters but also for information as simple as the solubility in water or the octanol/water partition coefficient. As most LOHC structures suggested so far are organic, uncharged chemicals, they are somehow volatile (with a technical tendency for using representatives of relatively low vapour pressure to allow easy hydrogen/LOHC separation), will have an affinity to organic phases (i.e., organic matter, biological membranes *etc.*) and their aqueous solubility will be somehow limited.

(1) On structural variability. Some chemicals are used as technical mixtures of several up to hundreds different compounds containing various impurities – the best example being crude oil derived fuels. This is – on a much lower level of structural complexity – also the case for the recently reported LOHC systems that use isomeric mixtures of e.g., dibenzyltoluenes. The presence of different regioisomers in the dibenzyltoluene mixture reduces greatly the melting point (down to 239 K for the hydrogen-lean mixture) but complicates toxicological evaluation of the mixture especially in combination with the extremely low water solubility of these compounds (Figure 3.1.4). It is possible that some of the structural forms have significantly higher environmental/health impacts than the others. Luckily, due to the constant large-scale production process from small building blocks the isomer mixture does not change significantly from batch-to-batch. This makes the issue of structural variability easier to handle than in e.g., the case of crude oil based fuels where crude oil from different origins is known to result in very different structural compositions of the fuel.

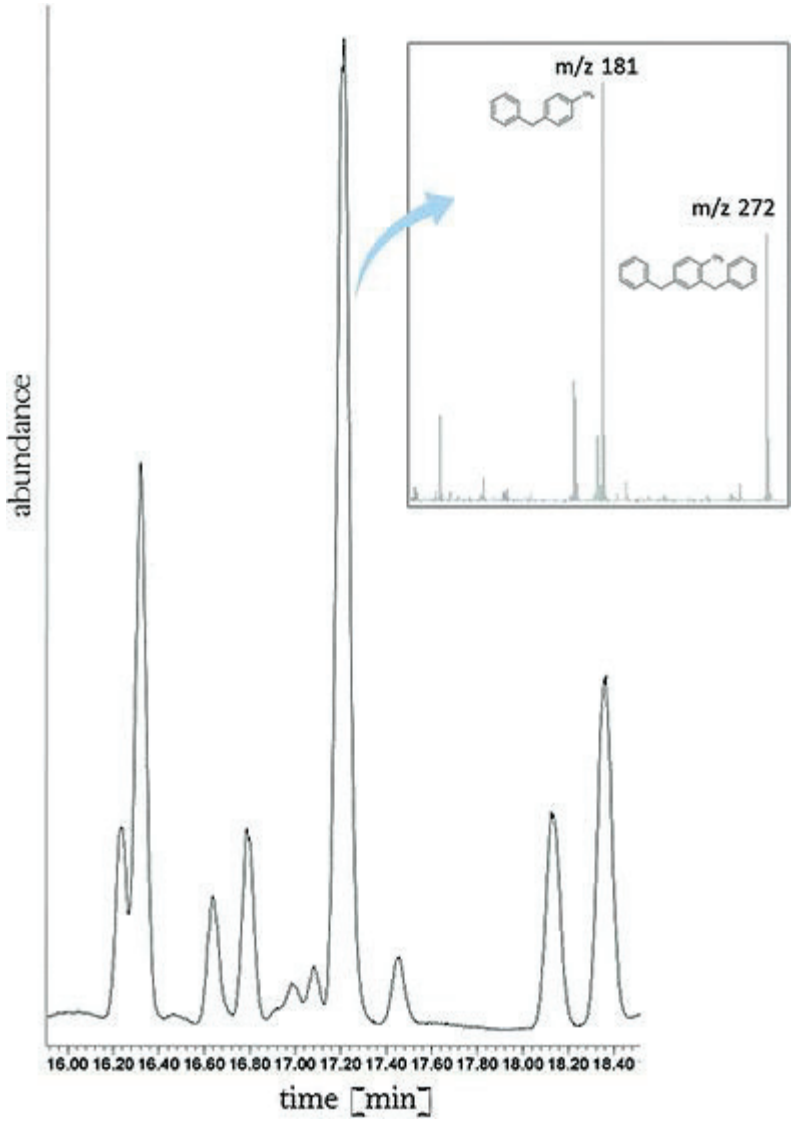


Figure 3.1.4. GC/MS spectrum of a commercial mixture of dibenzyltoluenes marketed as heat transfer oil under commercial trade names, e.g., Marlotherm SH; spectrum was obtained in Total Ion Current (TIC) mode and shows exemplary the molecular ion *m/z* 272 and the base ion *m/z* 181.

In all future scenarios of LOHC application, the LOHC molecules are expected to be produced in technical quantities and technical qualities. This means that the LOHC systems will also include some amounts of contaminants. Apart from impurities in the starting material, the working LOHC systems will always contain a mixture of hydrogenated, partially hydrogenated and hydrogen-lean compounds depending on the degree of hydrogen-loading.

For example, our initial examination of the LOHC system NEC/H_{12}-NEC revealed that octahydro compounds are dominant in partially dehydrogenated mixtures but some small amounts of the hexahydro form are also present (Figure 3.1.5). It is worth noting that the physicochemical properties of those two forms are quite different, as an example the K_{ow} of octahydro-*N*-ethylcarbazole is approximately one order of

magnitude lower than K_{ow} of hexahydro-*N*-ethylcarbazole. Even though most reported LOHC systems show very high stability under operation conditions, the fact that they are meant to be recycled many times in hydrogen charging and uncharging cycles suggests that some degree of breakdown or aging should be expected. This effect can be simulated by catalytic dehydrogenation under very harsh temperature conditions (> 543 K) for the NEC/H_{12}-NEC system and leads to NEC dealkylation to carbazole. The properties of all those diverse structures in the LOHC system can be very different therefore they should all actually be assessed as separate entities or mixtures prior to a large scale use of these systems.

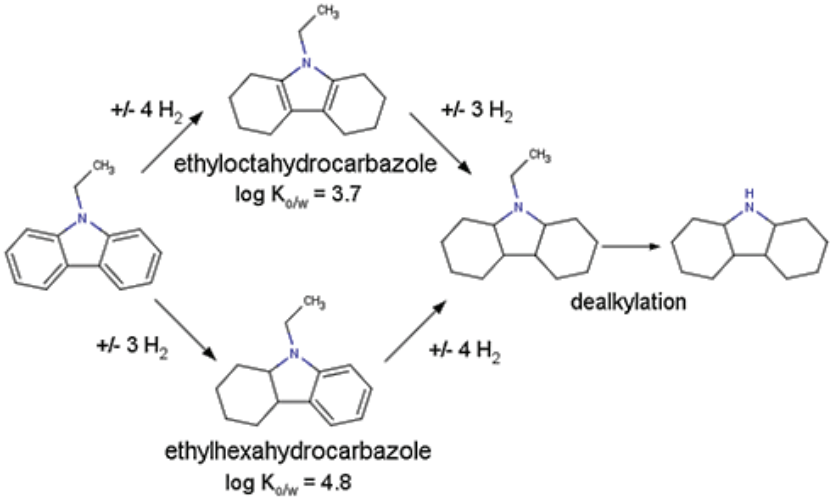


Figure 3.1.5. Hydrogenation/dehydrogenation cycle of *N*-ethylcarbazole (NEC) to perhydro-*N*-ethylcarbazole (H_{12}-NEC) through the two intermediates octahydro-*N*-ethylcarbazole (dominant) and hexahydro-*N*-ethylcarbazol (minor intermediate); on the very right carbazole is shown as the product of undesired dealkylation observable during catalytic dehydrogenation under very harsh temperature conditions (> 543 K).

(2) Solubility and partitioning. From the (eco)toxicological testing perspective substances having low water solubility and high octanol water partition coefficient are particularly difficult to deal with experimentally. The main reason for this is the inability to maintain constant concentration throughout the whole period of the test. If water solubility is not known it is impossible to make test solution by direct weighing. Even if solubility in pure water is known this information can only be taken as indication as in real aqueous environments, at the very least, some inorganic salts are present that would influence (reduce) the solubility. Of course the preparation of test solutions at maximum saturation is possible by either using the generator column method[46] or by "loading" biphasic systems. However, in these cases it is to be expected that some portion of the test compound will be adsorbed on the test vessel (depending on the material used) causing a decrease in real concentration and

resulting in an unknown bioavailable concentration.[47]

Due to these complications it is necessary to confirm in tests with poorly water soluble compounds the real concentration that was available during the toxicology test. This very often, and most certainly in the case of some of the proposed LOHC structures, requires sophisticated analytics as the nominal concentrations in the test can be in the μg L^{-1} to ng L^{-1} range (predicted aqueous solubility of H_{18}-MSH is in ng L^{-1} range[48]) and become even lower as the result of sorption. An order of magnitude differences depending on composition of medium can be expected as shown in our results with the NEC/H_{12}-NEC system using different contact times and different biological assays (Figure 3.1.6).

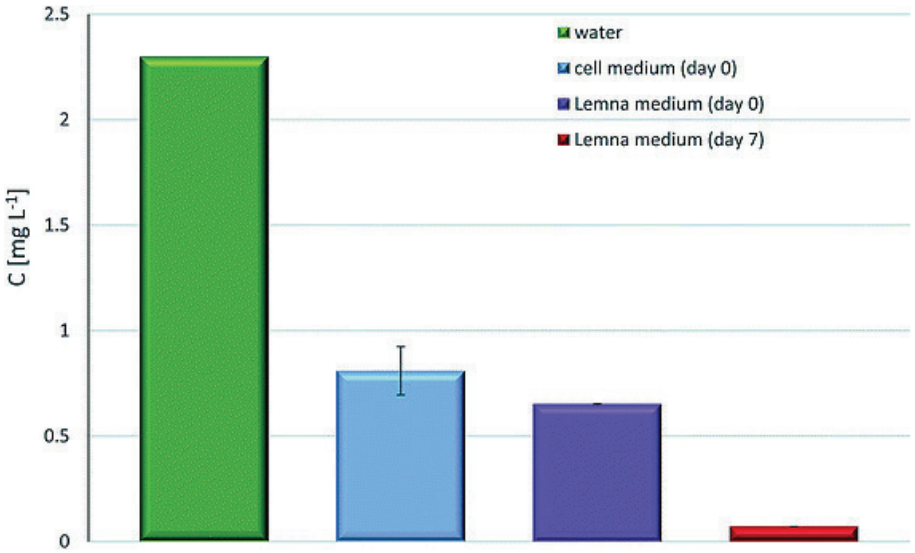


Figure 3.1.6. Variability in concentration of *N*-ethylcarbazole in different aqueous test media: water solubility (estimated from fragments), experimentally measured solubility in *Lemna minor* (duckweed) – test medium at the beginning and the end of the test, medium used for cytotoxicity testing with rat leukemia cell line containing 1% of DMSO as co-solvent.

The environmental distribution should be taken into account at the very beginning of hazard assessment as it defines the possible routes of release and exposure. Substances that are well water soluble and non-volatile e.g., inorganic salts will be predominantly released with water streams and will remain dissolved to a large extent. Their main route of exposure will be via water and to some extent diet. Substances that are poorly water soluble e.g., neutral, organic compounds like LOHCs compounds, might be released with water streams but after that they will most probably find some kind of a sink (e.g., sediments) and become adsorbed which can decrease their bioavailability. Their main route of exposure will be via diet and the exposure via water will be of limited importance. Considering distribution will therefore be of importance in selecting the most meaningful tests and most realistic

routes of exposure.

The main route of exposure in acute aquatic toxicity tests is passive diffusion through integumentum. Therefore only the fraction of test compounds that is truly dissolved in water can exert the toxic effect in this way.[49] On the other hand higher K_{ow} results in higher affinity to hydrophobic phases, including biological membranes, and implies higher toxicity. Many potential LOHCs can be classified as poorly water soluble based on predictions as for most of them basic physicochemical properties like aqueous solubility or K_{ow} are missing.

In extreme cases the aqueous solubility might be so low that the highest concentration which can be obtained in water/medium is too low for any acute toxic effects to be observed, so that it is not possible to obtain a full dose–response curve and derive an half maximal effective concentration (EC_{50}) value. In fact for some LOHCs solubility can hardly be measured as in the case of H_{18}-MSH whose predicted aqueous solubility lies in low ng L^{-1} range.[48] From this extremely low water solubility it does not necessarily follow, however, that the compound is not toxic. For very hydrophobic compounds (log $K_{ow} > 5$) chronic toxicity cannot be excluded even if there are no observable effects in acute tests as the compound might not have been sufficiently taken up by the test organism during the test duration. As they might be accumulating in living organisms these types of compounds have to be investigated for chronic effects. It is conceivable that poorly soluble compounds can form biphasic systems in the environment like e.g., oil spills forming droplets or layers on the surface of water. It is also possible that during prolonged exposure the compound will be concentrated in hydrophobic phases of living organisms (like fatty tissue, biological membranes) as a result of partitioning or will be ingested as droplets or in the particle bound form (sorbed on humic matter or biomass on which they feed) and build up in the body. In such a case the amount of compound that acts upon the test subjects might significantly exceed the water solubility limit. Building the concentration up in a longer food chain – biomagnification – might have even more pronounced ecological effects and can influence humans directly. Therefore, it is advisable to include the chronic test or the multi-generations test in addition to acute tests in the toxicity evaluation especially for poorly water soluble substances as the same processes take place in the environment.

(3) Preliminary insights into ecotoxicity and biodegradability of LOHCs. To give a first impression of the ecotoxicity of selected LOHC structures, Figure 3.1.7 shows dose response curves obtained in the acute test with *Daphnia magna* (water flea) for three forms of quinaldine (aromatic: Quin-2Me, partially hydrogenated: Quin-2Me-pH, fully hydrogenated: Quin-2Me-H10).

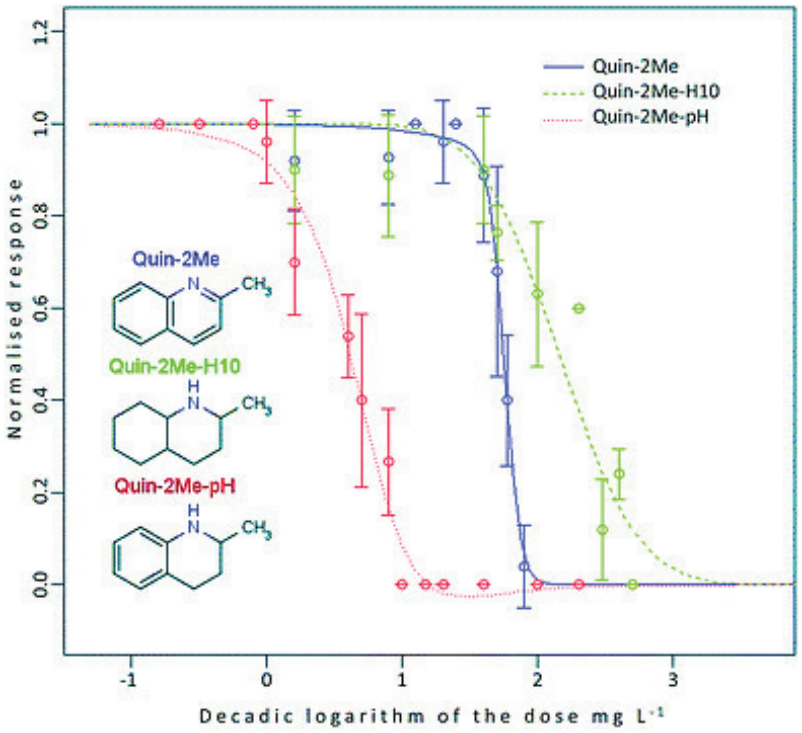


Figure 3.1.7. Dose–response curve of *Daphnia magna* (48 hours test) to fully hydrogenated (Quin-2Me-H10, green line), partially hydrogenated (Quin-2Me-pH, red line) and dehydrogenated (Quin-2Me, blue line) forms of quinaldine.

Table 3.1.1 summarizes EC_{50} values for the same compounds obtained experimentally and predicted using QSAR. What is evident is that EC_{50} values differ by around two orders of magnitude for the different hydrogenated forms indicating differences in their toxicities. Nevertheless in this example the EC_{50}s are rather high (meaning low acute toxicity towards this organism) and in general one to three orders of magnitude higher than a cut-off value of 0.1 mg L^{-1} for classification as 'T'.[50] This is the first indication that those compounds would not be classified as environmentally toxic in PBT assessment based on the Daphnia test. However, tests with algae and/or fish are still necessary to make definite conclusion. Moreover, the QSAR model used for prediction works quite well for aromatic and partially hydrogenated quinaldines but seems to overestimate the toxicity of the fully hydrogenated compound by an order of magnitude. To further illustrate this point, literature EC_{50} values experimentally obtained in the same test system using loadings (water accommodated fraction) of diesel fuel and gasoline are also given.[51] Based on this comparison aromatic (Quin-2Me) and fully hydrogenated (Quin-2Me-H10) forms of quinaldine show toxicity comparable to diesel fuel. The EC_{50} value of the partially hydrogenated form (Quin-2Me-pH) is nearly an order of magnitude lower, which makes it more toxic than the fully H_2 loaded and unloaded forms and approximately as toxic as gasoline. However, none of the compounds listed in Table 3.1.1 has to be classified as "T" based on the results from the *Daphnia magna* test.

Table 3.1.1. Experimental and ECOSAR predicted EC_{50} values (including confidence intervals) for three forms of quinaldine in the acute (48 hours) *Daphnia magna* test. For the sake of comparison experimentally measured EC_{50} values for diesel fuel no. 2 and natural gasoline are also given.

Compound	**Measured EC_{50}** [mg L^{-1}]	**Predicted EC_{50}** [mg L^{-1}]
Quin-2Me	56 (53–59)	17
Quin-2Me-pH	2.7 (2.3–3.2)	5.1
Quin-2Me-H10	204 (155–204)	10
Diesel fuel no. 2[b]	138[a]	n/a
Natural gasoline[c]	4.5[a]	n/a

[a] Source of data: European Chemical Agency.[51]

[b] Diesel fuel no. 2 (CAS 68476-34-6).

[c] Natural gasoline (CAS 8006-61-9), n/a – not available.

As mentioned before another important element of environmental assessment is PBT evaluation which includes biodegradability. This is to make sure that target compounds can be biologically degraded in a reasonable time frame and will not be accumulating in e.g., surface waters. Additional information that can be derived from the biodegradation test is an indication that target compounds can be degraded during standard wastewater treatment. An inoculum used for e.g., ready biodegradability testing is often a diluted microbial community obtained from an activated sludge aeration tank – a core part of wastewater treatment. Therefore positive results of biodegradation testing indicate that removal in the wastewater treatment plant will most probably be possible although no assumptions regarding degradation rates or time frames can directly be made.[52]

There is a set of rules of thumb allowing to "guesstimate" if compounds will be degradable or not. Aromatic forms of LOHC structures presented here generally do not possess structural features that are considered to hinder biodegradability (high degree of halogenation, more than 3 aromatic rings, excessive branching *etc.*), except the presence of the heteroatom.[53] The heteroatom itself is usually not very problematic unless it is substituted like in the case of *N*-ethylcarbazole.[54] Indeed, our preliminary biodegradation study revealed that Quin-2Me containing unsubstituted nitrogen in the ring is degradable to a high extent but *N*-ethylcarbazole bearing an ethyl group on N atom does not show any biodegradation even though its unsubstituted parent compound carbazole is known to be degradable (Figure 3.1.8).[55]

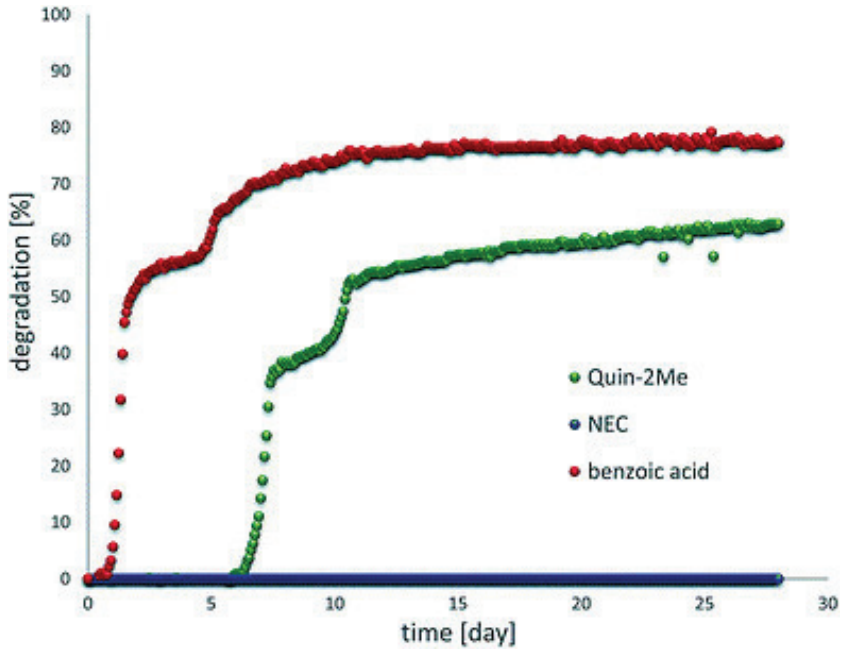


Figure 3.1.8. Biodegradation test with a diluted microbial community derived from activated sludge aeration tank – comparison of *N*-ethylcarbazole (NEC), quinaldine (Quin-2Me) and benzoic acid (positive control).

(4) Filling the gaps – QSAR for preliminary assessment. Performing all the required tests for all potential LOHC structures is extremely time- and resource-consuming. Therefore, some insight into potential hazards can be obtained from already existing data, QSARs or other "non-REACH" fast screening tests which might reveal certain specific modes of action at the early stage. For LOHC structures those alternative ways are currently very important since there are significant gaps in our knowledge regarding their environmental impact. When reliable PTB indicators obtained via testing are not available, it is possible to use QSAR as a screening tool to obtain indicators. Table 3.1.2 shows PBT/vPvB screening parameters generated using EPISuite™[48] for the hydrogen-lean, partially hydrogenated and fully hydrogenated forms of *N*-ethylcarbazole. Based on these parameters only the partially hydrogenated *N*-ethylcarbazole (H_8-NEC) could raise some potential concerns in this regard since its predicted K_{ow} is higher than 4.5 which is a threshold for classifying it as potentially bioaccumulative. For the hydrogen-lean, aromatic form (NEC) and the fully hydrogenated, heterocyclic form (H_{12}-NEC) the likelihood of being classified as PBT, based solely on QSARs, would be rather low. It is worth noting, however, that in a preliminary ready biodegradability test performed within this work no biodegradation was observed for NEC within 4 weeks (see Figure 3.1.8) yet model design for predicting results of that test (Biowin3) gave "weeks" as the expected time frame (Table 3.1.2). It seems therefore that this model overestimates the biodegradability of NEC as compared to our experimental results. All the Biowin models predict the susceptibility to biodegradation in following order NEC >

H_8-NEC > H_{12}-NEC. The higher biodegradability for hydrogen-lean and partially hydrogenated NEC is in line with QSAR since paraffins are usually degradable better than olefins and aromatics better than cycloalkanes.[56] Nevertheless taking into account that the biodegradability of NEC predicted by the model is already 'optimistic' it might be the case that for the other two forms the degradability will be slower than indicated by QSAR. Therefore real-life testing, especially under more realistic conditions (e.g., inherent biodegradability or aerobic sludge treatment simulation tests with higher biomass content or longer testing period) is required especially for high production volume chemicals since the predictive power of QSAR can vary. This is especially true for T indicators (since they are usually modelling only hydrophobicity based toxicity – so called baseline toxicity). Some aromatic LOHC compounds may exert specific modes of toxic action. Among these are genotoxicity by DNA intercalation or adduct formation characteristic for PAHs (polyaromatic hydrocarbons) due to their relatively planar geometry or possibility to be metabolically activated to electrophilic species (epoxides or radical cations).[57] DNA adduct formation was previously shown to occur for many PAHs including naphthalene which was previously suggested as the LOHC chemical but currently abandoned due to obvious safety concerns.[58]

Table 3.1.2. PBT screening for three hydrogenation forms of ethyl-carbazoles using QSAR*.

	NEC	**H_8-NEC**	**H_{12}-NEC**
Persistence assessment			
Biodegradation probability[a]	Biodegrades fast	Biodegrades fast	Does not biodegrade fast
Ultimate biodegradation time frame[b]	Weeks	Weeks–months	Weeks–months
Ready biodegradation probability[c]	Not readily degradable	Not readily degradable	Not readily degradable
P indicator	Not P[1]	Not P[1]	Not P[1]
Bioaccumulation assessment			
Log K_{ow}[d]	4.33	5.85	3.44
B indicator	Not B[2]	Potentially B/vB[2]	Not B[2]
Toxicity assessment			
EC_{50} [mg L^{-1}] algae[e]	1.8	0.15	7.3
EC_{50} [mg L^{-1}] daphnia[e]	1.0	0.05	5.6
EC_{50} [mg L^{-1}] fish[e]	1.5	0.06	8.7
T indicator	Presumably not T[3]	Potentially T[3]	Presumably not T[3]

* Models used for generating screening data: [a] Biowin2,[48] [b] Biowin3,[48] [c] Biowin6,[48] [d] KOWWIN,[48] [e] ECOSAR,[48] (data for baseline toxicity).

Decision making criteria: (1) for classifying as P the outcome of Biowin2 has to be "does not

biodegrade fast" and Biowin3 predicted the biodegradation time frame ≥ months or outcome of Biowin2 has to be "does not biodegrade fast" and Biowin6 predicted the ultimate biodegradation time frame ≥ months; (2) for classifying as not B K_{ow} < 4.5; (3) for classifying as potentially T the EC_{50} or LC_{50} < 0.1 mg L^{-1} in the algae, daphnia or fish test. [50]

The higher the quantity of a given chemical circulating on the markets the more data has to be gathered for its risk assessment. In the case of LOHC technology the amount of carrier needed and the potential for release will be relatively high if the technology becomes a technical success. Therefore, it is particularly important to choose a system which does not raise significant concerns in terms of PBT/CMR assessment. In general any QSAR derived indicators are 'class specific' and will perform relatively good if the chemical in question is structurally similar to the training set used to establish that QSAR. In this context 'similar' very often means having similar K_{ow} at least for ecotoxicity assessment. Since the universe is not made of both water and octanol one can easily imagine that the reality can be much more complex. In terms of compliance with REACH QSAR derived data are so far only admissible as the supporting information (SI), therefore generation of test data cannot be avoided.

LOHCs suggested to date are a group of structurally diverse chemicals therefore it is difficult to make general statements with regard to their overall environmental and health impact. Further testing is needed for most structures of interest.

Conclusion

A proper communication of risk associated with using LOHC compounds to the general public is a key in gaining social acceptance for a future LOHC-based energy and hydrogen transport. This new technology promises a link between unsteady renewable electricity production and a CO_2-free energy supply for stationary and mobile applications and thus offers multiple benefits for the society.

Many different organic molecules can serve as potential LOHC structures. Most of them are uncharged organics, thus volatile, flammable and lipophilic – but so are the gasoline and diesel fuels that we use with great success every day.

In order to facilitate broad introduction of LOHC-based hydrogen distribution systems, all precautions have to be taken to select not only the carrier that performs technically the best but also the carrier that is least toxic and most environmentally friendly.

Since LOHCs are supposed to be a cleaner alternative to fossil fuels, the latter are a good reference point for assessing the 'greenliness' of LOHC – in this comparison it should be still taken into account that fuels are burned while the LOHC systems act like a "deposit bottle" for hydrogen. Conventional fossil fuels, like diesel fuel or

gasoline, usually contain hundreds of species, their composition is mostly unknown and defined on the basis of boiling point range only. Even though individual components of crude oil can be quite toxic (e.g., naphthalene) and reading the Material Safety Data Sheet of diesel no. 2 gives every layman the creeps, those products are circulating on the market since years in billions of tonnes. History knows numerous accidents involving fossil fuels that had catastrophic and far reaching consequences. Despite obvious and multiple risks associated with fossil fuels and high uncertainty linked with their unknown composition our civilisation relies heavily on them simply because of the lack of better options and the overruling socio-economic benefit. One important and unquestioned benefit of LOHC systems in comparison with crude oil based fuels is that the amount of components in LOHCs is limited and known which makes the assessment and risk management much less complex.

If, in addition to being more sustainable than fossil fuels, selected LOHCs can be shown in the future to have much better environmental profiles this would be already a giant improvement. A clear first point in favour of the LOHC systems *vs.* fossil fuels is the fact that the amount of components in LOHCs is limited and known which makes the assessment and risk management much less complex than in the case of crude oil with varying compositions depending on the oil origin.

Several challenges have to be faced when assessing the risk of LOHCs which result mostly from high K_{ow} of some LOHCs as reported throughout this manuscript. However, at the current stage of development (eco)toxicological screening and environmental fate assessment of potential candidates should not only aim to exclude chemicals with enhanced hazards, but should also derive design criteria for better LOHC structures with regard to the ecotox profile. Testing should be focused on especially CMR (carcinogenic, mutagenic or toxic for reproduction) assessment as this is of high importance and very difficult to model or predict via QSAR. The avoidance of CMR chemicals would already give LOHCs an enormous competitive advantage in comparison to fossil fuels that contain carcinogens. The data from CMR and PBT assessment as well as economic data should be used for sound risk-benefit and socio-economic analyses. The results of the latter should decide on the type of LOHC system that should be industrially used and, finally, on the degree the LOHC technology should be applied in a future hydrogen-based economy.

Acknowledgements

We would like to thank the whole UFT Team, especially Dr Jürgen Arning for helpful discussion and Ulrike Bottin-Weber for their experimental support. The authors from Bremen would like to acknowledge financial support of Universität Bremen and European Union 7FP COFOUND BREMEN-TRAC Fellowship Program under the grant number 600411 and the German Federal Foundation for the Environment (Deutsche Bundesstiftung Umwelt (DBU). The authors from Erlangen would like to acknowledge support from the Bavarian Hydrogen Center and the Cluster of Excellence “Engineering of Advanced Materials”.

References

(1) B. D. Solomon and A. Banerjee, *Energy Policy*, 2006, **34**, 781–792.

(2) M. S. Dresselhaus and I. L. Thomas, *Nature*, 2001, **414**, 332–337.

(3) European Comission, *A Roadmap for Moving to a Competitive Low Carbon Economy in 2050*, 2011.

(4) I. Dincer, *Renewable Sustainable Energy Rev.*, 2000, **4**, 157–175.

(5) M. Grätzel, *Philos. Trans. R. Soc., A*, 2007, **365**, 993–1005.

(6) A. Evans, V. Strezov and T. J. Evans, *Renewable Sustainable Energy Rev.*, 2009, **13**, 1082–1088.

(7) N. Mayer, N. Kreifels and B. Burger, *Kohleverstromung zu Zeiten nidriger Böresnstrompreise – Kurzstudie*, 2013.

(8) U. Eberle, B. Müller and R. Von Helmolt, *Energy Environ. Sci.*, 2012, **5**, 8780.

(9) D. U. Eberle and D. R. von Helmolt, *Energy Environ. Sci.*, 2010, **3**, 689.

(10) J. Van Mierlo and G. Maggetto, *Energy Convers. Manage.*, 2006, **47**, 2748–2760.

(11) *European Hydrogen and Fuel Cell Technology Platform - Strategic Research Agenda*, 2005.

(12) C. Liu, F. Li, L.-P. Ma and H.-M. Cheng, *Adv. Energy Mater.*, 2010, **22**, 28–62.

(13) J. Yang, A. Sudik, C. Wolverton and D. J. Siegel, *Chem. Soc. Rev.*, 2010, **39**, 656–675.

(14) L. Schlapbach and A. Züttel, *Nature*, 2001, **414**, 353–358.

(15) E. Robert, H. Heinz, L. Jean-Francois, L. Laura, M. Heiko and R. David, *Well - to - Wheels Analysis of Future Automotive Fuels and Powertrains in the European Context*, 2014.

(16) M. Felderhoff, C. Weidenthaler, R. Von Helmolt and U. Eberle, *Phys. Chem. Chem. Phys.*, 2007, **9**, 2643–2653.

(17) D. Teichmann, W. Arlt and P. Wasserscheid, *Int. J. Hydrogen Energy*, 2012, **37**, 18118–18132.

(18) S. Satyapal, J. Petrovic, C. Read, G. Thomas and G. Ordaz, *Catal. Today*, 2007, **120**, 246–256.

(19) B. Bogdanović and M. Schwickardi, *J. Alloys Compd.*, 1997, **253–254**, 1–9.

(20) F. Schüth, B. Bogdanović and M. Felderhoff, *Chem. Commun.*, 2004, 2249–2258.

(21) T. Q. Hua, R. K. Ahluwalia, J.-K. Peng, M. Kromer, S. Lasher, K. McKenney, K. Law and J. Sinha, *Int. J. Hydrogen Energy*, 2011,**36**, 3037–3049.

(22) B. Loges, A. Boddien, F. Gärtner, H. Junge and M. Beller, *Top. Catal.*, 2010, **53**, 902–914.

(23) S. Saxena, S. Kumar and V. Drozd, *Int. J. Hydrogen Energy*, 2011, **36**, 4366–4369.

(24) T. Kobayashi and H. Takahashi, *Energy Fuels*, 2004, **18**, 285–286.

(25) P. Kaiser, R. B. Unde, C. Kern and A. Jess, *Chem. Ing. Tech.*, 2013, **85**, 489–499.

(26) G. P. Pez, A. Scott, A. C. Cooper, and H. Cheng, US 7101530, 2006.

(27) G. P. Pez, A. C. Cooper and R. Scott, *Pat. Appl. Publ.*, 2008, 1–23.

(28) US Department of Energy, *Hydrogen and Fuel Cell Program*.

(29) A. C. Cooper, K. M. Campbell and G. P. Pez, *16th World Hydrog. Energy Conf.*, 2006.

(30) C. M. Araujo, D. L. Simone, S. J. Konezny, A. Shim, R. H. Crabtree, G. L. Soloveichik and V. S. Batista, *Energy Environ. Sci.*, 2012, **5**, 9534–9542.

(31) K. Müller, J. Völkl and W. Arlt, *Energy Technol.*, 2013, **1**, 20–24.

(32) F. Sotoodeh, B. J. M. Huber and K. J. Smith, *Appl. Catal., A*, 2012, **419–420**, 67–72.

(33) C. Wan, Y. An, G. Xu and W. Kong, *Int. J. Hydrogen Energy*, 2012, **37**, 13092–13096.

(34) F. Sotoodeh and K. J. Smith, *Can. J. Chem. Eng.*, 2013, **91**, 1477–1490.

(35) M. Amende, C. Gleichweit, K. Werner, S. Schernich, W. Zhao, M. P. A. Lorenz, O. Höfert, C. Papp, M. Koch, P. Wasserscheid, M. Laurin, H.-P. Steinrück and J. Libuda, *ACS Catal.*, 2014, **4**, 657–665.

(36) M. Amende, C. Gleichweit, S. Schernich, O. Höfert, M. P. A. Lorenz, W. Zhao, M. Koch, K. Obesser, C. Papp, P. Wasserscheid, H.-P. Steinrück and J. Libuda, *J. Phys. Chem. Lett.*, 2014, **5**, 1498–1504.

(37) D. Teichmann, W. Arlt, P. Wasserscheid and R. Freymann, *Energy Environ. Sci.*, 2011, **4**, 2767–2773.

(38) N. Brückner, K. Obesser, A. Bösmann, D. Teichmann, W. Arlt, J. Dungs and P. Wasserscheid, *ChemSusChem*, 2013, **7**, 229–235.

(39) J. N. Dumont and T. W. Schultz, *Bull. Environ. Contam. Toxicol.*, 1979, **22**, 159–166.

(40) S. Hodoshima, S. Takaiwa, A. Shono, K. Satoh and Y. Saito, *Appl. Catal., A*, 2005, **283**, 235–242.

(41) N. Kariya, A. Fukuoka and M. Ichikawa, *Appl. Catal., A*, 2002, **233**, 91–102.

(42) M. Taube, D. W. T. Rippin, D. L. Cresswell and W. Knecht, *Int. J. Hydrogen Energy*, 1983, **8**, 213–225.

(43) H. Y. Zhao, S. T. Oyama and E. D. Naeemi, *Catal. Today*, 2010, **149**, 172–184.

(44) R. Yamaguchi, C. Ikeda, Y. Takahashi and K.-i. Fujita, *J. Am. Chem. Soc.*, 2009, **131**, 8410–8412.

(45) H. Boudet, C. Clarke, D. Bugden, E. Maibach, C. Roser-Renouf and A. Leiserowitz, *Energy Policy*, 2013, **65**, 57–67.

(46) OECD, *Guideline for Testing of Chemicals 105-Water Solubility*, 1995.

(47) OECD, *Guidance Document on Aquatic Toxicity Testing of Difficult Substanes and Mixtures*, 2000, vol. 23.

(48) US EPA *Estimation Programs Interface SuiteTM for Microsoft® Windows EPI SuiteTM V. 4.1*, 2014.

(49) H. Rufi, P. R. Fisk, A. E. Girling, J. M. H. King, R. Länge, E. X. Lejeune, N. Stelter, C. Stevens, P. Suteau, J. Tapp, J. Thus, D. J. Versteeg and H. J. Niessen, *Ecotoxicol. Environ. Saf.*, 1998, **39**, 72–77.

(50) ECHA, *Chapter R.11 PBT Assess.*, 2012.

(51) Source: European Chemicals Agency, http://echa.europa.eu/.

(52) OECD, *Guideline Fortesting of Chemicals 301-Ready Biodegradability*, 1992.

(53) R. S. Boethling, E. Sommer and D. DiFiore, *Chem. Rev.*, 2007, **107**, 2167–2820.

(54) B. Philip, M. Hoff, F. Germa, B. Schink, D. Beimborn and V. Mersch-Sudermann, *Environ. Sci. Technol.*, 2007, **41**, 1390–1398.

(55) C. Zhao, Y. Zhang, X. Li, D. Wen and X. Tang, *J. Hazard. Mater.*, 2011, **190**, 253–259.

(56) P. A. Vieira, R. B. Vieira, S. Faria, E. J. Ribeiro and V. L. Cardoso, *J. Hazard. Mater.*, 2009, **168**, 1366–1372.

(57) R. Benigni and C. Bossa, *Chem. Rev.*, 2011, **111**, 2507–2536.

(58) M. Kapuci, Z. Ulker, S. Gurkan and L. Alpsoy, *Toxicol. Ind. Health*, 2014, **30**, 82–89 .

‡ Supporting Information

S3.1.1. Liquid Organic Hydrogen Carriers.

The aromatic forms of LOHC: MSH, Quin-2Me, NEC were purchased from Hydrogenious Technologies GmbH, Erlangen (www.hydrogenious.net). The partially hydrogenated and fully hydrogenated forms, that is H_{18}-MSH, H_x-NEC, Quin-2Me-pH and Quin-2Me-H10 were prepared by catalytic hydrogenation reaction using literature proceedings.[25]

S3.1.2. 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).

S3.1.3. Solubility in media.

For the media/water solubility assessment, a so called generator column method was used according to OECD 105.[1] 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^{-1} to 280 °C, ramp 35 °C min^{-1} to 320 °C hold 1 min; detector temperature 320 °C; column pressure 2 bar, column flow 3.7 mL min^{-1} .

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 $NaHCO_3$, 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_3, 1.25 mM $Ca(NO_3)_2$, 0.66 mM KH_2PO_4, 0.072 mM K2HPO4, 0.41 mM MgSO4, 1.94 µM H3BO3, 0.63 µM ZnSO4, 0.18 µM Na_2MoO_4, 0.91 µM $MnCl_2$, 2.81 µM $FeCl_3$, 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.

S3.1.4. 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 µm) from CS Chromatographie Service, Langerwehe, Germany were used. The GC method parameters were: inlet temperature 280 °C, split-less injection of 1 µL, oven program: 100 °C hold 3 min, ramp 15 °C 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 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.

S3.1.5. 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.[2] 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 O_2 L^{-1}. Two replicates were run for each compound accompanied by two blanks and two positive controls (benzoic acid).

S3.1.6. 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).[3] Marvin software was used for drawing, displaying and characterizing chemical structures, Marvin 6.3.1, 2014, available from ChemAxon (http://www.chemaxon.com).[4]

References

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

3.2. Manuscript 2

Zhang, Y. Q.; Stolte, S.; Alptekin, G.; Rother, A.; Diedenhofen, M.; Filser, J.; Markiewicz, M. (2017) '**Mobility and adsorption of liquid organic hydrogen carriers (LOHCs) in soil.**'

Submitted manuscript to the journal – Environmental Science & Technology.

Page: 66–95

Contributions of Zhang, Y. Q.:

- Experimental design for all tests;
- Experimental preparation and related calculations;
- Performance of the experiments, included the soil column leaching test, around 50% of the HPLC screening test, initial preparation for adsorption batch equilibrium experiment;
- Software estimations by Marvinsketch;
- Adsorption modelling;
- Data processing and all statistical analysis;
- Preparation of all Figures (expect the graphic of COSMO screening charge density) and Tables; and
- Manuscript preparation and writing.

Stolte, S. participated the development of the topic and gave many advices to this research. Alptekin, G. performed the adsorption batch equilirium experiment and the initial data analysis regarding to this test under the guidance of Zhang, Y. Q. and Markiewicz, M.. Alptekin, G. also performed the test of HPLC screening together with Zhang, Y. Q. by the support of Rother, A.. Rother, A. supported the soil column leching test. Diedenhofen, M. did the computational prediction for the physicochemical parameters (S_w, K_{ow}, and K_{oc}) of LOHCs and the screening charge density by COSMO. Filser, J. commented and gave advices on the manuscript. Markiewicz, M. guided the research and the preparation of the manuscript. Each co-author contributed to the revisions of the manuscript.

Mobility and adsorption of liquid organic hydrogen carriers (LOHCs) in soil‡

Ya-Qi Zhang[a], Stefan Stolte[a,b], Gizem Alptekin[a], Alica Rother[a], Michael Diedenhofen[c], Juliane Filser[d], and Marta Markiewicz[a]*

[a] UFT - Centre for Environmental Research and Sustainable Technology, Department Sustainable Chemistry, University of Bremen, Leobener Straße, D-28359, Bremen, Germany.

[b] Department of Environmental Analysis, Faculty of Chemistry, University of Gdańsk, ul. Wita Stwosza 63, 80-308 Gdańsk, Poland.

[c] COSMO*logic* GmbH and Co. KG, Imbacher Weg 46, 51379 Leverkusen, Germany.

[d] UFT - Centre for Environmental Research and Sustainable Technology, Department General and Theoretical Ecology, Faculty 2 (Biology/Chemistry), Leobener Straße, D-28359, Bremen, Germany.

Abstract

Liquid organic hydrogen carriers (LOHCs) constitute a novel energy system with an attractive technological performance. However, the potential behavior and fate of these compounds in the environment have not yet been investigated. In this study, the adsorption properties of 13 technologically promising LOHC candidates, including indoles, quinaldines, carbazole derivatives, benzyltoluenes and dibenzyltoluene, together with their partially hydrogenated forms, were first investigated and compared based on their organic carbon-water partition coefficients (K_{oc}) determined via HPLC screening. The adsorption of the quinaldines including H_2-rich, H_2-lean and partially hydrogenated forms, was further estimated by investigating the soil-water partition coefficient (K_d) via adsorption batch equilibrium experiments and modeling as well as column leaching tests. The log K_{oc} values of the LOHC systems generally increased in the order indoles < quinaldines < carbazole derivatives < benzyltoluenes < dibenzyltoluene. The batch equilibrium experiments and Freundlich isotherm modeling performed for the quinaldine-based LOHC system showed that the partially hydrogenated form adsorbed the strongest to the soil. The highest retention was also found for this chemical in the column leaching tests, wherein the H_2-rich form was determined to be the most mobile, indicating that it had the highest leaching capacity.

Key words: adsorption, K_{oc}, K_d, leaching, mobility, PAHs, N-PAHs

Introduction

Liquid organic hydrogen carrier (LOHC) systems are promising alternatives developed in recent years to support and improve the current options for energy storage and transport[1,2,3]. Unlike traditional energy systems in which fossil fuels are the main energy source, hydrogen that is covalently bound to LOHCs is used as the energy vector[1,2]. The hydrogen used in LOHC systems can be produced either from fossil fuels by steam reforming or by water electrolysis[2,4] using renewable energy sources (RE, Figure 3.2.1), with the latter considered to be more attractive[5]. Renewable resources, such as wind, sun, biomass and hydropower, which are inexhaustible and allow for significant reduction in greenhouse gas emissions, will play an important role in future energy systems[6]. However, their strong dependence on the weather and geographic conditions leads to intermittent (overproduction or shortage) energy production[1,7,8] and impedes the transition to fully RE systems. In addition, RE is currently limited to stationary facilities, and the advancements in mobile applications have been limited[7]. Coupling RE with LOHC systems by storing energy in the form of H_2 is expected to alleviate these problems.

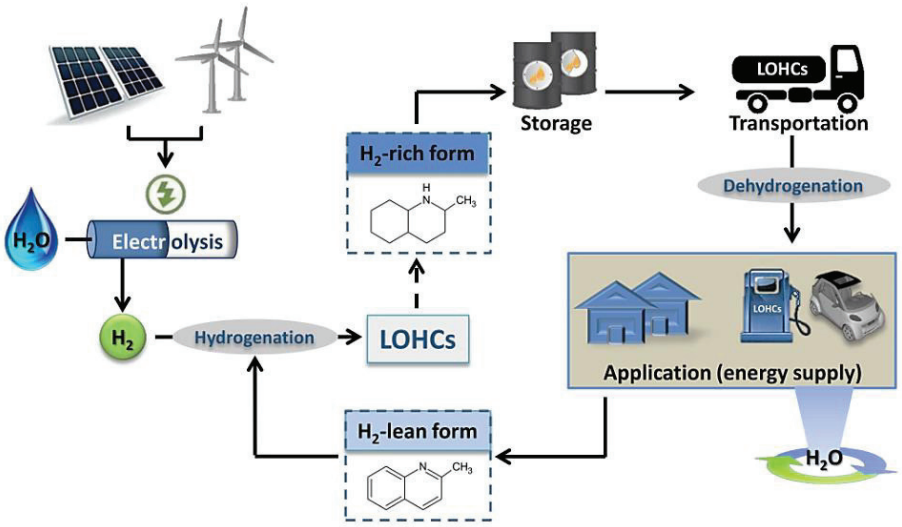


Figure 3.2.1. Distribution of energy in the form of H_2 via hydrogenation/dehydrogenation of the LOHC systems.

Two main factors have caused hydrogen to be studied intensively as a superior energy vector over fossil fuels. First, the high gravimetric energy density (120 MJ kg^{-1})[7] of hydrogen is three times larger than that of petroleum[3]. Second, hydrogen has inherently lower emissions, where the only exhaust gas after combustion is water vapor, as opposed to the SO_2, NO_x, CO, CO_2 and volatile organic compounds (VOCs) produced from fossil fuels[9], which contribute to global warming, air pollution, acid precipitation, ozone depletion, etc.[9]. These advantages make hydrogen an attractive power source for both stationary and mobile applications. However, the volumetric

energy density of hydrogen is very low – 10.8 kJ L^{-1} – under ambient conditions[7], and this density must be significantly increased to make hydrogen a viable energy storage option. This improvement can currently be achieved commercially by compression under high pressures (up to 700 bar)[4] or by liquefaction at low temperature (-253 °C)[4]. However, these technologies create concerns related to safety, storage capacity and complexity of infrastructure[4,7]. These drawbacks can largely be alleviated by using LOHC-based technologies. LOHC systems usually consist of a tandem of organic molecules, one of which is H_2-rich (loaded), and the other is H_2-lean (unloaded or spent). Cycloalkanes, nitrogen-substituted heterocycles, methane, methanol (CH_3OH), formic acid (HCOOH), etc.[3,7,10,11] have been proposed as potential H_2-rich LOHCs. The hydrogen storage capacity of these compounds ranges from 1.7 to 12.6 wt%[3]. The incorporation of nitrogen into the cyclic ring reduces the dehydrogenation enthalpy[3] and improves the thermodynamics and kinetics of dehydrogenation[4,7], which renders nitrogen-substituted heterocycles particularly promising candidates[3]. Indoles[3,4], quinaldines[3,4], and carbazole derivatives[2,3,4,10], as well as isomeric mixtures of benzyltoluene or dibenzyltoluene[11,12] with hydrogen storage capacities ranging from 5 to 7 wt%[3,7], have been recently proposed as the most promising potential candidates.

The compounds forming the LOHC tandem can be reversibly converted by catalytic hydrogenation and dehydrogenation reactions[5] (Figure 3.2.1). Briefly, the LOHC is loaded with hydrogen to give the H_2-rich form via catalytic hydrogenation in an industrial facility. The H_2-rich LOHC can then be stored for long periods or transported under ambient temperature and pressure. To obtain energy, hydrogen is released from the loaded carrier via catalytic dehydrogenation and utilized to power devices, e.g., fuel cells or internal combustion engines[13]. The spent LOHC must then be transported back to the regeneration facility, where it can be reloaded with hydrogen and returned to the cycle. Note that the LOHC chemicals are not consumed in the process and can be subjected to multiple hydrogenation-dehydrogenation cycles, which is very different from fossil fuels[2]. Another benefit of LOHC systems is that they can be implemented using the existing infrastructure, such as ships, ports[2], oil tanks, pipelines, and fueling stations[1,10], that were developed for the transport, distribution and processing of fossil fuels due to the similarities in the physicochemical properties of LOHCs to those of fossil fuels[2,3]. This reuse of existing infrastructure is also economically attractive due to the lower required investment costs[3,7], and it allows for a smooth transition between conventional and LOHC energy systems[1,3].

The technological advantages and disadvantages related to particular LOHC candidates, especially in automotive applications, have recently been reported in detail by many studies[3,10,11,12,14].

The considerable application potential of LOHC systems opens the global markets to this technology. The delivery of LOHC-based hydrogen to hydrogen fueling stations is expected to become possible within the next few years[4]. A tank containing approximately 80 kg of LOHCs is required to achieve a driving range of ca. 700 km[4]. The full-scale application of this technology and the complete replacement of fossil

fuels would require tens of thousands of tons of LOHC chemicals to be circulated worldwide[7], which makes their release into the environment very likely. The potential influence that these chemicals may have on the environment after release (e.g., through leaks, accidental spills during production, transport, and fueling) should therefore be considered before the LOHCs become available in the public market. More specifically, the environmental hazard potential of these chemicals should be known before the technology enters commercial use to assure that the LOHC systems with both the best performance and least harm to the environment are chosen. However, experimental data regarding the environmental impacts (in terms of toxicity, bioavailability, biodegradability, mobility, etc.) of the LOHCs are too scarce to make a proper assessment. Environmental legislation requires a broad set of information to evaluate and authorize the usage of chemicals that are produced and used on such a large scale[15].

Because the transportation, distribution and use of LOHCs are very similar to those of conventional fossil fuels, all environmental components, i.e., soil, water and air, are likely to be affected. Among these components, soil is of particular importance since it usually acts as a sink for anthropogenically produced organic chemicals, especially those that are hydrophobic and moderately volatile to non-volatile[16]. Once in the soil, organic contaminants may undergo volatilization, biotransformation, biodegradation, and leaching[17], as well as adsorption (chemical or physical binding to soil organic matter, for example)[17,18] and sequestration[19] within the soil. Among these processes, adsorption is particularly important in defining the mobility of chemicals in soil, i.e., how far they can spread from the point of release and what concentrations can be expected in adjacent ground and surface waters. The greatest concern of contaminant mobility is the likelihood of the chemicals reaching the groundwater. Adsorption also defines the extent of exposure to soil-dwelling organisms for which uptake is not based on the consumption of soil particles; this includes plants since the adsorbed fraction is largely unavailable for uptake through the pore water. Clearly, these questions are of immediate interest, as the contamination of drinking water supplies or the uptake by edible plants has a direct effect on humans. From the ecotoxicological point of view, adsorption might reduce the toxicity towards soil-dwelling organisms (those with soil pore water as the dominant route of exposure). In the absence of testing data, the toxicity in soil is often predicted using aquatic toxicity and sorption coefficients[20]. However, very little data is currently available for either of those parameters, thus making predictions difficult.

The properties of the soil (the amount and nature of the organic and inorganic fractions), composition of the pore water and physicochemical properties of the organic contaminant[17] influence its sorption behavior in soil[16]. The interactions between the soil and contaminants can involve hydrophobic forces, covalent binding, ion-exchange[16,18,21,22,23], hydrogen bonding, π–π interactions, etc.[24,25]. For soils containing more than a 0.2% organic carbon, hydrophobic forces have been shown to dominate the interactions with organic pollutants[24]. Nevertheless, charge interactions are also important, especially for permanently charged or ionizable organics. Among

the most important properties of organic contaminants that influence the soil distribution are hydrophobicity (often expressed as the octanol–water partition coefficient – log K_{ow}), polarity and acidity/basicity (pK_a value)[24,26]. The affinity of contaminants for the soil or their mobility in the environment is most frequently assessed using two methods: column leaching and adsorption batch equilibrium[27]. The soil-water partition coefficient (K_d), which is usually obtained from a batch equilibrium test and describes the partitioning of chemicals between the soil (both organic and inorganic components) and pore water, is the parameter most frequently used to define the mobility of chemicals in soil[24]. Due to the abovementioned significance of the interactions between the contaminants and organic matter, the partition coefficient is often normalized to the organic carbon content and denoted as K_{oc}. The soil behaviors of many polycyclic aromatic hydrocarbons (PAHs) and nitrogen-substituted PAHs (N-PAHs), which share structure similarities with LOHCs, have been studied[22, 28, 29, 30, 31, 32]. However, data on the behavior and fate of the potential LOHC compounds remain scarce.

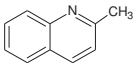
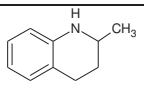
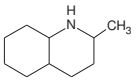
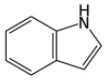
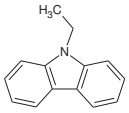
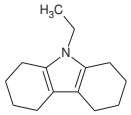
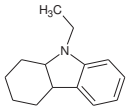
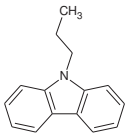
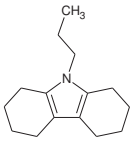
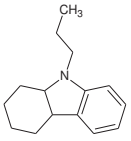
The present study therefore aimed to characterize the behaviors of several promising LOHC candidates: quinaldines, indoles, carbazole derivatives, benzyltoluenes and dibenzyltoluene, by investigating their adsorption to and mobility in standard soil. To this end, the organic carbon-water partition coefficients (K_{oc}) were evaluated with the classification of the mobility. The K_d, adsorption isotherms and modeling as well as leaching capacities were investigated using the quinaldine-based LOHC system as a cross-check. The relationships between these parameters as well as their significance in the assessment of the environmental hazards of LOHCs were discussed.

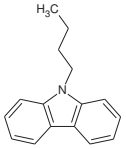
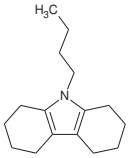
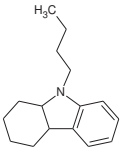
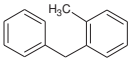
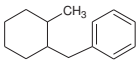
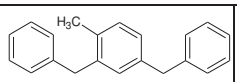
Materials and methods

All details regarding the chemicals and the experimental procedures are given in the Supporting Information (SI).

Materials. The LOHC systems tested in the present study, including indoles, quinaldines, carbazole derivatives, MLHs and MSH (Table 3.2.1), were provided by the research group of Prof. Dr. Peter Wasserscheid, Institute of Chemical Reaction Engineering, Friedrich-Alexander University of Erlangen-Nürnberg, Germany. Two batches (briefly named as soil I and soil II) of standard soil RefeSol 01-A-004, loamy sand (Table S3.2.1) (soil I: pH ($CaCl_2$) = 5.41, organic carbon 1.21%, sand 76.7%, silt 17.2%, clay 6.1%; soil II: pH ($CaCl_2$) = 5.33, organic carbon 0.80%, same texture as soil I) were ordered from Fraunhofer IME, Schmallenberg, Germany. The soils were dried for 24 h at room temperature and 2.0 mm-sieved before tests. Detailed information on all the materials is available in SI section S3.2.1.

Table 3.2.1. Physicochemical properties of the LOHC candidates.

LOHC name	Abbrev.	Formula	Chemical structure	MW [g mol^{-1}]	Log S_w [a] (25°C) [mg L^{-1}]	Log K_{ow} [a]	Log K_{oc} [a]
Quinaldines							
2-Methylquinoline	Quin-2Me	$C_{10}H_9N$		143.2	3.95	2.45	2.18
Tetrahydro-2-methylquinoline	Quin-2Me-pH	$C_{10}H_{13}N$		147.2	2.66	3.04	2.48
Decahydro-2-methylquinoline[b]	Quin-2Me-H10	$C_{10}H_{19}N$		153.3	3.86	3.25	2.53
Indoles							
Indole	Indole	C_8H_7N		117.2	4.05	2.32	2.21
Indoline	Indoline	C_8H_9N		119.2	3.71	2.04	1.94
Carbazole derivatives							
9-Ethyl-9H-carbazole	Car-2	$C_{14}H_{13}N$		195.3	1.09	4.42	3.36
9-Ethyl-octahydrocarbazole	Car-2-pH	$C_{14}H_{21}N$		203.3	0.36	5.19	3.26
9-Ethyl-hexahydrocarbazole[b]		$C_{14}H_{19}N$		201.3	0.97	4.60	3.08
9-Propyl-9H-carbazole	Car-3	$C_{15}H_{15}N$		209.3	0.54	4.96	3.61
9-Propyl-octahydrocarbazole	Car-3-pH	$C_{15}H_{23}N$		217.4	-0.19	5.72	3.50
9-Propyl-hexahydrocarbazole[b]		$C_{15}H_{21}N$		215.3	0.41	5.16	3.36

LOHC name	Abbrev.	Formula	Chemical structure	MW [g mol^{-1}]	Log S_w[a] (25°C) [mg L^{-1}]	Log K_{ow}[a]	Log K_{oc}[a]
Carbazole derivatives							
9-Butyl-9H-carbazole	Car-4	$C_{16}H_{17}N$		223.3	-0.001	5.50	3.89
9-Butyl-octahydrocarbazole	Car-4-pH	$C_{16}H_{25}N$		231.4	-0.77	6.27	3.79
9-Butyl-hexahydrocarbazole[b]		$C_{16}H_{23}N$		229.4	-0.04	5.58	3.62
Benzyltoluenes							
Benzyltoluene	MLH	$C_{14}H_{14}$		182.3	0.69	4.78	3.34
Benzyltoluene (partially hydrogenated)[b]	MLH-pH	$C_{14}H_{20}$		188.3	0.02	5.46	3.65
Dibenzyltoluene							
Dibenzyltoluene[b]	MSH	$C_{21}H_{20}$		272.4	-1.37	6.84	4.52

[a] Calculated by COSMO-RS (SI section S3.2.2); S_w, water solubility.

[b] Mean values of the components (includes *cis* and *trans* isomers) in mixtures.

Determination of log K_{oc}. The log K_{oc} values of the LOHCs (Table 3.2.1) were first predicted by the conductor-like screening model for realistic solvation (COSMO-RS, COSMO*logic* GmbH and Co. KG, Leverkusen, Germany, SI section S3.2.2) to estimate the retention times. Subsequently, the K_{oc} values of the H_2-lean (aromatic) and partially hydrogenated LOHC chemicals were determined by high-performance liquid chromatography (HPLC) screening following OECD guideline 121[33]. The H_2-rich forms of the LOHC chemicals were not investigated due to their lack of UV activity. The HPLC instrument (Hewlett Packard system Series 1100, Agilent Technologies, Waldbronn, Germany) was equipped with an autosampler and a UV-vis detector (Agilent Technologies, Waldbronn, Germany) at a wavelength of 210 nm. A normal-phase cyanopropyl column (ZORBAX CN, 5 µm, 4.6 mm ID × 150 mm, Agilent) was used, and the flow rate was 1.0 mL min^{-1}. The

mobile phase for HPLC was a mixture of methanol and citrate buffer (pH 6.0) (0.01 M, consisting of 11.5 mL of a 0.1 M $C_6H_8O_7 \cdot H_2O$ solution plus 88.5 mL of a 0.1 M $C_6H_5O_7Na_3 \cdot 2H_2O$ solution in a total volume of 1 L) with a ratio of 55/45% v/v. Sodium nitrate was used as a non-retained substance to measure the dead-time of the HPLC system. Ten reference compounds were used for calibration. The calibration and calculation of log K_{oc} are described in detail in SI section S3.2.3. The LOHCs were then assigned to a relative mobility class based on the K_{oc}.

Determination of K_d and modeling of the adsorption isotherms. The quinaldine-based LOHC system was chosen as an example for cross-check. This particular LOHC system was chosen since its components are ionizable within the environmental pH range, suggesting that their partitioning behavior might not conform to the commonly used models. The adsorption isotherms in soil I were established for 5 concentrations (2.5, 5.0, 10, 25, and 50 mg L^{-1}) following the batch equilibrium protocol given by OECD guideline 106[34]. Two independent tests with triplicates of each concentration were conducted for every compound. One gram dry weight (dw) of soil was equilibrated in 9 mL of 0.01 M $CaCl_2$ (in 40-mL glass vials with polytetrafluoroethylene (PTFE)-lined screw caps) by agitation on a horizontal shaker (240 rpm, compact flat orbital shaker, IKA® HS 260 control, IKA®-Werke GmbH and Co. KG, Staufen, Germany) at room temperature for 24 h. A 1-mL aliquot of the quinaldine stock solution (in 0.01 M $CaCl_2$) was added to obtain a soil/liquid ratio of 1:10, and the mixture was shaken for 6 days. The liquid phase was then transferred to a 15-mL centrifuge tube (with printed graduations and plug caps from VWR, Germany) and centrifuged at 3,000 g for 15 min at 20°C (Labofuge 400R, Thermo Scientific Heraeus, Schnackenberg, Germany). Any soil residues in the supernatant were further removed by filtration through a Pasteur pipette packed with glass wool (2.5 cm). One quantity control per concentration and one blank control were simultaneously prepared (details are provided in SI section S3.2.4). The contents of the filtrates were extracted using a liquid-liquid extraction, and the amount of test substance was measured using gas chromatography mass spectrometry (GC/MS) analysis.

The amount of quinaldine adsorbed to the soil was calculated by subtracting the amount remaining in the aqueous phase after equilibration from the amount initially added. The concentrations of the test compounds in the soil phase (q_e) were plotted against the concentrations in the liquid phase (C_e), and K_d was thus calculated as the slope of the linear portion of the isotherm. Freundlich (Eq. (1)) and Langmuir (Eq. (2)) models were used to fit the experimental data as follows:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \tag{1}$$

$$\frac{1}{q_e} = \frac{1}{K_L \cdot q_m} \cdot \frac{1}{C_e} + \frac{1}{q_m} \tag{2}$$

The Freundlich constant (K_f), Freundlich exponent $1/n$, Langmuir constant (K_L) and maximum adsorption capacity (q_m) were then obtained. The coefficient of determination (R^2) was also calculated for both fits to describe the goodness of fit.

Leaching in soil columns. The leaching of quinaldines in soil II was tested in columns according to OECD guideline 312[35]. Columns made of carbon steel (3.8 cm inner diameter and 35 cm in length) were sealed at both ends with polyvinylchlorid (PVC) caps (each 1.15 cm long). A steel capillary was inserted through each cap to provide an inlet and an outlet. Air-dried soil was uniformly packed in the columns to a height of approximately 28 cm followed by a pre-wetting procedure. Quinaldine was then spiked into the top of the soil columns using a small amount of soil as a vector as follows. The quinaldine was first dissolved in 10 mL of acetone and then spiked into 20 g of soil. The acetone was allowed to evaporate over 24 h, after which atrazine (Table S3.2.2), which served as the reference compound, was added to the spiked soil and thoroughly mixed. The soil matrix was then added carefully and evenly to the top of the soil column and covered with a round piece of filter paper. Atrazine was used as the reference compound in all tests and was added to the two columns containing the test compound. Additionally, two columns containing only atrazine were run for 648 h. The total concentrations of the LOHC and atrazine in the soil column were 100 and 20 mg kg^{-1} dw soil, respectively. Artificial rain (0.01 M $CaCl_2$) was simulated by the dropwise addition of water to the top of the column with the aid of a peristaltic pump (Spetec, Perimax 12, Erding, Germany) at a flow rate of 0.108 mL min^{-1} for 172 h (Quin-2Me-H10), 648 h (Quin-2Me) or 720 h (Quin-2Me-pH). The leachates were collected from the bottom of the column every 24 or 48 h and passed through filters made of glass wool packed in Pasteur pipettes. Two independent leaching tests were performed for each quinaldine, with two replicates (columns) performed for each test. The quinaldines in the leachates were extracted by liquid-liquid extraction and analyzed by GC/MS. The amount of quinaldine in the leachate was calculated as the percentage of the total mass (mass%) that was originally added and plotted against the multiples of the pore volume (PV). The breakthrough was determined as the PV point at which the substance first appeared at the outlet.

Another set of K_d values was determined from the column leaching experiments according to the method proposed by the Environmental Protection Agency (EPA)[36]. Here, the K_d values were directly calculated from the retardation factor (R_f, the ratio of the pore water velocity to the contaminant velocity, i.e., V_p/V_c) and factors related to the soil properties (n, total porosity; ρ_b, bulk density) using the equation $K_d = \left[\left(R_f - 1\right) \times n\right]/\rho_b$. All the details of the soil column preparation steps and calculation of K_d are available in SI section S3.2.5.

Liquid-liquid extraction system. The concentrations of extracted quinaldines were determined via liquid-liquid extraction followed by GC/MS analysis. All the steps are described in detail in SI section S3.2.6.

GC/MS analysis. The concentrations of quinaldines in the extracts were analyzed on a gas chromatograph (HP® GC system 6890N) equipped with a mass selective detector (HP® MS 5973, Agilent, Waldbronn, Germany). Further details of the setup and calibration parameters are given in SI section S3.2.7.

Statistical analysis. Regression analysis was performed for the comparison between COSMO-RS (see SI section S3.2.2) and HPLC methods in terms of K_{oc} values and for the evaluation of isotherm models. Significance of difference between two data sets (i.e., the K_d of H_2-rich and H_2-lean forms of the quinaldines that obtained in adsorption batch test) were analyzed by generalized linear model analysis using the software R (version 3.1.1) (https://www.r-project.org). $P > 0.05$ was considered not significant.

Results

Log K_{oc} of LOHCs. The log K_{oc} values of the test compounds ranged from 1.17 to 5.41 (Table 3.2.2). The partially hydrogenated forms of the carbazole derivatives, MLHs and MSH were found to exist as technical mixtures containing at least two components (Table S3.2.3); therefore, the total mean of all the components was used. The log K_{oc} values were well regressed to those predicted by COSMO-RS (SI section S3.2.2 and Figure S3.2.1; $R^2 = 0.92$). The LOHCs were assigned to a mobility class according to their log K_{oc} values and McCall's scale of mobility in soil[37]. According to this scale[37], indoline, which had the lowest log K_{oc}, was classified as highly mobile in soils (class I: log K_{oc} 1.70–2.18). Quin-2Me, Quin-2Me-pH and indole fell into the "moderately mobile" category (class II: log K_{oc} 2.18–2.70). The remaining compounds were characterized by notably higher log K_{oc} values, which increased in the following order: MLH < Car-2 < Car-2-pH < Car-3 < Car-3-pH < Car-4 ≈ Car-4-pH < MSH < MLH-pH. Thus, these compounds were assigned to the lowest mobility class (class V: log K_{oc} > 3.70) and labeled "immobile". Moreover, the carbazole derivatives containing extended alkyl chains tended to have higher log K_{oc} values and lower mobilities.

Table 3.2.2. Log K_{oc} (n = 3, ± SD) determined by HPLC screening and the prediction of mobility according to McCall's soil mobility scale[37].

LOHC	**Indoline**			**Quin-2Me**		**Quin-2Me-pH**		**Indole**	
Log K_{oc}	1.71 ± 0.01			2.19 ± 0.02		2.36 ± 0.004		2.36 ± 0.004	
Mobility	------ Highly mobile ------ (Class I: log K_{oc} 1.70–2.18)			------ Moderately mobile ------ (Class II: log K_{oc} 2.18–2.70)					
LOHCs	**MLH**	**Car-2**	**Car-2-pH**[a]	**Car-3**	**Car-3-pH**[a]	**Car-4**	**Car-4-pH**[a]	**MSH**	**MLH-pH**[a]
Log K_{oc}	3.85 ± 0.07	4.27 ± 0.01	4.31 ± 0.06	4.61 ± 0.05	4.63 ± 0.05	5.01 ± 0.01	5.01 ± 0.07	5.38 ± 0.22	5.41 ± 0.23
Mobility	------ Immobile ------ (Class V: log K_{oc} > 3.70)								
K_{oc}	Low ---------- High								

[a] Mean values ± SD of the components (includes *cis* and *trans* isomers) in mixtures.

Summarized results of K_d. The soil-water partition coefficients (K_d) obtained from the adsorption equilibrium batch experiments (batch-K_d) and soil column leaching tests (column-K_d) are shown in Table 3.2.3.

Table 3.2.3. Summarized K_d, log D and pK_a (of the conjugated acid) values of the quinaldines.

	Batch-K_d [mL g^{-1}][a]		**Column-K_d**[b]	**Log D**	**pK_a**
	K_d	R^2	[mL g^{-1}]	at pH 5.4[c]	(25°C)[c]
Quin-2Me	2.03 ± 0.12	0.99	1.00 ± 0.40	2.07	5.15 (5.7–5.8)[38]
Quin-2Me-pH	6.57 ± 0.39	0.99	1.88 ± 0.43	2.23	4.88
Quin-2Me-H10	2.42 ± 0.43	0.91	0.23 ± 0.03	-0.88	10.75

[a] Based on the adsorption batch equilibrium experiment in soil I (n = 6, ± SD).

[b] Based on the soil column leaching test in soil II (n = 4, ± SD).

[c] Estimated using MarvinSketch 14.10.6.0.

K_d and adsorption modeling of the quinaldines. The quinaldines were chosen as an example LOHC system for further investigation of the soil-water partitioning. The sorption isotherms and K_d values were established using batch equilibrium experiments. The isotherms are shown in Figure 3.2.2; those of Quin-2Me and Quin-2Me-H10 were concave but without a well-defined plateau. Interestingly, the isotherm of Quin-2Me-pH appeared to be convex, though the number of data points was not sufficient to confirm that feature. The fully hydrogenated form had a slightly higher (though not significant, p = 0.77) K_d than that of the dehydrogenated form

(2.42 vs. 2.03 mL g^{-1}), whereas the K_d of the partially hydrogenated quinaldine was substantially higher than both, with a value of 6.57 ± 0.39 mL g^{-1} (Table 3.2.3).

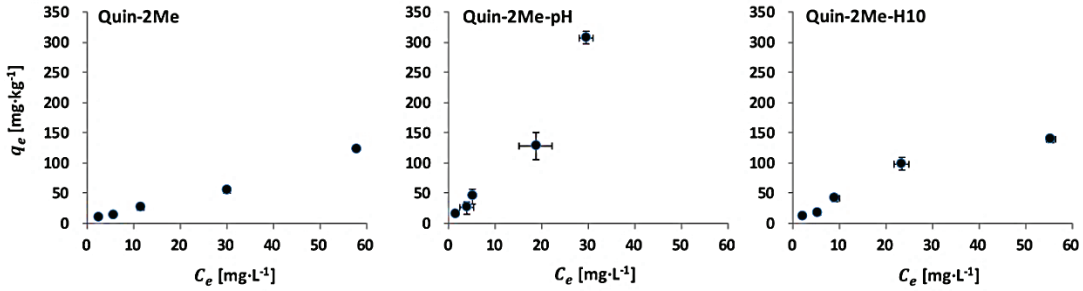


Figure 3.2.2. Adsorption isotherms (n = 6, ± SD) of the quinaldines in soil I.

Since K_{oc} is the soil-water partition coefficient normalized to the amount of organic matter in soils according to the empirical equation $K_{oc} = K_d/f_{oc}$, K_{oc} can be easily calculated from the measured K_d values for soil I, giving values of 168 mL g^{-1}, 543 mL g^{-1} and 200 mL g^{-1} for Quin-2Me, Quin-2Me-pH and Quin-2Me-H10, respectively.

Furthermore, the adsorption isotherms were fitted using Freundlich and Langmuir models (Figure 3.2.3), and the fitting parameters are listed in Table 3.2.4. The highest adsorption coefficients estimated by both models (K_f = 9.20 and K_L = 0.081 mL g^{-1}) were found for Quin-2Me-pH, which was followed by Quin-2Me-H10 (5.72 mL g^{-1}) and Quin-2Me (4.19 mL g^{-1}) in the Freundlich model or by Quin-2Me (0.074 mL g^{-1}) and Quin-2Me-H10 (0.050 mL g^{-1}) in the Langmuir model. The data were better fit by the Freundlich model (R^2 = 0.95–0.97) than the Langmuir adsorption model (R^2 = 0.89). Comparatively low values of the Freundlich exponent (the non-linearity constant, $1/n$) were observed for Quin-2Me and Quin-2Me-H10, while a higher $1/n$ value (0.96) was found for Quin-2Me-pH.

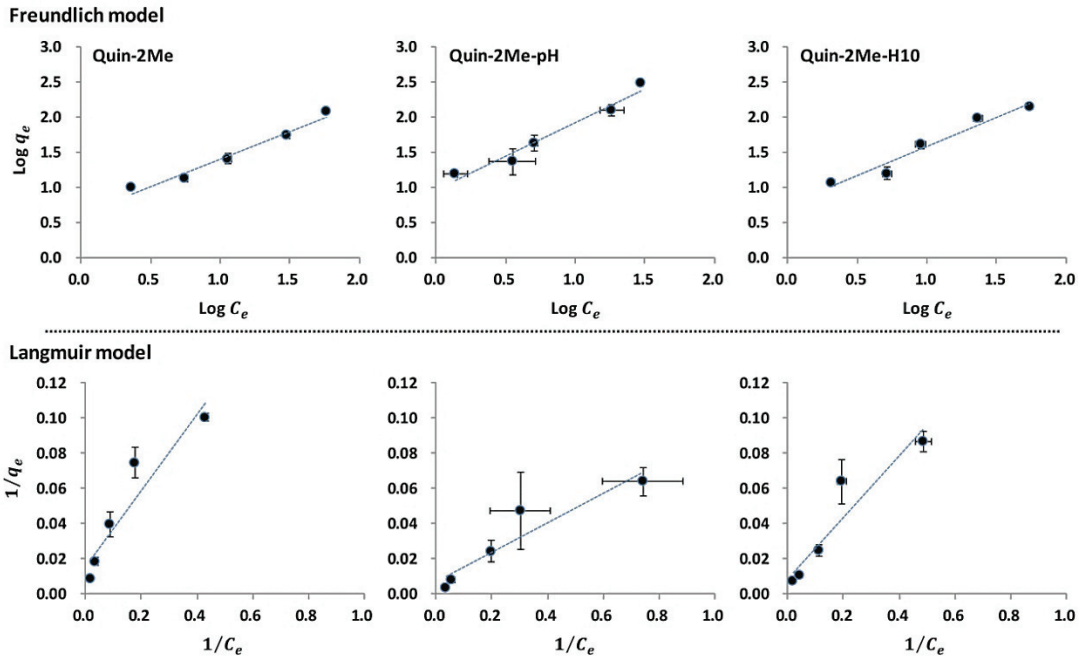


Figure 3.2.3. Freundlich and Langmuir adsorption modeling (n = 6, ± SD) of the quinaldines in soil I.

Table 3.2.4. Parameters of the Freundlich and Langmuir modeling of the quinaldines.

	Freundlich				Langmuir			
	Model	K_f [mL g^{-1}]	$1/n$	R^2	**Model**	K_L [mL g^{-1}]	q_m [mg kg^{-1}]	R^2
Quin-2Me	$\log q_e = 0.6218 + 0.7875 \log C_e$	4.19	0.79	0.97	$\frac{1}{q_e} = 0.2148 \frac{1}{C_e} + 0.016$	0.074	62.50	0.89
Quin-2Me-pH	$\log q_e = 0.9637 + 0.9629 \log C_e$	9.20	0.96	0.96	$\frac{1}{q_e} = 0.0842 \frac{1}{C_e} + 0.0068$	0.081	147.06	0.89
Quin-2Me-H10	$\log q_e = 0.7574 + 0.8296 \log C_e$	5.72	0.83	0.95	$\frac{1}{q_e} = 0.1747 \frac{1}{C_e} + 0.0087$	0.050	114.94	0.89

In the subsequent discussion, the hydrophobicity as well as the ionizability of the LOHC compounds will be important. Therefore, the octanol-water partition coefficients corrected for ionization at the pH of soil I (log D) are given in Table 3.2.3. Comparable values were obtained for Quin-2Me and Quin-2Me-pH (2.07 and 2.23), whereas a much lower value was calculated for Quin-2Me-H10 (-0.88).

Leaching of the quinaldines in soil columns. The amount of quinaldines collected in the leachates, expressed as the percentage of the total mass (mass%) spiked at the

beginning of the test, was plotted against the amount of solution pumped through the columns (expressed as multiples of the PV) (Figure 3.2.4). Breakthrough was achieved the fastest for Quin-2Me-H10 (Figures 3.2.4 C and S3.2.2 C), with approximately 65% of the total mass ultimately collected in the leachate. The breakthrough of Quin-2Me (Figures 3.2.4 A and S3.2.2 A) occurred later than of Quin-2Me-H10 with a lag of ca. 7–9 PV, and only approximately 45–60% of this compound was ultimately collected in the effluent of the column. Quin-2Me-pH behaved very differently from the other quinaldines, with more than 99% of the total spiked mass retained on the column at the end of the test (Figures 3.2.4 B and S3.2.2 B). Atrazine, which was used as the reference compound and run either alone in a separate column (Figures S3.2.3 A and S3.2.4 A) or in the same column with the quinaldines (Figures S3.2.3 B–D and S3.2.4 B–D), showed a relatively constant leaching behavior with breakthrough occurring at 4–6 PV (or approximately 48–72 h), and more than 90% was collected in the leachate at the end of the test.

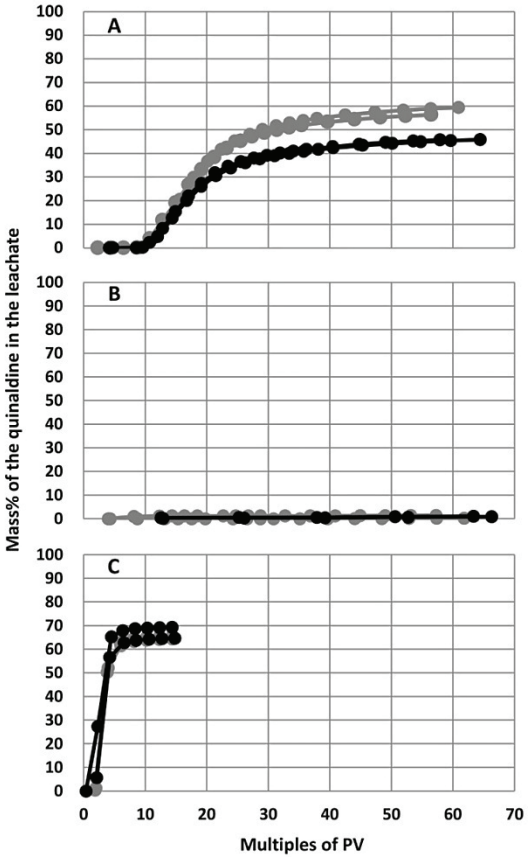


Figure 3.2.4. Breakthrough curves for Quin-2Me (**A**), Quin-2Me-pH (**B**) and Quin-2Me-H10 (**C**) in two independent experiments (gray and black), each with two columns packed with soil II. "PV": pore volume.

The column K_d values calculated according to the laboratory column method proposed by the EPA[36] yielded values of 1.00 ± 0.40 mL g^{-1}, 1.88 ± 0.43 mL g^{-1} and

0.23 ± 0.03 mL g^{-1} for Quin-2Me, Quin-2Me-pH and Quin-2Me-H10, respectively (Table 3.2.3). These values were 2–10 times lower than those measured in the batch equilibrium experiment. The corresponding partition coefficient of the reference compound, atrazine, in the leaching experiments was also determined, either together with the test compounds or alone. The values were of the same order of magnitude as the quinaldines and ranged from 0.46 mL g^{-1} to 1.39 mL g^{-1} (Table S3.2.4).

Discussion

Interaction of LOHCs with soil.

In this study, we characterized the adsorption and mobility of several potential LOHCs, including indoles, quinaldines, carbazole derivatives, benzyltoluenes and dibenzyltoluene, as well as some of their partially hydrogenated forms, in standard soils. The potential mobility of these compounds in the soil ranged from highly mobile to immobile, and the log K_{oc} values spanned five orders of magnitude (1.71–5.41) (Table 3.2.2). Partitioning into the organic matter of soil plays an important role in the retention of many chemicals in soil[39,40,41] in that the mobility decreases with increasing K_{oc}[32,42]. Therefore, indoline, Quin-2Me, Quin-2Me-pH and indole would likely be transferred farther and deeper into the soil than the carbazole derivatives, MLHs and MSH; the compounds with the highest K_{oc} values are more likely to be accumulated at the soil surface where they were released. The log K_{oc} values obtained for the H_2-lean and partially hydrogenated LOHCs using the HPLC method showed a good correlation with the COSMO-RS-predicted log K_{ow} values ($R^2 = 0.94$, Figure 3.2.5). The correlation also fit well to the K_{ow}-based K_{oc} model established by Baker *et al.*[43] ($R^2 = 0.94$, Figure S3.2.5).

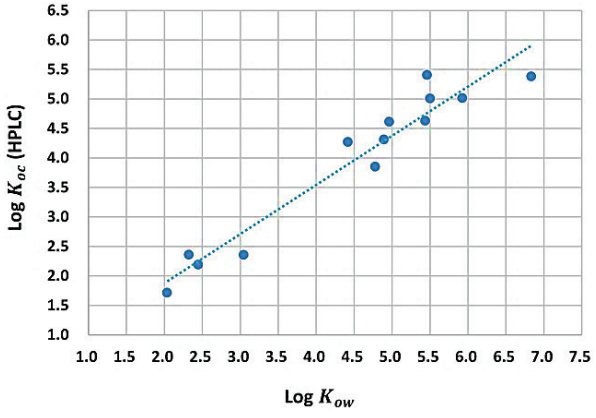


Figure 3.2.5. Relationship between log K_{oc} (n = 13, HPLC) and the COSMO-RS-predicted log K_{ow} values.

The influence of the alkyl chain length on K_{oc} was also found for the carbazole homologues. Longer alkyl chains correspond to greater interactions between the compound and the organic matter of soil through London dispersion forces[44]. Similar phenomena have also been reported for imidazolium-based compounds; rings substituted with longer alkyl chains interacted with soil much more strongly while compounds with shorter chains were comparatively more mobile in soils[45].

The soil partition coefficients of the quinaldine-based LOHC system measured using the batch equilibrium tests (batch-K_d) in soil I followed the order Quin-2Me-pH > Quin-2Me-H10 ≥ Quin-2Me, in which a factor of 2–3 difference exists between the partition coefficients of the first two compounds, and those of the latter two compounds are very similar (no significant difference, with p = 0.77). The order of affinities of the quinaldines for soil II determined from the column experiments (column-K_d) is as follows: Quin-2Me-pH > Quin-2Me >> Quin-2Me-H10. The K_d values obtained for the batch tests are generally higher than those from the column tests, which can be partially explained by the higher content of organic matter in soil I vs. soil II (1.21% vs. 0.8%).

To further aid the comparison, both the batch-K_d and column-K_d values were converted to K_{oc} values using the organic matter contents of soil I and II to yield $K_{oc,batch}$ and $K_{oc,column}$, respectively. For Quin-2Me, the $K_{oc,HPLC}$ value (155 mL g^{-1}, obtained using HPLC) matches well to $K_{oc,batch}$ (168 mL g^{-1}) and $K_{oc,column}$ (125 mL g^{-1}) as well as to $K_{oc,predicted}$ (151 mL g^{-1}, COSMO-RS-based), with less than a factor of 1.4 difference between the values. In addition, all three quinaldines are organic bases and can be present either in their neutral or protonated[16,22,42] forms depending on their pK_a values (Table 3.2.3) and the pH of the surroundings. Generally, the neutral forms (the screening charge densities indicating the polarities of the neutral forms are given in Figure S3.2.6) tend to interact with soils mostly via hydrophobic interactions, while the ionized forms mostly interact through electrostatic interactions[18]. Quin-2Me-pH showed the highest affinity for the soils, with $K_{oc,batch}$ = 543 mL g^{-1}, $K_{oc,column}$ = 235 mL g^{-1} and $K_{oc,HPLC}$ = 229 mL g^{-1}; this compound was also strongly retained in the column experiment. At the pH values of the tests (pH 5.33 and 5.41), only 23–26% of this compound is protonated, and it has the highest pH-dependent octanol-water coefficient among the quinaldines (log D = 2.23, Table 3.2.3), corresponding to the highest hydrophobicity under the test conditions. In contrast, Quin-2Me is protonated to larger extent (35–40%, or 66–75% if based on the pK_a 5.7–5.8[38]) at the pH of the tests, which leads to a lower log D value (2.07). This is likely the cause of the reduced soil partitioning of Quin-2Me, as evidenced by its lower K_{oc} values.

Quin-2Me-H10 is fully protonated at a pH of 5.33–5.41. As a result, it is significantly less hydrophobic than the other two members of the LOHC system, as evidenced by its lowest log D value (-0.88). Therefore, this compound would be expected to interact with soils through electrostatic interactions rather than hydrophobic interactions. The sorption of Quin-2Me-H10 on negatively charged soil sites[22] might be the reason why the amount adsorbed is comparable to that of the

much more hydrophobic Quin-2Me compound (no significant difference in the batch-K_d values). The fact that Quin-2Me-H10 moved through the soil column relatively quickly and was minimally retained ($K_{oc,column}$ = 29 mL g^{-1}) was therefore rather unexpected in this context. However, this phenomenon might be explained by the fact that soil column is a much more dynamic system than that of the batch tests, with a lower solid-to-liquid ratio and a much larger amount of competing ions (Ca^{2+} in this case). Competition for sorption sites has been observed between Ca^{2+} and the cations of ionic liquids[44], and suppression of the adsorption of nitro-aromatics has also been found in the presence of Ca^{2+} [18]. The absence of such a large difference between the partition coefficients obtained from the batch tests and the column experiments for the other two members of the quinaldine-based LOHC system supports this assumption. These other two compounds should have been adsorbed mostly through hydrophobic interactions; therefore, the presence of competing cations had a smaller impact on their sorption than on the sorption of Quin-2Me-H10.

Although the column leaching experiment was not performed for the other LOHC compounds in this study, i.e., indoles, carbazole derivatives, MLHs and MSH, their soil affinities (excluding the fully hydrogenated forms) could be estimated based on their K_{oc} values, especially since these compounds are unlikely to be ionized within the environmentally relevant pH range. The order of affinities of these compounds would then follow the order of their K_{oc} values, i.e., indoles would be the most leachable/mobile, while MLH, the carbazole derivatives, MSH and MLH-pH may be retained in the soil to higher extent. Nevertheless, further confirmation of the affinities of these compounds by batch equilibrium and leaching experiments is recommended given the variables discussed above.

Method comparison for partitioning-based adsorption.

Three typical methods for determining the adsorption potential of organic contaminants in soils were applied in this study. The HPLC screening method is the simplest, but the assumption that it adequately represents the potential for interaction with the soil was not fully supported in the current study. The estimated K_{oc} value was quite accurate for Quin-2Me, whereas a larger discrepancy was observed for Quin-2Me-pH.

Both column leaching and batch equilibrium tests are commonly conducted in the laboratory to evaluate sorption properties. The duration of the batch equilibrium test is much shorter than that of the column test, especially when investigating strongly adsorbing substrates (such as Quin-2Me-pH in this study)[45]. Moreover, due to efficient mixing, the adsorption equilibrium is generally achieved faster in the batch equilibrium test because macroscopic mass transfer is not hindered[45]. Therefore, the batch equilibrium test is often treated as the "worst case scenario"[45]. However, the effluent concentrations during percolation are underestimated in the batch equilibrium test[27], as shown here for Quin-2Me-H10. Column leaching represents a dynamic system that is supposed to simulate the downward movement of chemicals through

soil[45]. Although this column experiment is more time consuming, it allows for an extended period of testing and a better simulation of the water flow through the porous soil profile due to a more realistic solid-to-liquid ratio[45]. Processes that occur in nature, such as particle-associated transport[32], and the presence of regions with immobile water or preferential flow[45] are also accounted for in column leaching tests, better matching the conditions of the experiment to those of the environment[45]. In such a test, the maximum adsorption equilibrium is not necessarily fully achieved in the dynamic process, even at the point of breakthrough[45], due to insufficient mixing and the adsorption/desorption processes of various, often competing, ions[45,46]. Therefore, the column-K_d values of the quinaldines tended to be lower than those measured in the batch tests. In addition, column leaching tests could provide more information for the assessment of the potential for groundwater contamination, which can eventually be correlated to the risk of human exposure via drinking water or contaminated crops[32].

The concentration used to spike the soil columns in the study was 100 mg kg^{-1}, which is considered the worst case scenario which might occur in the environment only through heavy contamination resulting from accidental spills. However, the localized leakage or spillage of these compounds is possible since they may eventually be produced and transported on a large scale and handled by citizens in the same way fossil fuels currently are. Furthermore, the spiked dose was high enough to ensure the detection of the leached quinaldines.

Environmental relevance.

According to the data presented in this work, a preliminary environmental hazard assessment of the LOHCs can be performed based on the estimated adsorption behavior and the corresponding possible exposure route of LOHCs in the soil. The mobility of LOHCs in soils can be predicted to a certain extent based on their K_{oc} values. Indoles and quinaldines (specifically Quin-2Me) are thereby expected to be more mobile and thus bioaccessible to soil organisms (at least those for which exposure to pore water is dominant) than the other LOHCs. Nevertheless, K_{oc} might not be a sufficient indicator for predicting the behavior of compounds for which hydrophobicity-driven partitioning does not govern the soil interactions (e.g., ionizable compounds). Therefore, batch equilibrium adsorption or column leaching experiments are still recommended. Based on the results of the leaching experiments performed in this study, two major components of the quinaldine-based LOHC system (i.e., Quin-2Me and Quin-2Me-H10) are expected to distribute farther from the release point and penetrate deeper into the soil. The rather low affinity of these two compounds for the soils studied here indicates a risk of groundwater contamination, especially in the case of Quin-2Me-H10, which is comparatively easily transported through soil. The exposure of soil-dwelling organisms through pore water is therefore anticipated to be the highest for Quin-2Me and Quin-2Me-H10 but rather limited for their partially hydrogenated forms. In contrast, Quin-2Me-pH is likely to be retained

in the upper layers of the soil and therefore presents a lower risk of groundwater contamination. Nevertheless, soil organisms that feed primarily on particles could still be very vulnerable to this compound. It is noteworthy that soil in the environment is highly heterogeneous and could represent more complex properties than the test soils in the study. Large variation of soil composition, from texture to minerals to varied quantity and quality of organic carbon has to be taken into consideration for further predictions, i.e., adsorption and mobility of the LOHCs and their accessibility to organisms will vary great according to local soil[47,48] and climatic conditions[49].

Acknowledgements

This work was financially supported by the University of Bremen and the European Union FP7 COFUND within Marie Curie Actions BremenTrac Program (grant agreement No.600411) and M8 Postoc-Initiatve PLUS (funded by the German Excellence Initiative) as well as the German Federal Foundation for the Environment (Deutsche Bundesstiftung Umwelt (DBU), Osnabrück/Germany). We would like to thank the entire UFT Team for the interdisciplinary cooperation. We acknowledge the working group of Prof. Dr. Peter Wasserscheid for providing the LOHCs chemicals. The authors would like to thank Dr. Jan Köser for the support on MATLAB.

References

(1) Teichmann, D.; Arlt, W.; Wasserscheid, P.; Freymann, R. A future energy supply based on Liquid Organic Hydrogen Carriers (LOHC). *Energy Environ. Sci.* **2011**, *4* (8), 2767–2773.

(2) Teichmann, D.; Arlt, W.; Wasserscheid, P. Liquid organic hydrogen carriers as an efficient vector for the transport and storage of renewable energy. *Int. J. Hydrogen Energy* **2012**, *37* (23), 18118–18132.

(3) Zhu, Q. L.; Xu, Q. Liquid organic and inorganic chemical hydrides for high-capacity hydrogen storage. *Energy Environ. Sci.* **2015**, *8*, 478–512.

(4) Preuster, P.; Papp, C.; Wasserscheid, P. Liquid Organic Hydrogen Carriers (LOHCs): toward a hydrogen-free hydrogen economy. *Acc. Chem. Res.* **2017**, *50* (1), 74–85.

(5) Geburtig, D.; Preuster, P.; Bösmann, A.; Müller, K.; Wasserscheid, P. Chemical utilization of hydrogen from fluctuating energy sources - catalytic transfer hydrogenation from charged Liquid Organic Hydrogen Carrier systems. *Int. J. Hydrogen Energy* **2016**, *41* (2), 1010–1017.

(6) Evans, A.; Strezov, V.; Evans, T. J. Assessment of sustainability indicators for renewable energy technologies. *Renew. Sustain. Energy Rev.* **2009**, *13* (5), 1082–1088.

(7) Markiewicz, M.; Zhang, Y. Q.; Bösmann, A.; Brückner, N.; Thöming, J.; Wasserscheid, P.; Stolte, S. Environmental and health impact assessment of Liquid Organic Hydrogen Carrier (LOHC) systems - challenges and preliminary results. *Energy Environ. Sci.* **2015**, *8* (3), 1035–1045.

(8) Teichmann, D.; Stark, K.; Müller, K.; Zöttl, G.; Wasserscheid, P.; Arlt, W. Energy storage in residential and commercial buildings via Liquid Organic Hydrogen Carriers (LOHC).

Energy Environ. Sci. **2012**, *5* (10), 9044–9054.

(9) Dincer, I. Renewable energy and sustainable development: a crucial review. *Renew. Sustain. Energy Rev.* **2000**, *4* (2), 157–175.

(10) Eblagon, K. M.; Rentsch, D.; Friedrichs, O.; Remhof, A.; Zuettel, A.; Ramirez-Cuesta, A. J.; Tsang, S. C. Hydrogenation of 9-ethylcarbazole as a prototype of a liquid hydrogen carrier. *Int. J. Hydrogen Energy* **2010**, *35* (20), 11609–11621.

(11) Fikrt, A.; Brehmer, R.; Milella, V. O.; Müller, K.; Bösmann, A.; Preuster, P.; Alt, N.; Schlücker, E.; Wasserscheid, P.; Arlt, W. Dynamic power supply by hydrogen bound to a liquid organic hydrogen carrier. *Appl. Energy* **2017**, *194*, 1–8.

(12) Müller, K.; Stark, K.; Emel'yanenko, V. N.; Varfolomeev, M. A.; Zaitsau, D. H.; Shoifet, E.; Schick, C.; Verevkin, S. P.; Arlt, W. Liquid Organic Hydrogen Carriers: thermophysical and thermochemical studies of benzyl- and dibenzyl-toluene derivatives. *Ind. Eng. Chem. Res.* **2015**, *54* (32), 7967–7976.

(13) Papp, C.; Wasserscheid, P.; Libuda, J.; Steinrück, H. P. Liquid organic hydrogen carriers: surface carbazole derivatives. *Chem. Rec.* **2014**, *14* (5), 879–896.

(14) Hu, P.; Fogler, E.; Diskin-Posner, Y.; Iron, M. A.; Milstein, D. A novel liquid organic hydrogen carrier system based on catalytic peptide formation and hydrogenation. *Nat. Commun.* **2015**, *6*, 6859.

(15) European Chemicals Agency. Guidance on information requirements and chemical safety assessment Part B: Hazard assessment. **2011**, No. December, 1–68.

(16) Anyanwu, I. N.; Semple, K. T. Fate and behaviour of nitrogen-containing polycyclic aromatic hydrocarbons in soil. *Environ. Technol. Innov.* **2015**, *3*, 108–120.

(17) Stokes, J. D.; Paton, G. I.; Semple, K. T. Behaviour and assessment of bioavailability of organic contaminants in soil: relevance for risk assessment and remediation. *Soil Use Manag.* **2005**, *21* (s2), 475–486.

(18) Bi, E.; Schmidt, T. C.; Haderlein, S. B. Environmental factors influencing sorption of heterocyclic aromatic compounds to soil. *Env. Sci Technol* **2007**, *41* (9), 3172–3178.

(19) Semple, K. T.; Morriss, A. W. J.; Paton, G. I. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. *Eur. J. Soil Sci.* **2003**, *54* (4), 809–818.

(20) Di Toro, D. M.; Zarba, C. S.; Hansen, D. J.; Berry, W. J.; Swartz, R. C.; Cowan, C. E.; Pavlou, S. P.; Allen, H. E.; Thomas, N. A.; Paquin, P. R. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ. Toxicol. Chem.* **1991**, *10* (12), 1541–1583.

(21) Liao, P.; Yuan, S.; Xie, W.; Zhang, W.; Tong, M.; Wang, K. Adsorption of nitrogen-heterocyclic compounds on bamboo charcoal: kinetics, thermodynamics, and microwave regeneration. *J. Colloid Interface Sci.* **2013**, *390* (1), 189–195.

(22) Thomsen, A. B.; Henriksen, K.; Grøn, C.; Møldrup, P. Sorption, transport, and degradation of quinoline in unsaturated soil. *Environ. Sci. Technol.* **1999**, *33* (17), 2891–2898.

(23) Sverdrup, L. E.; Kelley, A. E.; Krogh, P. H.; Nielsen, T.; Jensen, J.; Scott-Fordsmand, J. J.; Stenersen, J. Effects of eight polycyclic aromatic compounds on the survival and reproduction of the springtail *Folsomia fimetaria L.* (Collembola, Isotomidae). *Environ. Toxicol. Chem.* **2001**, *20* (6), 1332–1338.

(24) Aboul-Kassim, T. A. T.; Simoneit, B. R. T. *Interaction mechanisms between organic*

pollutantsand solid phase systems; Springer Berlin Heidelberg: Berlin Heildelberg, 2001.

(25) Liu, D.; Gui, J.; Sun, Z. Adsorption structures of heterocyclic nitrogen compounds over Cu(I)Y zeolite: a first principle study on mechanism of the denitrogenation and the effect of nitrogen compounds on adsorptive desulfurization. *J. Mol. Catal. A Chem.* **2008**, *291* (1–2), 17–21.

(26) Doucette, W. J. Quantitative structure-activity relationships for predicting soil-sediment sorption coefficients for organic chemicals. *Environ. Toxicol.* **2003**, *22* (8), 1771–1788.

(27) Grathwohl, P.; Susset, B. Comparison of percolation to batch and sequential leaching tests: theory and data. *Waste Manag.* **2009**, *29*, 2681–2688.

(28) Witt, G.; Bartsch, C.; Liehr, G. A.; Thiele, R.; McLachlan, M. S. Using solid-phase microextraction to evaluate the role of different carbon matrices in the distribution of PAHs in sediment-porewater systems of the Baltic Sea. *J. Soils Sediments* **2010**, *10* (7), 1388–1400.

(29) Xiao, F.; Pignatello, J. J. π^{+}-π Interactions between (hetero)aromatic amine cations and the graphitic surfaces of pyrogenic carbonaceous materials. *Environ. Sci. Technol.* **2015**, *49* (2), 906–914.

(30) Li, S.; Turaga, U.; Shrestha, B.; Anderson, T. A.; Ramkumar, S. S.; Green, M. J.; Das, S.; Cañas-Carrell, J. E. Mobility of polyaromatic hydrocarbons (PAHs) in soil in the presence of carbon nanotubes. *Ecotoxicol. Environ. Saf.* **2013**, *96*, 168–174.

(31) Yang, L.; Jin, M.; Tong, C.; Xie, S. Study of dynamic sorption and desorption of polycyclic aromatic hydrocarbons in silty-clay soil. *J. Hazard. Mater.* **2013**, *244–245*, 77–85.

(32) Revitt, D. M.; Balogh, T.; Jones, H. Soil mobility of surface applied polyaromatic hydrocarbons in response to simulated rainfall. *Environ. Sci. Pollut. Res.* **2014**, *21* (6), 4209–4219.

(33) OECD. Test No. 121: Estimation of the adsorption coefficient (Koc) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC). *OECD Guidel. Test. Chem.* **2001**, No. January, 1–11.

(34) OECD. Test No. 106: Adsorption -- desorption using a batch equilibrium method. *OECD Guidel. Test. Chem.* **2000**, No. January, 1–44.

(35) OECD. Test No. 312: Leaching in soil columns. *OECD Guidel. Test. Chem.* **2004**, No. April, 1–15.

(36) EPA. Understanding variation in partition coefficient, Kd, values: review of geochemistry and available Kd values for americium, arsenic, curium, iodine, neptunium, radium, and technetium. *Environ. Prot. Agency* **2004**, *III* (July).

(37) McCall, P. J.; Swann, R. L.; Laskowski, D. A.; Unger, S. M.; Vrona, S. A.; Dishburger, H. J. Estimation of chemical mobility in soil from liquid chromatographic retention times. *Bull. Environ. Contam. Toxicol.* **1980**, *24* (1), 190–195.

(38) Jones, G. *The Chemistry of Heterocyclic Compounds, Quinolines*; John Wiley & Sons, 2009; Vol. 32.

(39) Tolls, J. Sorption of veterinary pharmaceuticals in soils: a review. *Environ. Sci. Technol.* **2001**, *35* (17), 3397–3406.

(40) Kuśmierz, M.; Oleszczuk, P.; Kraska, P.; Pałys, E.; Andruszczak, S. Persistence of polycyclic aromatic hydrocarbons (PAHs) in biochar-amended soil. *Chemosphere* **2016**,

146, 272–279.

(41) Weissenfels, W. D.; Klewer, H. J.; Langhoff, J. Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity. *Appl. Microbiol. Biotechnol.* **1992**, *36* (5), 689–696.

(42) Kobetičová, K.; Bezchlebová, J.; Lána, J.; Sochová, I.; Hofman, J. Toxicity of four nitrogen-heterocyclic polyaromatic hydrocarbons (NPAHs) to soil organisms. *Ecotoxicol. Environ. Saf.* **2008**, *71* (3), 650–660.

(43) Baker, J. R.; Mihelcic, J. R.; Luehrs, D. C.; Hickey, J. P. Evaluation of estimation methods for organic carbon normalized sorption coefficients. *Water Environ. Res.* **1997**, *69* (2), 136–145.

(44) Stepnowski, P.; Mrozik, W.; Nichthauser, J. Adsorption of alkylimidazolium and alkylpyridinium ionic liquids onto natural soils. *Environ. Sci. Technol.* **2007**, *41* (2), 511–516.

(45) Markiewicz, M.; Jungnickel, C.; Cho, C.-W.; Stolte, S. Mobility and biodegradability of an imidazolium based ionic liquid in soil and soil amended with waste sewage sludge. *Environ. Sci. Process. Impacts* **2015**, *17* (8), 1462–1469.

(46) Kördel, W.; Bernhardt, C.; Derz, K.; Hund-Rinke, K.; Harmsen, J.; Peijnenburg, W.; Comans, R.; Terytze, K. Incorporating availability/bioavailability in risk assessment and decision making of polluted sites, using Germany as an example. *J. Hazard. Mater.* **2013**, *261*, 854–862.

(47) Matzke, M.; Thiele, K.; Müller, A.; Filser, J. Sorption and desorption of imidazolium based ionic liquids in different soil types. *Chemosphere* **2009**, *74* (4), 568–574.

(48) Styrishave, B.; Hartnik, T.; Christensen, P.; Andersen, O.; Jensen, J. Influence of soil type and organic matter content on the bioavailability, accumulation, and toxicity of α-cypermethrin in the springtail *Folsomia candida*. *Environ. Toxicol. Chem.* **2010**, *29* (5), 1084–1090.

(49) Vancampenhout, K.; Wouters, K.; De Vos, B.; Buurman, P.; Swennen, R.; Deckers, J. Differences in chemical composition of soil organic matter in natural ecosystems from different climatic regions - a pyrolysis-GC/MS study. *Soil Biol. Biochem.* **2009**, *41* (3), 568–579.

‡ Supporting Information

S3.2.1. Materials.

The LOHC compounds with different degrees of hydrogenation used in the study included quinaldines (2-methylquinoline, tetrahydro-2-methylquinoline, decahydro-2-methylquinoline), indoles (indole and indoline), carbazole derivatives (9-ethyl-9H-carbazole, 9-propyl-9H-carbazole, 9-butyl-9H-carbazole and the respective partially hydrogenated forms), MLH (benzyltoluene and partially hydrogenated forms) and MSH (dibenzyltoluene). The partially hydrogenated LOHC

chemicals were technical mixtures (excluding the quinaldine-based) obtained from the hydrogenation reaction before full conversion was achieved; they contained a mixture of the respective H_2-lean forms at different levels of hydrogenation, including the H_2-lean and H_2-rich compounds as well as intermediate structures.

Citric acid ($C_6H_8O_7 \cdot H_2O$, > 99.5%) was obtained from Acros Organics, New Jersey, USA. Sodium nitrate ($NaNO_3$, 99.5%) was purchased from Riedel-deHaën, Seelze, Germany. Methanol (HPLC grade) was from VWR Chemicals, France. Anhydrous sodium sulfate (Na_2SO_4, 99.9%) was purchased from VWR, Belgium. Tri-sodium citrate dihydrate ($C_6H_5O_7Na_3 \cdot 2H_2O$, 99%) and dichloromethane (DCM, GC grade ≥ 99.8%) were purchased from Merck, Darmstadt, Germany. Atrazine (analytical grade, Table S3.2.2), acetone (HPLC grade ≥ 99.8%), quinoline (GC grade = 98%) and naphthalene (GC grade ≥ 99%) were purchased from Sigma-Aldrich (Steinheim, Germany). Unless specifically stated, deionized water filtered through a Carbonit NFP Premium-9 water filter (Heidenheim, Germany) with a pore size of 0.45 µm was used in this study.

The information of the soils was obtained in the product sheet from Fraunhofer IME, Schmallenberg, Germany. Both soils were in the same type but of different batches, which were collected from Schmallenberg, Nordrhein-Westfalen, Germany. No pesticide has been applied to the soils during the previous two years or the application of fertilizers since the one year before collection.

Table S3.2.1. Properties of the test soils. "%" indicates w/w.

	Sand [%]	**Silt** [%]	**Clay** [%]	**OC**[a] [%]	**Total (N)** [g kg^{-1}]	**pH($_{CaCl_2}$)**	**CEC**[b] [mmol kg^{-1}]	**WHC** [g kg^{-1}]	**Applied in the test**
Soil I	76.7	17.2	6.1	1.21	0.79	5.41	9.90	291	batch[c]
Soil II	76.7	17.2	6.1	0.80	0.71	5.33	17.90	291	leaching[d]

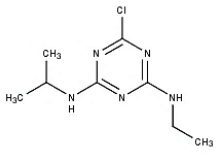
[a] Organic carbon.

[b] Cation exchange capacity.

[c] Adsorption batch equilibrium experiment.

[d] Soil column leaching test.

Table S3.2.2. Physicochemical properties of atrazine.

	Formula	**Chemical structure**	**MW** [g mol^{-1}]	**Water solubility** (25°C)[a] [mg L^{-1}]	**Log K_{ow}**[a]	**Log K_{oc}**[a]
Atrazine	$C_8H_{14}ClN_5$		45.0	214.1	2.82	2.61

[a] Predicted by Estimation Programs Interface Suite™ v4.1 (https://www.epa.gov/).

S3.2.2. The COSMO-RS model and calculations.

COSMO-RS (Conductor like Screening Model for Realistic Solvation, COSMO*logic* GmbH and Co. KG, Leverkusen, Germany)[1] is a statistical thermodynamics model for calculation of the chemical potential of a compound in solution. Thus, it can be used to predict all equilibrium properties that can be derived from the chemical potential, e.g., the octanol–water partition coefficient used in this study.

The COSMO-RS model uses the information of an underlying quantum chemical COSMO[2] calculation that minimizes the energy of the molecule in the surroundings of a perfect conductor. This artificial state is used as the reference state for the COSMO-RS calculation. The so-called screening charge density, σ, which describes the response of the conductor to the charge distribution of the solute at the solute-solvent boundary, is the most important descriptor used in the statistical thermodynamic procedure of COSMO-RS and contains information about the polarity of the solute. This σ value can also be used to fit quantitative structure-property relationship models for the partitioning between less well-defined phases, such as the soil sorption (K_{oc}) model used in this work[3].

All COSMO-RS calculations were performed with the COSMO*therm* (Version C3.0 Release 17.01) program (COSMO*logic* GmbH and Co. KG, Leverkusen, Germany, http://www.cosmologic.de). The BP_TZVP_C30_1701 parameterization was used for the K_{oc} predictions. All other results were obtained with the BP_TZVPD_FINE_C30_1701 parameter set and the standard conformer treatment. The TURBOMOLE V7.1 program package (TURBOMOLE GmbH, http://www.turbomole.com) was used for the quantum chemical calculations. All structures were optimized with the BP86 functional[4,5,6] and the TZVP basis set[7] using the COSMO model with standard cavity. Single point calculations were performed with the BP functional and the def2-TZVPD[8] basis set with iso-radii COSMO cavity using the optimized structures.

S3.2.3. Determination of log K_{oc}.

The reference substances for HPLC calibration were divided into two groups based on the predicted K_{oc} values to shorten the time of analysis and also for better integrations of the signals. Group A consisted of acetanilide, acetophenone,

4-chloroaniline, toluene, ethylbenzene, biphenyl, phenanthrene and fluoranthene, and group B contained 4-methoxyphenol and cinnamyl alcohol. The retention time of each compound was measured on three separate days with three injections each day, and the averages of the measurements were calculated. The retention times of the reference substances (t_R) and non-retained substance (t_0) were used to calculate the capacity factor (k) of each reference substance according to Eq. (S1)[9]. The calibration

was established by linear regression with the literature log K_{oc} values of the reference substances as a function of log k.

$$k = \frac{t_R - t_0}{t_R} \qquad (S1)$$

The retention times of the LOHCs under the same HPLC settings were used to calculate their capacity factors, and these in turn were used to estimate the K_{oc} values from the calibration curve. The solutions of the reference substances and LOHC analytical samples were prepared in the mobile phase

S3.2.4. Determination of K_d and adsorption isotherm modeling.

An optimum soil/liquid ratio of 1:10 and equilibration time of 6 days were determined from preliminary experiments. The adsorption isotherms were investigated for 5 concentrations. One control containing only the test substance in 10 mL of 0.01 M $CaCl_2$ and no soil was prepared for each concentration to account for possible losses (e.g., sorption on the test vessels). One blank run (1 g dw of soil and 10 mL of 0.01 M $CaCl_2$ solution without a test substance) was prepared in parallel for each test.

S3.2.5. Leaching in soil columns.

Soil was packed in columns in small portions with a spoon and pressed with a plunger to obtain uniform packing. The columns were filled with soil to a height of approximately 28 cm until the top of the soil was level. The soil columns were subsequently pre-wetted from bottom to top with 0.01 M $CaCl_2$ and then allowed to drain. The prepared columns were then spiked with the quinaldines.

The K_d values of the quinaldines from the column leaching tests were calculated using the relationship $K_d = \left[\left(R_f - 1\right) \times n\right]/\rho_b$ [10]. The R_f (retardation factor) is the ratio of V_p (assumed to be the pore water velocity) to V_c (the contaminant velocity). The parameters n and ρ_b indicate the total porosity and bulk density, respectively, of soil II and were calculated for each test column. To obtain V_p, the PV (in mL) of the packed soil in each column was first calculated by subtracting the volume of the soil particles (calculated from the particle density and soil weight) from the total volume of soil that was packed into the column. The time that the artificial rain passed the pores in the soil column (t_0) was thus calculated as PV/0.108 (0.108 mL min^{-1} was the flow rate). V_p (cm h^{-1}) was presumed to be the velocity of the artificial rain passing through the column and thus was the quotient of the height of soil packed in each column (L_s, cm) divided by t_0. V_c was the division of L_s by the time in which the first portion of each quinaldine was collected and detected (t_q).

S3.2.6. Liquid-liquid extraction system.

A 10-mL aliquot of the quinaldine samples was subjected to extraction. Quinoline, which served as a surrogate standard, was prepared in water, and a 10 µL aliquot of this solution was spiked into the aqueous sample to give a final concentration of 1.0 mg L^{-1}. Then, 2 mL of dichloromethane was added, and the sample was vortexed for 45 s. The organic phase was subsequently transferred to a new vial, and approximately 0.3 g of anhydrous sodium sulfate (Na_2SO_4) was added to remove the remaining water. A 1-mL portion of the extract was transferred to a GC vial, and 10 µL of naphthalene in dichloromethane (internal standard, 2.0 g L^{-1}) was spiked into the final sample. The concentrations were measured by GC/MS.

S3.2.7. GC/MS analysis.

The samples (2 µL) were injected in pressure-pulsed splitless mode with the aid of an autosampler. The capillary column (CS, FS-Supreme-5ms column, 0.25 mm ID × 30 m, 0.25 µm film thickness) was operated at a pressure of 2.01 atm with a flow rate of 3.0 mL min^{-1} using helium as the carrier gas. The GC method parameters were as follows: inlet temperature: 250°C; oven program: 100°C, hold 0.6 min, ramp to 150°C at 20°C min^{-1}, ramp to 300°C at 35°C min^{-1}, hold 1 min. The MS source and the temperature of the quadrupole were set to 230°C and 150°C, respectively. The MS detector was operated in positive ion mode using an electron ionization energy of 70 eV. Spectra were recorded in full scan mode with a scan rate of 3.0 scans s^{-1}. The results were evaluated with Chemstation software (Agilent Technologies, Waldbronn, Germany). The concentrations of each component in the extract were determined using the peak area normalized by the internal standard (naphthalene) and an eleven-point calibration series (containing naphthalene, quinoline, atrazine, Quin-2Me, Quin-2Me-pH and Quin-2Me-H10, with the latter five at concentrations ranging from 0.5 to 100 mg L^{-1} in dichloromethane).

Table S3.2.3. Log K_{oc} values (n = 3, ± SD) determined by HPLC screening for the components in each mixture.

LOHCs	**Car-2-pH**		**Car-3-pH**		**Car-4-pH**		**MLH**	
Log K_{oc}	4.27 ± 0.01	4.35 ± 0.01	4.59 ± 0.01	4.67 ± 0.01	4.96 ± 0.01	5.06 ± 0.01	3.78 ± 0.01	3.85 ± 0.01
LOHCs	**MLH**	**MLH-pH**						**MSH**
Log K_{oc}	3.92 ± 0.01	5.10 ± 0.03	5.28 ± 0.03	5.34 ± 0.03	5.43 ± 0.03	5.53 ± 0.03	5.76 ± 0.03	5.11 ± 0.03
LOHC	**MSH**							
Log K_{oc}	5.20 ± 0.03	5.28 ± 0.03	5.35 ± 0.03	5.44 ± 0.03	5.54 ± 0.03	5.76 ± 0.04		

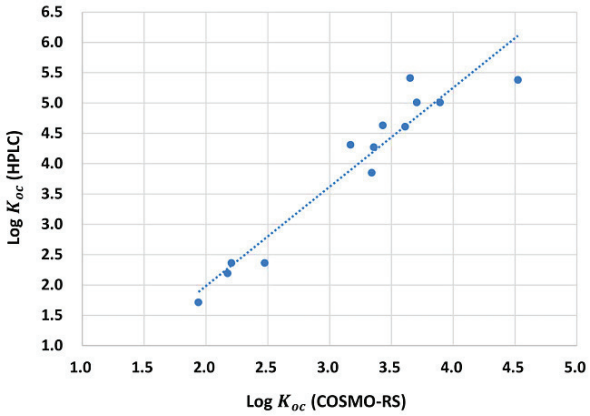


Figure S3.2.1. Relationship (R^2 = 0.92) between log K_{oc} (n = 13, HPLC) and COSMO-RS-predicted log K_{oc}.

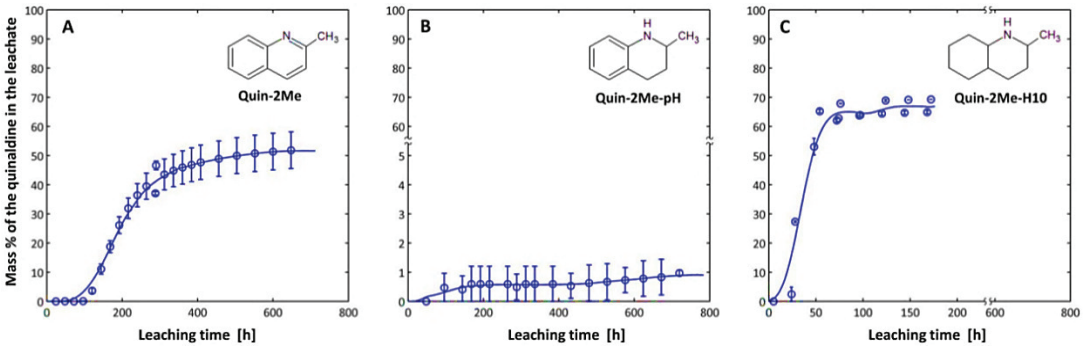


Figure S3.2.2. Breakthrough curves (mass% in the leachate, n = 4, ± SD) of Quin-2Me (**A**), Quin-2Me-pH (**B**) and Quin-2Me-H10 (**C**) in soil columns (soil II) over 648, 720, and 172 h, respectively.

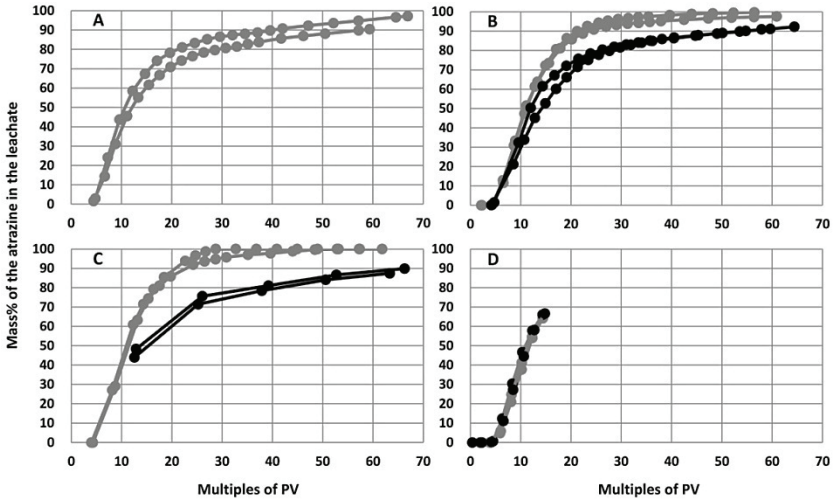


Figure S3.2.3. Breakthrough curves for the column leaching of pure atrazine independently in two columns (**A**) or together with Quin-2Me (**B**), Quin-2Me-pH (**C**) and Quin-2Me-H10 (**D**) in two independent experiments (gray and black), each with two columns of soil II.

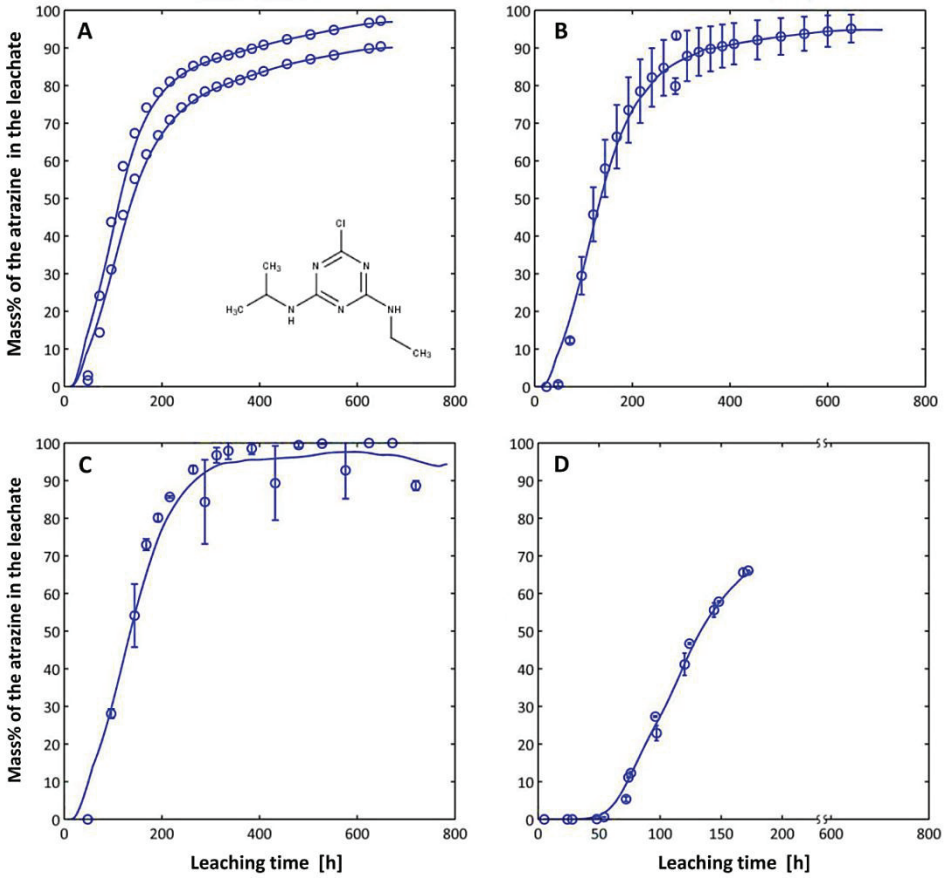


Figure S3.2.4. Breakthrough curves (mass% in the leachate) of pure atrazine alone (**A**) or in the presence of Quin-2Me (**B**), Quin-2Me-pH (**C**) and Quin-2Me-H10 (**D**) (n = 4, ± SD) in soil columns (soil II) over 648, 648, 720, and 172 h, respectively.

Table S3.2.4. K_d values of atrazine extrapolated from the column leaching test in soil II.

	Column-K_d [mL g^{-1}]
Atrazine (with Quin-2Me)	0.46
Atrazine (with Quin-2Me-pH)	1.39
Atrazine (with Quin-2Me-H10)	0.82
Atrazine (pure)	0.61
Average	0.82

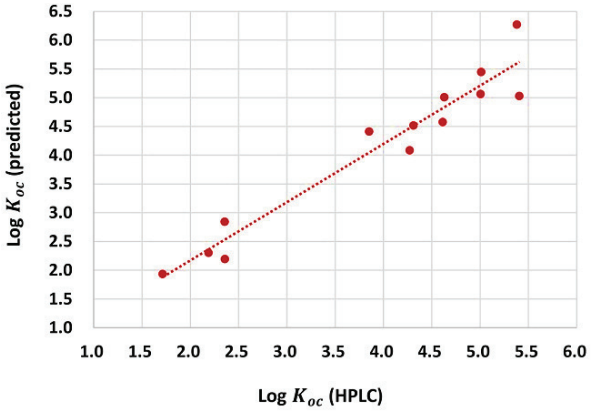


Figure S3.2.5. Relationship (R^2 = 0.94) between log K_{oc} (n = 13, HPLC) and the K_{ow}-based K_{oc} model proposed by Baker *et al.*[11].

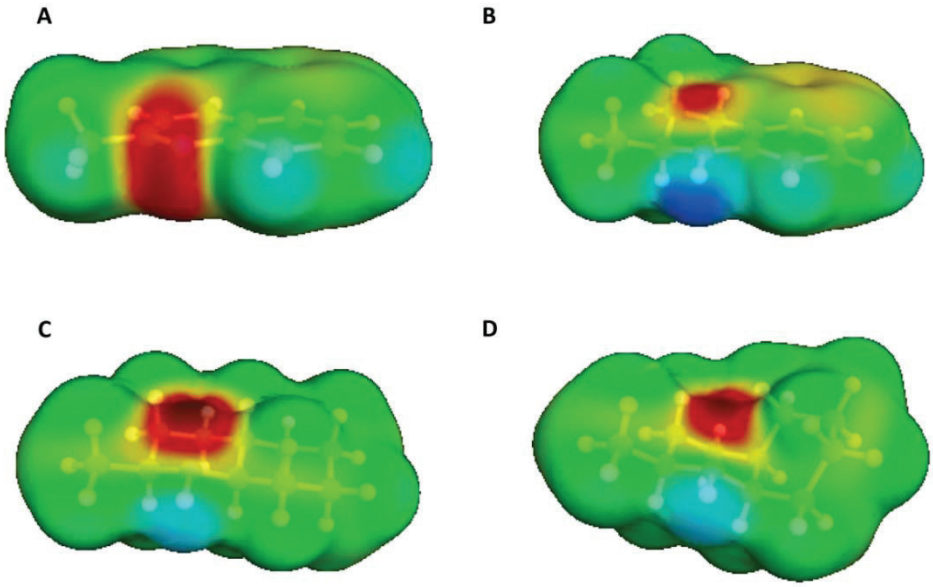


Figure S3.2.6. COSMO screening charge density (σ) on the surfaces of Quin-2Me (**A**), Quin-2Me-pH (**B**), *trans*-Quin-2Me-H10 (**C**), and *cis*-Quin-2Me-H10 (**D**).

References

(1) Eckert, F.; Klamt, A. Fast Solvent Screening via Quantum Chemistry: COSMO-RS Approach. *AIChE J.* **2002**, *48* (2), 369–385.

(2) Klamt, A.; Schüürmann, G. COSMO: a new approach to dielectric screening in solvents with explicit expressions for the screening energy and its gradient. *J. Chem. Soc., Perkin Trans. 2* **1993**, No. 5, 799–805.

(3) Klamt, A.; Eckert, F.; Diedenhofen, M. Prediction of soil sorption coefficients with a conductor-like screening model for real solvents. *Environ. Toxicol. Chem.* **2002**, *21* (12), 2562–2566.

(4) Becke, A. D. Density-functional exchange-energy approximation with correct asymptotic behavior. *Phys. Rev. A* **1988**, *38* (6), 3098–3100.

(5) Vosko, S. H.; Wilk, L.; Nusair, M. Accurate spin-dependent electron liquid correlation energies for local spin density calculations: a critical analysis. *Can. J. Phys.* **1980**, *58* (8), 1200–1211.

(6) Perdew, J. P.; Yue, W. Accurate and simple density functional for the electronic exchange energy: generalized gradient approximation. *Phys. Rev. B* **1986**, *33* (12), 8800–8802.

(7) Schäfer, A.; Huber, C.; Ahlrichs, R. Fully optimized contracted Gaussian basis sets of triple zeta valence quality for atoms Li to Kr. *J. Chem. Phys.* **1994**, *100* (8), 5829–5835.

(8) Rappoport, D.; Furche, F. Property-optimized Gaussian basis sets for molecular response calculations. *J. Chem. Phys.* **2010**, *133* (13), 0–11.

(9) OECD. Test No. 121: Estimation of the adsorption coefficient (Koc) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC). *OECD Guidel. Test. Chem.* **2001**, No. January, 1–11.

(10) EPA. Understanding variation in partition coefficient, K_d, values: review of geochemistry and available K_d values for americium, arsenic, curium, iodine, neptunium, radium, and technetium. *Environ. Prot. Agency* **2004**, *III* (July).

(11) Baker, J. R.; Mihelcic, J. R.; Luehrs, D. C.; Hickey, J. P. Evaluation of estimation methods for organic carbon normalized sorption coefficients. *Water Environ. Res.* **1997**, *69* (2), 136–145.

3.3. Manuscript 3

Zhang, Y. Q.; Markiewicz, M.; Filser, J.; Stolte, S. (2017) "**Toxicity of a quinaldine-based Liquid Organic Hydrogen Carrier (LOHC) system toward soil organisms *Arthrobacter globiformis* and *Folsomia candida*.**"

Submitted manuscript to Environmental Science & Technology, **considered for publication after major revisions**. Those revisions are already introduced in the following manuscript.

Page: 97–124

Contributions of Zhang, Y. Q.:

- Experimental design, preparation and calculations for all tests;
- Performance of all experiments, included organism culturing, ecotoxicity tests, microscopic observation and sample extractions;
- Computational predictions by EPI;
- Data processing and all statistical analysis;
- Preparation of all Figures and Tables; and
- Manuscript preparation and writing.

Markiewicz, M. contributed to the development of the topic and provided suggestions on the chemical analysis of the LOHCs; Filser, J. gave comments on the ecotoxicity test with Collembola and the manuscript; Stolte, S. guided this research and the preparation of the manuscript. Each co-author contributed to the revisions of the manuscript.

Toxicity of a quinaldine-based Liquid Organic Hydrogen Carrier (LOHC) system toward soil organisms *Arthrobacter globiformis* and *Folsomia candida*‡

Ya-Qi Zhang[a], Marta Markiewicz[a], Juliane Filser[b], and Stefan Stolte[a,c]*

[a] UFT - Centre for Environmental Research and Sustainable Technology, Department Sustainable Chemistry, University of Bremen, Leobener Straße, D-28359, Bremen, Germany.

[b] UFT - Centre for Environmental Research and Sustainable Technology, Department General and Theoretical Ecology, Faculty 2 (Biology/Chemistry), Leobener Straße, D-28359, Bremen, Germany.

[c] Department of Environmental Analysis, Faculty of Chemistry, University of Gdańsk, ul. Wita Stwosza 63, 80-308 Gdańsk, Poland.

Abstract

The study aims to establish a preliminary environmental assessment of a quinaldine-based LOHC system composed of hydrogen-lean, partially hydrogenated and fully hydrogenated forms. We examined their toxicity toward the soil bacteria *Arthrobacter globiformis* and the Collembola *Folsomia candida* in two exposure scenarios, with and without soil, to address differences in the bioavailability of the compounds. In both scenarios, no or only slight toxicity toward soil bacteria was observed at the highest test concentration (EC_{50} > 3397 µmol L^{-1} and > 4892 µmol kg^{-1} dry weight soil). The effects of the three quinaldines on *F. candida* in soil were similar, with EC_{50} values ranging from 2119 to 2559 µmol kg^{-1} dry weight soil based on nominal concentrations. Additionally, corrected pore-water-concentration-based EC_{50} values were calculated by equilibrium partitioning using soil/pore-water distribution coefficients. The tests without soil (simulating pore-water exposure) revealed higher toxicity, with LC_{50} values between 78.3 and 161.6 µmol L^{-1} and deformation of the protective cuticle. These results assign the compounds to the category "harmful to soil organisms". Potential risks toward the soil environment of the test compounds are discussed on the basis of predicted no-effect concentrations.

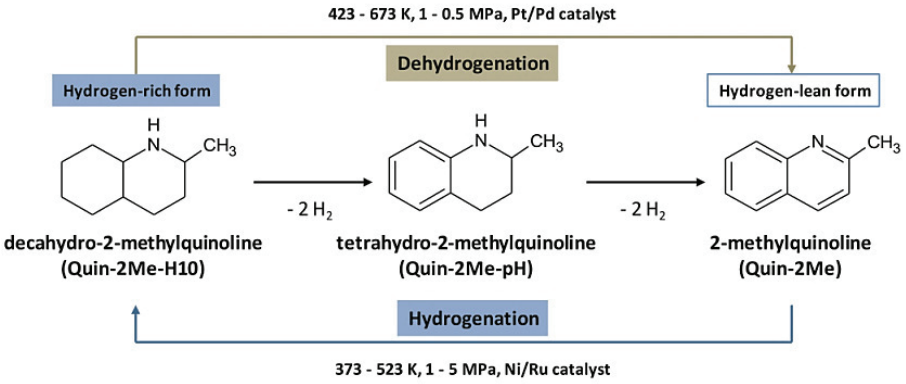
Keywords: bioavailability, PNECs, hazard assessment

Introduction

Rapid economic development has vastly increased worldwide energy demand in recent decades. Fossil fuels have been important energy sources for generations, but their reserves are estimated to be depleted within the next 100 years[1]. The limited availability of fossil fuels and all of the related environmental problems due to their use (e.g., emissions of pollutants such as SO_2, NO_x, smut, CO, CO_2 and volatile organic compounds (VOCs)[2]) have prompted the research and development of renewable and green energy resources. The amount of energy that can be harvested from renewable sources such as wind, solar, biomass or hydropower is high and could satisfy global energy demand (514 EJ in 2008 and could increase to 600–800 EJ by 2030[3]), one hundred times over[2,3]. However, serious obstacles remain because the production, output and storage of most renewable energies is geographically limited and strongly dependent on weather and environmental conditions[4]. In particular, energy systems with a high proportion of energy from wind and solar sources have a highly variable feed into the electrical grid, characterized by periods of overproduction that may cause low or even negative energy prices[5].

Alternatively, hydrogen is considered a very promising energy vector[6,7,8] with an excellent gravimetric energy density of 120 MJ kg^{-1} [6,8], which is three times that of petroleum[8]. Attractive from an environmental viewpoint, molecular hydrogen can be produced using renewable resources such as solar and wind energy via electrolysis of water and without direct CO_2 emissions[6,7]. The volumetric energy density of hydrogen, however, is very low; 1 L contains only 10.8 kJ of energy under ambient conditions[9]. To increase the volumetric energy density of hydrogen without compromising its gravimetric energy density, it is either stored in a gaseous state under very high pressures of 200 to 700 bar[6] (350 to 700 bar for automobile applications[7]) or in a liquid state at an extremely low temperature of -253 °C[6,7]. These technologies require specialized and dedicated infrastructures for hydrogen storage and distribution, which compromises economic efficiency, another important aspect of developing future energy systems[6]. Especially in the automobile industry, the design of special tanks and new fueling stations will be required[10]. Moreover, both compression and liquefaction are energy-intensive. They involve the loss of hydrogen by evaporation or boil-off[10]; in addition, because they involve working with molecular hydrogen, they are problematic in terms of safety[6,11]. In this context, liquid organic hydrogen carriers (LOHCs) represent a promising option to store and transport hydrogen in a chemically bound form[7,8,12] (Scheme 1). LOHCs can be used in both mobile and stationary systems[7,10,12] to supply energy to vehicles, buildings, mobile electronic equipment, etc. Generally, LOHCs are loaded with hydrogen (Scheme 1) to obtain an energy-rich carrier via catalytic hydrogenation when an excess of conventional or especially renewable energy is available. These compounds are then stored or transported to places where energy is currently needed. The hydrogen is catalytically released from the carrier through dehydrogenation[12,13], and the energy produced can be harnessed (e.g., using an internal combustion engine or a fuel cell), leaving the

LOHC compound in its energy-lean state[9]. During this process, the carrier is not consumed, and it can be reloaded with H_2 and recycled for further reactions[7,12].



Scheme 3.3.1. Simplified hydrogenation/dehydrogenation under typical reaction conditions[9] using quinaldines as examples. A partially hydrogenated intermediate form (Quin-2Me-pH) can be presented as a result of incomplete hydrogenation or dehydrogenation.

The gravimetric hydrogen storage capacity of most LOHCs ranges between 6 and 7.0 wt% H_2[9]. The carriers can be handled safely[13] under ambient conditions (temperature and pressure)[7]. Because of their physicochemical similarities to diesel fuel, they can be stored and transported using existing infrastructures[7,8,10,11] such as tanks, pipelines, trucks, ships and fueling stations[8,10]. Compared to battery technologies, LOHCs stand out for their higher energy storage capacities[6], rapid loading and unloading[6,14], and lack of irreversible capacity loss[14].

Various compounds have been studied for potential use as LOHCs; the most extensively researched ones are those based on *N*-ethylcarbazole[7,9,15] and dibenzyltoluene (MSH)[7,9]. Among all candidates, quinaldine (2-methylquinoline) is currently considered a particularly promising candidate because of its lower heat requirement for hydrogenation/dehydrogenation compared with the requirements for most of the other candidates—a quality that makes reactions possible at even milder temperatures[9]. LOHC-based hydrogen logistics over shorter- and longer-distance transports to Europe have been evaluated recently; in each case, a large amount of LOHCs was included[6,7]. LOHC-based hydrogen delivery to hydrogen fueling stations has also been predicted to become a realistic technology within the upcoming three to five years[6]. Given their promising properties, quinaldines would be produced at multi-ton scale in the case of successful implementation[7]. To cover a distance of 100 km, an average personal vehicle requires approximately 15 kg of decahydro-2-methylquinoline, which is twice the mass of currently used fossil fuels required to travel the same distance. Substantial amounts of LOHC compounds (hydrogen-rich, hydrogen-lean as well as partially hydrogenated compounds generated as by-products) would have to circulate on the market. Hence, the possibility exists for substantial amounts of such compounds to be released into the

environment, such as during production, usage, transportation and/or via leakage or accidental spills[9]. From the viewpoint of preventive environmental protection, studying the environmental impact of quinaldines in the early stage of development, prior to their introduction into the market, is important[9,13]. However, studies thus far have been focused on the development of chemical processes and the improvement of technical performance, whereas little is known about the environmental hazards that quinaldines may pose[9,13].

Soils are a sink for many anthropogenic chemicals[16], in particular fuels[17]. Therefore, investigating the toxicity and behavior of the quinaldine-based LOHC system in soils is highly relevant.

The aim of the present study is to conduct a proactive hazard assessment of the three forms of quinaldines that comprise the typical LOHC system (Scheme 1) in terrestrial environments: 2-methylquinoline (dehydrogenated, Quin-2Me), tetrahydro-2-methylquinoline (partially hydrogenated, Quin-2Me-pH), and decahydro-2-methylquinoline (fully hydrogenated, Quin-2Me-H10). The toxicity of the quinaldines was investigated in modified standard ecotoxicity tests using two model soil organisms representing bacteria, *Arthrobacter globiformis* (2-h exposure) and micro-arthropods Collembola *Folsomia candida* (with 14 or 28 days of exposure), with different exposure scenarios in both cases (with and without soil), to assess the influence of the test compounds.

Materials and Methods

All details regarding the culturing of the organisms, the experimental procedures and the chemicals used are provided in Supporting Information (SI).

Materials. Quinaldines (Table 3.3.1) were provided by the working group of Prof. Dr. Peter Wasserscheid, Institute of Chemical Reaction Engineering, Friedrich-Alexander University of Erlangen-Nürnberg, Germany. Detailed information of all the materials is given in SI section S3.3.1.

Table 3.3.1. Physicochemical properties of the quinaldines.

	Quin-2Me	Quin-2Me-pH	Quin-2Me-H10
Chemical formula	$C_{10}H_9N$	$C_{10}H_{13}N$	$C_{10}H_{19}N$
Molecular weight (MW) [g mol^{-1}]	143.2	147.2	153.3
Boiling point (°C)[a]	257.7	245.5	225.5
Log K_{ow}[a]	2.69	2.97	2.97
K_d [mL g^{-1}][b]	2.03	6.57	2.42
Log K_{oc}[c]	2.22	2.91	2.30
Water solubility (25°C)[a] [µmol L^{-1}]	16535	3885	31116
pK_a (25°C)[d]	5.9 ± 0.4	5.2 ± 0.4	10.9 ± 0.4

[a] Predicted in Estimation Programs Interface (EPI) Suite™ v4.1.

[b] Soil/water partitioning coefficient measured in the same artificial soil by adsorption batch equilibrium experiment in our previous work[18].

[c] Calculated using K_d by using the empirical formula $K_{oc} = \frac{K_d}{OC\%}$, where OC% equals to 0.0121 (organic fraction of the test soil).

[d] Estimated using Scifinder (calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2015 ACD/Labs)).

Purity of the quinaldines. GC/MS (gas chromatography-mass spectrometry) analysis was used to identify the purity of the three components of the quinaldine-based LOHC.

Culturing and synchronization of organisms. The test organisms used here are part of standard protocols for assessing effects of contaminants in soils and sediments. Two test scenarios were selected to represent the most relevant exposure pathways in soil, a) soil pore-water, and b) solid matrix. Test endpoints are dehydrogenase activity of the bacteria, survival and deformation of the Collembola in soil pore-water, and reproduction of the Collembola in soil.

The culture of *A. globiformis* was prepared according to DIN 38412 L48[19], with slight modifications. Before the test, 1 mL of the stock culture of the bacteria was thawed and cultured in growth medium B (DMS-B) for 20 h. Cultures whose cell density was adjusted to an optical density (OD) of 0.3–0.4 were used for the ecotoxicity tests. A detailed description of the procedure is provided in SI section S3.3.2. The Collembola *F. candida* were cultured according to OECD guideline 232[20]; however, the culturing substrate was reduced by half of the overall amounts, with the mixture consisting of 200 g plaster of Paris, 25 g activated charcoal and 180 mL distilled water. Adult Collembola of 11–12 day-old and 55–56 day-old were used for the reproduction test in soil and the pore-water exposure survival test, respectively. Further details are available in SI section S3.3.3.

Contact test with *A. globiformis* in pore-water exposure scenario. Bacteria were exposed (2 h) to quinaldines with concentrations of 6983–7.0 µmol L^{-1} (1000–1 mg

L^{-1}), 3397–6.8 μmol L^{-1} (500–1 mg L^{-1}) and 6523–6.5 μmol L^{-1} (1000–1 mg L^{-1}) for Quin-2Me, Quin-2Me-pH and Quin-2Me-H10, respectively. In brief, the test was performed following the method of Engelke *et al.*[21]; however, 500 μL of either bacterial suspensions or DMS-B were added after the quinaldine solution. The reaction with resazurin was conducted by shaking with a frequency of 150 min^{-1} for 40 min. Further details are provided in SI section S3.3.4.

Survival inhibition test with *F. candida* in pore-water exposure scenario. The tests were performed in accordance with the method of Houx *et al.*[22]. Quin-2Me, Quin-2Me-pH and Quin-2Me-H10 with the test concentrations ranging from 100–0.5 mg L^{-1}, corresponding to 698.3–3.5 μmol L^{-1}, 679.3–3.4 μmol L^{-1} and 652.3–3.3 μmol L^{-1}, respectively, were prepared in water. The numbers of Collembola that survived after 14 days were counted; the change of the body surface was observed by a stereo zoom microscope (Olympus SZX12) at the 16 × magnification. Additional details are presented in SI section S3.3.5.

Contact test with *A. globiformis* in soil exposure scenario. Bacteria were exposed to Quin-2Me, Quin-2Me-pH and Quin-2Me-H10 with the test concentrations ranging from 750–10 mg kg^{-1} dw soil, corresponding to 5237–69.8 μmol kg^{-1}, 5095–67.9 μmol kg^{-1}, and 4892–65.2 μmol kg^{-1} dw soil, respectively, for 2 h. The method used was based on that of Engelke *et al.*[21]; however, 500 μL of either bacterial suspensions or DMS-B were added. The testing procedure, including test soil preparation, is described in SI section S3.3.6.

Reproduction inhibition test with *F. candida* in soil exposure scenario. Test soils containing Quin-2Me, Quin-2Me-pH or Quin-2Me-H10 with the test concentrations ranging from 1000–50 mg kg^{-1} dw soil, corresponding to 6983–349.2 μmol kg^{-1}, 6794–339.7 μmol kg^{-1} or 6523–326.2 μmol kg^{-1} dw soil, respectively, were prepared. A reproduction inhibition test was performed according to OECD guideline 232[20] but miniaturized[23]. The soil pH was measured in 0.01 M $CaCl_2$, and the Collembola were extracted by flotation in 100 mL of water. The number of adults and juveniles were photorecorded using a Canon EOS 600D and counted in ImageJ v1.48 software. All the steps are described in detail in SI section S3.3.7.

Concentration determination. Additional soil test samples were prepared for the concentration measurements via extraction; the samples were prepared following EPA guideline 3550c[24]. They were prepared the same way as for the ecotoxicity test but without inoculating organisms. Pore-water concentrations (C_q) in soil samples for each test concentration were calculated from the nominal concentrations of the test substance by integrating the soil/water partitioning coefficient (K_d)[25,26]. Dose-response curves (pore-water concentration based) were re-established (effects against C_q) and pore-water concentrations based on EC_{50} and EC_{10} values were thus extrapolated. Concentrations of liquid samples from the pore-water exposure test were determined via extraction by liquid-liquid extraction followed by GC/MS analysis. Details of the procedure and calculations according to the equilibrium partitioning are available in SI section S3.3.8.

GC/MS analysis. A gas chromatograph (HP® GC system 6890N) equipped with a 30 m × 0.25 mm (ID), 0.25 µm thickness, FS-Supreme-5ms column (CS) was interfaced to a mass-selective detector (HP® MS 5973, Agilent, Waldbronn, Germany). The setup and calibration parameters as well as the limit of detection (LOD) and the limit of quantification (LOQ) of the method are described in SI section S3.3.9.

Statistical analysis. All data are presented as the means ± SD of values from at least two independent experiments with at least two replicates of each concentration. Significance was analyzed by the paired *t*-test with GLM (generalized linear model) analysis using the software R (version 3.1.1) (https://www.r-project.org), and $p > 0.05$ was considered insignificant. Dose-response curves, the LC_{10} and EC_{10} values as well as the LC_{50} and EC_{50} values with the confidence limits for each quinaldine were estimated by the "drfit" model in R.

Results

Throughout the following text, all results (except for the concentration determination) are given in µmol L^{-1} and µmol kg^{-1} dw soil. The corresponding mass concentrations (mg L^{-1} and mg kg^{-1} dw soil) are presented in the SI (Table S3.3.2 and S3.3.3; Figure S3.3.1 and S3.3.2). Identified by GC/MS analysis, the three forms of the quinaldines were single compounds: 2-methylquinoline (i.e., quinaldine, Quin-2Me), tetrahydro-2-methylquinoline (Quin-2Me-pH), and decahydro-2-methylquinoline (Quin-2Me-H10).

Concentration determination. The results in µmol L^{-1} and µmol kg^{-1} dw soil are presented in SI Table S3.31. In the pore-water exposure tests, the concentrations of the quinaldines at the test beginning (C_{t0}) and the nominal concentrations (C_N) showed rather small differences for both organisms, ranging between 81.3 and 117.1% (Table 3.3.2). The differences between the concentrations at the test end (C_{tE}) and test beginning (C_{t0}) for *Arthrobacter* (2 h) were small (89.5–113.2%), whereas for the 14-day test with Collembola, deviations as large as 43.7% were found for Quin-2Me-pH at 100 mg L^{-1}.

In the soil tests, much larger deviations between C_N and C_{t0} were observed (19.8–76.1%). After 28 days in the Quin-2Me-pH test with Collembola, no test compound was detected (0% remained in C_{tE}). Neither in the pore-water exposure test nor in the soil exposure test was a clear dependence of test compound recovery on the test concentrations observed. Related data were not determined for the tests with *A. globiformis*.

Table 3.3.2. Nominal concentrations (C_N) and measured concentrations at the test beginning (C_{t0}) and end (C_{tE}) in the pore-water (part A, n = 3) or soil (part B, n = 4) exposure scenario. Deviations between concentrations are shown in %; "n.a." means data not available.

Part-A		**Pore-water exposure** [mg L^{-1}]				
Organism	**Quinaldines**	**C_N**	**C_{t0}**	**C_{t0} vs. C_N (%)**	**C_{tE}**	**C_{tE} vs. C_{t0} (%)**
A. globiformis	**Quin-2Me**	1000	812.6 ± 12.6	81.3	727.2 ± 7.9[a]	89.5
		500	449.6 ± 7.3	89.9	461.1 ± 14.7[a]	102.6
		50	43.2 ± 2.4	86.4	48.9 ± 4.7[a]	113.2
	Quin-2Me-pH	500	529.1 ± 2.2	105.8	519.8 ± 7.7[a]	98.2
	Quin-2Me-H10	1000	949.8 ± 13.1	105.0	936.3 ± 11.0[a]	98.6
		500	443.7 ± 20.3	88.7	441.6 ± 20.6[a]	99.5
		50	48.2 ± 0.8	96.4	47.5 ± 0.4[a]	98.5
F. candida	**Quin-2Me**	100	91.5 ± 2.3	91.5	74.8 ± 9.2[b]	81.7
		10	8.8 ± 0.2	88.0	10.6 ± 0.2[b]	120.5
	Quin-2Me-pH	100	117.1 ± 2.1	117.1	51.2 ± 1.0[b]	43.7
		10	11.3 ± 0.2	113.0	8.7 ± 0.6[b]	77.0
	Quin-2Me-H10	100	95.7 ± 2.4	95.7	74.3 ± 2.5[b]	77.6
		10	9.0 ± 0.2	90.0	7.5 ± 0.2[b]	83.3
Part B		**Soil exposure** [mg kg^{-1} dw soil]				
Organism	**Quinaldines**	**C_N**	**C_{t0}**	**C_{t0} vs. C_N (%)**	**C_{tE}**	**C_{tE} vs. C_{t0} (%)**
A. globiformis		n.a.	n.a.	n.a.	n.a.[a]	n.a.
F. candida	**Quin-2Me**	1000	672.1 ± 12.4	67.2	572.5 ± 4.0[c]	85.2
		400	304.5 ± 4.1	76.1	260.4 ± 18.6[c]	85.5
	Quin-2Me-pH	1000	396.8 ± 4.4	39.7	0.0 ± 0.0[c]	0.0
		400	167.4 ± 7.5	41.8	0.0 ± 0.0[c]	0.0
	Quin-2Me-H10	1000	253.3 ± 2.5	25.3	260.1 ± 2.7[c]	102.7
		400	79.1 ± 4.8	19.8	84.9 ± 11.0[c]	107.4

[a] After 2 h.

[b] After 14 days.

[c] After 28 days.

Summarized results of toxicity. The effects in terms of LC_{50}, LC_{10}, EC_{50} and EC_{10} values on the growth of *A. globiformis* and the survival or reproduction of *F. candida* in pore-water or the soil exposure tests are shown in the Table 3.3.3.

Table 3.3.3. LC_{10}, LC_{50}, EC_{10} (only for the Collembola) and EC_{50} values of the quinaldines toward the two organisms in the pore-water (µmol L^{-1}) and soil (µmol kg^{-1} dw soil) tests. Values were given with 2.5% and 97.5 % confidence intervals in the brackets. "n.a." indicates data not available.

	A. globiformis		***F. candida***					
	Pore-water exposure	**Soil exposure**	**Pore-water exposure**		**Soil exposure**		**Calculated pore-water**	
	EC_{50}[a]	EC_{50}[a]	LC_{50}[a]	LC_{10}[a]	EC_{50}[a]	EC_{10}[a]	EC_{50}[b]	EC_{10}[b]
Quin-2Me	> 6983	> 5237	78.8 (67.8–n.a.)	21.2	2559 (2388–n.a.)	526.9	1183 (1104–n.a.)	243.7
Quin-2Me-pH	> 3397	> 5095	78.3 (71.1–88.9)	30.9	2357 (2191–2570)	696.1	235.0 (218.4–256.2)	69.4
Quin-2Me-H10	≥ 4892	> 4892	161.6 (129.8–200.7)	22.4	2119 (1984–2257)	760.5	830.4 (777.3–884.2)	298.0

[a] Effective and lethal concentrations are based on the nominal concentrations.

[b] Effective concentrations are based on the K_d-corrected soil pore-water concentrations (µmol L^{-1}).

Toxicity toward *A. globiformis*. None of the test compounds in the soil reached 50% inhibition of bacterial growth, even at the highest test concentration (Figure S3.3.1). The same holds true for Quin-2Me and Quin-2Me-pH in the pore-water exposure test at the highest test concentrations of 6983 and 3397 µmol L^{-1}, respectively, whereas toxicity was found for Quin-2Me-H10 when concentrations exceeded 652.3 µmol L^{-1}, with $EC_{50} \geq 4892$ µmol L^{-1}. Moreover, stimulation of bacterial growth was observed in both test scenarios at concentrations greater than 698.3 and 679.3 µmol L^{-1} of Quin-2Me and Quin-2Me-pH, respectively, and in the whole concentration range in the soil for all test compounds.

Toxicity toward *F. candida*. A reduction in survival of Collembola in the pore-water exposure scenario was observed after 14 days (Figure 3.3.1 A) with LC_{50} values of 78.8 µmol L^{-1} (Quin-2Me), 78.3 µmol L^{-1} (Quin-2Me-pH) and 161.6 µmol L^{-1} (Quin-2Me-H10) (Table 3.3.3). Moreover, individual Collembola turned partly brownish and, in some cases, fluid exudation on the body surface was observed (Figure 3.3.2).

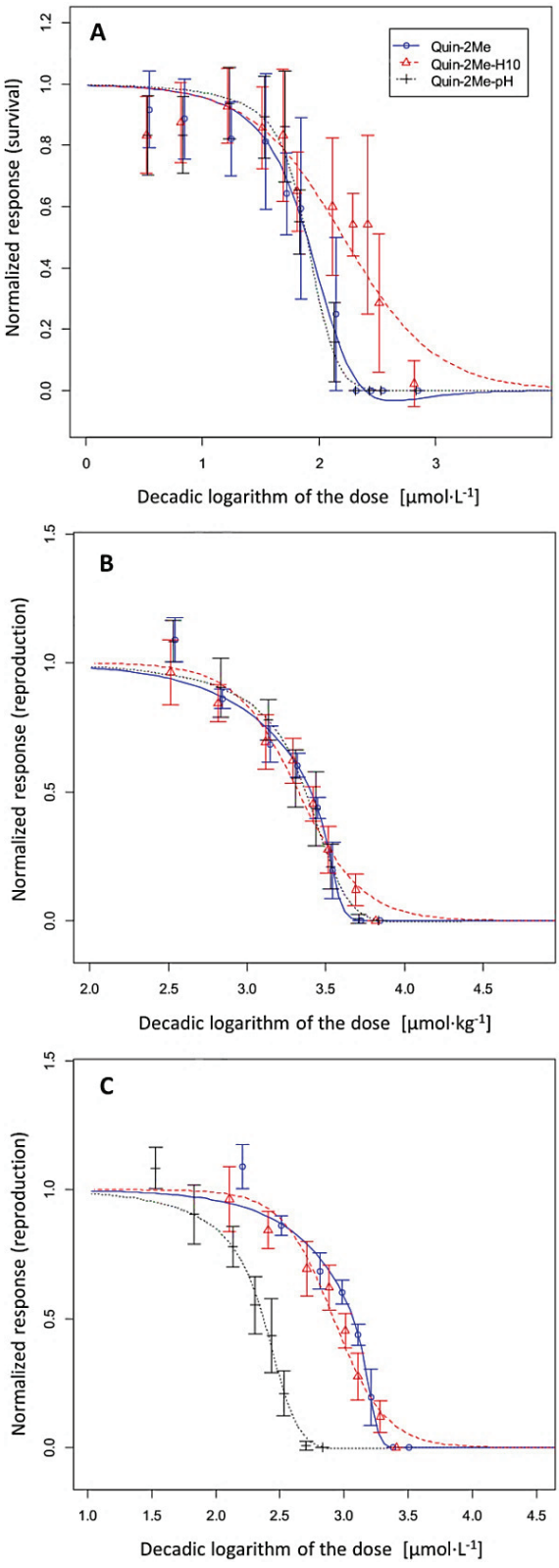


Figure 3.3.1. Effects of the quinaldines on *F. candida* in pore-water (**A**: nominal concentrations, n = 12) and soil (**B and C**: n = 16) exposure scenarios. **B**: nominal concentrations in soil; **C**: calculated soil pore-water concentrations.

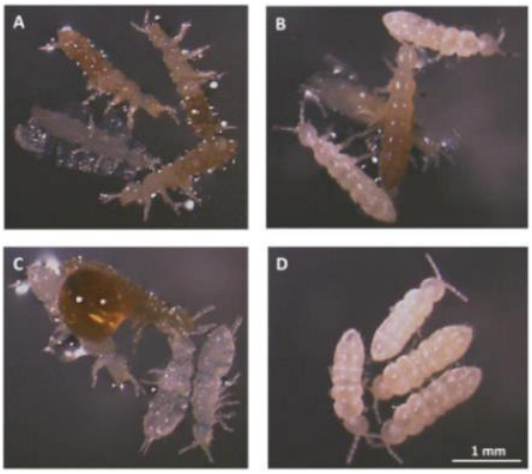


Figure 3.3.2. Microscopic observation (16 × magnification) of *F. candida* exposed to the quinaldines in the pore-water scenario (same observations in Quin-2Me-pH as in Quin-2Me). **A**: Quin-2Me at 698.3 µmol L^{-1}; **B**: Quin-2Me at 69.8 µmol L^{-1}; **C**: Quin-2Me-H10 at 652.3 µmol L^{-1}; **D**: negative control.

In the reproduction inhibition test in the soil (28 days), the EC_{50} values (nominal concentrations based) of the three test compounds ranged between 2119 and 2559 µmol kg^{-1} dw soil (Table 3.3.3) but were not significantly different from each other ($p > 0.5$) (Figure 3.3.1 B). Because large differences between the nominal concentration and the measured C_{t0} or C_{tE} were observed, indicating significant sorption to soil, the exposure concentration was adjusted to the pore-water concentration (Figure 3.3.1 C). The pore-water concentration was calculated on the basis of the equilibrium partitioning using the measured soil/pore-water distribution coefficient (K_d)[18]. As a result, the dose-response curves shifted to lower concentrations and a general decrease of EC_{50} values (235.0–1183 µmol L^{-1}, Table 3.3.3) was observed. In addition, larger differences in toxicity among the three chemicals forming the LOHC system became apparent, and the order of toxicity changed to Quin-2Me-pH > Quin-2Me-H10 ≥ Quin-2Me. The partially hydrogenated form (Quin-2Me-pH), previously having an EC_{50} value similar to the remaining two compounds, had the lowest EC_{50} value, exhibiting significantly greater toxicity than the other two (Table 3.3.3).

Discussion

Concentration determination.

In the pore-water exposure test, the deviation between the measured and nominal concentrations (C_{t0} vs. C_N) was rather small; the observed difference is likely due to fast sorption of the test compounds to the test vessels. The higher loss in the pore-water exposure test with Collembola (14 days) compared to the test with bacteria

(2 h) might be due to additional adsorption of the test compounds to test vessels because of the longer test duration. All three compounds have similar boiling points (Table 3.3.1); therefore, the greater loss of Quin-2Me-pH (only 43.7% remained) observed at the highest test concentration with Collembola was most likely due to the higher adsorption of this compound to the test vessel rather than to evaporation.

Extraction of quinaldines from soil (C_{t0} vs. C_N) with organic solvents gave poor recovery rates, indicating that the bioaccessibility of these compounds in soil pore-water is low. Because the recovery is lower at the end of the test, the chemicals likely adsorbed to test vessels to some extent and/or diffused deeply into the soil matrix (e.g., soil organic matters or particles) and were occluded[27], thereby becoming less extractable by solvents and less accessible to organisms via pore-water[27]. In particular, the complete loss of Quin-2Me-pH in the extracts can be explained by its higher affinity to the test soil. The K_d value of Quin-2Me-pH (6.57 mL g^{-1} [18], Table 3.3.1) is approximately 3 times higher than those of Quin-2Me (2.03 mL g^{-1}) and Quin-2Me-H10 (2.42 mL g^{-1}), indicating stronger sequestration into the soil matrix[28].

Toxicity and the mode of action.

In this study, *Arthrobacter* was found to be less sensitive to the quinaldines than *F. candida*, which might be contributed to the shorter exposure duration in the former. Nonetheless, Collembola have been reported to be more sensitive to exposure to polycyclic aromatic hydrocarbons (PAHs) or their nitrogen-substituted derivatives (N-PAHs) than many other terrestrial organisms such as earthworms[26,29,30,31], enchytraeids[32] and bacteria[32,33]. The springtail *Folsomia fimetaria* has been found to be more vulnerable to N-PAHs than soil-nitrifying bacteria[32]. In oil-polluted soil, neither bacterial numbers nor the activity of the microbial community were influenced by elevated PAH concentrations[33]. Moreover, *F. candida* is one of the most sensitive springtails[29]. All of the aforementioned information supports our observations regarding differences in species sensitivity. Furthermore, members of the genus *Arthrobacter* have been reported to be able to use various organic compounds, including xenobiotics[34]. Quinaldine can be used by *Arthrobacter nitroguajacolicus* as the sole source of carbon and energy[34]. Utilization of alkylpyridines (2.15 mmol L^{-1})[35] and carbazoles (40 mg L^{-1})[36] by *Arthrobacter* sp. has also been reported. The observed stimulation of bacterial growth may thus be explained by the ability of these compounds to serve as primary C, N, and energy sources for bacteria.

In the present study, different exposure scenarios, test durations and endpoints were used to determine adverse effects on *F. candida* (pore-water, 14 days, mortality vs. soil, 28 days, reproduction inhibition). The Collembola reacted with greater sensitivity to the quinaldines in the pore-water test setup despite the shorter test duration and the less sensitive endpoint in this test. While the exposure of soil-dwelling organisms to organic compounds mainly occurs through the digestion of soil particles or diffusion from pore-water through the body surface[37], *F. candida* does not usually ingest soil particles or ingests them only to a limited extent[37]. Uptake from

pore-water through body surface (cuticle)[38,39,40] or the ventral tube[29] is therefore assumed to be more significant[37]. The portion of the chemicals bound to soil is not available for uptake in this manner. In this context, the greater sensitivity observed in the pore-water setup was expected because, in the absence of soil, the test chemicals could be considered fully bioavailable. Moreover, starvation stress (animals were not fed) most likely contributed to the increased sensitivity in the pore-water exposure test, though the adults in the controls (no quinaldines) remained vigorous throughout the test.

Narcosis has been suggested to be the main mode of toxic action for different PAHs and N-PAHs toward various soil invertebrates[25,26,28,32,41,42]. For instance, Sverdrup *et al.*[32] have demonstrated in the Collembola *Folsomia fimetaria* that the toxicity of 8 PAHs correlated well with log K_{ow}. However, no comprehensive data sets of LC_{50} values (or EC_{50} values) in *Folsomia candida* exist for PAHs and N-PAHs that might allow us to establish meaningful quantitative structure-activity relationships (QSARs) based on log K_{ow}. Nevertheless, similar effects or lethal concentrations have been observed for all three compounds which representing similar log K_{ow} values between 2.69 and 2.97 (Table 3.3.1). However, a different soil exposure scenario is observed when pore-water concentrations are taken into account (the reproduction assay). Whereas the EC_{50} values of Quin-2Me and Quin-2Me-H10 are still similar, the EC_{50} value of Quin-2Me-pH is 3 to 5 times lower, which is a result of the high affinity of the partially hydrogenated compound for soil, which causes a shift of the dose-response curve toward lower concentrations. Moreover, the affinity toward soil is not only based on lipophilicity, at least in the case of Quin-2Me-pH[18].

In the pore-water exposure scenario, the LC_{50} value of Quin-2Me-H10 is significantly ($p < 0.001$) higher than the other two despite the almost equivalent K_{ow} values, which can be explained by its protonation. With a pK_a value of nearly 11 (Table 3.3.1), Quin-2Me-H10 is fully protonated and accordingly less lipophilic at the medium pH of 5. Thus, this observation does not contradict the assumed narcosis but shows that log K_{ow} for ionized compounds is not the proper physicochemical parameter for such estimations[43].

Additionally, the observed malformations (Figure 3.3.2) and the brownish vesicles that extrude liquids might indicate a disturbance of membrane integrity that corresponds to a lipophilicity-based mode of action. Similar observations were made for benzo[h]quinoline and 1,7-phenanthroline which caused physical changes in the earthworm *Eisenia fetida*—changes such as secretion of yellowish fluids, body lesions, and body ruptures that can be attributed to autolytic cell destruction[44].

Preliminary hazard assessment.

The EC_{50} values of the quinaldines to Collembola in soil were between 324.9 and 366.4 mg kg^{-1} dw soil (Table S3.3.2). These compounds are therefore classified as "harmful" ($100 < EC_{50} \leq 1000$ mg kg^{-1} dw soil) to terrestrial life, with long-lasting

effects[45]; this classification is based on acute categories according to the JRC report (Science and Policy Report by the Joint Research Centre, the European Commission's in-house science service)[46]. Because quinaldines are potential substitutes for fossil fuels, we further compared their toxicity to the effects of crude oil on the reproduction of *F. candida* (28 days); the EC_{50} values of the quinaldines and crude oil (EC_{50} = 210.4 mg kg^{-1}, OC% = 0.39 and EC_{50} = 880.3 mg kg^{-1}, OC% = 2.1)[17] are within the same order of magnitude. These values are based on nominal concentrations and are not corrected for adsorption in soil; therefore, they are expected to be lower, indicating that crude oil is more toxic than the quinaldines. Applying the categories from the aquatic assessment and in accordance with the JRC report[46], the results of the pore-water exposure tests for exposure to Quin-2Me (LC_{50} = 11.3 mg L^{-1}), Quin-2Me-pH (LC_{50} = 11.5 mg L^{-1}) and Quin-2Me-H10 (LC_{50} = 24.8 mg L^{-1}) (Table S3.3.2) reveal that these quinaldines are classified as "harmful" ($10 < LC_{50} \leq 100$ mg L^{-1}) to non-aquatic organisms. In a study that assessed the aquatic toxicity of the three quinaldines with water flea *Daphnia magna* showed the EC_{50} values as follows: Quin-2Me (EC_{50} = 56 mg L^{-1}), Quin-2Me-pH (EC_{50} = 2.7 mg L^{-1}), and Quin-2Me-H10 (EC_{50} = 204 mg L^{-1})[9]. Quin-2Me can thereby be classified as acute 3 ($10 < EC_{50} \leq 100$ mg L^{-1}), whereas Quin-2Me-pH was in the acute 2 category ($1 < EC_{50} \leq 10$ mg L^{-1}) according to the GHS (Globally Harmonised System)[47].

Environmental relevance.

The data presented here for the toxicity of quinaldines toward two typical soil organisms give preliminary information regarding the acceptable exposure levels in the environment. Predicted no-effect concentrations (PNECs) were calculated on the basis of the EC_{10} values and by applying an assessment factor of 100, in accordance with the European Chemicals Agency (ECHA) methods[48] for a single long-term toxicity test based on a single trophic level. The PNECs (Tables 3.3.4 and S3.3.3) derived from nominal concentrations are in the range 5.3–7.6 μmol kg^{-1} dw soil (0.8–1.2 mg kg^{-1} dw soil), and the PNECs based on calculated pore-water concentrations are 0.7–3.0 μmol L^{-1} (0.1–0.5 mg L^{-1}).

Table 3.3.4. PNECs derived from EC_{10} values (nominal or calculated pore-water based) in soil with an assessment factor of 100.

	PNECs (nominal) [μmol kg^{-1} dw soil]	**PNECs (calculated pore-water)** [μmol L^{-1}]
Quin-2Me	5.3	2.4
Quin-2Me-pH	7.0	0.7
Quin-2Me-H10	7.6	3.0

Little is known thus far about the actual production scale of quinaldines. Nevertheless, a car with a tank of 80 kg LOHCs would be needed for a maximum

driving range of ca. 700 km[6]. An LOHC demonstration unit for LOHC-based hydrogen logistics based on road transport in Stuttgart, Germany, has a total storage capacity of 1000 L of H18-DBT (perhydrodibenzyltoluene, another candidate LOHC)[6]. Hence, LOHC concentrations substantially greater than their PNECs are likely to be present in cases of local site-spills or leakages. Even if the hazards of the quinaldines to terrestrial organisms are moderate or even low, the risk might still be considerable. In particular, the high adsorption of Quin-2Me-pH to soil may increase the exposure risk to organisms such as earthworms that primarily feed on soil solids. Nevertheless, quinaldines are less hazardous or at least no worse for the environment (e.g., the 28-day reproduction of *F. candida*) in terms of EC_{50} values than typical N-PAHs[41] such as naphthalene (11.3–52.4 mg kg^{-1} dw soil), pyrene (21.2 mg kg^{-1} dw soil), phenanthrene (45.8 mg kg^{-1} dw soil), quinoline (75.0 mg kg^{-1} dw soil) and acridine (312.7 mg kg^{-1} dw soil). However, both more realistic soil environments and additional species need to be investigated in detail, along with the degradation and half-life in soil, for a reliable hazard and risk assessment of these compounds.

Acknowledgements

This work was financially supported by the University of Bremen and the European Union FP7 COFUND within Marie Curie Actions BremenTrac Program (grant agreement No.600411) and M8 Postoc-Initiatve PLUS, funded by the German Excellence Initiative. We would like to thank the entire UFT Team for the interdisciplinary cooperation. We thank the working group of Prof. Dr. Peter Wasserscheid for providing the quinaldines and helpful discussion. We are also grateful to M.Sc Elaheh Daghighi Masouleh for her help with microscope observation and imaging.

References

(1) Shafiee, S.; Topal, E. When will fossil fuel reserves be diminished? *Energy Policy* **2009**, *37* (1), 181–189.

(2) Dincer, I. Renewable energy and sustainable development: a crucial review. *Renew. Sustain. Energy Rev.* **2000**, *4* (2), 157–175.

(3) Moriarty, P.; Honnery, D. What is the global potential for renewable energy? *Renew. Sustain. Energy Rev.* **2012**, *16* (1), 244–252.

(4) Dresselhaus, M.S.; Thomas, I. L. Alternative energy technologies. *Nature* **2001**, *414* (November), 332–337.

(5) Eberle, U.; Müller, B.; von Helmolt, R. Fuel cell electric vehicles and hydrogen infrastructure: status 2012. *Energy Environ. Sci.* **2012**, *5* (10), 8780–8798.

(6) Preuster, P.; Papp, C.; Wasserscheid, P. Liquid Organic Hydrogen Carriers (LOHCs): toward a hydrogen-free hydrogen economy. *Acc. Chem. Res.* **2017**, *50* (1), 74–85.

(7) Teichmann, D.; Arlt, W.; Wasserscheid, P. Liquid Organic Hydrogen Carriers as an efficient vector for the transport and storage of renewable energy. *Int. J. Hydrogen Energy* **2012**, *37* (23), 18118–18132.

(8) Zhu, Q. L.; Xu, Q. Liquid organic and inorganic chemical hydrides for high-capacity hydrogen storage. *Energy Environ. Sci.* **2015**, *8*, 478–512.

(9) Markiewicz, M.; Zhang, Y. Q.; Bosmann, A.; Brückner, N.; Thöming, J.; Wasserscheid, P.; Stolte, S. Environmental and health impact assessment of Liquid Organic Hydrogen Carrier (LOHC) systems - challenges and preliminary results. *Energy Environ. Sci.* **2015**, *8* (3), 1035–1045.

(10) von Wild, J.; Friedrich, T.; Cooper, A.; Toseland, B.; Muraro, G.; TeGrotenhuis, W.; Wang, Y.; Humble, P.; Karim, A. Liquid Organic Hydrogen Carriers (LOHC): an auspicious alternative to conventional hydrogen storage technologies. *18th World Hydrog. Energy Conf.* **2010**, *78*, 189–197.

(11) Papp, C.; Wasserscheid, P.; Libuda, J.; Steinrück, H. P. Liquid organic hydrogen carriers: surface science studies of carbazole derivatives. *Chem. Rec.* **2014**, *14* (5), 879–896.

(12) Teichmann, D.; Stark, K.; Müller, K.; Zöttl, G.; Wasserscheid, P.; Arlt, W. Energy storage in residential and commercial buildings via Liquid Organic Hydrogen Carriers (LOHC). *Energy Environ. Sci.* **2012**, *5* (10), 9044–9054.

(13) Teichmann, D.; Arlt, W.; Wasserscheid, P.; Freymann, R. A future energy supply based on Liquid Organic Hydrogen Carriers (LOHC). *Energy Environ. Sci.* **2011**, *4* (8), 2767–2773.

(14) Driscoll, P. F.; Deunf, E.; Rubin, L.; Arnold, J.; Kerr, J. B. Electrochemical redox catalysis for electrochemical dehydrogenation of liquid hydrogen carrier fuels for energy storage and conversion. *J. Electrochem. Soc.* **2013**, *160* (7), 3152–3158.

(15) Müller, K.; Geng, J.; Arlt, W. Reversible vs. irreversible conversion of hydrogen: how to store energy efficiently? *Energy Technol.* **2013**, *1* (1), 42–47.

(16) Anyanwu, I. N.; Semple, K. T. Fate and behaviour of nitrogen-containing polycyclic aromatic hydrocarbons in soil. *Environ. Technol. Innov.* **2015**, *3*, 108–120.

(17) Reinecke, A. J.; van Wyk, M.; Reinecke, S. A. The influence of soil characteristics on the toxicity of oil refinery waste for the springtail Folsomia candida (Collembola). *Bull. Environ. Contam. Toxicol.* **2016**, *96* (6), 804–809.

(18) Zhang, Y.-Q.; Stolte, S.; Alptekin, G.; Rother, A.; Diedenhofen, M.; Filser, J.; Markiewicz, M. Mobility and adsorption of liquid organic hydrogen carriers (LOHCs) in soil. (Yet unpublished work).

(19) DIN. German standard methods for the examination of water, waste water and sludge bio-assays (group L)— Toxicity test with Arthrobacter globiformis for contaminated solids (L48). DIN 38412 L48. *Dtsch. Inst. für Normung* **2002**.

(20) OECD. Test No.232: Collembolan reproduction test in soil. *OECD Guidel. Test. Chem.* **2009**, No. September, 1–19.

(21) Engelke, M.; Köser, J.; Hackmann, S.; Zhang, H.; Mädler, L.; Filser, J. A miniaturized solid contact test with Arthrobacter globiformis for the assessment of the environmental impact of silver nanoparticles. *Environ. Toxicol. Chem.* **2014**, *33* (5), 1142–1147.

(22) Houx, N. W. H.; Dekker, A.; Van Kammen-Polman, A. M. M.; Ronday, R. Acute toxicity test for terrestrial hazard assessment with exposure of Folsomia candida to pesticides in an aqueous medium. *Arch. Environ. Contam. Toxicol.* **1996**, *30* (1), 9–14.

(23) Filser, J.; Wiegmann, S.; Schröder, B. Collembola in ecotoxicology-any news or just boring routine? *Appl. Soil Ecol.* **2014**, *83*, 193–199.

(24) U. S. Environmental Protection Agency. EPA Method 3550C: ultrasonic extraction. *U. S.*

Environ. Prot. Agency **2007**, No. February, 1–17.

(25) Sverdrup, L. E.; Kelley, A. E.; Krogh, P. H.; Nielsen, T.; Jensen, J.; Scott-Fordsmand, J. J.; Stenersen, J. Effects of eight polycyclic aromatic compounds on the survival and reproduction of the springtail Folsomia fimetaria L. (Collembola, Isotomidae). *Environ. Toxicol. Chem.* **2001**, *20* (6), 1332–1338.

(26) Kobetičová, K.; Bezchlebová, J.; Lána, J.; Sochová, I.; Hofman, J. Toxicity of four nitrogen-heterocyclic polyaromatic hydrocarbons (NPAHs) to soil organisms. *Ecotoxicol. Environ. Saf.* **2008**, *71* (3), 650–660.

(27) Semple, K. T.; Doick, K. J.; Jones, K. C.; Burauel, P.; Craven, A.; Harms, H. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. *Environ. Sci. Technol.* **2004**, *38* (12), 228A–231A.

(28) Kobetičová, K.; Šimek, Z.; Brezovský, J.; Hofman, J. Toxic effects of nine polycyclic aromatic compounds on Enchytraeus crypticus in artificial soil in relation to their properties. *Ecotoxicol. Environ. Saf.* **2011**, *74* (6), 1727–1733.

(29) Fountain, M. T.; Hopkin, S. P. Folsomia candida (Collembola): A "standard" soil arthropod. *Annu. Rev. Entomol.* **2005**, *50* (1), 201–222.

(30) Eom, I. C.; Rast, C.; Veber, A. M.; Vasseur, P. Ecotoxicity of a polycyclic aromatic hydrocarbon (PAH)-contaminated soil. *Ecotoxicol. Environ. Saf.* **2007**, *67* (2), 190–205.

(31) Droge, S. T. J.; Paumen, M. L.; Bleeker, E. A. J.; Kraak, M. H. S.; Van Gestel, C. A. M. Chronic toxicity of polycyclic aromatic compounds to the springtail Folsomia candida and the enchytraeid Enchytraeus crypticus. *Environ. Toxicol. Chem.* **2006**, *25* (9), 2423–2431.

(32) Sverdrup, L. E.; Nielsen, T.; Krogh, P. H. Soil ecotoxicity of polycyclic aromatic hydrocarbons in relation to soil sorption, lipophilicity, and water solubility. *Environ. Sci. Technol.* **2002**, *36* (11), 2429–2435.

(33) Van Gestel, C. A. M.; Van der Waarde, J. J.; Derksen, J. G. M.; Van der Hoek, E. E.; Veul, M. F. X. W.; Bouwens, S.; Rusch, B.; Kronenburg, R.; Stokman, G. N. M. The use of acute and chronic bioassays to determine the ecological risk and bioremediation efficiency of oil-polluted soils. *Environ. Toxicol. Chem.* **2001**, *20* (7), 1438–1449.

(34) Jones, D.; Keddie, R. M. *The genus Arthrobacter*; Springer: New York, 2006.

(35) O'Loughlin, E. J.; Sims, G. K.; Traina, S. J. Biodegradation of 2-methyl, 2-ethyl, and 2-hydroxypyridine by an Arthrobacter sp. isolated from subsurface sediment. *Biodegradation* **1999**, *10* (2), 93–104.

(36) Seo, J. S.; Keum, Y. S.; Cho, I. K.; Li, Q. X. Degradation of dibenzothiophene and carbazole by Arthrobacter sp. P1-1. *Int. Biodeterior. Biodegrad.* **2006**, *58* (1), 36–43.

(37) Styrishave, B.; Hartnik, T.; Christensen, P.; Andersen, O.; Jensen, J. Influence of soil type and organic matter content on the bioavailability, accumulation, and toxicity of α-cypermethrin in the springtail Folsomia candida. *Environ. Toxicol. Chem.* **2010**, *29* (5), 1084–1090.

(38) Schmidt, S. N.; Smith, K. E. C.; Holmstrup, M.; Mayer, P. Uptake and toxicity of polycyclic aromatic hydrocarbons in terrestrial springtails-studying bioconcentration kinetics and linking toxicity to chemical activity. *Environ. Toxicol. Chem.* **2013**, *32* (2), 361–369.

(39) Domene, X.; Alcañiz, J. M.; Andrés, P. Ecotoxicological assessment of organic wastes using the soil collembolan Folsomia candida. *Appl. Soil Ecol.* **2007**, *35* (3), 461–472.

(40) Peijnenburg, W.; Capri, E.; Kula, C.; Liess, M.; Luttik, R.; Montforts, M.; Nienstedt, K.; Römbke, J.; Sousa, J. P.; Jensen, J. Evaluation of exposure metrics for effect assessment of soil invertebrates. *Crit. Rev. Environ. Sci. Technol.* **2012**, *42* (17), 1862–1893.

(41) Bleeker, E. A. J.; Wlegman, S.; Droge, S. T. J.; Kraak, M. H. S.; Van Gestel, C. A. M. Towards an improvement of the risk assessment of polycyclic (hetero)aromatic hydrocarbons. *Report* **2003**, No. 1, 27–32.

(42) Sverdrup, L. E.; Jensen, J.; Kelley, A. E.; Krogh, P. H.; Stenersen, J. Effects of eight polycyclic aromatic compounds on the survival and reproduction of Enchytraeus crypticus (Oligochaeta, Clitellata). *Environ. Toxicol. Chem.* **2002**, *21* (1), 109–114.

(43) Hermens, J. L. M.; de Bruijn, J. H. M.; Brooke, D. N. The octanol-water partition coefficient: strengths and limitations. *Environ. Toxicol. Chem.* **2013**, *32* (4), 732–733.

(44) Anyanwu, I. N.; Semple, K. T. Effects of phenanthrene and its nitrogen-heterocyclic analogues aged in soil on the earthworm Eisenia fetida. *Appl. Soil Ecol.* **2016**, *105*, 151–159.

(45) Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals. *Environmental hazards: classification criteria for the terrestrial environment (Un/sceghs/12/inf.5)*; 2006.

(46) Hartmann, N. B.; Stefania, G.; Sokull-Klüttgen, B. *Review of available criteria for non-aquatic organisms within PBT/vPvB frameworks Part II : Toxicity assessment*; European Union, 2014: Luxembourg, 2014.

(47) GHS. Globally harmonized system of classification and labelling of chemicals (GHS)(4th Edition). *United Nations*. New York 2011.

(48) European Chemicals Agency. Guidance on information requirements and chmical safety assessment. Chapter R.10: characterisation of dose [concentration]-response for environment. *Eur. Chem. Agency* **2008**, No. May, 1–65.

‡ Supporting Information

S3.3.1. Materials.

Acetone (HPLC grade ≥ 99.8%) purchased from Sigma-Aldrich (Steinheim, Germany) was used to prepare stock solutions of quinaldines. The dichloromethane (DCM, GC grade ≥ 99.8%) used as the extraction solvent was obtained from Merck (Darmstadt, Germany). The surrogate (SSt) and internal (ISt) standards used in the extraction procedure, i.e., quinoline (GC grade, 98%) and naphthalene (GC grade, ≥ 99%), respectively, were purchased from Sigma-Aldrich (Steinheim, Germany). Snap-cap clear glass vials with polyethylene (PE) lids of 30 mL (75 × 28 mm) volume for the ecotoxicity test with *F. candida* in soil were purchased from VWR (Germany). Sterilized 24-well tissue culture plates (flat bottom with lid) and 96-well micro-test plates were acquired from Sarstedt (Nümbrecht, Germany). The artificial soil RefeSol 01-A-004 (loamy sand) was ordered from Fraunhofer IME (Schmallenberg, Germany). The basic information of the soil obtained in the product sheet was shown below:

pH_{CaCl2} 5.41; sand 76.7%, silt 17.2%, clay 6.1%; organic carbon (OC) 1.21%, total (N) 0.79 g kg^{-1}; cation exchange capacity 9.90 mmol kg^{-1}. The collection location was Schmallenberg, Nordrhein-Westfalen, Germany. There were no pesticides applied during the previous two years or the application of fertilizers since one year before collection. Sampling was combined subsamples (afterwards unified) collected by the aid of a driller (5–30 cm in diameter). The soil was dried at room temperature for 24 h and 2.0 mm-sieved before each test. The water used in this study, unless specifically stated otherwise, was deionized and filtered with a Carbonit NFP Premium-9 water filter with a pore size of 0.45 µm (Heidenheim, Germany). The soil, medium, water and containers for the culturing and ecotoxicity tests with *A. globiformis* were previously autoclaved at 121 °C for 20 min.

S3.3.2. Culturing of *Arthrobacter globiformis*.

The bacterial strain was obtained from the German Collection of Microorganisms (DSMZ, serial number 2014). The stock cultivation was prepared according to DIN 38412 L48[1]. Briefly, the original strain of 1 mL suspension was first cultured in 50 mL of growth medium A (DMS-A: 10.0 g peptone from casein, 5.0 g yeast extract, 5.0 g glucose, 5.0 g NaCl in 1000 mL sterilized water) on a horizontal shaker (Heidolph Tiramax 1000) at 30 °C in the dark with a frequency of 150 min^{-1} for 8 h. Afterwards, 1 mL of the bacterial suspension was transferred into another 50 mL of DMS-A medium and incubated for 16 h. The cell density of the bacteria was then determined in a 96-well plate with 300 µL of suspension per well, as measured using a microplate reader (Wallace VICTOR2 1420 multilabel counter, Perkin Elmer). The OD was preferably ≥ 0.6 at 620 nm. One-milliliter aliquots to be used as stocks (500 µL of the bacterial suspension in 500 µL 87% glycerin) were stored in sterile cryogenic vials at -20 °C. One day before the test, 1 mL of the deep frozen stock was gently thawed at 4 °C for 1 h and cultured in growth medium B (DMS-B, 1:3 dilution of DMS-A with sterilized water) following the procedure of DIN 38412 L48[1] except that the incubation duration was extended to 20 h. Cultures of cell density with OD 0.3–0.4 were ready for the growth inhibition test in both the soil and pore-water exposure scenarios, each of which was conducted for 2 h.

S3.3.3. Culturing of *Folsomia candida*.

Collembola are perennially cultured in our laboratory. They were originally obtained in the 1990s from the research group of Prof. Achazi at the Freie Universität Berlin. Culture substrate for synchronization was prepared in accordance with OECD guideline 232[3] but was reduced by half of the overall amounts, with the mixtures consisting of 200 g plaster of Paris, 25 g activated charcoal and 180 mL distilled water. The substrates were divided into plastic Petri dishes to a ~0.5 cm layer. Thirty adults from the stock culture were transferred to the dishes, and baker's yeast was added to serve as food. The dishes were then placed in a dark chamber at room

temperature. After 3-days of laying eggs, the adults and food were removed and the eggs were hatched for 7 to 8 days until massive amounts of juveniles were observed. After being fed with yeast, they were kept in a controlled climate chamber (Versatile environmental test chamber, MLR-350H, Sanyo, Japan) with a humidity of 85% at 20 ± 1 °C in 12:12-h light-dark cycles (600 Lux) for 11–12 days, at which point they were collected for the reproduction test in soil for 28 days. Cultures were kept moist and aerated with controls/adjustments every three days. Moldy food was removed, and sufficient fresh food was provided. Collembola for the survival test in pore-water exposure (14 days) were adults with an age of 55–56 days and were synchronized in the same manner but cultured in a dark chamber after hatching at a constant temperature of 15 °C.

S3.3.4. Contact test with *A. globiformis* in pore-water exposure scenario.

The bacteria were exposed to quinaldines prepared in sterilized water instead of soil, as suggested by Engelke *et al.*[2]. Quinaldine stock solutions of 500 µL with different concentrations were diluted with 500 µL DMS-B per well in 24-well plates in triplicate to obtain testing series with concentrations 6983–7.0 µmol L^{-1} (6983, 6285, 5237, 3492, 1746, 698, 349, 69.8, and 7.0 µmol L^{-1}, i.e., 1000 , 900, 750, 500, 250, 100, 50, 10, and 1 mg L^{-1}), 3397–6.8 µmol L^{-1} (3397, 1698, 679, 340, 67.9, and 6.8 µmol L^{-1}, i.e., 500, 250, 100, 50, 10, and 1 mg L^{-1}) and 6523–6.5 µmol L^{-1} (6523, 5871, 4892, 3262, 1631, 652, 326, 65.2, and 6.5 µmol L^{-1}, i.e., 1000, 900, 750, 500, 250, 100, 50, 10, and 1 mg L^{-1}) for Quin-2Me, Quin-2Me-pH and Quin-2Me-H10, respectively. Paired plates (with and without bacteria) for all treatments were prepared as described by Engelke *et al.*[2] except that 500 µL of either bacterial suspension or DMS-B were added. After 2 h of incubation, samples were dyed with resazurin (45 mg L^{-1}) but were incubated and shaken at 150 min^{-1} and 30 °C for 40 min. The samples were then centrifuged at 3000g for 5 min at 20 °C. Supernatant aliquots of 3 × 300 µL per well were transferred to each well of the 96-well micro-plates. Each test was repeated twice. Inhibition of bacterial growth by quinaldines, as represented by the decrease of the dehydrogenase activity of the bacteria, was determined in terms of the reduction of resazurin according to the decrease in the OD values[1] at 620 nm[2]; the survival rates were thus calculated. Benzyldimethylhexadecylammonium chloride (Sigma-Aldrich, Steinheim, Germany) was used as a positive control and tested in parallel in each test to ensure the validity.

S3.3.5. Survival inhibition test with *F. candida* in pore-water exposure scenario.

Tests were performed in accordance with the procedure of Houx *et al.*[4]. Similar setups have been reported with *F. candida*[4], and the adaptation of the pore-water test with the soil-dwelling Collembola was also demonstrated[4]. Quin-2Me, Quin-2Me-pH and Quin-2Me-H10 were prepared in water to create a concentration series of 100–0.5 mg L^{-1} (i.e., 100, 50, 40, 30, 20, 10, 7.5, 5.0, 2.5, 1.0, and 0.5 mg L^{-1}), corresponding to

698.3–3.5 µmol L^{-1} (i.e., 698.3, 349, 279, 210, 140, 69.8, 52.4, 34.9, 17.5, 7.0, and 3.5 µmol L^{-1}), 679.3–3.4 µmol L^{-1} (i.e., 679.3, 340, 272, 204, 136, 67.9, 51.0, 34.0, 17.0, 6.8, and 3.4 µmol L^{-1}) and 652.3–3.3 µmol L^{-1} (i.e., 652.3, 326, 261, 196, 131, 65.2, 48.9, 32.6, 16.3, 6.5, and 3.3 µmol L^{-1}), respectively. Two milliliters of the resulting solutions was then added to the wells of a 24-well plate in triplicate. Pure water was used as the negative control. Four adult Collembola were then placed in each well. The plates were covered with Parafilm and kept in a dark chamber at a constant temperature of 15 °C for 14 days. The Collembola were aerated every 3 days, and the numbers of live animals were recorded. Four repetitions were performed for each test. The effects of the quinaldines were calculated as the numbers of live individuals per well in the plates normalized by the numbers in the negative controls. Individuals were further observed with a stereo zoom microscope (Olympus SZX12, at × 16 magnification) equipped with a Sony DXC-9100P color video camera.

S3.3.6. Contact test with *A. globiformis* in soil exposure scenario.

The 10 mL solutions of the concentration series for each quinaldine were prepared in acetone. Two milliliters of solution were added to 2.5 g soil in a 100 mL glass beaker. The solvent control was prepared by adding 2 mL pure acetone. Spiked soil was then mixed by hand-shaking to form a homogeneous spiked matrix. After a 24 h evaporation of acetone, 7.5 g of pure soil was added to reach the testing concentrations of 750–10 mg kg^{-1} dw soil (i.e., 750, 500, 250, 100, 50, and 10 mg kg^{-1} dw soil), corresponding to 5237–69.8 µmol kg^{-1} (i.e., 5237, 3492, 1746, 698, 349, and 69.8 µmol kg^{-1}), 5095–67.9 µmol kg^{-1} (i.e., 5095, 3397, 1698, 679, 340, and 67.9 µmol kg^{-1}), and 4892–65.2 µmol kg^{-1} (i.e., 4892, 3262, 1631, 652, 326, and 65.2 µmol kg^{-1}) dw soil for Quin-2Me, Quin-2Me-pH and Quin-2Me-H10, respectively. The negative control (with neither quinaldine nor acetone) was prepared by weighing 10 g soil directly in a beaker. Sterilized water was added to each beaker to reach 50% water holding capacity (WHC). After the mixture was thoroughly mixed with a spatula, 0.6 (± 0.02) g spiked soil was added per well in 24-well plates with triplicate for each concentration. Paired plates (with and without bacteria) for all treatments were prepared according to the procedure of Engelke *et al.*[2]. To each well, 600 µL sterilized water was added followed by 500 µL of either bacterial suspensions or DMS-B. The test was repeated twice. The incubation procedures and effect determinations were performed as described in the pore-water exposure test. Positive control – benzyldimethylhexadecylammonium chloride was tested in parallel in each soil test to ensure the validity.

S3.3.7. Reproduction inhibition test with *F. candida* in soil exposure scenario.

Quinaldines were prepared in acetone and soil was spiked as previously described, but by gently pouring 10 mL solutions into 12 g autoclaved soil in 600 mL glass beakers. In the solvent control, only 10 mL pure acetone was added. After the evaporation of

acetone, 38 g pure soil was added to the matrix to obtain the final testing concentrations of 1000–50 mg kg^{-1} dw soil (i.e., 1000, 750, 500, 400, 300, 200, 100, and 50 mg kg^{-1} dw soil), corresponding to 6983–349.2 µmol kg^{-1} (i.e., 6983, 5237, 3492, 2793, 2095, 1397, 698, and 349.2 µmol kg^{-1}), 6794–339.7 µmol kg^{-1} (i.e., 6794, 5095, 3397, 2717, 2038, 1359, 679, and 339.7 µmol kg^{-1}) and 6523–326.2 µmol kg^{-1} (i.e., 6523, 4892, 3262, 2609, 1957, 1305, 652 and 326.2 µmol kg^{-1}) dw soil for Quin-2Me, Quin-2Me-pH and Quin-2Me-H10, respectively. The negative control (without either quinaldine or acetone) was prepared by weighing 50 g soil directly in a beaker. Water was added to reach 50% WHC and samples were thoroughly mixed with a spatula. The toxicity test was performed according to OECD guideline 232[3] but was miniaturized according to a previously described method[5]. Aliquots of 10 g spiked soil were placed into 30 mL glass vials with snap caps in 4 replicates; to each vial, four adult Collembola were added. A few grains of baker's yeast were provided on the top to serve as a food source. Additional samples were prepared using 0.01 M $CaCl_2$ for pH measurements. Test vials were placed in the same climate chamber under the same conditions as those used for the culturing. Aeration, water and food were supplemented twice a week. After 28 days, Collembola were extracted by flotation following OECD guideline 232[3] but using 100 mL instead of 200 mL water. Individuals were recorded by photograph (Canon EOS 600D with an EFS 18–135 mm lens) and photos were processed using the ImageJ v1.48 software, wherein the numbers of adults and juveniles were counted. The test was repeated four times. The effects of quinaldines on the reproduction of Collembola were calculated as the numbers of juveniles produced per test vial normalized by the numbers in the negative controls.

S3.3.8. Concentration determination.

Additional samples were prepared in the same way as those for the ecotoxicity tests, but without inoculating organisms in order to determine the concentrations. The soil samples were extracted following the EPA guideline 3550c[6]. Duplicate of 2 g spiked soil were collected into 20 mL GC headspace vials to which 1 mL quinoline (2.0 g L^{-1} in DCM, SSt) was then added. The pH of the soil samples of Quin-2Me-H10 was adjusted to 12 by addition of 1 M NaOH before the quinoline was added. After brief hand-mixing, 2 g anhydrous sodium sulfate (Na_2SO_4) was added and thoroughly mixed with the soil by hand-shaking until oversaturation was achieved. Nine milliliters of DCM was immediately added, and the samples were tightly closed for extraction using double-extraction in an ultrasonic ice-water bath (50/60 Hz, Bandeln SONOREX SUPER RK106, Germany) for 30 min. The supernatant was filtered through a Pasteur pipette packed with glass wool and was then collected. Another 10 mL of DCM was added to the same soil sample for the second extraction. The supernatant was collected into the same vial after filtration. Aliquots of 1 mL of each extract were transferred to GC vials and spiked with 10 µL naphthalene (2.0 g L^{-1} in DCM, ISt), then analyzed using GC/MS. Blanks (pure soil and DCM) and quality controls (quinaldines diluted in DCM) were prepared and extracted in parallel.

Additional 2 g soil samples were collected in duplicate for determining the water content at 70 °C for 24 h.

Pore-water concentrations (C_q, µmol L^{-1} or mg L^{-1}) were calculated from the nominal concentrations for each test concentration (C_N, µmol kg^{-1} or mg kg^{-1} dw soil) used in soil ecotoxicity tests by including the equilibrium partitioning[7,8]. For each of the test concentrations, the total mass of quinaldine that had been originally spiked (M_N) was consumed, where M_N represents the mass of the test substance in the soil (M_S) and that of the test substance in the liquid (M_q) phase, i.e., $M_N = M_S + M_q$, with further substitutions using $M_N = C_N \times m_s$, $M_S = C_S \times m_s$ and $M_q = C_q \times V_q$, where C_S is the concentration of the test substance in the soil phase and m_s is the mass of the soil phase (referenced to 50 g dw soil, i.e., the total amount of the soil matrix after spiking). Correspondingly, C_q and V_q are the concentration of quinaldines in the liquid phase and the volume of the liquid (6.6 mL water was added to reach 50% WHC), respectively. According to the equilibrium partitioning relationship, namely, $K_d = C_S / C_q$, C_S was afterwards substituted by $C_q \times K_d$. Hence, the pore-water concentrations (C_q) were calculated, the dose-response curve for each quinaldine was re-established using effects vs. C_q, and the pore-water-concentration-based EC_{50} values and EC_{10} values were extrapolated in R.

Samples from the pore-water exposure tests were extracted in triplicate by liquid-liquid extraction. Samples were first diluted in water to approximately 1.0 mg L^{-1} to a final volume of 10 mL. The pH of the diluted samples of Quin-2Me-H10 was adjusted to 12 by addition of 1 M NaOH. Quinoline prepared in a water volume of 10 µL (1.0 g L^{-1}) was added as SSt. Two milliliters of DCM was then added, and the resultant mixture was shaken by hand and vortexed for 45 s. The organic phase was subsequently transferred, and approx. 0.3 g anhydrous Na_2SO_4 was applied to remove the residual water. Aliquots of 1 mL were transferred to GC vials to which 10 µL naphthalene (ISt, 2.0 g L^{-1} in DCM) was then added. The concentrations of the resultant solutions were then measured by GC/MS.

S3.3.9. GC/MS analysis.

The concentration of quinaldines in the extracts were determined using a gas chromatograph (HP® GC system 6890N) equipped with a mass-selective detector (HP® MS 5973, Agilent, Waldbronn, Germany). Two microliter aliquots of the samples were injected in pressure pulse splitless mode with the aid of an autosampler. The capillary column (FS-Supreme-5ms column (CS), 30 m × 0.25 mm (ID), 0.25 µm film thickness) was operated at a pressure of 2.01 atm with He as the carrier gas flowing at 3.0 mL min^{-1}. The GC parameters were as follows: inlet temperature 250 °C; oven program 100 °C hold 0.6 min, ramp at 20 °C min^{-1} to 150 °C, ramp at 35 °C min^{-1} to 300 °C, and hold 1 min; the MS ionization source and the quadrupole were maintained at 230 °C and 150 °C, respectively. The MS detector was operated in the positive ion mode, using an ionization energy of 70 eV. The spectrum was recorded in full scan mode with a scan rate of 3.0 scans s^{-1}. The results were evaluated

with Chemstation software (Agilent Technologies). The concentrations of each component in the extracts were determined using the internal standard (naphthalene) normalized peak area and an eleven-point calibration using standards containing naphthalene, quinoline, Quin-2Me, Quin-2Me-pH and Quin-2Me-H10 with the latter four chemicals in concentrations ranging from 0.5 to 100 mg L^{-1} in DCM. The LODs of the measurements for quinoline, Quin-2Me, Quin-2Me-pH and Quin-2Me-H10 were 0.18, 0.19, 0.17 and 0.64 mg L^{-1}, respectively (corresponding to 1.4, 1.3, 1.2 and 4.2 μmol L^{-1}, respectively); the LOQs of the method for these same compounds were 0.55, 0.57, 0.51 and 1.91 mg L^{-1}, respectively (corresponding to 4.3, 4.0, 3.5, and 12.5 μmol L^{-1}, respectively).

Table S3.3.1. Nominal concentrations (C_N) and measured concentrations (µmol L^{-1} and µmol kg^{-1} dw soil) at the test beginning (C_{t0}) and end (C_{tE}) in the pore-water (part A, n = 3) or soil (part B, n = 4) exposure scenario. Deviations between concentrations are shown in %; "n.a." means data not available.

Part A		**Pore-water exposure** [µmol L^{-1}]				
Organism	**Quinaldines**	**C_N**	**C_{t0}**	**C_{t0} vs. C_N (%)**	**C_{tE}**	**C_{tE} vs. C_{t0} (%)**
A. globiformis	**Quin-2Me**	6983	5675 ± 88.0	81.3	5078 ± 55.2[a]	89.5
		3492	3140 ± 51.0	89.9	3220 ± 102.7[a]	102.6
		349.2	301.7 ± 16.8	86.4	341.5 ± 32.8[a]	113.2
	Quin-2Me-pH	3397	3594 ± 14.9	105.8	3531 ± 52.3[a]	98.2
	Quin-2Me-H10	6523	6196 ± 85.5	105.0	6108 ± 71.8[a]	98.6
		3262	2894 ± 132.4	88.7	2881 ± 134.4[a]	99.5
		326.2	314.4 ± 5.2	96.4	309.8 ± 2.6[a]	98.5
F. candida	**Quin-2Me**	698.3	639.0 ± 16.1	91.5	522.3 ± 64.2[b]	81.7
		69.8	61.5 ± 1.4	88.0	74.0 ± 1.4[b]	120.5
	Quin-2Me-pH	679.3	795.5 ± 14.3	117.1	347.8 ± 6.8[b]	43.7
		67.9	76.8 ± 1.4	113.0	59.1 ± 4.1[b]	77.0
	Quin-2Me-H10	652.3	624.3 ± 15.7	95.7	484.7 ± 16.3[b]	77.6
		65.2	58.7 ± 1.3	90.0	48.9 ± 1.3[b]	83.3
Part B		**Soil exposure** [µmol kg^{-1} dw soil]				
Organism	**Quinaldines**	**C_N**	**C_{t0}**	**C_{t0} vs. C_N (%)**	**C_{tE}**	**C_{tE} vs. C_{t0} (%)**
A. globiformis		n.a.	n.a.	n.a.	n.a.[a]	n.a.
F. candida	**Quin-2Me**	6983	4693 ± 86.6	67.2	3998 ± 27.9[c]	85.2
		2793	2126 ± 28.6	76.1	1818 ± 129.9[c]	85.5
	Quin-2Me-pH	6793	2696 ± 29.9	39.7	0.0 ± 0.0[c]	0.0
		2717	1137 ± 51.0	41.8	0.0 ± 0.0[c]	0.0
	Quin-2Me-H10	6523	1652 ± 16.3	25.3	1697 ± 17.6[c]	102.7
		2609	516.0 ± 31.3	19.8	553.8 ± 71.8[c]	107.4

[a] After 2 h.

[b] After 14 days.

[c] After 28 days.

Table S3.3.2. LC_{10}, LC_{50}, EC_{10} (only for the Collembola) and EC_{50} values of the quinaldines toward the two organisms in the pore-water (mg L^{-1}) and soil (mg kg^{-1} dw soil) tests. Values were given with 2.5% and 97.5 % confidence intervals in the brackets. "n.a." indicates data not available.

	A. globiformis		***F. candida***					
	Pore-water exposure	**Soil exposure**	**Pore-water exposure**		**Soil exposure**		**Calculated pore-water**	
	EC_{50} [a]	EC_{50} [a]	LC_{50} [a]	LC_{10} [a]	EC_{50} [a]	EC_{10} [a]	EC_{50} [b]	EC_{10} [b]
Quin-2Me	> 1000	> 750	11.3 (9.7–n.a.)	3.0	366.4 (341.9–n.a.)	75.5	169.5 (158.2–n.a.)	34.9
Quin-2Me-pH	> 500	> 750	11.5 (10.5–13.1)	4.5	347.0 (322.5–378.3)	102.5	34.6 (32.1–37.7)	10.2
Quin-2Me-H10	≥ 750	> 750	24.8 (19.9–30.8)	3.4	324.9 (304.1–346.0)	116.6	127.3 (119.2–135.5)	45.7

[a] Effective and lethal concentrations are based on the nominal concentrations.

[b] Effective concentrations are based on the K_d-corrected soil pore-water concentrations (mg L^{-1}).

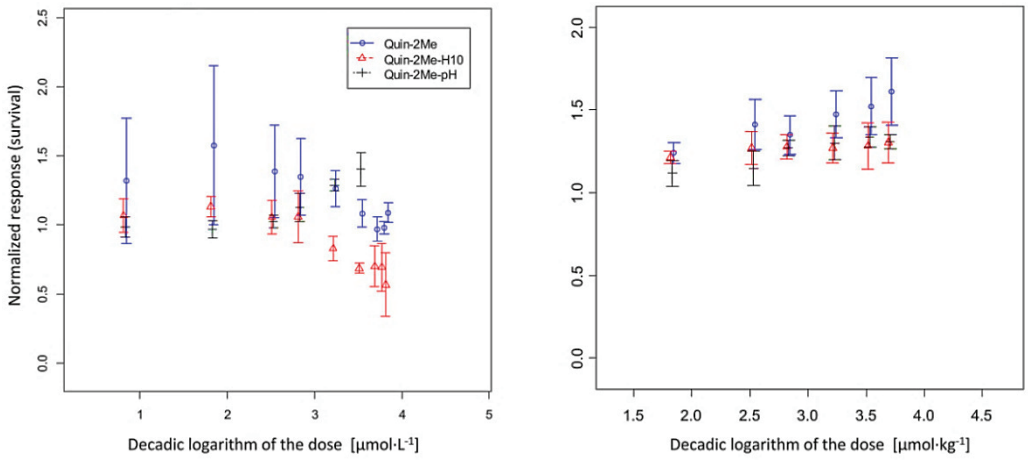


Figure S3.3.1. Effects (nominal concentrations, µmol L^{-1} and µmol kg^{-1} dw soil) of the quinaldines on *A. globiformis* in pore-water (**left**, n=6) or soil (**right**, n=6) exposure test.

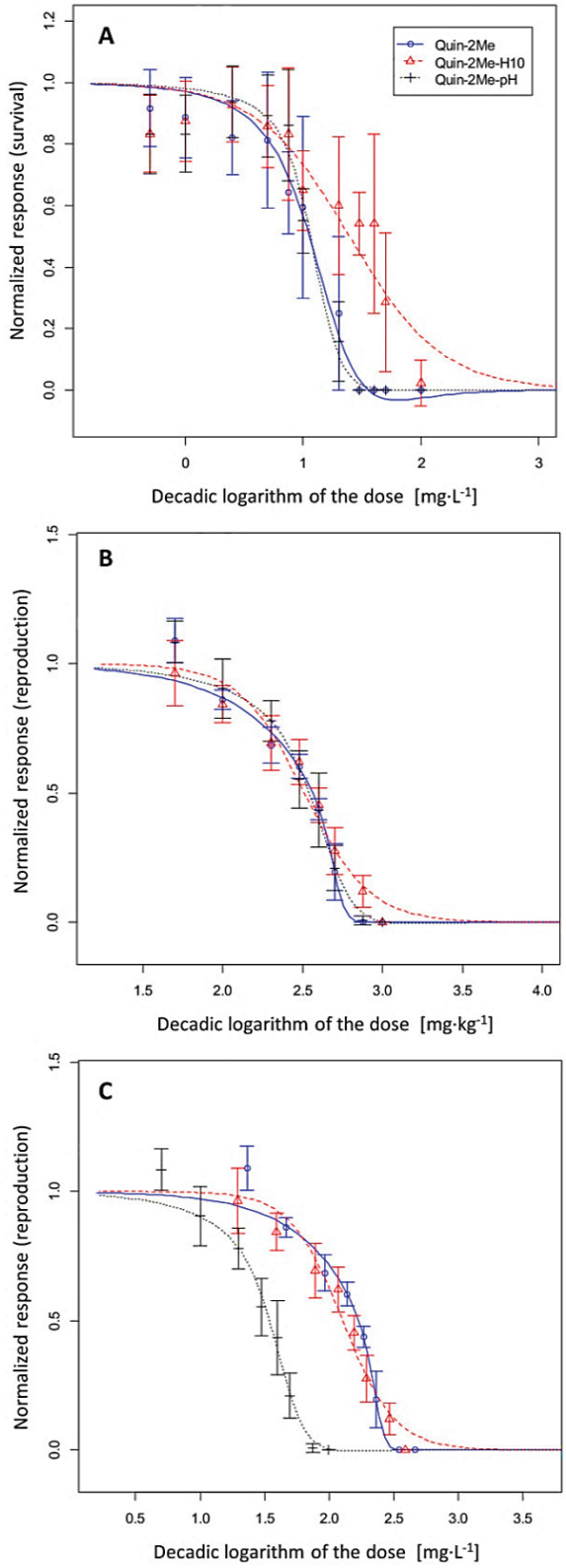


Figure S3.3.2. Effects (in mg L^{-1} or mg kg^{-1} dw soil) of the quinaldines on *F. candida* in pore-water (**A**: nominal concentrations, n = 12) and soil (**B and C**:, n=16) exposure scenarios. **B:** nominal concentrations in soil; **C**: calculated soil pore-water concentrations.

Table S3.3.3. PNECs derived from EC_{10} values (nominal or calculated pore-water based) in soil with an assessment factor of 100.

	PNECs (nominal) [mg kg^{-1} dw soil]	**PNECs (calculated pore-water)** [mg L^{-1}]
Quin-2Me	0.8	0.3
Quin-2Me-pH	1.0	0.1
Quin-2Me-H10	1.2	0.5

References

(1) Engelke, M.; Köser, J.; Hackmann, S.; Zhang, H.; Mädler, L.; Filser, J. A miniaturized solid contact test with *Arthrobacter globiformis* for the assessment of the environmental impact of silver nanoparticles. *Environ. Toxicol. Chem.* **2014**, *33* (5), 1142–1147.

(2) OECD. Test No.232: Collembolan reproduction test in soil. *OECD Guidel. Test. Chem.* **2009**, No. September, 1–19.

(3) Houx, N. W. H.; Dekker, A.; Van Kammen-Polman, A. M. M.; Ronday, R. Acute toxicity test for terrestrial hazard assessment with exposure of *Folsomia candida* to pesticides in an aqueous medium. *Arch. Environ. Contam. Toxicol.* **1996**, *30* (1), 9–14.

(4) Filser, J.; Wiegmann, S.; Schröder, B. Collembola in ecotoxicology-any news or just boring routine? *Appl. Soil Ecol.* **2014**, *83*, 193–199.

(5) U. S. Environmental Protection Agency. EPA Method 3550C: ultrasonic extraction. *U. S. Environ. Prot. Agency* **2007**, No. February, 1–17.

(6) Sverdrup, L. E.; Kelley, A. E.; Krogh, P. H.; Nielsen, T.; Jensen, J.; Scott-Fordsmand, J. J.; Stenersen, J. Effects of eight polycyclic aromatic compounds on the survival and reproduction of the springtail *Folsomia fimetaria L.* (Collembola, Isotomidae). *Environ. Toxicol. Chem.* **2001**, *20* (6), 1332–1338.

(7) Kobetičová, K.; Bezchlebová, J.; Lána, J.; Sochová, I.; Hofman, J. Toxicity of four nitrogen-heterocyclic polyaromatic hydrocarbons (NPAHs) to soil organisms. *Ecotoxicol. Environ. Saf.* **2008**, *71* (3), 650–660.

3.4. Manuscript 4

Markiewicz, M.; **Zhang, Y. Q.**; Empl, M. T.; Lykaki, M.; Thöming, J.; Wasserscheid, P.; Bösmann, A.; Steinberg, P.; Stolte, S. (2017) '**Preliminary hazard assessment of quinaldine and alkylcarbazole based liquid organic hydrogen carriers**.'

Prepared manuscript for submission to Energy & Environmental Science.

This manuscript was just completed rencently, and it has not been reviewed by all the co-authors. Paticularly for the mutagenicity tests with intestinal bacteria *Salmonella thypimurium*, the description regarding the test procedure, results and related discussion has not been well developed and structured. However, it will not influence the understanding of the presented thesis in which the discussion was established on soil and aquatic organisms.

Page: 126–157

Contributions of Zhang, Y. Q.:

- Support in developing the research questions and experimental planning;
- Experimental performance of the acute tests with *R. subcapitata* and corresponding method description and data analysis;
- Preparation of the introduction and part of the results and discussion sections for the initial draft of the manuscript; and
- Revisions of the manuscript.

The rest of the manuscript was prepared by the co-authors.

Preliminary hazard assessment of quinaldine and alkylcarbazole based Liquid Organic Hydrogen Carriers‡

Marta Markiewicz[a]*, Ya-Qi Zhang[a], Michael T. Empl[b], Marianna Lykaki[a], Jorg Thöming[c], Pablo Steinberg[b], Peter Wasserscheid[d], and Stefan Stolte[a,e]

[a] UFT - Centre for Environmental Research and Sustainable Technology, Department Sustainable Chemistry, University of Bremen, Leobener Straße, D-28359, Bremen, Germany.

[b] Institute for Food Toxicology and Analytical Chemistry, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany.

[c] UFT - Centre for Environmental Research and Sustainable Technology, Department Chemical Engineering-Recovery and Recycling, University of Bremen, Leobener Straße, D-28359, Bremen, Germany.

[d] Department of Chemical and Bioengineering, Friedrich-Alexander-University of Erlangen-Nürnberg, Egerlandstr. 3, 91058 Erlangen, Germany.

[e] Department of Environmental Analysis, Faculty of Chemistry, University of Gdańsk, ul. Wita Stwosza 63, 80-308 Gdańsk, Poland.

Abstract

Due to the finite nature and scarcity of crude oil based fuels increasing attention is directed towards renewable energy. To obtain fully functional renewable energy systems, a way to compensate for their spatiotemporal fluctuations is necessary. The Liquid Organic Hydrogen Carrier (LOHC) systems are promising from the technological point of view yet their environmental and health impacts have not been considered in greater detail. Hereby we present a proactive, comparative environmental impact assessment of LOHC systems based on three alkylcarbazoles and quinaldine including H_2-rich, H_2-lean and intermediate (partially hydrogenated) forms of each of them. The paper reports on their enzyme inhibition (acetylcholinesterase), mutagenicity (Ames test) and cytotoxicity (IPC-81 cell line). The aquatic toxicity of test compounds towards marine bacterium (*Vibrio fischeri*), green algae (*Raphidocelis subcapitata*), aquatic plant (*Lemna minor*) and water flea (*Daphnia magna*) was also assessed. Moreover, their biodegradability was screened using inoculum from wastewater treatment plant. To put the results in a bigger picture we have incorporated the comparison with diesel oil in a preliminary environmental impact assessment. In this context our results suggest that the quinaldine-based LOHC system is comparable with diesel oil. The alkylcarbazoles seem to be more toxic and

poorly biodegradable. Nevertheless, due to less complex composition, the assessment of the LOHC systems carries much lower levels of uncertainty. Additionally thanks to more favourable physicochemical properties (e.g., higher boiling point) the safety of handling and transportation is also higher.

Introduction

Global utilization of fossil fuels, such as coal, oil, and natural gas, has tremendously increased in recent decades to meet the energy needs caused by the rapid economic development. However, growing demand for the fossil energy raises concerns regarding not only their limited availability but also environmental and human health impacts. Burning of fossil fuels accounts for one of the biggest sources of air pollution and recovering of fossil fuels has undeniable negative environmental consequences.[1] World largest economies direct their attention towards renewable energy to reduce the dependence on fossil fuels.[2–4] Theoretically, solely solar energy available on Earth could cover energy needs of our civilisation.[1] Practically, even all types of renewable energies taken together so far cannot form a stable energy system. This is due to intermittent nature of such energy sources i.e., spatial and temporal limitations in energy generation or inability to connect them into one system providing stable continuous output. Since we cannot control the sun or wind, we have to find a way to store the energy when it is present in excess to use it in the time when it is needed. This is by no means an easy task.

In current concepts of future energy economy a special place is taken by hydrogen – a clean, high energy density resource (gravimetric energy density of hydrogen equals 120 MJ kg^{-1}).[5,6] Considerable efforts are being invested in optimizing the process of hydrogen generation by water splitting using renewable energies.[7] Nevertheless, developing an economic way of hydrogen production is only a half of success since storage and distribution of hydrogen are not a trifle. Due to low density of hydrogen (91 g m^{-3})[5] its volumetric energy density is also low (10.9 kJ kg^{-1}). Therefore, any form of storage essentially includes lowering the energy content-to-volume ratio. Several methods can be applied to achieve that including compression, liquefaction, physisorption on high surface area materials or chemisorption in form of various hydrides.[8,9] Unfortunately, all of these methods suffer from drawbacks, including low storage capacity, high mass of the storage system, slow loading/unloading and, hydrogen losses that hamper their direct implementation.[10] Therefore yet another system based on storage of hydrogen chemically bound in Liquid Organic Hydrogen Carrier (LOHC) was proposed.

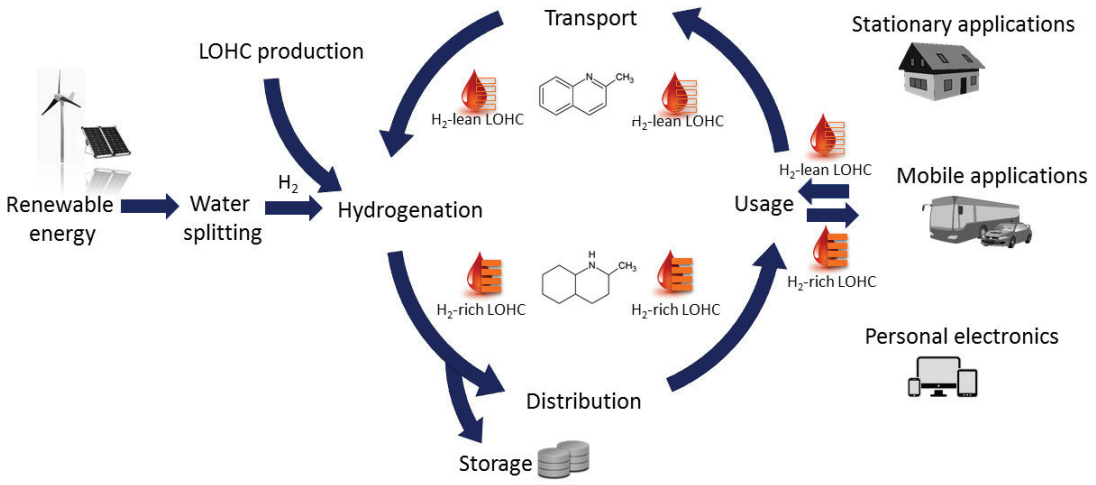


Figure 3.4.1. Schematic representation of LOHC cycle using the quinaldine-based LOHC system as an example.

LOHC system consists of a tandem of organic compounds in which one, H_2-lean usually aromatic or heteroaromatic compound is hydrogenated to another H_2-rich cyclic or heterocyclic equivalent. Hydrogen is drawn from the loaded H_2-rich form in a catalytic reaction. In the process, liquid carrier is reverted to H_2-lean form and can be hydrogenated again closing the cycle (Figure 3.4.1). Two main advantages over conventional fuels are immediately visible. First, the energy stored in LOHC carrier might come from renewable sources. Second, the carrier is not spent in the process but releases hydrogen and is fed back into the cycle where it undergoes multiple hydrogenation-dehydrogenation rounds. Since the carrier, with regard to properties defining handling, is very similar to diesel, it could be stored, transported and distributed using similar infrastructure which reduces costs of implementation of LOHC technology. In some aspects the LOHC carriers (see Table 3.4.1) are superior to currently used fuels e.g., they have usually higher boiling points, which means lower evaporation (less atmospheric pollution, higher safety during handling), and their composition is much better defined than it is in case of fossil fuels. This facilitates all kinds of evaluation, standardisation or quality control. Demonstration units of LOHC-based energy storage were recently launched in Stuttgart[*] and Arzberg[†], Germany. Additionally, commercially available solutions employing LOHC technology include off-grid storage/generator units (66 to 792 kW) for buildings, renewable energy farms and shipping industry as well as water desalination units, air-conditioning units and a spectacular CO_2-free energy autonomous megayacht.[‡]

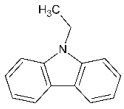
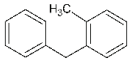
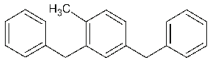
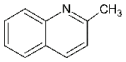
In principle, any molecule having unsaturated bond could serve as a LOHC. However, technological feasibility and safety concerns limit the choice, and so far only a few LOHC systems were investigated (Table 3.4.1). The most established one

[*] Fraunhofer Institute of Industrial Engineering, www.iao.fraunhofer.de.
[†] Areva GmbH, de.areva.com.
[‡] H2-Industries SE: www.h2-industries.com.

is based on ethylcarabzole.[11–13] Yet the high melting point of ethylcarbazole and limited thermal stability of that LOHC system stimulated further search for more suitable carriers e.g., homologues substituted with longer alkyl chains (propyl and butyl). The melting points of propyl- and butylcarbazole (H_2-lean forms) are 49 °C and 59 °C respectively[14] so still not low enough. The hydrogenated equivalents remain liquid at much lower temperatures (e.g., perhydro-ethylcarbazole solidifies at -84 °C[14]) and the melting points of partially hydrogenated species are somewhere between these values as was shown on the example of ethylcarbazole.[14] Intentionally preventing full dehydrogenation of dodecahydro-alkylcarbazoles, even though counterproductive in terms of amount of H_2 obtained, might be desired technologically since the mixtures do not solidify as readily as pure H_2-lean alkylcarbazoles. As a result, a mixture of the molecules with different levels of hydrogenation (e.g., see Table 3.4.2 for the structures of partially hydrogenated alkylcarbazoles) might be used. Both hydrogenation and dehydrogenation are catalytic processes requiring elevated temperature known to result in a certain level of carrier degradation (e.g., transalkylation or dealkylation), especially in the case of heteroaromatic carriers.[15] All three carbazole-derivatives are prone to dealkylation. Additionally, each methyl group added to the molecule acts as a ballast decreasing the H_2 storage capacity.[15]

Recently Brückner et al. examined the applicability of heat transfer fluid composed of isomers of benzyl- or dibenzyltoluene in hydrogen storage.[16] This LOHC system has higher H_2 storage capacity with all of the components being liquid down to -40 °C yet it requires higher temperatures to achieve complete dehydrogenation (see Table 3.4.1 for details on properties of different LOHC systems). Another LOHC system based on quinaldine (2-methylquinolines) gained significant interest due to the low dehydrogenation temperature (Table 3.4.1).[17] The temperature of dehydrogenation of decahydroquinaldine is low enough that it could be supplied by a waste heat of a polymer electrolyte membrane fuel cell, which is not the case for any of the previously mentioned LOHC systems.[18]

Table 3.4.1. Basic properties of LOHC systems currently under development and diesel.

	LOHC system				Diesel
	Ethyl-carbazole	**Benzyl-toluene**	**Dibenzyl-toluene**	**Quinaldine**	
Structure					Mixture of paraffins, olefins, naphthenes and aromatics[a]
Melting point [°C]	70[14]	-30[16]	-39 to -34[16]	-9 to -2[19]	-40 to 6[a]
Boiling point [°C]	270[16]	280[16]	390[16]	248[19]	141 to 462[a]
kg fuel per 100km driving range	17.3[b]	15.2[b]	16.1[b]	15.3[b]	7.3[5 c]
H_2 carrying capacity [wt%]	5.8	6.2	6.2	6.6	n/a
Dehydrogenation temperature [°C]	200–230[20]	250–270[16]	270–290[16]	138[21]	n/a

[a] European Chemical Agency (ECHA) for automotive diesel oil CAS68334-30-5 (https://echa.europa.eu/).

[b] Assuming density of 0.85 kg L^{-1}.

[c] Assuming consumption of 1 kg H_2 per 100 km and zero losses.

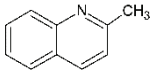
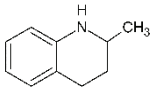
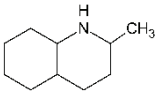
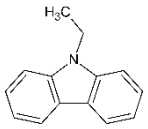
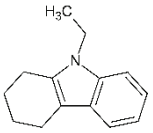
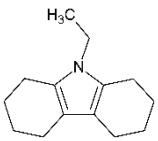
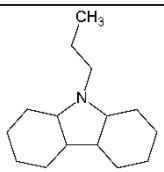
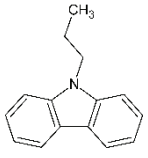
The annual world fuel oil demand was estimated to be 5.5×10^{12} L in 2016.[22] If all of it were to be replaced by any of the above mentioned LOHC systems, 3.3–3.7 × 10^{13} L of the carrier would be needed.[*] Even if the LOHC technology would only be implemented in niche application, the carrier chemicals would be handled, processed, stored and transported on a multi-ton scale with the citizens having access to large quantities. Consequently, there is a possibility that the carrier might be released into the environment by leakage or accidental spill as it was many times the case for fossil fuels.[6] The LOHC-based energy storage is still a young technology, which requires research and development efforts to optimise its performance to commercially attractive levels. This opens up a possibility to design the carriers for increased operational and environmental safety proactively according to the rules of green chemistry.[23]

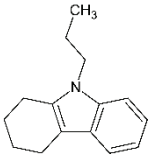
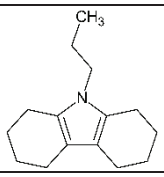
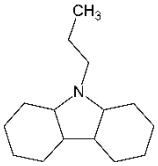
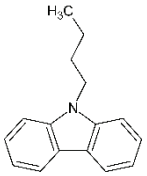
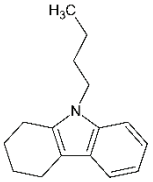
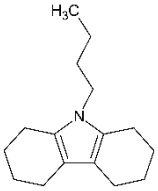
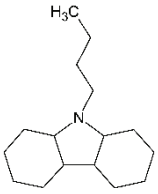
Since there is a choice of at least six viable LOHC systems described in the literature (Table 3.4.1), the one with the best technological performance but also the

[*] Assuming energy density of diesel oil equal to 47 MJ kg^{-1} and energy density of LOHC listed in Table 3.4.1 to be between 6.96 and 7.90 MJ kg^{-1}.

lowest environmental and health impacts should be chosen. To achieve this, the possible impacts on human health and the environment have to be investigated and evaluated first – the process known as a hazard assessment. Hereby we present a preliminary assessment of (eco)toxicity including mutagenicity as well as biodegradability of four possible LOHC systems: three based on alkylcarbazoles and one based on quinaldine.

Table 3.4.2. Basic properties of chemicals comprising carbazole- and quinaldine-based LOHCs.

Sample acronym	**Name**	**Structure**	**Formula MW** [g mol^{-1}]	**Log D** (pH 7.4)	**pK$_b$**
Quin-2Me	Quinaldine		$C_{10}H_9N$ 143.2	2.59 [24] 2.26[a]	4.94 [25] 5.15[a]
Quin-2Me-pH	Tetrahydro-quinaldine		$C_{10}H_{13}N$ 147.2	2.35[a]	4.88[a]
Quin-2Me-H10	Decahydro-quinaldine		$C_{10}H_{19}N$ 153.3	-0.84[a]	10.75[a]
Car-2	Ethylcarbazole		$C_{14}H_{13}N$ 195.3	3.67[a]	-[a]
Car-2-pH	Tetrahydro-ethylcarbazole (Car-2-H4)		$C_{14}H_{17}N$ 199.3	3.87[a]	-[a]
	Octahydro-ethylcarbazole (Car-2-H8)		$C_{14}H_{21}N$ 203.3	4.07[a]	-[a]
Car-2-H12	Dodecahydro-ethylcarbazole		$C_{14}H_{25}N$ 207.4	0.09[a]	11.69[a]
Car-3	Propylcarbazole		$C_{15}H_{15}N$ 209.3	4.19[a]	-[a]

Sample acronym	Name	Structure	Formula MW [g mol^{-1}]	Log D (pH 7.4)	pK$_b$
Car-3-pH	Tetrahydro-propylcarbazole (Car-3-H4)		$C_{15}H_{19}N$ 213.3	4.39[a]	-[a]
	Octahydro-propylcarbazole (Car-3-H8)		$C_{15}H_{23}N$ 217.3	4.59[a]	-[a]
Car-3-H12	Dodecahydro-propylcarbazole		$C_{15}H_{27}N$ 221.4	0.58[a]	11.97[a]
Car-4	Butylcarbazole		$C_{16}H_{17}N$ 223.3	4.64[a]	-[a]
Car-4-pH	Tetrahydro-butylcarbazole (Car-4-H4)		$C_{16}H_{21}N$ 227.3	4.84[a]	-[a]
	Octahydro-butylcarbazole (Car-4-H8)		$C_{16}H_{25}N$ 231.3	1.02[a]	4.90[a]
Car-4-H12	Dodecahydro-butylcarbazole		$C_{16}H_{29}N$ 235.4	1.02[a]	12.09[a]

[a] Calculated using ChemAxon.

The LD_{50} of orally administered compound in rat is 1230 mg kg^{-1} and higher than 5000 mg kg^{-1} [26] for Quin-2Me and Car-2 respectively, which generally falls correspondingly within in acute oral toxicity category 4 and 5 (the second lowest and lowest category) according to GHS (Globally Harmonised System).[27,28] So far, the knowledge about the effects and behaviour of the components of alkylcarbazole and quinaldine, or all the other, LOHC systems in the environment is scarce. In the

absence of the testing data some insight into environmental and health hazard can be gained from the analysis of physicochemical properties or read-across with structural analogues. The hydrophobicity expressed as octanol-water partition coefficient (log K_{ow}) for neutral compounds or octanol-water distribution coefficient (pH dependent log D) for ionisable compounds is used to estimate the affinity for biological membranes. From such values the minimum toxicity (baseline toxicity) of chemicals deprived of specific modes of toxic action can be derived. Table 3.4.2 summarises the most important parameters influencing the toxicity of LOHC systems under investigation.

While toxicity and mutagenicity largely depend on hydrophobicity of the molecule the latter is very sensitive to the presence and positions of substituents. (Remark: The paragraph regarding the background information of mutagenicity has not yet been provided by the co-author and will be added before submission.)

In case of broad scale implementation of hydrogen mobility based on LOHCs a hazard assessment taking into account at the very least the physicochemical properties, stability, (eco-)toxicity, biodegradability and bioaccumulation potential would need to be conducted. Four potential LOHC systems based on ethyl-, propyl- and butylcarbazole as well as quinaldine were included in this work. For each LOHC system three forms of the carrier were assessed: H_2-lean (aromatic), H_2-rich (perhydrogenated) and partially hydrogenated (where the hydrogenation reaction was stopped before full hydrogenation could occur). The partially hydrogenated (or partially dehydrogenated if one wishes) samples were prepared and tested since carriers are intended to be used multiple times so that some amount of impurities is unavoidable. Moreover, as mentioned above, it is sometimes technologically advantageous prevent complete dehydrogenation to avoid solidification of the H_2-lean carrier. We have therefore investigated H_2-rich and H_2-lean molecules as well as mixtures containing technical grade partially hydrogenated carriers. We have focused on the aquatic environment since it is likely to be the most affected compartment. We based the evaluation on (eco)toxicity: (i) acute/subchronic toxicity investigations in six biological test models following an increasing complexity of the test subjects: enzymes (acetylcholine esterase), mammalian cell lines (IPC-81 promyelocytic leukemia rat cells), bacteria (*Vibrio fischeri*), unicellular algae (*Raphidocelis subcapitata*), freshwater vascular plant (*Lemna minor*) and invertebrate (*Daphnia magna*). We have additionally investigated biodegradability since the persistence in the environment often leads to the concentration build-up so that even relatively non-toxic compounds can have detrimental impact on the environment. Finally, we have included a bacterial mutagenicity assessment (*Salmonella typhimurium*) since some PAHs are known to be mutagenic.[29] Applications of several test systems allows us to form an ecotoxicological profile of the LOHC systems based on different modes of action and on different levels of biological organisation.

Materials and Methods

Chemicals. The 2-methylquinoline (quinaldine, Quin-2Me, CAS 91-63-4), N-ethylcarbazole (Car-2, CAS 86-28-2), N-propylcarbazole (Car-3, CAS 1484-10-2) and N-butylcarbazole (Car-4, CAS 1484-08-8) were supplied by the research group of professor Peter Wasserscheid, Institute of Chemical Reaction Engineering, University of Erlangen, Germany. Tetrahydro-2-methylquinoline (Quin-2Me-pH), decahydro-2-methylquinoline (Quin-2Me-H10), dodecahydro-N-ethylcarbazole (Car-2-H12, CAS 146900-30-3), dodecahydro-N-propylcarbazole (Car-3-H12, CAS 1612790-27-8), dodecahydro-N-butylcarbazole (Car-4-H12, CAS 1612790-29-0) and mixtures of partially hydrogenated carbazoles (abbreviated in text as Car-2-pH, Car-3-pH and Car-4-pH) were prepared by catalytic hydrogenation according to the procedures described elsewhere.[19] Carbendazim (CAS 10605-21-7, Sigma Aldrich), 1-octyl-3-methylimidazolium chloride (CAS 64697-40-1, Merck GmbH), sodium chloride (Sigma Aldrich), 3,5-dichlorophenol (CAS 591-35-5, Merck GmbH), potassium dichromate (CAS 7778-50-9, Sigma Aldrich), cetyldimethylbenzylammonium chloride (C_{16}-benzalkonium chloride, CAS 122-18-9, Sigma Aldrich) and sodium benzoate (CAS 532-32-1, Sigma Aldrich) were used as positive controls in biodegradation and toxicity testing.

Solution preparation. The water/medium solubility of all tested chemicals was screened using shake-flask method.[30] The solutions of chemicals having water solubility above 100 mg L^{-1} (these were all forms of quinaldine LOHC system as well as all H_2-rich forms of the alkylcarbazoles) were prepared by directly weighing and dissolving in test medium followed by visual inspection of the solution for particles or droplets. The solutions of all remaining chemicals with water solubility lower than 100 mg L^{-1} (all H_2-lean and partially hydrogenated alkyl-carbazoles) were prepared using generator column method.[30] Briefly test substances were deposited on glass beads by vacuum evaporation from the solution in hexane. The beads were placed in generator column and medium was circulated through for 24 hours in thermostatic chamber. Such solution was used as a stock solution in tests as well as for concentration determination. The concentration of the stock solution was determined after liquid-liquid extraction.

Analytical methods.

Qualitative analysis. The HP series 6890N GC with HP 5973 MSD was used to analyse the composition of samples and to confirm the identity of the test compounds. A FS-supreme-5ms column (length = 30m, id = 0.25mm, film thickness 0.5µm) from CS Chromatographie Service, Langerwehe, Germany was used. The GC method parameters were: inlet temperature 280 °C, split-less injection of 1 µL, oven program: 100 °C hold 0.6 min, ramp 20 °C min^{-1} to 210 °C for the analysis of the quinaldine-based LOHC system and: inlet temperature 250 °C, split-less injection of 1 µL, oven program: 100 °C hold 0.6 min, ramp 20 °C min^{-1} to 150 °C no hold, ramp

35 °C min^{-1} to 300 °C hold 1 min for the analysis of the alkylcarbazole-based LOHCs. The MSD was working in electron ionisation positive ion mode, using energy of 70 eV in both cases with MS source at 230 °C and quadrupole at 150 °C. Spectrum was recorded in full scan mode. The stock solutions in hexane were injected. The identity of target LOJC compound is discussed in more detail in Supporting Information file (SI).

Quantitative analysis. The concentration in the test media was determined analytically. The aliquots of test solutions (10 mL of quinaldines and fully hydrogenated alkylcarbazoles or 40 mL of alkylcarbazoles) were spiked with the surrogate standard (quinoline for quinaldine LOHC system and phenathrene for all the other compounds) and extracted with 2 mL of dichloromethane (quinaldines) or hexane (alkyl-carbazoles). The tests solutions of fully hydrogenated compounds (quinaldines and carbazoles) were alkalised using 1M NaOH before extraction. Anhydrous sodium sulfate was added to remove water residues. The extract was transferred into a GC vial and the concentrations were determined by GC/FID (quinaldines) or GC/MS (carbazoles).

Concentration of the quinaldines was measured using GC/FID (HP 6890 series) with split-less injection of 1 μL. The same column type was used. GC method parameters: inlet temperature 250 °C, oven program 40 °C hold 0.6 min, ramp 20 °C min^{-1} to 150 °C, ramp 35 °C min^{-1} to 300 °C hold 1 min; detector temperature 320 °C. Concentration of the alkylcarbazoles was measured using the same method that was used for qualitative analysis. Limits of detection and quantification (LOD/LOQ) are presented in SI. Due to complex composition and low aqueous solubility of partially hydrogenated samples of alkylcarbazoles only the most abundant compound in the mixture was used for quantification namely Car-2-H12 in Car-2-pH samples, Car-3-H4 in Car-3-pH samples and Car-4-H4 in Car-4-pH samples.

Ecotoxicity tests.

Ecotoxicological impact of LOHC chemicals was investigated in up to six test systems with increasing complexity of the test subject starting from enzymes, through cell lines, bacteria, unicellular algae, vascular plants and invertebrates. In all tests pH was monitored and adjusted if needed to exclude pH caused toxicity. The co-solvents used were proved to be non-toxic in the concentrations used. The testing methodology is described below for each test system.

Cytotoxicity tests using IPC-81 cell line. The colorimetric WST-1 assay with IPC-81 promyelocytic leukemia rat cell line was used to investigate the influence of LOHC chemicals on cell viability. The test is based on spectrophotometric assessment of intensity of red colour caused by enzymatic reduction of WST-1 reagent (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) which is inversely proportional to cytotoxicity. The WST-1 test was carried out in RPMI medium (with L-glutamine, without $NaHCO_3$, supplemented with

1% penicillin–streptomycin and 1% glutamine, pH 7) with 10% horse serum at 37 °C in atmosphere containing 5% CO_2. The exact procedure is described in detail elsewhere[31]. The test was repeated on three different days using three independently prepared solutions. Each dose response curve was thus recorded for at least 9 parallel dilution series on three different 96-well plates. Solutions were prepared directly in medium containing 1 % (v/v) of DMSO as a co-solvent and diluted in 1:1 series with medium. Testing ranges were: 0.5 to 900 mg L^{-1} for Quin-2Me, 0.1 to 300 mg L^{-1} for Quin-2Me-pH and 0.7 to 1500 mg L^{-1} for Quin-2Me-H10. Carbendazim was used as a positive control and tested in regular intervals to ensure the validity of obtained results.

AChE inhibition. The inhibition of acetylcholinesterase (AChE) activity was measured using a colorimetric assay based on the reduction of the DTNB dye (5,5′-dithio-bis-(2-nitrobenzoic acid) by thiocholine enzymatically formed from the acetylthiocholine iodide.[32] A dilution series of the substances in phosphate buffer (20 mM, pH 8.0) was prepared directly in the microplates, DTNB (2 mM, 0.185 mg mL^{-1} $NaHCO_3$ in phosphate buffer) and the enzyme (0.2 U mL^{-1}, 0.25 mg L^{-1} bovine serum albumin in phosphate buffer) were added and the reaction started by the addition of acetylcholine iodide (2 mM in phosphate buffer). The final test concentrations were 0.5 mM each of DTNB and acetylcholine iodide and 0.05 U mL^{-1} acetylcholinesterase. Each plate contained blanks (no enzyme) and controls (no toxicant). The enzyme kinetics was measured at 405 nm at 30^{-s} intervals in a microplate-reader (MRX Dynatech) for 5 min. Enzyme activity was expressed as OD min^{-1} from the linear regression. Stock solutions of test compounds were prepared in 2 mM phosphate buffer in concentration four times higher than highest test concentration. The testing range was 0.6–1065 mg L^{-1} for Quin-2Me and Quin-2Me-pH and 0.6 to 1015 mg L^{-1} for Quin-2Me-H10. 1-Octyl-3-methylimidazolium chloride was used as a positive control.

Luminescence inhibition test with *Vibrio fischeri*. This test with the marine luminescent bacterium *Vibrio fischeri* was performed according to DIN EN ISO 11348-2.65. The freeze-dried bacteria were purchased from Dr Lange GmbH (Düsseldorf, Germany). Each substance was tested three times, using independently prepared solutions, with two replicates at each concentration level and accompanied by at least 4 controls (2% NaCl solution in phosphate buffer). All solutions were prepared directly in phosphate-buffered (0.02 M, pH 7.0, including 2% sodium chloride). The tests were performed at 15 °C using thermostats (LUMIStherm, Dr Lange GmbH, Düsseldorf, Germany). The freeze-dried bacteria were rehydrated according to the producer's test protocol, and then 500 µL aliquots of the bacteria solution were pre-incubated for 15 min at 15 °C. After measuring the initial luminescence, 500 µL of the samples were added. The bioluminescence was measured again after an incubation time of 30 min using a luminometer (LUMIStox 300, Dr Lange GmbH, Düsseldorf, Germany). The toxicity of the samples was expressed as a percentage inhibition compared to the controls. Luminescent bacteria assays were conducted with addition of 1% methanol (v/v) as co-solvent. The range of

concentrations tested covered 0.03 to 240 mg L^{-1} for Quin-2Me, 0.015 to 110 mg L^{-1} for Quin-2Me-pH and 0.035 to 285 mg L^{-1} for Quin-2Me-H10. Sodium chloride (7.5 g L^{-1}) was used as a positive control.

Immobilisation test 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 72 h-old, were placed in 10 mL of mineral medium (controls) or solution of test substances in mineral medium. The number of immobilized (dead) organisms was checked after 24 and 48 h. The toxicity of the test compound was expressed as percentage of not affected organisms compared to the controls. All substances were tested in three independent experiments (five concentrations, five replicates) in the following test ranges: Quin-2Me 0.3 to 200 mg L^{-1}, Quin-2Me-pH 0.2 to 200 mg L^{-1}, Quin-2Me-H10 0.32 to 500 mg L^{-1}. Potassium dichromate was used as positive control and tested in regular intervals to ensure the validity of the results.

Growth inhibition test with *Lemna minor*. Test was conducted according to OECD 221 guideline.[33] The plants were grown in Erlenmeyer flasks in sterilised Steinberg medium (pH 5.5 ± 0.2), in a climate chamber with a constant temperature of 24 ± 2 °C illuminated continuously with a maximum of 125 µE m^{-2} s^{-1}. The assays were performed in plastic six-well plates incubated in the same conditions for seven days. All substances were tested three times, on different days using independently prepared solutions, with three replicates at each concentration level and a minimum of six controls (pure Steinberg medium) for each test. The test was started with one plant having three fronds in each sample. The endpoint was the inhibition of growth rate based on frond area calculated in relation to the controls. The frond area (mm^2) was determined using Scanalyzer software from Lemnatec GmbH (Aachen, Germany). The Steinberg medium contained: 3.46 mM KNO_3, 1.25 mM $Ca(NO_3)_2$, 0.66 mM KH_2PO_4, 0.072 mM K_2HPO_4, 0.41 mM $MgSO_4$, 1.94 µM H_3BO_3, 0.63 µM $ZnSO_4$, 0.18 µM Na_2MoO_4, 0.91 µM $MnCl_2$, 2.81 µM $FeCl_3$, 4.03 µM EDTA; pH 5.5 ± 0.2. Testing range was 3.5 mg L^{-1} to 2.42 g L^{-1} for Quin-2Me, 3.4 mg L^{-1} to 1.29 g L^{-1} for Quin-2Me-pH and 1 mg L^{-1} to 2 g L^{-1} for Quin-2Me-H10. C_{16}-benzalkonium chloride was used as a positive control and tested in regular intervals to ensure the validity of the results.

Growth inhibition test with *Raphidocelis subcapitata*. The tests were performed following the OECD 201 guideline,[34] using slightly modified light conditions (light : dark cycle of 14:10 h instead of a continuous illumination). Cell culture flasks (Nunc EASY flasks, 25 cm^2, obtained from VWR, Hannover, Germany) were filled with 20 mL of test solution containing medium and test substance (test solutions) or only medium (controls). The test setup was validated using 3,5-dichlorophenol as the reference chemical. Algae in exponential growth phase were exposed to Quin-2Me, Quin-2Me-pH, or Quin-2Me-H10 in a testing range of 0.5–100 mg L^{-1} prepared in the OECD 201 medium (pH = 8.1 ± 0.2). Algae cell counts at the test beginning of the

test were set to 2.5×10^4 cells mL^{-1}. Samples were placed on orbital shakers at 150 rpm for 72 h in a climate chamber (Thermostatschrank ET 618-4/135, Sanyo) at 22 ± 1 °C with a light:dark cycle of 14:10 h. Cell counts after 72 h were recorded by a cell counting chamber (Neubauer-improved, depth 0.100 mm, 0.0025 mm^2) and light microscope (Zeiss, Germany). Eight concentration levels were tested in three replicates with six controls for each test and each test was performed 2 times. The growth rate of algae at the end of test was expressed as percentage of cell counts compared to the controls (absence of test compound). 3,5-dichlorophenol was used as a positive control and tested in regular intervals to ensure the validity of obtained results.

Amest test for mutagenicity. The test solutions were prepared by dissolving the test compound in dimethylsulfoxide in highest possible concentration using shake flask method (OECD 105).[30] (Remark: The description of the test procedure in detail has not yet been provided by the co-author and will be added before submission).

Ultimate biodegradation test. Ultimate biodegradation was measured by manometric respirometry method according to OECD guideline 301F using automated, thermostatically controlled OxiTop© set (from WTW GmbH, Weilheim, Germany).[35] 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 4–7 days prior to use. Test lasted at least 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 O_2 L^{-1}. Two replicates were run for each compound accompanied by two blanks and two positive controls (benzoic acid).

Data processing and analysis. 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). LemnaTec software was used for plant phenotyping in test with *Lemna minor*. Marvin software was used for drawing, displaying and characterizing chemical structures, Marvin 6.3.1, 2014, available from ChemAxon (http://www.chemaxon.com).

Results and Discussion

Purity of test compounds.

Some samples of LOHC chemicals were products of hydrogenation, and the exact composition, especially of partially hydrogenated samples, was unknown. Therefore, we have analysed them using GC/MS technique to confirm their identity and establish purity (see Table 3.4.3). All H_2-lean and H_2-rich samples were single compounds (as shown in Table 3.4.2). Additionally, partially hydrogenated sample of quinaldine-based LOHC system contained only tetrahydroquinaldine (Table 3.4.2).

On the contrary, samples of partially hydrogenated alkylcarbazoles were mixtures of several different compounds (see Table 3.4.2 and 3.4.3 and SI for more details). For the quantification of partially hydrogenated samples the most abundant compound was used i.e., Car-2-H12 for Car-2-pH, Car-3-H4 for Car-3-pH and Car-4-H4 for Car-4-pH. Therefore, concentrations in Table 3.4.4 and Table **3.4.5** refer to the concentrations of this compound in partially hydrogenated mixtures.

Table 3.4.3. Composition of the partially hydrogenated alkylcarbazoles.

Acronym	**Composition including name (abbreviation) and amount** [%][a]
Car-2-pH	70% Dodecahydroethylcarbazole (Car-2-H12)
	13% Tetrahydroethylcarbazole (Car-2-H4)
Car-3-pH	54% Tetrahydropropylcarbazole (Car-3-H4)
	11% Propylcarbazole (Car-3)
	7 % Dodecahydrpropylcarbazole (Car-3-H12)
	7% Octahydroproplycarbazole (Car-3-H8)
	5% Hexahydropropylcarbazole (Car-3-H6)
Car-4-pH	54% Tetrahydrobutylcarbazole (Car-4-H4)
	37% Butylcarbazole (Car-4)

[a] Calculated based on the GC/MS analysis, as a percent area under the peak for a given component of the mixture in relation to summed areas of all components observed.

Cytotoxicity, AChE inhibition and mutagenicity

The summary of the results of cytotoxicity, acetylcholinesterase inhibition and mutagenicity tests – expressed as half-maximal effective/inhibitory concentrations (EC_{50}/IC_{50}) – is given in Table 3.4.4.

The cytotoxicity tests using cell lines measure the so called basal cytotoxicity, i.e., the toxicity towards common functions and structures of cells. The magnitude of cytotoxic effects is often similar when tested in different cell lines, using different endpoints and exposure times, sometimes even across species.[36] The EC_{50} values for all tested compounds in this test varied by four orders of magnitude and were within 0.78–842 mg L^{-1} (Table 3.4.4). In the quinaldine-based LOHC system, the cytotoxicity towards the IPC-81 cells follows the trend: Quin-2Me ≥ Quin-2Me-pH > Quin-2Me-H10, yet the differences in EC_{50} values are rather small (Table 3.4.4). The exact EC_{50} for Quin-2Me-pH could not be fitted since only approximately 50% effect was achieved at the highest tested concentration therefore the EC_{50} was reported as approximately 480 mg L^{-1}. The alkylcarbazoles showed generally larger spread in cytotoxicity between the three forms and were two to three orders of magnitude more toxic than the quinaldines. Among the alkylcarbazoles the highest EC_{50} (lowest cytotoxicity) was found for perhydrogenated forms where EC_{50} values were within 59–72 mg L^{-1}. All these values can be considered moderate when compared to a biocide carbendazim (EC_{50} 10 mg L^{-1}) used as positive control or common organic

solvent ethanol (EC_{50} 32 g L^{-1}) on the other side of the toxicity spectrum.[37]

Table 3.4.4. Results of IPC-81 cell line cytotoxicity, acetylcholinesterase (AChE) inhibition and mutagenicity (*Salmonella typhimurium*) test, EC_{50}/IC_{50} with 2.5% and 97.5 % confidence intervals in the brackets are given in mg L^{-1}. Same values in µmol L^{-1} are given in SI Table S3.4.2. (Remark: The specific data for the test with *Salmonella thypimurium* has not yet been provided by the co-author and will be added before submission).

Compound	**IPC-81**	**AChE**
	EC_{50} and IC_{50} [mg L^{-1}] (confidence interval)	
Quin-2Me	313 (292–328)	61 (54–67)
Quin-2Me-pH	≥ 480 (n.d.)	≥ 147 (n.d.)
Quin-2Me-H10	842 (734–990)	90 (80–104)
Car-2	> 0.92[a]	n.d.
Car-2-pH	9.1[b] (8.3–10.0)	n.d.
Car-2-H12	60 (45–81)	n.d.
Car-3	> 0.24[a]	n.d.
Car-3-pH	0.78[c] (0.68–0.87)	n.d.
Car-3-H12	72 (62–85)	n.d.
Car-4	> 0.44[a]	n.d.
Car-4-pH	0.85[d] (0.78–0.94)	n.d.
Car-4-H12	59 (53–68)	n.d.
Positive control	10.0 (7.3–14.2)[e]	9.8 (9.2–10.5)[f]

[a] Averaged maximum solubility in test medium. [b] Quantified as Car-2-H12 (Table 3.4.2 and 3.4.3). [c] Quantified as Car-3-H4 (Table 3.4.2 and 3.4.3). [d] Quantified as Car-4-H4 (Table 3.4.2 and 3.4.3). [e] Carbendazim. [f] Octylmethylimidazolium chloride.

The partially hydrogenated alkylcarbazoles were one to two orders of magnitude more cytotoxic than their perhydrogenated counterparts and showed the following trend of toxicity Car-3-pH ≈ Car-4-pH > Car-2-pH with EC_{50} between 0.78 and 9.1 mg L^{-1}. The partially hydrogenated propyl- and butyl-substituted species turned out to be more toxic than the positive control, indicating generally high cytotoxicity. The

EC_{50} for Car-2, Car-3, and Car-4 were not obtained since no effect at the highest tested concentration was observed due to very low medium solubility of these compounds.

The enzyme AChE catalyses the hydrolysis of a neurotransmitter, acetylcholine, in the synaptic cleft of the nervous system. The structure of AChE is highly conservative among species; therefore AChE inhibition is often used in screening for neurotoxic potential of chemicals.[38] The inhibitory potential of the quinaldines towards AChE was low ranging from 61 to approximately 147 mg L^{-1} especially when compared to the positive control 1-octyl-3-methylimidazolium chloride (IC_{50} = 9.8 mg L^{-1}) or a known inhibitor of that enzyme – carbamate insecticide aldicarb (IC_{50} = 0.93 mg L^{-1}).[38] Again only approximate IC_{50} was obtained for Quin-2Me-pH (IC_{50} ~ 147 mg L^{-1}). The inhibitory action of quinaldines puts them in the following sequence of toxicity: Quin-2Me > Quin-2Me-H10 > Quin-2Me-pH.

(Remark: The following paragraph has not yet been commented and modified by the co-authors who have performed these tests).

The *in vitro* bacterial mutagenicity test, so called Ames test, is often used as a first stage of screening to mutagenic potential of chemicals. From the regulatory perspective, a positive result of Ames test triggers further testing yet not necessarily indicates mutagenicity or carcinogenicity *in vivo*. We have not observed significant mutagenicity in the Ames test using *Salmonella typhimurium* strains TA100 and TA98 with and without metabolic activation for any of the members of investigated LOHC systems. Nevertheless, more extensive testing including other *Salmonella* strains would be necessary. Eisentraeger et al.[39] and Nagao et al.[40] found that quinoline and several of its monomethylated derivatives were mutagenic with *Salmonella typhimurium* strains TA100 and TA98 but only after metabolic activation (2-methylquinoline was not tested). Debnath observed the mutagenic activity of quinoline and various substituted quinolines after S9 mix activation only in strains TA98.[24] The 2-methylquinoline however was shown to be not mutagenic in *Salmonella typhimurium*[41–43] but mutagenic in *Salmonella enterica subsp. enterica* (umu test).[44] It was also not toxic against the *Salmonella* bacterium up to the concentrations of 5 g L^{-1}.[43] 2-methylquinoline was only weakly mutagenic under this conditions.[24]

Aquatic ecotoxicity

Aquatic organisms at different trophic levels including the luminescent marine bacterium, limnic green algae, aquatic plant and crustacean were used for investigating potential adverse effects towards aquatic organisms and effect concentrations were determined (Table 3.4.5).

The luminescence inhibition with *Vibrio fischeri* is a fast, cost-effective frequently

used test thanks to which a large amount of data for comparison is available. It is also the representative marine organism within our test battery. The EC_{50} values towards *V. fischeri* were one to two orders of magnitude lower for Quin-2Me and Quin-2Me-pH as compared to cytotoxicity and AChE inhibition tests. Additionally, there is a bigger variation between particular members of the LOHC system with more than one order of magnitude differences in their inhibitory activity. The potency order of Quin-2Me-pH > Quin-2Me >> Quin-2Me-H10 follows the order of log D values (Table 3.4.2). Not more than 40% luminescence inhibition was recorded for Quin-2Me-H10 at the highest attainable concentration therefore the EC_{50} was reported as higher than this concentration. Among the alkylcarbazoles, the EC_{50} values were calculated only for Car-2-pH and Car-3-pH and were within the same order of magnitude as for Quin-2Me-pH. Among all the other members of the alkylcarbazoles-based LOHC systems the H_2-lean forms had very low solubility in the test medium (below 1 mg L^{-1}) and H_2-rich forms did not show any activity even at high concentrations (up to 431–457 mg L^{-1}). Therefore, the EC_{50} values were reported as higher than the highest concentrations soluble in test media.

Table 3.4.5. Results of acute aquatic ecotoxicity test with marine bacterium (*Vibrio fischeri*), green algae (*Raphidocelis subcapitata*), duckweed (*Lemna minor*) and water fleas (*Daphnia magna*). EC_{50} with 2.5% and 97.5 % confidence intervals in brackets are given in mg L^{-1}. Same values in µmol L^{-1} are given in SI Table S3.4.3.

Compound	***Vibrio fischeri***	***Raphidocelis subcapitata***	***Lemna minor***	***Daphnia magna***
	EC_{50} values [mg L^{-1}] (confidence interval)			
Quin-2Me	19 (16–22)	43 (-)	42 (36–48)	56 (54–59)
Quin-2Me-pH	7.4 (6.2–8.9)	17 (14–20)	51 (46–59)	2.7 (2. –3.2)
Quin-2Me-H10	> 306	52 (50–55)	~1000	155 (120–191)
Car-2	> 0.36[a]	n.d.	> 0.65[a]	> 0.38[a]
Car-2-pH	2.5[b] (2.0–2.82)	n.d.	n.d.	n.d.
Car-2-H12	> 431[a]	n.d.	258 (254–263)	60 (50–76)
Car-3	> 0.06[a]	n.d.	> 0.40[a]	> 0.36[a]
Car-3-pH	4.3[c] (3.8–4.8)	n.d.	n.d.	n.d.
Car-3-H12	> 440[a]	n.d.	> 240	10.2 (8.3–12.6)
Car-4	> 0.03[a]	n.d.	> 0.063[a]	> 0.16[a]
Car-4-pH	> 2.9[a,d]	n.d.	n.d.	n.d.

Compound	*Vibrio fischeri*	*Raphidocelis subcapitata*	*Lemna minor*	*Daphnia magna*
	EC_{50} values [mg L^{-1}] (confidence interval)			
Car-4-H12	> 457[a]	n.d.	112 (n.d.)	9.6 (7.7–11.8)
Reference compound	See below[e]	1.6[f] (1.3–1.9)	6.6[g] (4.5–10.2)	1.1[h] (0.96–1.17)
Diesel oil	-	22–78[i]	-	13–210[i]

[a] Averaged maximum solubility in test medium. [b] Quantified as Car-2-H12 (Table 3.4.2 and 3.4.3). [c] Quantified as Car-3-H4 (Table 3.4.2 and 3.4.3). [d] Quantified as Car-4-H4 (Table 3.4.2 and 3.4.3). [e] 7.5% (w/w) NaCl (luminescence inhibition between 40 and 60% is expected). [f] 3,5-dichlorophenol. [g] Benzalkonium chloride. [h] Potassium dichromate. [i] American Petroleum Institute (API) for automotive diesel oil CAS 68334-30-5[45]. n.d. – not determined.

The green algae are primary producers and therefore are very important link in the food chains in aquatic environments. Together with crustaceans, they are usually the most sensitive species in our test battery and in general. For that reason, these organisms are often used for regulatory purposes. The *Raphidocelis supcapitata* showed in this case sensitivity comparable with the other four organisms comprising the test battery. The EC_{50} values obtained in this test for quinaldines put them in the following order of toxic potency Quin-2Me-pH > Quin-2Me > Quin-2Me-H10 which again mirrors their log D (Table 3.4.3). The EC_{50} values indicate moderate toxicity in comparison to the positive control. Similar EC_{50} values were found for structural analogues of Quin-2Me quinoline, missing a methyl group, (EC_{50} = 60.9 mg L^{-1}) and 6-methylquinoline, differing in the position of methyl group, (EC_{50} = 33.2 mg L^{-1}) towards green algae *Desmodesmus subcapitatus*.[39]

Lemna minor (duckweed) is a vascular aquatic plant. It was included in the test battery due to the relatively long duration (7 days) which can be treated as semi-chronic test. For Quin-2Me and Quin-2Me-pH the EC_{50} of 42 mg L^{-1} and 51 mg L^{-1} respectively were observed. H_2-rich form was significantly less toxic than the other two with a much higher EC_{50} values of approximately 1000 mg L^{-1}. None of the H_2-lean forms of alkylcarbazoles elicited any significant growth inhibition in the test with *L. minor* yet the test concentrations were very low due to poor water solubility of these compounds. The Car-2-H12 had a relatively high EC_{50} value of 258 mg L^{-1}. The exact EC_{50} of Car-3-H12 was not determined but approximately 50% growth rate inhibition was observed at the highest tested concentration of 240 mg L^{-1}. Despite the fact that the butyl-homologue had lower medium solubility the full range of effects was observed and the EC_{50} was determined to be 112 mg L^{-1}. This puts the compounds in the following order of toxicities Car-4-H12 > Car-3-H12 > Car-2-H12 mirroring the hydrophobicity (log D).

There were clear differences in the toxicity towards the water flea (*Daphnia magna*) for the three quinaldines. The EC_{50} of Quin-2Me was 56 mg L^{-1} so within the same

order of magnitude as for all the other organisms within aquatic test battery (Table 3.4.5) and somewhat higher than measured by Eisentraeger et al. for structurally similar quinoline and 6-methylquinoline (the with EC_{50} values equal 14.7 mg L^{-1} and 8.6 mg L^{-1} respectively).[39] The perhydrogented form turned out to be an order of magnitude less toxic (EC_{50} = 155 mg L^{-1}). *D. magna* seems to be particularly sensitive to the partially hydrogenated quinaldine, as was *V. fischeri*, with an EC_{50} of only 2.7 mg L^{-1}. Perhydro-alkylcarbazoles were moderately to considerably toxic towards water flea with toxicity increasing with the length of the alkyl substituent and EC_{50} values between 9.6 and 60 mg L^{-1} which follows the log D values (Table 3.4.3). No effects were observed for H_2-lean molecules due to their limited solubility in the test medium.

Range of toxicity and preliminary insight into the mechanism of toxic action

The cytotoxicity of the quinaldines was rather low showing that the IPC-81 cell line was generally less sensitive than the aquatic organisms. The fully or partially hydrogenated alkylcarbazoles showed up to three orders of magnitude higher cytotoxicity than any of the quinaldines. The partially hydrogenated samples turned out to be particularly toxic with EC_{50} values close to or below 1 mg L^{-1}.

The following trends in toxicity were observed in the test battery. In the aquatic ecotoxicity tests for the quinaldine-based LOHC system, the partially hydrogenated sample was usually the most toxic. The *V. fischeri* and *D. magna* seem to be particularly sensitive to this compound with the EC_{50} of only 4.7 and 2.7 mg L^{-1} respectively. Based on the *V. fischeri* test the partially hydrogenated samples were also the most toxic forms of alkylcarbazoles-based LOHC systems which additionally showed highest toxicity within the entire test set. The H_2-rich species were usually the least toxic. The perhydrogenated alkylcarbazoles were more toxic than perhydro-quinaldine (based on *D. magna* and *L. minor* tests). The H_2-lean species of alkylcarbazoles-based LOHC systems where very poorly soluble in test media and did not elicit any observable effects in any of the tests. Testing of partially hydrogenated samples shows that the hazard might be higher than expected from testing of pure H_2-rich and H_2-lean LOHC chemicals. This is especially important for alkylcarbazoles where it is probable that partially hydrogenated species will be intentionally used. In such case probably more detailed tests are needed to resolve the toxicity of each particular partially hydrogenated form.

The order of the toxicity generally follows the order of hydrophobicity suggesting rather baseline toxicity than specific mode of toxic action. All LOHC chemicals tested here are organic bases. The H_2-rich species have predicted dissociation constants (pk_b) above pH 10 and are therefore positively charged in all test media. As the result the log D (which takes ionisation into account) is significantly lower than log K_{ow} (which only considers the neutral species). Consequently, the H_2-rich LOHC compounds are the least hydrophobic and were therefore least toxic. In case of quinaldines the partially hydrogenated species has the highest hydrophobicity which

is also in line with highest toxicity of this compound (*ref. to the manuscript in subchapter 3.2*). The situation is more complicated in case of the partially hydrogenated samples of alkylcarbazoles which are actually mixtures of several compounds at different levels of hydrogenation often including (H_2-rich and -lean species). Thanks to the method of solution preparation, each of these forms is theoretically expected to be present at its maximum water solubility at the beginning of the test. The combined action of such a mixture elicits stronger effects than single H_2-lean and H_2-rich compounds.

Inhibition of the AChE was the only test system which showed different trend in toxic potency of quinaldines which does not follow the order of hydrophobicity as indicated by log D (Table 3.4.2). The Quin-2Me-H10, being considerably less toxic in most of the aquatic tests, showed higher AChE inhibition potential than Quin-2Me-pH, which was in turn usually most toxic. This can be explained by the specific interaction of quaternary nitrogen in the structure of Quin-2Me-H10 with the active site of AChE. The potent AChE inhibitors often possess positively charged nitrogen in their structure which allows them to interact more with the active site of the AChE. This was shown for quinolinium cations possessing a positive charge, which were inhibiting AChE much more than it could be explained based solely on their lipophilicity.[38] Even though Quin-2Me-H10 is less hydrophobic its pk_b value is much higher than that of the other two forms (Table 3.4.2). Under test pH it carries a positive charge on the nitrogen whereas the two other forms are uncharged.

No toxicity up to the solubility limit is stated when there is a lack of observable toxic effect for poorly water-soluble compounds (like in the case of H_2-lean alkylcarbazoles) according to the so called "solubility cut-off". This concept is based on the belief that highly hydrophobic compounds do not dissolve well enough to cause toxic effects.[46] It was shown however, that even very hydrophobic substances can exert aquatic toxicity and they definitely can contribute to the toxicity when present in mixtures.[47] Different issues often stand behind the "solubility cut-off" including kinetic aspects (too short duration of standard aquatic tests), fast excretion or metabolisation of test chemicals or poorly controlled exposure.

Due to high hydrophobicity of H_2-lean and partially hydrogenated alkylcarbazoles (see Table 3.4.2) we were expecting that the exposure concentration might decrease especially in the longer test i.e., IPC-81, *Daphnia magna* (both 48h long) and *Lemna minor* (7 days). In the latter two test systems the volume of the test solution was large enough to measure the concentration of the H_2-lean compounds at the end of the test would it remain within +/-20%. Nevertheless, in all *L. minor* tests the concentration at the end of the test fell below the limit of quantification (below 1% of initial concentration). The losses were lower in *Daphnia magna* test, probably as a combined effect of shorter duration, lower temperature and lack of illumination, yet still remained above 40%. Unstable exposure, i.e., test concentration changing by more than 20 %, precludes making meaningful quantitative conclusions since the effect or the lack of thereof cannot be clearly assigned to the starting concentration. Yet the confirmation of the exposure stability is not always performed or is sometimes

impossible due to analytic limitations leading to underestimation of toxicity. In such cases, strategies that limit the fluctuations of exposure (e.g., use of passive dosing or medium renewal) should be used to confirm the result. These are however time consuming and were outside of scope of our preliminary assessment. As a result, the lack of toxic effects observed for H_2-lean forms of alkylcarbazoles cannot be treated as an explicit proof of inherent lack of toxicity. In the assessment for regulatory purposes however, such results are used due to lack of data. As an example, Car-2 was assigned to chronic aquatic toxicity category 2 within REACH (European Union, the Registration, Evaluation, Authorisation and Restriction of Chemicals regulation) based on an aquatic exposure fish test conducted in the concentration range of 1–500 mg L^{-1} even though it is not soluble in such high concentrations.*

Biodegradability

Biodegradability of all three forms of quinaldines as well as H_2-rich and H_2-lean alkylcarbazoles was tested according to manometric respirometry procedure.[35] Only Quin-2Me was biodegradable in this test system (Figure 3.4.2). Neither of the remaining chemicals caused any oxygen consumption in the manometric system and therefore no degradation was measured (results not shown). In four out of six replicates, Quin-2Me actually fulfilled the criterion of ready degradability (at least 60% degradation within 10 days window).[35] In those cases a lag phase of four to nine days is followed by rapid degradation. One of the replicates did not show any appreciable degradation even though the validity criteria were fulfilled.[35] These differences are a natural consequence of variability in composition of microbial inoculum. Nevertheless, the extent of degradation was generally high confirming that Quin-2Me is degradable.

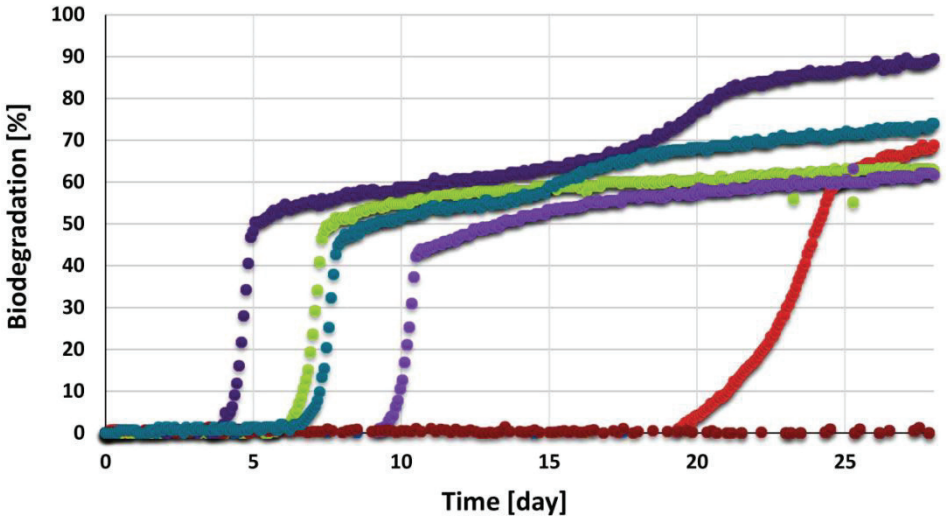


Figure 3.4.2. Ultimate biodegradation of Quin-2Me, ten replicates tested in five independent tests

* According to ECHA, details are available from http://echa.europe.eu/.

are shown in different colours.

The degradation of methylquinolines under aerobic conditions is much faster than degradation under denitrifying conditions, which often does not occur or proceeds slower and is incomplete.[48,49] The degradation pathway of methylquinolines under aerobic conditions often begins with hydroxylation of carbon atom adjacent to nitrogen.[50] This is however not possible in case of 2-methylquinoline (quinaldine) studied here since this position is occupied by the methyl group.[48,51] In this case hydroxylation in position 4 usually occurs leaving 2-methyl-4(1H)quinolinone.[49,52,53] Direct oxidation of the methyl group was also reported by Dembek et al. leading to the formation of quinaldinic acid and further oxidation.[54] After initial hydroxylation further degradation usually proceeds through the dioxygenolytic cleavage of benzene ring[55] even though an interesting pathway that proceeds through cleavage of pyridine ring and release of carbon monoxide molecule was observed.[52,53] These results combined with our findings suggest that Quin-2Me will be degraded rather quickly and to high extent. Interestingly tetrahydro-2-methylquinoline (Quin-2Me-pH) – one of our compounds of interest – was detected as one of main degradation products of 2-methylquinoline. Quin-2Me-pH was initially accumulated in the degradation batch but was also later degraded to some extent suggesting higher resistance to degradation but not persistence.[49] The fact that the conditions used in our work were far less conducive than in the test of Wang (lower biomass, lack of adaptation or active aeration) allows us to suspect that Quin-2Me-pH is indeed not recalcitrant even if it did not show any appreciable degradability in our tests. Cyclic alkanes like Quin-2Me-H10 are usually more resistant towards biodegradation than their linear equivalents but the presence of alkyl substituent on cycloalkane ring facilitates biodegradation.[56] It was also shown that cycloalkanes can be co-metabolised i.e., degraded in the presence of other compounds serving as sources of energy due to the low substrate specificity of enzymes responsible for degradation.[57–59] Therefore, degradability of partially or fully hydrogenated equivalents will be slower and further research under more favourable conditions is necessary to exclude possibility of environmental persistence.

We found no data for degradation of any of the members of alkylcarbazole LOHC systems in standard biodegradation tests. A simpler homologue carbazole is rather recalcitrant but can be degraded by adapted microbial species/communities.[60]

Preliminary hazard assessment

To put the results presented here in a bigger picture we have made a comparison of environmental hazard assessment of the four LOHC systems with diesel oil using thresholds of Globally Harmonised System for Labelling of Chemicals (GHS) as a guideline (Table 3.4.6). The GHS system is a widely adopted yet voluntary system developed to assure clear communication of hazard associated with usage of chemicals. Additionally, many countries have their own regulatory authorities that have the power to restrict usage of chemicals if the hazard associated with them is

deemed unacceptable. In European Union, the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation lays out the rules of hazard management. The GHS entails three categories of acute aquatic toxicity. Substances are assigned to categories based on EC_{50} values obtained in acute test with green algae and/or aquatic plants (often *Lemna minor*) and/or crustacean and/or fish. The ranges for each category are shown at the bottom of Table 3.4.6 with their respective colour code. If data on more than one organism are available, the most sensitive one is used to assign the compound to the category. For the cases where ecotoxicity was lower than the threshold for category 'acute 3' we have marked it in green as not requiring labelling. In the absence of chronic toxicity data the REACH legislation set a screening threshold on toxicity towards aquatic organisms (including algae, crustaceans and fish) at $EC_{50} \leq 0.1$ mg L^{-1} to identify toxic substances.

Table 3.4.6. A colour-coded simulation of hazard classification of LOHC systems and diesel oil including toxicity (T) and persistence (P).

Compound	Hazard			
	T	Rationale	P	Rationale *
Quin-2Me	acute 3	EC_{50} (*L. minor*) = 42 mg L^{-1}	not P	biodegradability > 60%
Quin-2Me-pH	acute 2	EC_{50} (*D. magna*) = 2.7 mg L^{-1}	P?	biodegradability ~ 0%
Quin-2Me-H10	acute 3	EC_{50} (*R. subcapitata*) = 52 mg L^{-1}	P?	biodegradability ~ 0%
Car-2		Not enough data	P?	biodegradability ~ 0%
Car-2-pH		Not enough data		Not enough data
Car-2-H12	acute 3	EC_{50} (*D. magna*) = 60 mg L^{-1}	P?	biodegradability ~ 0%
Car-3		Not enough data	P?	biodegradability ~ 0%
Car-3-pH		Not enough data		Not enough data
Car-3-H12	acute 3	EC_{50} (*D. magna*) = 10.2 mg L^{-1}	P?	biodegradability ~ 0%
Car-4		Not enough data	P?	biodegradability ~ 0%
Car-4-pH		Not enough data		Not enough data
Car-4-H12	acute 2	EC_{50} (*D. magna*) = 9.6 mg L^{-1}	P?	biodegradability ~ 0%
Diesel oil	acute 2	EC_{50} (*D. magna*) = 13 mg L^{-1}	not P	biodegradability > 60%
Colour code				
T (aquatic toxicity)	acute 1 EC_{50} < 1 mg L^{-1}	acute 2 EC_{50} 1–10 mg L^{-1}	acute 3 EC_{50} 10–100 mg L^{-1}	not labelled EC_{50} > 100 mg L^{-1}
P (persistence)	not biodegradable (persistent?)	not readily biodegradable	readily biodegradable	

* Biodegradability tested according to OECD 301 ready biodegradability procedure.

For the LOHCs tested here, *Daphnia magna* is often the most sensitive species (Table 3.4.6). Using the GHS thresholds most of the LOHC chemicals fall into 'acute 2' or 'acute 3' category. The Quin-2Me-pH is close to the threshold of the GHS highest aquatic toxicity category ('acute 1'). Unlike in the case of alkylcarbazoles, the Quin-2Me-pH is not a deliberately introduced component of the LOHC system but could be perceived as an impurity that can be present in rather low amount as a result of unintentional incomplete dehydrogenation. Moreover, based on our results, none of the LOHC chemicals would need to be labelled toxic according to REACH screening

criteria though some members, especially of alkylcarbazole, LOHC systems would probably need to be tested in more detail. For all H_2-lean forms of alkylcarbazoles no EC_{50} was observed in aquatic test systems yet due to considerable differences between nominal and real exposure concentration we have decided that the amount of data is not sufficient to assign them to any T category. Quin-2Me was shown to be biodegradable when tested according to ready biodegradability OECD 301F test procedure. Other LOHC chemicals did not show appreciable levels of biodegradation therefore their persistence cannot be excluded. They were marked as potentially persistent and require more testing for definite classification. Among the LOHC chemicals for which we gathered enough data to make the assessment only the Quin-2Me-pH and Car-4-H12 showed toxicity comparable to diesel oil, whereas H_2-lean and H_2-rich quinaldine as well as H_2-rich ethyl- and butylcarbazoles showed lower toxicity. In terms of biodegradability only Quin-2Me was readily biodegradable as was diesel oil. All other LOHC chemicals seem to be less degradable and potentially persistent.

Comparison with fossil fuels

To be considered greener alterative in terms of environmental hazard the LOHC systems should be at least not worse than the fossil fuels they are meant to replace. Surprisingly little scientific data are available regarding environmental toxicity of fossil fuels probably due to their complex variable composition and poor water solubility, making testing difficult. The EC_{50} (48h) of automotive diesel oil towards *Daphnia magna* and *Raphidocelis subcapitata* were reported to be within 13–210 mg L^{-1} and 22–78 mg L^{-1} based on water accommodated fraction (Table 3.4.5)[45]. The water accommodated fraction (WAF) is often used in testing poorly water soluble compounds especially mixtures of unknown composition. WAF is obtained for water or medium at equilibrium with certain amount (loading) of poorly soluble substance and thus it not necessarily represents the truly dissolved amount. The toxicity of quinaldine LOHC system towards green algae and crustaceans is comparable to that of diesel oil apart from partially hydrogenated form. The EC_{50} values of perhydro-propyl and especially perhydro-butylcarbazole towards *Daphnia magna* are considerably lower than the EC_{50} of diesel oil, meaning higher toxicity of these LOHC systems. In regard to potential persistence fossil fuels are generally biodegradable to high extent[61] and seem to be better than LOHCs.

Apart from environmental hazard, there are also physicochemical hazards associated with chemicals related to their e.g., explosive, oxidising, flammable etc. properties. All LOHC discussed here are less volatile than diesel oil therefore posing lesser danger of fire or explosion. Since LOHC chemicals have less complex composition much lower batch-to-batch variability in composition is expected than in case of diesel fuel. This also means that uncertainty of the evaluation is lower. On the other hand, the fact that LOHC chemicals are recycled brings up the issue of carrier degradation.

Conclusions

Within the suggested proactive, comparative environmental impact assessment of LOHC systems a preliminary evaluation of four potential LOHC systems was made. Low to moderate (eco)toxicity was observed for LOHC system based on quinaldine. High biodegradability (> 60%) was observed for Quin-2Me, whereas no significant degradation occurred for Quin-2Me-pH and Quin-2Me-H10. This was accompanied by low cytotoxicity, moderate AChE inhibition and lack of mutagenicity for all quinaldines tested. No EC_{50} value was observed in any of the test systems for H_2-lean forms of alkylcarbazoles prohibiting hazard classification and indicating the need for more advanced testing. The H_2-rich forms showed moderate ecotoxicity that was generally higher than quinaldines. High cytotoxicity was observed for partially hydrogenated alkylcarbazoles with the toxic effect increasing with the chain length. The perhydrogenated alkylcarbazoles showed moderate cytotoxicity which was again higher than in the case of quinaldines. None of tested alkylcarbazoles was biodegradable. The lack of observable biodegradation does not necessarily mean that these LOHC chemicals are persistent but that additional tests under less stringent conditions than the ready biodegradability test, e.g., with higher density of (pre-adapted) microbial inoculum or addition of another source of carbon, are needed. Nevertheless, the degradation timeframe will most definitely be longer than for Quin-2Me or diesel oil.

Based on results presented above, quinaldine-based LOHC system can be considered not worse than currently available energy system based on fossil fuels in terms of (eco)toxicity. Nevertheless, the fact that H_2-rich form did not undergo biodegradation raises concerns of potential persistence, which is a rather significant drawback. The LOHC systems based on alkylcarbazoles are generally more toxic and less biodegradable. Additionally considerable hydrophobicity of H_2-lean and partially hydrogenated forms of alkylcarbazoles (log D 3.6–4.8) indicates they might be bioaccumulative. Undeniable benefits come from the fact that LOHC energy systems operate on renewable energies (even though they can be implemented also using conventional energy sources). Additionally, some of the physicochemical properties of LOHCs are more favourable in the context of safety of handling and transportation e.g., higher boiling points mean less loss due to evaporation, lower flammability and inhalatory exposure to vapours. Based on results gathered here the quinaldine-based LOHC system seems to exhibit environmental hazard to some degree higher that e.g., automotive diesel oil on account of potential persistence of H_2-rich form. Alkylcarbazole based LOHC systems tested here pose higher hazard that both quinaldine-based LOHC system and diesel oil due to resistance to biodegradation of all forms and considerable (eco)toxicity of especially partially hydrogenated forms.

Acknowledgements

The authors thank Ms Ulrike Bottin-Weber and Ms Alica Rother for conducting the

ecotoxicity tests and GC analysis. We would also like to acknowledge the financial support of Universität Bremen and the European Union FP7 COFUND within Marie Curie Actions BremenTrac Program (grant agreement No.600411) and M8 Postoc-Initiatve PLUS, funded by the German Excellence Initiative.

References

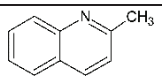
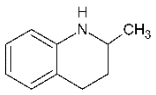
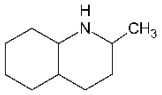
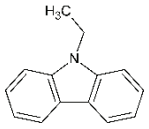
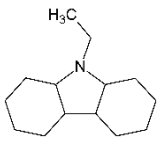
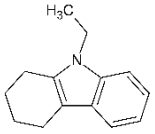
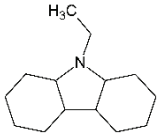
(1) M. S. Dresselhaus and I. L. Thomas, *Nature*, 2001, **414**.
(2) European Comission, *A Roadmap for moving to a competitive low carbon economy in 2050*, 2011.
(3) S. Satyapal, J. Petrovic, C. Read, G. Thomas and G. Ordaz, *Catal. Today*, 2007, **120**, 246–256.
(4) S. Dutta, *J. Ind. Eng. Chem.*, 2014, **20**, 1148–1156.
(5) A. Züttel, A. Remhof, A. Borgschulte and O. Friedrichs, *Philos. Trans. A. Math. Phys. Eng. Sci.*, 2010, **368**, 3329–42.
(6) M. Markiewicz, Y. Q. Zhang, A. Bösmann, N. Brückner, J. Thöming, P. Wasserscheid and S. Stolte, *Energy Environ. Sci.*, 2015, **8**, 1035–1045.
(7) A. A. Ismail and D. W. Bahnemann, *Sol. Energy Mater. Sol. Cells*, 2014, **128**, 85–101.
(8) D. J. Durbin and C. Malardier-Jugroot, *Int. J. Hydrogen Energy*, 2013, **38**, 14595–14617.
(9) U. Eberle, M. Felderhoff and F. Schüth, *Angew. Chemie Int. Ed.*, 2009, **48**, 6608–6630.
(10) L. Schlapbach and A. Züttel, *Nature*, 2001, **414**, 353–358.
(11) A. C. Cooper, K. M. Campbell and G. P. Pez, *16th World Hydrog. Energy Conf.*, 2006, 3.
(12) Air Products and Chemicals Inc., 2006, US 7101530.
(13) D. Teichmann, W. Arlt, P. Wasserscheid and R. Freymann, *Energy Environmnetal Sci.*, 2011, **4**, 2767–2773.
(14) K. Stark, P. Keil, S. Schug, K. Müller, P. Wasserscheid and W. Arlt, *J. Chem. Eng. Data*, 2016, **61**, 1441–1448.
(15) C. Papp, P. Wasserscheid, J. Libuda and H. P. Steinrück, *Chem. Rec.*, 2014, **14**, 879–896.
(16) N. Brückner, K. Obesser, A. Bösmann, D. Teichmann, W. Arlt, J. Dungs and P. Wasserscheid, *ChemSusChem*, 2013, **7**, 229–235.
(17) T. He, Q. Pei and P. Chen, *J. Energy Chem.*, 2015, **24**, 587–594.
(18) S. Mekhilef, R. Saidur and A. Safari, *Renew. Sustain. Energy Rev.*, 2012, **16**, 981–989.
(19) N. T. Brückner, *PhD thesis*, 2015, 206.
(20) D. Teichmann, K. Stark, K. Müller, G. Zöttl, P. Wasserscheid and W. Arlt, *Energy Environ. Sci.*, 2012, **5**, 9044–9054.
(21) P. Preuster, C. Papp and P. Wasserscheid, *Acc. Chem. Res.*, 2017, **50**, 74–85.
(22) BP, *BP Statistical Review of World Energy About this review Contents*, 2016.
(23) P. T. Anastas and J. C. Warner, *Green Chemistry, Theory and Practice* , Oxford University Press, 2000.
(24) A. K. Debnath, R. L. Lopez de Compadre and C. Hansch, *Mutat. Res. Toxicol.*, 1992, **280**, 55–65.
(25) A. Albert, R. Goldacre and J. Phillips, *J. Chem. Soc.*, 1948, 2240.
(26) *European Chemical Agency, registration dossier (ethylcarbazole), http://echa.europa.eu/*, .
(27) G. Collin and H. Höke, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH

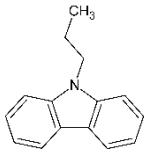
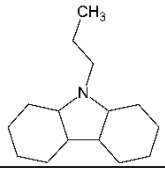
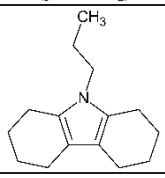
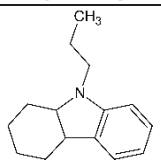
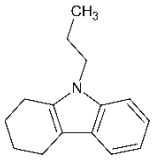
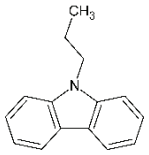
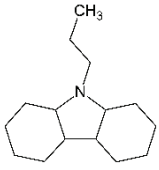
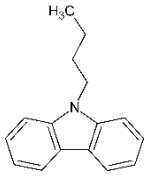
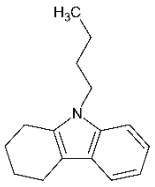
Verlag GmbH & Co. KGaA, Weinheim, Germany, 2000.
(28) *Globally Harmonized System of Classification and Labelling of Chemicals (GHS): Fourth Revised Edition*, 2011.
(29) R. Pahlman and O. Pelkonen, *Carcinogenesis*, 1987, **8**, 773–778.
(30) OECD, *Guidelines for testing of chemicals 105: Water solubility*, 1995.
(31) J. Ranke, K. Mölter, F. Stock, U. Bottin-Weber, J. Poczobutt, J. Hoffmann, B. Ondruschka, J. Filser and B. Jastorff, *Ecotoxicol. Environ. Saf.*, 2004, **58**, 396–404.
(32) F. Stock, J. Hoffmann, J. Ranke, R. Störmann, B. Ondruschka and B. Jastorff, *Green Chem.*, 2004, **6**, 286–290.
(33) OECD, *Guidelines for the Testing of Chemicals 221: Lemna sp. Growth Inhabition Test*, .
(34) OECD, *Guidelines for the Testing of Chemicals, 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test*, .
(35) OECD, *Guidelines for testing of chemicals 301: Ready Biodegradability*, 1992.
(36) C. Clemedson and B. Ekwall, *Toxicol. Vitr.*, 1999, **13**, 657–663.
(37) S. Stolte, J. Arning, U. Bottin-Weber, A. Müller, W.-R. Pitner, U. Welz-Biermann, B. Jastorff and J. Ranke, *Green Chem.*, 2007, **9**, 760–767.
(38) J. Arning, S. Stolte, A. Böschen, F. Stock, W.-R. Pitner, U. Welz-Biermann, B. Jastorff and J. Ranke, *Green Chem.*, 2008, **10**, 47.
(39) A. Eisentraeger, C. Brinkmann, H. Hollert, A. Sagner, A. Tiehm and J. Neuwoehner, *Environ. Technol. Chem.*, 2008, **27**, 1590–1596.
(40) M. Nagao, T. Yahagi, Y. Seino, T. Sugimura and N. Ito, *Mutat. Res. Mol. Mech. Mutagen.*, 1977, **42**, 335–341.
(41) D. A. Kaden, R. A. Hites and W. G. Thilly, *CANCER Res.*, 1979, **39**, 4152–4159.
(42) E. J. La Voie, J. Defauw, M. Fealy, B. M. Way and C. A. McQueen, *Carcinogenesis*, 1991, **12**, 217–220.
(43) P. Bowden, K.-T. Chung and A. W. Andrews, *J. Natl. Cancer Inst.*, 1976, **57**, 921–924.
(44) C. Brinkmann and A. Eisentraeger, *Environ. Sci. Pollut. Res. Int.*, 2008, **15**, 211–217.
(45) The American Petroleum Institute Petroleum HPV Testing Group, 2012, 1–152.
(46) H. Könemann, *Toxicology*, 1981, **19**, 209–221.
(47) P. Mayer and F. Reichenberg, *Environ. Toxicol. Chem.*, 2006, **25**, 2639–2644.
(48) J. Aislabie, A. K. Bej, H. Hurst, S. Rothenburger and R. M. Atlas, *Appl. Environ. Microbiol.*, 1990, **56**, 345–51.
(49) L. Wang, Y. Li and D. Yang, *Process Biochem.*, 2010, **45**, 919–928.
(50) J.-P. Kaiser, Y. Feng and J.-M. Bollag, *Microbilogical Rev.*, 1996, **60**, 483–498.
(51) S. S. Johansen, D. Licht, E. Arvin, H. Mosbæk and A. B. Hansen, *Appl. Microbiol. Biotechnol.*, 1997, **47**, 292–300.
(52) H.-K. Hund, A. de Beyer and F. Lingens, *Biol. Chem. Hoppe. Seyler.*, 1990, **371**, 1005–1008.
(53) I. Bauer, A. de Beyer, B. Tshisuaka, S. Fetzner and F. Lingens, *FEMS Microbiol. Lett.*, 1994, **117**, 299–304.
(54) G. Dembek, T. Rommel, F. Lingens and H. Höke, *FEBS Lett.*, 1989, **246**, 113–116.
(55) S. Fetzner, *Appl. Microbiol. Biotechnol.*, 1998, **49**, 237–250.
(56) W. Fritsche and H. Martin, in *Biotechnology, Environmnetalprocesses II*, eds. H.-J. Rehm and G. Reed, Wiley-VCH Verlag GmbH, Weinheim, Germany, 2000, vol. 14, pp. 146–164.

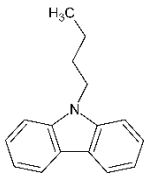
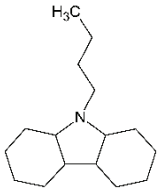
(57) S. H. Ko and J. M. Lebeault, *J. Appl. Microbiol.*, 1999, **87**, 72–79.
(58) J. J. Perry, *Microbiol. Rev.*, 1979, **43**, (59-72).
(59) H. W. Beam and J. J. Perry, *Arch. Mikrobiol.*, 1973, **91**, 87–90.
(60) C. Zhao, Y. Zhang, X. Li, D. Wen and X. Tang, *J. Hazard. Mater.*, 2011, **190**, 253–259.
(61) R. Marchal, S. Penet, F. Solano-Serena and J. P. Vandecasteele, *Oil Gas Sci. Technol. – Rev. IFP*, 2003, **58**, 441–448.

‡ Supporting Information

Table S3.4.1. Details of the analytical methods for the quantification of LOHC chemicals using GC/MS.

Abbreviation of LOHC	Rt [min]	LOD/LOQ [mg L^{-1}]	Structure	m/z*
Quin-2Me	4.02	0.08/0.23		**143**
Quin-2Me-pH	4.32	0.08/0.24		**147**
Quin-2Me-H10	3.14	0.32/0.96		**153**
Car-2	5.85	0.04/0.11		180, **195**
Car-2-pH	4.58 (69.8%)	0.50/1.49		**164,** 192, 207
	5.81 (12.13%)	-		171, **199**
Car-2-H12	4.58	0.13/0.39		**164,** 192, 207

Abbreviation of LOHC	**Rt** [min]	**LOD/LOQ** [mg L^{-1}]	**Structure**	**m/z***
Car-3	6.09	0.04/0.13		180, **209**
Car-3-pH	4.92 (6.56 %)	-		**178,** 192, 221
	5.74 (6.79%)	-		188, **217**
	5.81 (5.24%)	-		171, 186, 199, **215**
	6.06 (54.23%)	0.66/1.97		**184,** 213
	6.09 (11.02%)	-		**180,** 209
Car-3-H12	4.92	0.76/2.27		178, **192**, 221
Car-4	6.38	0.04/0.12		180, **223**
Car-4-pH	6.32 (53.6%)	0.54/1.63		**184,** 227

Abbreviation of LOHC	Rt [min]	LOD/LOQ [mg L^{-1}]	Structure	m/z*
Car-4-pH	6.38 (37.3%)	-		**180,** 223
Car-4-H12	5.28	0.70/2.09		**192,** 235
PHE (internal standard)	5.65	0.34/1.01	-	178

* Base ion (the most abundant ion in the spectrum) is marked in bold, structures are structures of corresponding molecular ions.

Table S3.4.2. Results of IPC-81 cell line cytotoxicity, acetylcholinesterase (AChE) inhibition and mutagenicity (*Salmonella typhimurium*) test, EC_{50} in µmol L^{-1} and mg L^{-1} with 2.5% and 97.5 % confidence intervals are given. (Remark: The specific data for the test with *Salmonella thypimurium* has not yet been provided by the co-author and will be added before submission).

Compound	IPC-81		AChE	
	µmol L^{-1}	mg L^{-1}	µmol L^{-1}	mg L^{-1}
Quin-2Me	2188 (2042–2291)	313 (292–328)	427 (380–468)	61 (54–67)
Quin-2Me-pH	≥3300 (n.d.)	≥ 480 (n.d.)	≥ 1000 (n.d.)	≥ 147 (n.d.)
Quin-2Me-H10	5495 (4786–6457)	842 (734–990)	589 (525–676)	90 (80–104)
Car-2	> 4.7[a]	> 0.92[a]	n.d.	n.d.
Car-2-pH	n.a.	9.1[b] (8.3–10.0)	n.d.	n.d.
Car-2-H12	291 (215–392)	60 (45–81)	n.d.	n.d.
Car-3	> 1.14[a]	> 0.24[a]	n.d.	n.d.
Car-3-pH	n.a.	0.78[c] (0.68–0.87)	n.d.	n.d.
Car-3-H12	327 (280–385)	72 (62–85)	n.d.	n.d.
Car-4	> 1.97[a]	> 0.44[a]	n.d.	n.d.
Car-4-pH	n.a.	0.85[d] (0.78 – 0.94)	n.d.	n.d.

Compound	IPC-81		AChE	
	µmol L^{-1}	mg L^{-1}	µmol L^{-1}	mg L^{-1}
Car-4-H12	251 (223–288)	59 (53–68)	n.d.	n.d.
Positive control	53 (38–74)[e]	10.0 (7.3–14.2)[e]	43 (40-46)[f]	9.8 (9.2–10.5)[f]

[a] Averaged maximum solubility in test medium. [b] Quantified as Car-2-H12 (Table 3.4.2). [c] Quantified as Car-3-H4 (Table 3.4.2). [d] Quantified as Car-4-H4 (Table 3.4.2). [e] Carbendazim. [f] Octylmethylimidazolium chloride.

Table S3.4.3. Results of acute aquatic ecotoxicity test with green algae (*Raphidocelis subcapitata*), duckweed (*Lemna minor*) and water flea (*Daphnia magna*). EC_{50} in µmol L^{-1} and mg L^{-1} with 2.5% and 97.5 % confidence intervals are given.

Compound	*Vibrio fischeri*		*Raphidocelis subcapitata*		*Lemna minor*		*Daphnia magna*	
	µmol L^{-1}	mg L^{-1}	µmol L^{-1}	mg L^{-1}	µmol L^{-1}	mg L^{-1}	µmol L^{-1}	mg L^{-1}
Quin-2Me	133 (113–156)	19 (16–22)	301 (-)	43 (-)	291 (254–334)	42 (36–48)	393 (375–411)	56 (54–59)
Quin-2Me-pH	50 (42–61)	7.4 (6.2–8.9)	118 (98–137)	17 (14–20)	348 (303–400)	51 (46–59)	18 (16–22)	2.7 (2.3–3.2)
Quin-2Me-H10	> 1998	> 306	339 (323–355)	52 (50–55)	~6524	~1000	1011 (784–1243)	155 (120–191)
Car-2	> 1.8[a]	> 0.36[a]	n.d.	n.d.	> 3.33 [a]	> 0.65[a]	> 1.96 [a]	> 0.38[a]
Car-2-pH	n.a.	2.5[b] (2.09–2.82)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Car-2-H12	> 2079[a]	> 431[a]	n.d.	n.d.	1242 (1225–1266)	258 (254–263)	291 (242–366)	60 (50–76)
Car-3	> 0.29[a]	> 0.06[a]	n.d.	n.d.	> 1.89 [a]	> 0.40[a]	> 1.72 [a]	> 0.36[a]
Car-3-pH	n.a.	4.3[c] (3.8–4.8)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Car-3-H12	> 1987	> 440[a]	n.d.	n.d.	> 1084	> 240	46 (38–57)	10.2 (8.3–12.6)
Car-4	> 0.15[a]	> 0.03[a]	n.d.	n.d.	> 0.56 [a]	> 0.063[a]	> 0.72[a]	> 0.16[a]

Compound	*Vibrio fischeri*		*Raphidocelis subcapitata*		*Lemna minor*		*Daphnia magna*	
	µmol L^{-1}	mg L^{-1}	µmol L^{-1}	mg L^{-1}	µmol L^{-1}	mg L^{-1}	µmol L^{-1}	mg L^{-1}
Car-4-pH	n.a.	> 2.9[a,d]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Car-4-H12	> 1945[a]	> 457[a]	n.d.	n.d.	478 (n.d.)	112 (n.d.)	41 (32–50)	9.6 (7.7–11.8)
Reference compound	See below[e]	See below[e]	9.9[f] (7.9–11.8)	1.6[f] (1.3–1.9)	17[g] (12–26)[c]	6.6[g] (4.5–10.2)	3.6[h] (3.2–4.0)	1.1[h] (0.96–1.17)
Diesel oil	-	-	-	22–78[i]	-	-	-	13–210[i]

[a] Averaged maximum solubility in test medium. [b] Quantified as Car-2-H12 (Table 3.4.2). [c] Quantified as Car-3-H4 (Table 3.4.2). [d] Quantified as Car-4-H4 (Table 3.4.2). [e] 7.5% (w/w) NaCl (luminescence inhibition between 40 and 60% is expected). [f] 3,5-dichlorophenol. [g] Benzalkonium chloride. [h] Potassium dichromate. [i] American Petroleum Institute (API) for automotive diesel oil CAS 68334-30-5[45]. n.d. – not determined. n.a. – not applicable, the value can not be calculated because substance is a mixture.

3.5. Manuscript 5

In the Appendix there is one published paper can be found. This paper is not in the main part of the thesis since it is not about LOHCs but nanopariticles. However, the overall theme “bioavailablity” and proactive hazard assessment of novel chemicals is also topic of the paper.

Published: **Zhang, Y.-Q.**, Dringen, R., Petters, C., Rastedt, W., Köser, J., Filser, J. and Stolte, S. (2016) ‘**Toxicity of dimercaptosuccinate-coated and un-functionalized magnetic iron oxide nanoparticles towards aquatic organisms**’, *Environ. Sci.: Nano*. Royal Society of Chemistry, 3(4), pp. 754–767. doi: 10.1039/C5EN00222B.

Impact factor: 6.1 (2016 Journal Citation Reports® (Clarivate Analytics, June 2017))

The article can be downloaded from:

http://pubs.rsc.org/-/content/articlehtml/2016/en/c5en00222b

Further details about this paper and the contributions of the authors are given in the **Appendix A.3**.

Chapter-IV Summarized Discussion

4 Summarized Discussion

In *subchapters 3.1 to 3.4*, the adsorption properties of LOHCs (in terms of the K_{oc}, K_d and leaching capacity), ecotoxicity in the soil and aquatic scenarios (including the estimation of bioavailability and mode of toxic action), and biodegradation were investigated. The following sections synthesize and discuss the information generated from these studies by addressing the hypotheses targeting to establish a preliminary environmental hazard assessment of LOHCs.

Due to environmental parameter variations (e.g., the content of organic matter and pH) and the different physicochemical properties of LOHCs, such as the molecular size, water solubility, hydrophobicity, and acid dissociation capacity, LOHC compounds of similar or even identical structures may behave differently and eventually lead to distinct adsorption, bioavailability and toxicity in soil and aquatic scenarios.

4.1 Adsorption behavior of LOHCs

Several basic physicochemical parameters were considered to be highly relevant for interpreting the results regarding the adsorption and mobility of LOHCs in this thesis. These parameters are the molecular weight (MW), Henry's law constant, boiling point (bpt), water solubility (S_w), acid dissociation constant (pK_a, conjugated acids), K_{ow}, log D, K_{oc} and K_d. Molecular descriptors are useful for the predictions of the behavior of PAHs (Kuśmierz *et al.*, 2016). Detailed descriptions of these parameters for each LOHC chemical can be found in **Tables 3 and 4** as well as in *subchapters 3.2–3.4*.

First, the comparatively low Henry's law constant (2.0×10^{-6}–1.0×10^{-4} atm-m^3 $mole^{-1}$, for e.g., quinaldines and carbazole derivatives) and high bpt (generally in a range of 200–400 °C) of the LOHC structures presented in the thesis increased the stability of the compounds in the test conditions (e.g., ambient temperature and pressure). Evaporation could be comparatively less relevant to the loss of these compounds during testing. For example, the similarity of the bpt values (225–257 °C) and the low Henry's law constants (2.13×10^{-6}–3.12×10^{-5} atm-m^3 $mole^{-1}$) of the three forms of the quinaldines supported the assumption that the differences in the recovered concentrations of these chemicals in the ecotoxicity tests, adsorption batch equilibrium experiments and soil column leaching tests were contributed to the distinct adsorption capacities between these compounds.

The K_{oc} and K_d (K_{oc}-derived and batch-test based) of the LOHCs are shown in

Table 3 and discussed in the following sections.

Table 3. Summarized carbon-water partition coefficient (K_{oc}, log) and soil-water partition coefficient (K_d, mL g^{-1}) of LOHCs.

	Indoles		Quinaldines			Carbazole derivatives	
LOHCs	**Indoline**	**Indole**	**Quin-2Me**	**Quin-2Me-pH**	**Quin-2Me-H10**	**Car-2**	**Car-2-pH**
Log K_{oc} [a]	1.71	2.36	2.19	2.36	3.25[b]	4.27	4.31
K_d	0.63[c]	2.76[c]	2.03 ± 0.12[d]	6.57 ± 0.39[d]	2.42 ± 0.43[d]	226.3[c]	247.5[c]
	Carbazole derivatives				**MLHs**		**MSHs**
LOHC	**Car-3**	**Car-3-pH**	**Car-4**	**Car-4-pH**	**MLH**	**MLH-pH**	**MSH**
Log K_{oc} [a]	4.61	4.63	5.01	5.01	3.85	5.41	5.38
K_d	497.8[c]	517.5[c]	1228.4[c]	1247.1[c]	86.0[c]	3089.0[c]	2911.2[c]

[a] Measured by HPLC screening (*subchapter 3.2: Table 3.2.2*).

[b] Estimated by Conductor like Screening Model for Realistic Solvation (COSMO-RS).

[c] Calculated by using the empirical formula $K_d = K_{oc} \times OC\%$, where OC% equals 0.0121 (organic carbon of the test soil I)

[d] Measured K_d (± SD) by adsorption batch equilibrium experiment.

4.1.1 Carbon-water partition coefficient (K_{oc})

The K_{oc} is often used as an indicator to describe the hydrophobic association of an organic contaminant with organic matter in adsorption processes. In this thesis, LOHCs were first evaluated in a screening check via software supported estimations. This approach was expected to be useful for providing an initial indication of the potential adsorption, and it was considered helpful for an initial ranking of the affinities of these compounds. The Estimation Programs Interface (EPI) Suite™ and Conductor like Screening Model for Realistic Solvation (COSMO-RS) were used for these preliminary simulations and predictions. The EPI Suite is easily available and commonly used for predictions of the physicochemical properties of chemicals and related characterizations in the environment. The model KOCWIN™ in the EPI Suite operates based on an extensive experimental dataset and derived Quantitative Structure–Activity Relationship (QSAR) equations using the K_{oc} values. Estimations of the chemical properties using COSMO-RS are based on the theory of quantum chemistry (quantum-based generation of charge density surfaces is used to predict the interactions between molecules), thermodynamics, and Quantitative Structure–Property Relationships (QSPR) derivation[*] (see *SI section S3.2.2 in subchapters 3.2*).

[*] COSMO*logic*: http://www.cosmologic.de/.

COSMO-RS has been used for the prediction of partitioning chemicals between water and organic phases, such as biological membranes (Klamt *et al.*, 2008), lipids (Geisler *et al.*, 2015) and organic solvents (Klamt *et al.*, 2016). The log K_{oc} values predicted by the two computational methods (**Table 2**; and *subchapter 3.2: Table 3.2.1*) were similar (R^2 = 0.85, p < 0.001). The predicted K_{oc} values were also useful for estimating the ranking of the potential retention times of LOHCs for the HPLC screening.

An instrumental analysis on the basis of chromatography provided more specific values. The analytical column of the HPLC system (Agilent Zorbax CN for normal-phase) was composed of dual phases*, including a non-polar fraction and a polar moiety. The two segments allow the LOHCs to engage in similar interactions as those observed with organic matter in soils (OECD, 2001). Consequently, these sections reflect adsorption processes, such as the diffusion of these chemicals into organic matter via hydrophobic forces. The resultant log K_{oc} values of the LOHC systems (**Table 3**; and *subchapter 3.2: Table 3.2.2*) were generally increased in the order of indoles < quinaldines < carbazole derivatives < MLHs < MSH. The HPLC-based log K_{oc} values were moderately well regressed to the EPI-based log K_{oc} (log $K_{oc,\,HPLC}$ = 1.0109 log $K_{oc,\,EPI}$ + 0.2678, R^2 = 0.85). However, an even higher correlation and higher determination coefficient were found between these values and the COSMO-RS-based K_{oc} (log $K_{oc,\,HPLC}$ = 1.6355 log $K_{oc,\,COSMO\text{-}RS}$ - 1.2887, R^2 = 0.92). Compared with the EPI Suite, the organic chemical properties predicted based on COSMO-RS enables the characterization of more specific structures (*subchapter 3.2: Table 3.2.1 and Figure S3.2.5*). These findings imply that both software are applicable for a preliminary prediction of the adsorption of LOHC structures in terms of K_{oc}. Therefore, these simulations can be applied to other new LOHC structures.

In addition, K_{oc} is usually treated as a function of the hydrophobicity (K_{ow}) of organic compounds (Weissenfels, Klewer and Langhoff, 1992). The HPLC-based log K_{oc} values in this thesis basically followed the order of log K_{ow} values as hypothesized (hypothesis I). However, indole, which was expected to have the lowest K_{oc}, demonstrated a higher value than both Quin-2Me and Quin-2Me-pH. Nevertheless, the measured log K_{oc} of the tested LOHCs were generally quantitatively in good regression to the COSMO-RS-based log K_{ow} values (log $K_{oc,\,HPLC}$ = 0.8339 log $K_{ow,\,COSMO\text{-}RS}$ + 0.2061, R^2 = 0.94, n = 13) (*subchapter 3.2: Figure 3.2.5*). Because K_{ow} values are typically easily available, this correlation may indicate the possibility of performing K_{ow}-based K_{oc} predictions for LOHCs. Moreover, high HPLC-based log K_{oc} values were found for the carbazole derivatives, MLHs and MSH, which are all chemicals with very low water solubility (*subchapter 3.2: Table 3.2.1*). Therefore, water solubility (S_w) was involved in controlling the affinity of LOHCs for organic matter. A quantitative analysis of the relationship between the HPLC-based log K_{oc} of the LOHCs and their log S_w (log $K_{oc,\,HPLC}$ =

* Non-polar fraction was primarily composed of Zorbax SIL particles (porous silica), and polar moiety consisted of cyanopropylsilane groups. The polar moiety was chemically bonded to the silica particles to establish a monolayer coating.

-0.7054 log $S_{w,\,COSMO\text{-}RS}$ + 4.7834, R^2 = 0.92, n = 13) was conducted, and the results showed that a lower S_w was correlated to a higher K_{oc}, which is consistent with hypothesis (hypothesis I). In addition, a high correlation was observed between the measured log K_{oc} values and the molecular weight (MW) of the LOHCs (log $K_{oc,\,HPLC}$ = 0.0261 MW - 1.099, R^2 = 0.93, n = 12), which is also consistent with hypothesis I. High coefficients of determination were obtained for the two relationships (except for with MLH-pH), which suggests that the two parameters are correlated with the K_{oc} values. This finding is also supported by a previous study in which an increased affinity of organic chemicals for soil organic matter was associated with the increment of MW according to a multilinear regression model that was established to predict the log K_{oc} of PAHs (Schüürmann, Ebert and Kühne, 2006). Hence, the MW and S_w can also be reasonably considered in a preliminary prediction for the K_{oc} and the adsorption potential (Revitt, Balogh and Jones, 2014) (Kuśmierz *et al.*, 2016). As a result, the K_{oc} values can be used to estimate the mobility of these LOHC structures. The ranking of mobility of the LOHCs according to McCall's soil mobility scale (McCall *et al.*, 1980) were assigned to three categories: "highly mobile" (indoline), "moderately mobile" (Quin-2Me, Quin-2Me-pH and indole) and "non-mobile" (Car-2, Car-2-pH, Car-3, Car-3-pH, Car-4, Car-4-pH, MLH, MLH-pH, and MSH) (*subchapter 3.2: Table 3.2.2*).

To investigate the adsorption and mobility potentials of the LOHCs, HPLC screening was performed because it provides a comparatively simple and fast method for ranking. However, the K_{oc} of the fully hydrogenated forms of the LOHCs cannot be determined via HPLC (unless equipped with mass spectrometry) because the absence of aromatic structures in these chemicals makes the undetectable via UV (ultra violet). Moreover, the hydrophobicity-dominant adsorption that was assumed in this method appeared inadequate for explaining the adsorption potential of ionizable LOHC structures, which was also observed in a further investigation of adsorption by using the quinaldine-based LOHC system as an example for a cross check. The K_d and leaching capacity obtained in the adsorption batch equilibrium experiment and the soil column leaching test, respectively, did not always correspond to the K_{oc} values (*subchapter 3.2: Table 3.2.3*). Instead of a slight difference of adsorption based on the measured and predicted log K_{oc} values, significant differences were found in the K_d values and the leaching capacities of the quinaldines in the soils (*subchapter 3.2: Tables 3.2.2 and 3.2.3*).

4.1.2 Acid dissociation constant (pK_a) and ionization corrected octanol-water partition coefficient (log D)

The discrepancy between the measured K_{oc} and K_d or the leaching capacity described in the previous section may be partly explained by LOHC systems that are consisting of alkaline compounds. These compounds present at different pH levels in either neutral or ionized forms could lead to adsorption by different mechanisms. Therefore, the pK_a (conjugated acid) and the log D of the LOHCs, as well as the

environmental pH must be considered before further interpretation of the K_d and leaching capacity.

Overall, alkaline compounds could be present primarily as non-ionized forms at pH values higher than their pK_a values (conjugated acids) and as cations (at the N atoms in LOHCs) at pH values lower than their pK_a values. This highlights the influence of the pK_a, log D, and pH values of the test conditions when evaluating the adsorption behavior of ionizable LOHCs. **Table 4** lists the log D, pK_a, and the degree of protonation (shown as %) of the LOHC structures (predicted by MarvinSketch 14.10.6.0[*]) in the test soils or media where the toxic effects of the quinaldines and carbazole derivatives on organisms were found. The pH values considered here ranged from 5 to 9, which represented the scope of the pH of both the test soil/media and a typical natural environment.

Table 4. pK_a values of the conjugated acid of LOHCs and the degree of protonation in different test soil/media of different pH values. "P." signifies protonation; and "n.a." indicates unavailable data due to no ionizable forms are found in the estimations by MarvinSketch.

LOHCs	Log D at pH 5–9	pK_a	P.[%] pH 5.0[a]	P.[%] pH 5.3/5.4[b]	P.[%] pH 5.5[c]	P.[%] pH 6.7[d]	P.[%] pH 7.0[e]	P.[%] pH 8.1[f]	P.[%] pH 9.0[g]
Quinaldines									
Quin-2Me	1.89–2.26	5.15	58.4	39.6/35.9	30.8	2.7	1.4	0.1	0.01
Quin-2Me-pH	2.10–2.35	4.88	43.0	26.1/22.7	19.3	1.5	0.75	0.06	0.01
Quin-2Me-H10	-0.88–0.61	10.75	100.0	100.0	100.0	100.0	100.0	99.8	98.3
Indoles									
Indole	2.07	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Indoline	1.34–1.49	4.61	29.1	16.1/13.8	11.5	0.81	0.41	0.03	0.0
Carbazole derivatives									
Car-2	3.67	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Car-2-pH	4.07	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	3.58–3.76	4.71	34.1	19.5/16.8	14.1	1.02	0.52	0.04	0.01
Car-2-H12	0.02–0.9	11.69	100.0	100.0	100.0	100.0	100.0	100.0	99.8
Car-3	4.19	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Car-3-pH	4.59	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	4.04–4.29	4.89	43.7	26.6/23.2	19.7	1.52	0.77	0.06	0.01

[*] MarvinSketch: https://www.chemaxon.com/products/marvin/marvinsketch/.

LOHCs	Log D at pH 5–9	pK_a	P. [%] pH 5.0[a]	P. [%] pH 5.3/5.4[b]	P. [%] pH 5.5[c]	P. [%] pH 6.7[d]	P. [%] pH 7.0[e]	P. [%] pH 8.1[f]	P. [%] pH 9.0[g]
Carbazole derivatives									
Car-3-H12	0.54–1.19	11.97	100.0	100.0	100.0	100.0	100.0	100.0	99.9
Car-4	4.64	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Car-4-pH	5.03	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	4.48–4.73	4.9	44.3	27.1/23.6	20.1	1.56	0.79	0.06	0.01
Car-4-H12	0.99–1.54	12.09	100.0	100.0	100.0	100.0	100.0	100.0	99.9
MLHs									
MLH	4.58	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MLH-pH	4.82	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MLH-H12	5.29	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MSHs									
MSH	6.67	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MSH-pH	7.15	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MSH-H18	7.62	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

[a] Pore water: filtered deionized water.

[b] Test soil I and II: RefeSol 01-A-004*.

[c] *Lemna* medium: Steinberg (OECD, 2006).

[d] *Vibrio* medium: DIN EN ISO 11348-2.65.

[e] *Daphnia* medium: ISO 6341 (OECD, 2004a).

[f] Algal medium: OECD TG 201 (OECD, 2011).

[g] Expected high threshold of the pH commonly occurring in nature.

Except for the MLH- and MSH-based LOHC systems, where the components appear to not ionize, all the other LOHC systems have different levels of protonation in their components, particularly with the fully hydrogenated forms and the quinaldine-based system. The overall trend of the partitioning (log D) is ordered as follows: indoles ≤ quinaldines < carbazole derivatives (-2, -3, -4) < MLHs < MSHs. The log D values between the paired H_2-lean, H_2-rich, and partially hydrogenated forms differed over the pH range in the general order of H_2-rich form < H_2-lean form < partially hydrogenated form (except for MLHs and MSHs). Correspondingly, potential variances in the adsorption and mobility between the LOHC forms are expected, which suggests that log D must be considered when describing the hydrophobicity and adsorption potential of ionizable LOHC structures.

* Specific information including the components and textures of the two test soils can be found in the supporting information *SI section S3.2.1 in subchapter 3.2*.

4.1.3 Soil-water partition coefficient (K_d) and leaching capacity

The quinaldine-based LOHC system was used to investigate the adsorption potential in terms of the K_d and leaching capacity by the adsorption batch equilibrium and soil column leaching experiment, respectively. Considering the ionizable potentials of the quinaldines (**Table 4**), the differences in the K_d values and the leaching potentials between these chemicals are discussed while considering the log D and pH effect.

The three components of the quinaldine-based LOHC system were predicted to be ionizable at different levels in the pH range of interest according to the environmental conditions (pH 5–9, **Table 4**). The protonation potentials of the quinaldines over the pH values were Quin-2Me-pH < Quin-2Me << Quin-2Me-H10 (almost 100% protonation) (**Table 4**). The lower the potential of being protonated corresponded to a higher partition coefficient (log D); thus, hydrophobicity is likely. This result was well correlated with the findings in the batch equilibrium isotherms, where Quin-2Me-pH had a higher batch-K_d (batch test-based) than did Quin-2Me, which was consistent with hypothesis II. On the other hand, ionic interactions (binding to the negative sites in soils, e.g., organic matter and minerals) (Thomsen *et al.*, 1999) rather than hydrophobicity may dominate (Chiou, McGroddy and Kile, 1998) (Bi, Schmidt and Haderlein, 2007) in the adsorption process of Quin-2Me-H10 in the test soils via charge-charge interactions. Ultimately, these interactions led to comparable adsorption capacities of Quin-2Me-H10 with Quin-2Me (no significant difference with p = 0.77, as shown in *subchapter 3.2*) because the latter adsorption was more likely to be driven by stronger hydrophobic forces (**Table 3**). This observation deviated from hypothesis II, in which Quin-2Me-H10 was anticipated to have a higher batch-K_d than Quin-2Me, because the influence of the ionization potential on octanol-water partitioning had not been considered in this hypothesis.

Further investigation of the adsorption of the quinaldines shifted from the static adsorption equilibrium system (batch test) to a dynamic process of soil column leaching. With less equilibration with the soils, lower soil/water ratios (Markiewicz, Jungnickel, *et al.*, 2015) and soil organic conent, as well as exchange with other ions (e.g., Ca^{2+}) (Stepnowski, Mrozik and Nichthauser, 2007) (i.e., conditions closer to that in nature), the adsorption of the quinaldines in the soil was not as efficient as in a static system, which resulted in K_d values (column-K_d) that were significantly lower than that obtained in the batch test (batch-K_d, as shown in *subchapter 3.2: Table 3.2.3*). Similar to the findings in the batch test, the adsorption and mobility governed by the log D with Quin-2Me-pH appeared to have the highest retention within the soil. This finding was consistent with hypothesis III, which posited that a higher affinity is correlated with lower mobility. Therefore, Quin-2Me-pH may have less potential to move more deeply into the soil layers to reach the groundwater than other analogs. The fully hydrogenated form, however, had a drastically low extrapolated column-K_d, which suggested that this chemical had the highest mobility through the soil layers (*subchapter 3.2: Figure 3.2.4*). This finding was inconsistent with hypothesis III, in which this chemical was expected to have low mobility. Faster

movement of this chemical through the soil layers than expected could be the result of reduced charge-charge interactions due to the competition of the constant input of Ca^{2+} (provided in the artificial rain) (Bi, Schmidt and Haderlein, 2007) for the negative-charged sites within the soil. Quin-2Me-H10 may thus be considered to spread faster and deeper into the soil layers than Quin-2Me (*subchapter 3.2: Figure 3.2.4*).

Although the adsorption (in terms of K_d) and leaching capacity of the other LOHC systems (i.e., indole-, carbazole derivative-, MLH- and MSH-based) were not investigated in as much detail compared with the quinaldines, evaluations of the adsorption potentials of these chemicals are possible based on the measured K_{oc} and the simulated log D values (**Tables 3 and 4**). According to the HPLC-based K_{oc} (**Table 3**), one can calculate the K_d values (except for the fully hydrogenated forms due to the lack of HPLC-based log K_{oc}) for these LOHC systems by using the empirical relationship of $K_d = K_{oc} \times f_{oc}$ (f_{oc}, the organic carbon content of the soil) (Schaffer *et al.*, 2012) (**Table 3**). In general, the indole-based LOHC system has a similar K_{oc} and log D values (**Tables 3 and 4**) to that of the quinaldine-based LOHC system (excluding the fully hydrogenated form), indicating possibly similar adsorption potentials over the pH ranges. The other three LOHC systems are mostly less ionizable and have much higher K_{oc} and log D values than the quinaldine-based LOHC system by up to several orders of magnitudes. Moreover, predictions of the adsorption behavior of these compounds may also possible simply based on the MW and S_w because of their close correlation to the adsorption potential (discussed in the former section). Therefore, the carbazole derivatives, MLHs and MSHs, are considered to have distinctively stronger adsorption, and their elevated retention in soils generally follows the trend of carbazole derivatives < MLHs < MSHs, indicating a much lower mobility in soil layers (i.e., ability to reach groundwater) than indoles and quinaldines. In addition, comparisons among the carbazole derivatives suggest that adsorption increases with prolonged alkyl chains. Furthermore, in each of the three carbazole derivative-based systems, the partially hydrogenated structure was expected to have a stronger affinity for soils.

4.2 Ecotoxicity of LOHCs

Differences in the adsorption potentials discussed above may correspondingly result in diverse degrees of bioavailability and thus distinct toxicity of the chemicals towards organisms. In this thesis, the soil ecotoxicity of LOHCs was investigated in the quinaldine-based LOHC system with the soil bacteria *A. globiformis* and Collembola *F. candida*. For both organisms, the soil pore water was expected to be the dominant route of exposure (sections 2.1.1 and 2.1.2). Therefore, partitioning via adsorption processes of the chemicals to soil pore-water fractions (e.g., K_d) was considered to be closely related to the observed toxicity. Two test scenarios (with and without soil) were investigated to identify the differences in bioavailability of these chemicals. The liquid-only exposure scenario represents a “worst case” within

the effect assessment and makes the assessment possible for the "pure" effects of the test chemicals without the interferences from the interaction to the soil, e.g., adsorption and the presence of other components in the soil (Stokes, Paton and Semple, 2005) .

The toxicity of the quinaldines to the growth of *A. globiformis* will not be discussed in the following sections because the bacteria showed little sensitivity to these chemicals. The quinaldines were not evidently toxic to this species in either the soil or pore-water exposure (without soils) test scenarios at 750 mg kg^{-1} dw soil and 500 mg L^{-1} (the highest test concentrations in the study), respectively (*subchapter 3.3: Tables 3.3.3 and S3.3.2*).

In the following sections, bioavailability and toxicity will be discussed for separate LOHC systems and compared between these systems by considering the adsorption potentials and partitioning (in terms of the log D and batch-K_d or the measured K_{oc}) of the chemicals in the corresponding test scenarios. Soil toxicity will be discussed in comparison to the available data regarding aquatic toxicity. For the LOHC structures or system toxicities that were not fully investigated in this thesis, the extent of the exposure and thus potential bioavailability of the chemicals will be discussed as a preliminary prediction.

4.2.1 Toxic effects of the quinaldine-based LOHC system

In the quinaldine-based LOHC system, overall, Quin-2Me-pH had the highest adsorption (in terms of the batch-K_d, column-K_d and mobility) observed in the adsorption batch equilibrium and soil column leaching tests (*subchapter 3.2: Table 3.2.3*). However, such high adsorption did not result in reduced toxicity of this chemical towards Collembola in the soil relative to its two analogs. This finding deviated from hypothesis IV, which posited that LOHCs of higher adsorption in soils would have lower toxicity due to consequently decreased bioavailability via pore-water fractions. Quin-2Me-pH had the highest toxicity towards the reproduction of Collembola in the soil (calculated-pore-water-concentration-based, EC_{50} = 34.6 mg L^{-1}) and was followed by toxicity of Quin-2Me-H10 (EC_{50} = 127.3 mg L^{-1}) and Quin-2Me (EC_{50} = 169.5 mg L^{-1}) (*subchapter 3.3: Tables 3.3.3 and S3.3.2*). Similar to Quin-2Me-pH, the slightly higher batch-K_d values for Quin-2Me-H10 than for Quin-2Me (**Table 3**) did not lead to lower toxicity of the former compared with the latter (*subchapter 3.3: Tables 3.3.3 and S3.3.2*). This observation was also contrary to hypothesis IV. Thus, the higher adsorption capacity of LOHCs does not necessarily mean that these chemicals have reduced effects on organisms in soils. Moreover, the soil pore-water concentrations (i.e., batch-K_d corrected concentrations) are not necessarily the original concentrations that were spiked in the soil. Using pore-water-based concentrations in the evaluation of soil toxicity appeared to avoid underestimating the toxicity that these chemicals may pose, particularly in comparison to the EC_{50} values that were estimated based on the nominal concentrations (*subchapter 3.3: Tables 3.3.3 and S3.3.2*).

In the aquatic ecotoxicity tests (soil was absent), the ranking of the toxicities of the quinaldines (toward bacteria *V. fischeri*, algae *R. subcapitata*, and daphnids *D. magna*) generally resulted in Quin-2Me-pH > Quin-2Me >> Quin-2Me-H10 (*subchapter 3.4: Table 3.4.5*), which was the same order of the log D of these chemicals (**Table 4**). These observations were consistent with the narcotic mode of toxic action, which is a theory that toxicity is governed by hydrophobicity (see Appendix A.4), and it is usually treated as the lowest level of toxicity that chemicals exert based on hydrophobicity. This observation was also consistent with hypothesis V, which posited that higher hydrophobicity is correlated with higher toxicity of the quinaldines in aquatic exposures. Although the correlations between the toxicity and hydrophobicity of the quinaldines in the pore-water exposure test (no soils) with Collembola were not significant (*subchapter 3.3: Table 3.3.3*), the surface damage found in the cuticle of Collembola when exposed to the quinaldines (*subchapter 3.3: Figure 3.3.2*) may demonstrate the mode of toxic action. This finding was consistent with those reported by Sverdrup et al., who demonstrated that narcosis leads to alterations in membrane integrity and fluidity (Sverdrup *et al.*, 2001).

The soil toxicity (expressed as batch-K_d corrected pore-water concentrations) and aquatic toxicity (LC_{50} or EC_{50} values obtained in the pore-water exposure or the aquatic test scenario) of the quinaldines toward Collembola and the aquatic organisms are compiled and given in **Figure 14**. A clear trend is shown regarding the toxicity of the quinaldines to the tested organisms. The effective concentrations varied between the three quinaldines by one to two orders of magnitude. Qui-2Me-pH generally had the lowest log LC/EC_{50} values (0.4–1.7), followed by Qui-2Me (1.0–2.3) and Qui-2Me-H10 (1.3–3.0). This finding may further support the aforementioned assumption with respect to narcosis of the quinaldines in which toxicity was likely controlled by pH-dependent hydrophobicity (i.e., log D). For each quinaldine, the differences in the log LC/EC_{50} values among different organisms spanned approximately 1.2 to 1.6 orders of magnitude.

Collembola was generally the most sensitive organism (pore-water exposure test scenario) to Quin-2Me (LC_{50} = 11.3 mg L^{-1}) and Quin-2Me-H10 (LC_{50} = 24.8 mg L^{-1}) (*subchapter 3.3: Table S3.3.2*; **Figure 14**, yellow dots), and *Daphnia* appeared to have the highest sensitivity to Quin-2Me-pH (EC_{50} = 2.7 mg L^{-1}) (*subchapter 3.4: Table 3.4.5*; **Figure 14**, red dots). Nevertheless, Collembola was still among the most sensitive test species (LC_{50} = 11.5 mg L^{-1}) (**Figure 14**, yellow dots) to this chemical. In general, *Lemna* had the lowest sensitivity to the quinaldine-based LOHC system (**Figure 14**, blue dots). The relatively higher sensitivity of *F. candida* compared with the other organisms may be related to the longer extended test period that was conducted (14-day test vs. 30-min to 7-day tests), and it might also indicate the necessity of conducting ecotoxicity tests with prolonged exposure periods (Baumann *et al.*, 2014) for these chemicals.

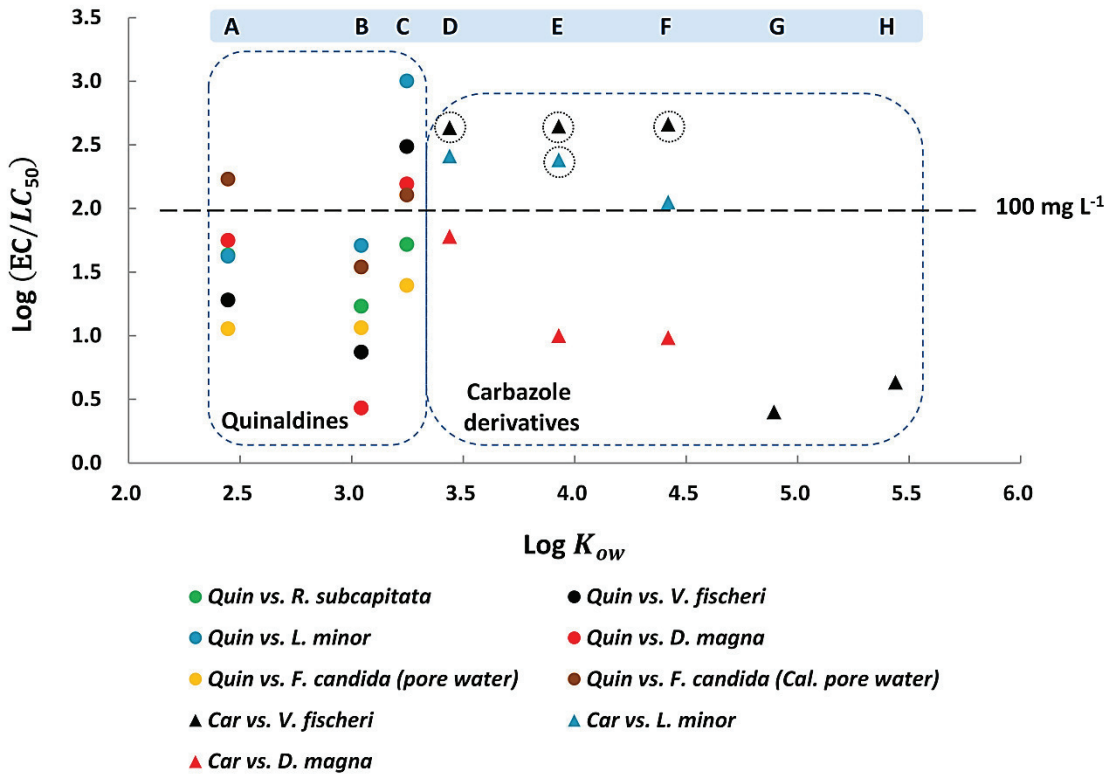


Figure 14. Summarized toxicity (EC_{50} or LC_{50} shown in log) of the quinaldines (**A**: Quin-2Me; **B**: Quin-2Me-pH; **C**: Quin-2Me-H10) and carbazole derivatives (**D**: Car-2-H12; **E**: Car-3-H12; **F**: Car-4-H12; **G**: Car-2-pH; **H**: Car-3-pH) towards the tested organisms in the soil and aquatic scenarios (mg L^{-1}). The soil organism was *F. candida* (pore-water and calculated soil pore water – Cal. pore water) and aquatic organisms were *V. fischeri*, *R subcaptitata*, *L. minor* and *D. magna*. K_{ow} values (without the correction by ionization) were predicted by COSMO-RS (*subchapter 3.2: Table 3.2.1*). Only the effective concentrations obtained without limited by water solubility are given. Dotted circle: EC_{50} or LC_{50} expressed as exceeding the highest test concentration.

Generally, the LC_{50} or EC_{50} values of the quinaldines obtained in the exposure scenarios without soils were lower than those in the soil test. Therefore, the liquid-only exposure scenario appeared to be a more sensitive test than the soil exposure scenario. This finding was consistent with hypothesis VI. Similar findings were reported by Hartnik et al. (Hartnik, Sverdrup and Jensen, 2008), who found the overall toxicity of pesticide alpha-cypermethrin towards four tested soil organisms in soils (includes *F. candida*, soil pore-water-based concentrations) was lower with one order of magnitude in EC_{50} than that of four typical aquatic organisms (includes *D. magna* (European Commission, 2004)). Compared with the liquid-only test, the exposure and thus bioavailability (when pore water is the dominant route of exposure) of the quinaldines might be comparatively lower in the soil test due to the partitioning of the chemicals to the soil solids. In the liquid-only test scenario, organisms could be considered to be fully exposed to the LOHCs, which would lead to increased uptake of the chemicals.

4.2.2 Toxic effects of the carbazole derivative-based LOHC system

Although the soil toxicity of the carbazole derivatives was not measured in this study, the extent of exposure of Collembola (or organisms with soil pore water as the dominant route of exposure) to these chemicals may be extrapolated based on their physicochemical properties (**Tables 3 and 4**; *subchapter 3.3: Table 3.3.1*). Organic compounds with log K_{ow} < 5.2 have been shown to strongly influence the survival or reproduction of Collembola (Sverdrup, Nielsen and Krogh, 2002) (Čvančarová, Křesinová and Cajthaml, 2013), whereas compounds with log K_{ow} ≥ 5.2 were reported to be non-toxic to Collembola due to limited water solubility (Čvančarová, Křesinová and Cajthaml, 2013) (i.e., reduced availability via pore water). Therefore, decreased partitioning of Car-3-pH, Car-4 and Car-4-pH to the pore-water fractions would be expected in comparison to the other components in this LOHC system, and the uptake and bioavailability of these chemicals in Collembola might be reduced. Moreover, carbazole (an analog of the derivatives) has been known to present a narcotic mode of toxic action on Collembola *Folsomia fimetari* (expressed as equilibrated pore-water concentrations in soils) (Sverdrup *et al.*, 2001). Nevertheless, soil ecotoxicity tests are still needed to avoid underestimating the toxicity which may occur when merely based on adsorption (in the case of Quin-2Me-pH as previously discussed). In addition, determining the effective concentrations by ecotoxicity tests enables a better recognition of hazards in terms of toxicity classification and PNECs.

On the other hand, carbazole derivatives were tested for aquatic toxicities with typical aquatic organisms (*V. fischeri*, *L. minor* and *D. magna*) (*subchapter 3.4: Table 3.4.5*). Not all forms of the carbazole derivatives were tested for aquatic toxicity towards the organisms. For certain EC_{50} values, the dehydrogenated forms had too low solubility in the test medium (< 1 mg L^{-1}) to be measured. Therefore, compared to the quinaldines, less data were available for specific EC_{50} values, and mostly were obtained for the hydrogenated forms.

Based on the data that were available (*subchapter 3.4: Table 3.4.5*), *D. magna* generally appeared to be the most sensitive species to the H_2-rich forms of the carbazole derivatives (Car-2-H12, Car-3-H12 and Car-4-H12 with EC_{50} values of one to two orders of magnitude lower) followed by *L. minor*, whereas *V. fischeri* were more likely to have the lowest sensitivity to these chemicals (**Figure 14**, black triangles). Toxicity was in an order consistent with that of hydrophobicity with respect to the log D values of the chemicals (**Table 4**) with Car-2-H12 < Car-3-H12 < Car-4-H12 (**Figure 14**). In addition, the generated data was used to compare the toxicity of the H_2-rich forms with the partially hydrogenated forms towards *V. fischeri* (*subchapter 3.4: Table 3.4.5*). Following the order of hydrophobicity (log D), partially hydrogenated forms generally had higher toxicity than the H_2-rich forms with a difference of two orders of magnitude (**Figure 14**, black triangles). These findings were consistent with hypothesis V; therefore, narcosis could be assumed as the mode of toxic action in these carbazole derivatives.

The carbazole derivative-based LOHC system may not be superior to the

quinaldines (at least the H_2-rich and partially hydrogenated forms), due to their slightly higher aquatic toxicity, with the EC_{50} values having a difference of one to two orders of magnitude. However, there might be decreased bioavailability of the chemicals to soil-dwelling organisms (species that are mostly affected by pore-water pathway of exposure, such as Collembola). Furthermore, the log EC_{50} values found for different carbazole derivatives spanned approximately two orders of magnitude, which is comparable to that found in the quinaldines mentioned in section 4.2.1 (**Figure 14**). The spread of the values among the components in a specific LOHC system or between different LOHC systems suggest that a comprehensive evaluation of the environmental hazards of these chemicals must involve tiered selection of test organisms from different trophic levels because of the diverse sensitivity of test organisms to LOHCs.

4.2.3 Prediction of the effects of the indole-, MLH- and MSH-based LOHC systems

Specific ecotoxicity of the three LOHC systems was not tested in this thesis. Nevertheless, the extent of bioavailability might be estimated on the basis of hydrophobicity and adsorption potentials (e.g., K_{oc}, K_d and log D). Indoles had comparable K_{oc} and log D values to that of the quinaldines (**Tables 3 and 4**) and with the log K_{ow} values lower than 5.2. Therefore, comparable partitioning potentials within soils and similar extents of exposure of Collembola might be anticipated. MLHs and MSHs, on the contrary, were expected to have much stronger adsorptions and extremely low water solubility. They may become far less bioavailable through soil pore water than other LOHCs to Collembola in soils; very limited bioavailability in exposure scenarios without soil could also be highly possible. Ecotoxicity experiments are still needed for further determination of these chemicals.

4.3 Environmental hazard assessment of LOHCs

The environmental impacts of the LOHCs were evaluated based on the classification of the observed toxicity and extrapolation of the PNECs. Further estimations in which the potential application volumes, the behavior (adsorption and mobility) in realistic environmental conditions (in terms of soil-aquatic network) and the fate of these chemicals are combined to allow for a better understanding of the potential risk of LOHCs. Comparing the risk among LOHCs, and between LOHC chemicals and current conventional energy systems, e.g., fossil fuels, can help identify and promote new energy sources for improved green energy development.

4.3.1. Toxicity classification and PNECs

Based on the observed toxicity, the quinaldine-based LOHC system is categorized as "harmful" (EC_{50} 100–1000 mg kg^{-1} dw soil) to non-aquatic organisms (Hartmann,

Stefania and Sokull-Klüttgen, 2014) (*subchapter 3.3: Table 3.3.3 and S3.3.2*) and classified as "acute 2" (EC_{50} 1–10 mg L^{-1}; Quin-2Me-pH) or "acute 3" (EC_{50} 10–100 mg L^{-1}; Quin-2Me and Quin-2Me-H10) to aquatic organisms (including the primary producer algae) (GHS, 2011)* (*subchapter 3.3*; *subchapter 3.4: Tables 3.4.5 and 3.4.6*). Similarly, the partially hydrogenated and H_2-rich forms of the tested carbazole derivatives could also be categorized. The resulting assignment of toxicity is similar to that of the quinaldines, thereby resulting in "acute 2" (Car-4-H12) or "acute 3" (Car-2-H12 and Car-3-H12). Furthermore, data obtained in this thesis allowed for the prediction of the PNECs (*subchapter 3.3: Tables 3.3.4 and S3.3.3*) for the quinaldines, which showed values between 0.8 and 1.2 mg kg^{-1} dw in soils and between 0.1 and 0.5 mg L^{-1} in the water phase.

4.3.2. Prediction of the application volumes

The real circulation volumes with respect to the production, transportation and application of the present LOHC systems currently are difficult to determine. For the potential application of LOHCs in automobiles, demonstration units have been conducted via trials (Preuster, Papp and Wasserscheid, 2017) with a road transport utility of 1,000 L LOHCs – H18-DBT (perhydrodibenzyltoluene) applied to power hydrogen cars in Stuttgart, Germany. The extent of LOHCs observed in the environment as a result of operating vehicles is also difficult to determine. Nevertheless, estimations can be performed by considering the gravimetric hydrogen capacity (H_2 wt%) of LOHC systems and the general consumption of LOHC by an operating vehicle (approximately with 1 kg H_2 per 100 km). The estimated mass of LOHCs potentially required to power a car per 100 km ranges between 15 and 60 kg (**Table 5**). Moreover, LOHC-based technology for energy storage and supply is recently commercially available by H_2-INDUSTRIES, which is a major energy storage solution supplier. A series of LOHC-based energy storage and release products have been designed and developed, such as "ePOWER", "eSTORE", and "eRELEASE". These products store and release large amounts of renewable energy in the form of H_2 loaded in LOHCs with different working capacities for off-grid electrical energy supply†. The nominal installed power ranges from 66 to 1,650 kW with 5 MWh electrical energy provided by 5000 L hydrogen-rich LOHCs‡.

* In GHS, the highest aquatic toxicity category is "acute 1" corresponded to $EC_{50} \leq 0.1$ mg L^{-1}.
† H_2 product by H_2-INDUSTRIES: http://www.h2-industries.com/products/.
‡ H_2-INDUSTRIES: http://www.h2-industries.com/technology/.

Table 5. Estimation of the amount of LOHCs that are required to operate a vehicle per 100 km.

LOHCs	H_2 capacity [wt%]	LOHCs per 100 km [kg]
Quinaldines	6.5[a]	15.4
Indoles	1.7[b]	58.8
Carbazole derivatives	5.8[a]	17.2
MLHs/MSHs	6.5[a]	15.4

[a] (Markiewicz, Zhang, *et al.*, 2015).

[b] (Zhu and Xu, 2015).

4.3.3. Potential impacts of LOHCs in soil-aquatic network in the environment

Considering that transportation commonly occur between soils and water phases in the environment, LOHCs in soils will most likely represent an important source of LOHC contamination for the aquatic environment. The effects of LOHCs in soils thus can also be expanded to organisms in aquatic environments (e.g., water phases adjacent to contact contaminated soils) through different types of translocation, including leaching through the soil layers, diffusion by surface run-off or driftage to the surface waters of streams, rivers and lakes (**Figure 3**). The comparatively higher leaching potentials of Quin-2Me-H10 and Quin-2Me (*subchapter 3.2: Figures 3.2.4 and S3.2.2*) may increase the potential for these LOHCs to contaminate groundwater. The less leachable/mobile LOHC, Quin-2Me-pH and perhaps the carbazole derivatives, MLHs and MSH, are most likely to be retained in the soil (surface), where they will be released. Based on the aforementioned application volumes of LOHCs (including the potential and current uses), the concentration of LOHCs in the environment will likely exceed the predicted PNECs that would occur during accidental spills or leakages. Moreover, soil and aquatic organisms may consequently be exposed to doses that are higher than the LC/EC_{50} values measured in this thesis.

The concentrations of LOHCs in the soil pore-water fractions and after leaching/drainage are estimated (**Tables 6 and 7**) by assuming 100 mg kg^{-1} dw soil as the initial amount that is released. This concentration is usually treated as a worst-case environmental contamination scenario, and it is also the nominal soil concentration of the quinaldines that approximately triggered EC_{10} in the reproduction of Collembola in this thesis (*subchapter 3.3: Table S3.3.2*). The fraction of LOHCs in the pore water was calculated based on equilibrium partitioning (for the calculation theory, see *subchapter 3.3: section S3.3.8*) using batch-K_d (for quinaldines) or HPLC-K_{oc}-based K_d values (for other LOHCs, see **Table 3**). The quantity of the quinaldines after leaching was considered according to i) the concentration at breakthrough and ii) the concentration when the leaching plateau was achieved (when no significant change in the concentrations of leachates occur).

Table 6. Estimated concentrations (mg L^{-1}) of the quinaldines (100 mg kg^{-1} dw soil are applied) in the soil pore-water fractions and leachates after partitioning or leaching within soil I or II, respectively.

Quinaldines	Pore water [mg L^{-1}]	Leaching through soil [mg L^{-1}]	
		At the breakthrough	At the plateau
Quin-2Me	46.3	0.05	8.1
Quin-2Me-pH	10.0	0.26	0.11
Quin-2Me-H10	39.2	7.8	72.6

Table 7. Estimated concentrations (mg L^{-1}) of the indoles, carbazole derivatives, MLHs and MSH (100 mg kg^{-1} dw soil are applied) in the soil pore-water fractions after partitioning within soil I.

	Indoles		Carbazole derivatives			
LOHCs	**Indole**	**Indoline**	**Car-2**	**Car-2-pH**	**Car-3**	**Car-3-pH**
Conc. [mg L^{-1}]	34.6	131.2	0.44	0.40	0.20	0.19
	Carbazole derivatives		**MLHs**		**MSHs**	
LOHCs	**Car-4**	**Car-4-pH**	**MLH**	**MLH-pH**	**MSH**	
Conc. [mg L^{-1}]	0.081	0.080	1.16	0.032	0.034	

In the quinaldine-based LOHC system, the estimated pore-water concentrations (**Table 6**) were generally lower or at least comparable to the concentrations that triggered 50% of the negative effects on the tested aquatic organisms (*subchapter 3.4: Table 3.4.5*). The estimated pore-water concentration of Quin-2Me (46.3 mg L^{-1}) and Quin-2Me-pH (10.0 mg L^{-1}) were significantly higher than the EC_{50} values toward *V. fischeri* and *D. magna*, respectively. Estimation of Quin-2Me-H10 in the pore water showed a concentration of 39.2 mg L^{-1}, which is less likely to represent as hazardous to the test aquatic organisms as its two equivalents. Furthermore, the calculated concentrations in the water phase after leaching through soils ranged from 0.05 to 7.8 mg L^{-1} and 0.11 to 72.6 mg L^{-1} in the two considered situations (**Table 6**). These concentrations are significantly lower than the EC_{50} values of the respective quinaldines toward the aquatic organisms (except for the level of Quin-2Me-H10 at the leaching plateau vs. algae). However, in both simulated scenarios (pore-water fraction and water phases after leaching), most of the estimated concentrations are higher than the PNECs that estimated for the quinaldines in water phase (0.1–0.5 mg L^{-1}), with differences at up to three orders of magnitude further indicating potential environmental risks of the quinaldines during transport in the compartments.

The available data for the carbazole derivatives (**Table 7**) did not allow for an overall comparison of all the structures and aquatic organisms presented. The estimated concentrations in the water phase after partitioning were in the range of 0.08–0.44 mg L^{-1}, which were several orders of magnitudes lower than the levels of the H_2-rich forms to trigger 50% inhibitions. The concentrations of the partially

hydrogenated forms were also lower than the EC_{50} values of the LOHCs toward the bacteria *V. fischeri* at more than one order of magnitude (*subchapter 3.4: Table 3.4.5*). Therefore, in such way, the carbazole derivatives seem unlikely to introduce the hazards to soil adjacent water phases as high as the quinaldines will do.

These evaluations however did not exclude that in the realistic environment concentrations of these LOHCs in the soil associated water phase will vary with regards to diverse soil properties (e.g., soil organic matter (SOM)) and many other natural conditions such as weather. To achieve an evaluation that is more reliable, realistic environmental conditions and processes must be considered as a fundamental background. SOM in the environment can have molecular sizes that range from single molecules to complex aggregates, and it may cover a diverse array of compositions (Schwarzenbach, Gschwend and Imboden, 2003) and quantities. Considering the structures and physicochemical properties of LOHCs, the adsorption and thus partitioning of the released LOHC chemicals between the soil matrix and water phases would vary based on interactions with SOM.

First of all, nonspecific interactions, such as the London dispersive interactions, are considered generally operating the overall attraction between LOHCs and SOM (e.g., between the alkyl chains and SOM, such as lipids and esters excreted by plants and animals) (Schwarzenbach, Gschwend and Imboden, 2003) (Jungnickel *et al.*, 2011). Moreover, SOM usually has exterior functional groups, such as carboxy-, hydroxyl-, phenoxy-, and carbonyl-substituted, and these groups display electron-rich regions and participate in a series of molecular interactions during the sorption processes. These regions are attractive to the cationized LOHCs (e.g., quinaldines and fully hydrogenated forms of other LOHCs, **Table 4**) often through nonspecific reactions, e.g., dipole-dipole interactions of these chemicals to humic acid and lignins. Specific polar interactions are also possible, such as H-bonding with different SOM e.g., fatty acids (Schwarzenbach, Gschwend and Imboden, 2003) (Jungnickel *et al.*, 2011). Ionic reactions may also occur simply by ionic bonding of the LOHC cationic molecules to negatively charged molecules, e.g., Cl^- or F^-, which are ubiquitous in mineral surfaces (Jungnickel *et al.*, 2011). However, for LOHC structures that are less likely to be ionized (i.e., neutral molecules, including most carbazole derivatives, MLHs, and MSHs, **Table 4**), hydrophobic interactions to SOM and penetration are expected to be more dominant in adsorption processes (Kördel *et al.*, 2013). In addition, the aromatic structure of the dehydrogenated and partially hydrogenated forms of LOHCs (**Table 2**) will lead to π-π interactions, which may potentially contribute to the adsorption of these chemicals to different SOM, such as humic and fulvic acids and black carbons (Schwarzenbach, Gschwend and Imboden, 2003) (Jungnickel *et al.*, 2011). In addition to chemical binding, physically governed restraints also occur and enable the adsorption and occlusion of LOHC chemicals in SOM or solid particles in nature. The occluded LOHCs also influence the partitioning of LOHCs between solid-aqueous phases. These compounds, however, have less opportunity to interact with organisms within a given period (Semple *et al.*, 2004).

Besides, in natural soils, the surface horizon (generally defined between 0 and 27

cm in depth) (Coleman, Crossley and Hendrix, 2004) accumulates amounts of SOM (Coleman, Crossley and Hendrix, 2004) (L. Yang *et al.*, 2013) that vary highly in quantities in different ecological landscapes. The concentration of SOM in topsoil generally ranges from 1% to 6%. Forest soil usually contains approximately 40% organic matter, whereas grassland and tundra generally hold 28% and 6% SOM, respectively (Vancampenhout *et al.*, 2009). Productive agricultural soil holds between 3 and 6% SOM (Greenland, 1980). Evidently, the organic content is often higher in nature than in the test soils in this thesis (1.21 and 0.8 %), which may imply that stronger interactions occur between LOHC chemicals and SOM via previously discussed reactions/mechanisms. Such interactions may lead to higher adsorption and retention of LOHCs in the soil environment than that observed in the lab-based batch equilibrium and soil column leaching tests. The overall LOHCs that reach water after the transfer from the soil to the aquatic environment would reduce and result in concentrations that are lower than the estimated concentrations (**Tables 6 and 7**). Nevertheless, the presented data may represent as reference and threshold values for preliminary evaluations in further studies.

Moreover, large numbers of soil organisms inhabit the upper horizons of soils (Coleman, Crossley and Hendrix, 2004) and the extended retention of certain LOHCs in soil (Quin-2Me-pH, carbazole derivatives, MLHs, and MSHs) may increase the contact and exposure of soil inhabitants to these chemicals. In addition to pore-water exposure, these LOHCs may also affect the organisms while binding to soil solids/particles (Tsibart and Gennadiev, 2013). Soil organisms that feed on solids/particles-associated substances may have increased exposure to these LOHCs, species such as earthworms and enchytraeids (Peijnenburg *et al.*, 2012); a cumulative effect is likely to occur due to their consumption of LOHC-bounded soil solids (strong sorbates, such as carbazole derivatives, MLHs, and MSHs) and the adsorption of these chemicals through their skin. However, it does not exclude the possibility that these relatively mobility-inert LOHCs might be transported to deeper soil layers than that had been observed in the study. Under the proper environmental conditions, such as a reduction of SOM, an increased pH, and the presence of competing ions (e.g., Ca^{2+}, especially for ionizable LOHCs), the initially adsorbed LOHCs would be dissociated from soil solids/particles due to the reduction of adsorption forces. Stronger water flows, such as heavy rainfall, could also increase dissociation due to the lack of time for chemicals to interact with SOM and soil solids (Li *et al.*, 2013), thus making these LOHCs become more available via water fractions.

Further evaluation of the risks of LOHCs after transportation from soils to aquatic scenarios must consider that co-transportation is likely to occur in bound forms with SOM or soil particles. Moreover, the estimated exposure concentrations in the aquatic environment may be further influenced by the dilution of chemicals in the surface water and the varied water solubility of the LOHC structures (especially in the carbazole derivatives, MLHs, and MSHs, which have limited solubility in water). In addition, the exposure levels in surface water may vary due to the partitioning processes that occur between the LOHCs and compartments, such as suspended

matter, sediment suspension and sediment (European Chemicals Agency, 2016), in aquatic systems. Specific investigations for these processes were not addressed in this thesis, however, the partitioning potentials with respect to the investigated partition coefficients, such as the K_{oc}, K_d, and log D values (simulated at several environmentally relevant pH levels) are expected to provide insights into the adsorption processes in the aquatic environment.

4.3.4. Environmental fate of LOHCs

LOHCs can be transferred while maintaining their original structures or probably as metabolites and degradation products. The rate at which the chemicals to be transformed governs the persistence and the fate of the chemicals in the environment. Persistent chemicals are considered to have enhanced potential to exert negative effects over expanded spatiotemporal scales (Boethling *et al.*, 2009). The persistence of LOHCs (the quinaldine- and carbazole derivative-based LOHC systems) was recently investigated by testing their biodegradation (*subchapter 3.4*). More than 60% of Quin-2Me was found to be degraded in activated sludge (obtained from wastewater treatment plants) after 28 days at a half-time of less than 10 days (*subchapter 3.4: Figure 3.4.2*). However, neither of the other two components of the quinaldines nor the carbazole derivatives showed biodegradability; there was nonetheless no evidence supporting the persistence of these compounds (*subchapter 3.4*). This finding suggests the reduced possibility of the LOHCs causing long-term effects and accumulating in organisms (to reach toxic threshold) and the environment. However, these chemicals may return to soils in the transported form in the effluents and/or by the sewage irrigation to soils, which still prevail in many countries. Quin-2Me-pH was the main biodegradation product of Quin-2Me (*subchapter 3.4*), which had generally higher toxicity to the soil and aquatic organisms (**Figure 14**) and lower biodegradability (*subchapter 3.4*). These findings further emphasize that a comprehensive understanding of the risks of LOHCs in the environment must involve long-term effects and diverse test conditions.

4.3.5. LOHCs as energy substitutes to fossil fuels and associated regulations

The comparison between LOHC systems showed that quinaldines may pose a higher environmental risk in (pore-)water phases via transfer from soils because of the generally lower adsorption, higher mobility and thus potentially higher concentrations of the chemicals after partitioning and leaching (discussed in section 4.3.3). Carbazole derivatives may be a lower risk to groundwater, however, they appeared to be more toxic to aquatic organisms when directly released to the aquatic environment. Carbazole derivatives may also be more bioavailable to organisms in which consumption of soil solids is the main uptake manner (discussed in 4.2.2). Despite the exposure potential and toxicity of the LOHCs discussed throughout the thesis, the LOHCs do not appear to be in the ‘bad’ category for

aquatic or soil organisms, especially compared with the toxicity of typical N-PAHs analogs (**Table 1 and Appendix Table AI**).

Moreover, the quinaldine-based LOHC system appears to represent an improvement over current energy supply systems, such as fossil fuels, in terms of environmental hazards. This improvement is shown in a comparison of the effects of both LOHC (H_2-rich and H_2-lean forms) and diesel fuel on aquatic organisms (*subchapter 3.4: Tables 3.4.5 and 3.4.6*). Due to the lack of specific data of the soil toxicity of fossil fuels (e.g., diesels or gasoline) towards *F. candida*, related comparisons were made on the effects of crude oil or petroleum contaminated soils on the anminals. Similarly, the quinaldine-based LOHCs are not expected to be more hazardous to the soil environment than crude oil or petroleum oil (Juvonen *et al.*, 2000) (Reinecke, van Wyk and Reinecke, 2016) (*subchapter 3.3*). The carbazole derivative-based LOHC system, however, appears to be slightly more toxic to aquatic organisms (Car-3-H12 and Car-4-H12 vs. *D. magna*) than diesel oil (*subchapter 3.4: Tables 3.4.5 and 3.4.6*).

Persistence in terms of degradation showed lower biodegradability of most of the quinaldines and carbazole derivatives than the diesel oil tested (> 60%, *subchapter 3.4*). Similar biodegradation rate of commercial diesel oil was also reported by Marchal et al. (Marchal *et al.*, 2003), who found a biodegradability of 73% in activated sludge from an urban waste water treatment plant. Gasoline showed a generally higher degradation rate exceeded 90% (Solano-Serena *et al.*, 1999).

However, the overall lower to comparable toxicity and weaker biodegradation of LOHCs relative to fossil fuels may not impede the use of LOHCs as potential energy substitutes. The original energy loaded in LOHCs is generated by renewable energy that is abundant and environmentally friendly. The energy storage and release processes *per se* are free from emissions of hazardous chemicals, such as harmful gas and heavy metals that commonly occur through the use of fossil fuels. High boiling points and low melting points of the LOHC chemicals also facilitate their safety regarding handling under ambient conditions (Teichmann, Arlt and Wasserscheid, 2012b) particularly life-cycle assessment is also considered a part of toxicity evaluation of chemicals. Moreover, compared with fossil fuels, LOHC chemicals present a lower quantity of and more specified components, meaning that risk assessments can be more easily determined through the investigation of a smaller scale of compounds (*subchapter 3.4*). These advantages promote the establishment of proper management and monitoring of the utilization of these compounds.

LOHCs are anticipated to be utilized as chemicals with a circulation volume of at least 10 tons or higher than 1,000 tons per year; therefore, an environmental regulatory framework for LOHCs is needed to facilitate the prudent use through monitoring and control (European Chemicals Agency, 2011). According to REACH, to achieve this purpose, information on the operational conditions, risk management measures, and release estimates on local and regional scales during utilization must be gathered to determine the basic input of these chemicals into the environment

(ECETOC, 1993) (European Chemicals Agency, 2016). These data are not yet available for LOHC chemicals but should be collected because the proposed LOHC-based technology has been become available on the market (see section 4.3.2). Hazard assessments will target risk characterizations and determinations for LOHC systems in the environment (European Chemicals Agency, 2012). Through the combination of behavioral and physicochemical properties of the chemicals and the environmental context, predicted environmental concentrations (PECs) can be extrapolated. A comparison of these values with the PNECs (derived from ecotoxicity tests) yields the risk characterization ratio (RCR), which is a factor for evaluating whether the specific environmental risks are adequately controlled (European Chemicals Agency, 2012). Chemicals are unlikely to be hazardous at a RCR < 1. Therefore, potential risk assessments of LOHCs can be ultimately developed in response to public concerns, technical developments, and legislation.

Chapter-V Conclusions and Perspectives

5. Conclusions and Perspectives

This thesis investigated the adsorption behavior and ecotoxicity of LOHC systems to establish a proactive assessment of the environmental hazards of these chemicals. The present structures are promising candidates of the current LOHC systems, which is a novel technology for the efficient and safe storage and supply of energy in the form of hydrogen. The affinity of these chemicals for organic matter in terms of the K_{oc} values was predicted using two software estimations and instrumental analyses via HPLC screening. LOHCs were set according to specific mobility classes, which ranged from highly (indoline) and moderately mobile (indoles and quinaldines) to non-mobile (carbazole derivatives, MLHs and MSH). Following hypothesis I, K_{oc} was strongly correlated with the basic physicochemical properties of the LOHCs, including the K_{ow}, S_w and MW. Further adsorption in the soil based on soil-water partitioning (K_d) was investigated in batch equilibrium experiments via the establishment of two types of isotherm models that used the quinaldine-based LOHC system as an example. Significantly different adsorption behavior was observed for Quin-2Me-pH, which had the highest K_d (batch-K_d), whereas Quin-2Me-H10 and Quin-2Me appeared to have similar adsorption potentials that deviated from hypothesis II. Overall, the leaching capacity through soil columns supported the findings and the dynamic adsorption processes were expected to be similar to that in the environment. However, Quin-2Me-H10 appeared to have the highest mobility through the soil columns, which was inconsistent with hypothesis III. The column-K_d values extrapolated in the leaching tests were lower than the batch-K_d because of the lower organic contents in the soil for leaching as well as the distinct intrinsic characteristics of the batch and leaching tests. The discrepancies between the observed adsorptions and what was expected may be related to the ionization potential of the quinaldines (particularly for the H_2-rich form); thus, ionization corrected octanol-water partitioning (i.e., log D) and pH was discussed to determine the adsorption potentials. In addition, potential adsorption mechanisms (hydrophobic forces vs. ionic interactions) were discussed to provide a better understanding of the distinct adsorptions. Compared with the LOHCs, which were less ionizable, ionized structures presented adsorption dominated by charge–charge interactions rather than hydrophobicity to organic matter.

The obtained adsorption and mobility characterization were integrated into a preliminary hazard assessment, which is the core of this thesis, in combination with the ecotoxicological effects. Toxicity was evaluated with typical soil and aquatic organisms in short- and long-term exposure periods with different test endpoints. Determining the toxicity based on the pore-water concentrations in the soil tests is

recommended to avoid underestimations and improve the comparison of toxicity between studies (nominal concentration-based values usually lead to increased deviations for dose-response relationships). Stronger adsorbed LOHCs did not show decreased toxicity, which contradicted hypothesis IV; nevertheless, hydrophobicity (log D)-governed toxicity was observed for the tested quinaldines and carbazole derivatives as initially hypothesized (hypothesis V). Such a correlation led to the assumption that narcosis was the mode of toxic action, although reliable QSARs could not be established for all LOHCs due to the limited available data points. Furthermore, consistent with hypothesis VI, the soil exposure scenario appeared less sensitive than the aquatic test scenario (no soil) due to the decreased bioavailability of the LOHCs from partitioning in soil solids. Generally, the Collembola and daphnids were the most sensitive species for the test series. The toxic effects of the quinaldine (soil and aquatic)- and carbazole derivative (aquatic)-based LOHC systems were assigned as “harmful” or “acute 2” and “acute 3”, with the latter LOHCs presenting slightly higher toxicity, although not all hydrogenated structures were measured for this system.

An evaluation of the potential impacts of the LOHC systems in the environment was conducted by considering the potential application volumes of these chemicals and realistic environmental conditions. Higher estimated concentrations of LOHCs in the environment than the PNECs indicated that these chemicals present certain environmental concerns. Therefore, the toxic effects and adsorption potentials were combined by considering the network between the soil and aquatic compartments and translocation, which was evaluated by estimating the amounts of LOHCs after partitioning and leaching from the soil to aquatic phase. The resulting concentrations were generally higher than the EC_{50} values for algae, bacteria and daphnids in the aquatic scenario although the situation might change in response to the diverse conditions (e.g., SOM and climates) in the realistic environment. In addition, not all the components of the quinaldines and carbazole derivatives showed ready biodegradability, though persistence of these chemicals was not sufficiently proved. Although LOHC systems demonstrate an improvement over the analogs (e.g., N-PAHs and fossil fuels), they should be adequately controlled by considering the potentially large circulation volumes that may be introduced into the market.

This thesis presents one of the first studies on the behavior and toxicity of LOHCs designed to provide a proactive environmental hazard assessment of these compounds. The data in this thesis are expected to constitute a starting point for the environmental risk assessment of LOHCs. To achieve a more comprehensive understanding of the environmental impacts of LOHCs, further studies are still necessary and the following suggestions may need to be considered.

I. Inclusion of more LOHC structures and diverse but relevant organisms

Laboratory experiments are generally the first step for most research questions. First, the observed adsorption and leaching capacity of LOHC systems, i.e., indoles,

carbazole derivatives, MLHs, and MSHs, may need to be investigated via adsorption tests in static equilibrium as the starting point. However, most LOHC structures, particularly those in the latter three clusters, are poorly water soluble. Thus, column leaching might be a better option because it presents a comparatively easier operation for that these compounds are prepared in soils by first dissolving them in organic solvents. Nevertheless, compared with the test with Quin-2Me-pH, a prolonged period of leaching time is probably required because of the distinctively higher K_{oc} values of those compounds. Obviously, each method presents advantages and disadvantages, and the use of each method alone or in combination with others to evaluate the adsorption of LOHCs in soils primarily depends on the properties of the compounds and the environmental issues under investigation. Such issues should be considered in the experimental design, and compromise may be necessary.

To assess the toxicity of indoles, carbazole derivatives, MLHs, and MSHs, soil bacteria and Collembola are recommended to be used for the preliminary evaluation. By using these species, toxicity comparisons with the quinaldines can be facilitated. Furthermore, performing these toxicity tests in soils is more operational than in liquid media, because of the extremely poor water solubility of those structures. Although aquatic tests was performed by the aid of passive dosing or column extraction for the solution preparation, addressing higher test concentrations, such as for the H_2-lean forms of carbazole derivatives, has been proved generally more difficult in the aquatic test scenario.

In the next study stage, the toxicity of LOHCs towards multispecies should be studied because of the enormous variety of soil-dwelling species in nature and the potential differences in sensitivity observed in the test organisms to LOHCs. However, the organisms for testing should be intentionally selected to avoid underestimating the effects that could occur in less relevant species because the manner of exposure differs among organisms (Kördel *et al.*, 2013). The following relevant factors should be considered: i) exposure route, ii) uptake manner (feeding behavior), and iii) habitat distribution in soils. For example, LOHC structures with lower K_{oc} and K_d values and higher water solubility may be more bioavailable to organisms with pore water as their dominant exposure pathway, whereas for other species, such as the earthworms, contaminants are digested through bonded to soil particles. Therefore, LOHCs with higher affinities for soil particles and organic matter would be more hazardous to these types of organisms. Furthermore, an integrated assessment that involves different organisms across trophic levels would promote a more realistic hazard assessment at the regulatory level (Peijnenburg *et al.*, 2012).

II. Narcosis determination

Although this thesis found a general link between the effects of the LOHCs (quinaldines and the H_2-rich forms of the carbazole derivatives) and their hydrophobicity (log D) and assumed narcosis, the quantitative-based relationship is

still unclear, which represents an obstacle for extrapolating narcosis for other LOHC structures. Narcosis based on one specific organism (being focused on one endpoint) should be extrapolated from the identification of reliable relationships by involving more compounds of this type (i.e., LOHC compounds).

III. Effects of the mixtures of different hydrogenated forms

In addition to evaluating single LOHC structures, dehydrogenated, fully hydrogenated, and intermediate forms that constitute specific LOHC systems should be tested in combination to determine their behavioral and toxic effects. Such tests are important because LOHCs technically co-occur in pairs in the energy storage and release processes, where reversible transformation between the paired structures occur, although the mediate forms usually appear due to incomplete hydrogenation with less amounts. Therefore, LOHCs enter the environment in mixed forms is very likely during e.g., accidental spills. The toxicity and adsorption behavior in mixed forms are expected to be more complex than that of single LOHC structures. Competitive sorption may occur within these constitutes with one form affecting the sorption and bioavailability of other forms.

IV. Long-term effects and toxicity spanning multigenerations

Long-term-based biological parameters are usually more sensitive than short-term parameters. For example, reproduction of *F. candida* is a more sensitive test endpoint than mortality. Similarly, increased toxicity in *D. magna* over an extended test period has also been reported (Baumann *et al.*, 2014). The reliability of toxic assessments could be dramatically minimized if the long-term effects are simply extrapolated from the results of short-term studies (ECETOC, 1990). Moreover, organisms will likely be subject to extended periods of exposure in realistic contaminated land and aquatic areas. Besides, the quinaldines and carbazole derivatives presented in the thesis were not all readily biodegradable within 28 days, though no proof of persistence, evaluation of the hazards in extended periods may be necessary. Although experiments with longer exposure are recommended, they may not be that necessary for all LOHC structures, especially those that are easily degraded in the environment (European Chemicals Agency, 2016). In addition, studies on organisms at the single-generation level may not address the long-term effects from the population level (Paumen *et al.*, 2008); therefore, tests established over multigenerations are recommended if possible.

V. Transformation products

Organic compounds are commonly transformed in the environment by either biotic or non-biotic processes (see introduction and **Figure 3**). The establishment of relevant studies depends on the conditions of the exposure scenario of concern.

Derivatives produced in these processes as either “degradation products” or “metabolites” (due to digestion by organisms) usually exhibit distinct physicochemical properties and thus show different adsorption behaviors, bioavailabilities, mode of toxic action, and toxic effects from their parent compounds. For example, the degradation products and derivatives of PAHs can be actively leached at a higher degree than the PAH parents (Srogi, 2007). These transformed compounds should be considered in the environmental hazard assessment for long-term effects of the parental chemicals, particularly when such transformation products are stable and/or treated as hazardous (European Chemicals Agency, 2016).

VI. Semi-field or field tests

The heterogeneous nature of soil in the realistic environment will lead to widely varying adsorption behaviors and toxicities. Pore-water mediated uptake can be modified by soil aging (B. Maliszewska-Kordybach, 2005) (Tsibart and Gennadiev, 2013) and speciation (Orecchio and Mannino, 2010) (Zhang and Fan, 2016). Consequently, further confirmation of the validity of the equilibrium partitioning theory is necessary for distinct environmental scenarios. The extrapolation of results from laboratory conditions to *in situ* environments should be carefully performed. Because of the high complexity of semi-field and field experiments, such tests are recommended only if data collected from previous laboratory studies have shown fundamental toxicity, retention and/or persistence. In addition, field-based investigations could start with the most promising candidate LOHC structures for industrial applications.

VII. Maintaining pace with the technological advancements of LOHCs

LOHC technology is still at an early stage of development; therefore, many studies are focused on improving its technical performance. New candidates or structures of attractive potentials are being continuously discovered. Therefore, currently promising structures, such as those studied in this thesis, may be outcompeted and replaced by new structures in the future. As such, environmental hazard assessment of LOHC systems must maintain the pace of the technological development of these structures through good cooperation with institutes of technology research and development and the industries that are producing or employing this technology. In addition, environmental hazard assessment should be performed by following updated international rules for appropriate chemical risk management and monitoring. Although new LOHC structures may emerge in the future, this thesis provides fundamental information to serve as a prototype for environmental hazard assessment of the chemicals of this type.

VII Appendix

A.1 Pros and cons of renewable energy

Renewable energy (RE) sources are considered paramount for supporting the current and future energy demand. For example, the annual solar energy input in the Sahara Desert is 8280 EJ per million km^2 and the electrical output is potentially 414 EJ per million km^2 based on 50% coverage of the collector surface and 10% conversion efficiency (Moriarty and Honnery, 2012). By 2050, the total electric energy produced from RE is estimated to be 34 EJ per annum (a), which is less than 0.003% of the sunlight that reaches land and less than 0.1% of the output from wind (Kalogirou, 2009). Furthermore, the RE from biomass is predicted to be 206 EJ/a by 2050, which is less than one tenth of what plants can convert from solar energy (3800 EJ/a). RE resources are considered to have the potential to present significant contributions to the energy needs throughout the world while reducing the energy dependence on fossil fuels. RE resources are particularly attractive because of their significantly lower GHS emissions compared with fossil fuels. Wind has the lowest CO_2 emissions (25 g kWh^{-1} CO_2), followed by hydropower and PHV (< 100 g kWh^{-1} CO_2) (Evans, Strezov and Evans, 2009). In the BLUE map scenario estimated by the Energy Technology Perspective (ETP) of the International Energy Agency, wind power, biomass, solar PHV, geothermal and hydropower contribute to 12%, 8%, 7%, 3% and 2% reductions of CO_2 emissions, respectively (Islam, Mekhilef and Saidur, 2013).

The energy supplied by RE systems is however intermittently produced, which represents an obstacle to its broad application worldwide. The storage of PHV is highly limited at night and during cloudy days (Evans, Strezov and Evans, 2009), and it is predicted that cloud cover will increase by as much as several percentages at latitudes higher than 50°N and 50°S (Patt, Pfenninger and Lilliestam, 2010). Moreover, wind power is limited because wind speeds must not be too high (> 25 m s^{-1}) or too low (< 3 m s^{-1}) (Evans, Strezov and Evans, 2009). In Germany, for example, produced wind energy is typically 3.5 TWh per month in the winter (in 2003–2009), although it decreases to nearly half during summer (Eberle, Müller and von Helmolt, 2012). Furthermore, global climate change is also influencing the total output of RE resources. Solar energy output suffers from sulfate aerosols that occur in the lower stratosphere because of the scatter of direct light, and every 1% reduction in the sunlight reaching the earths' surface generally results in a 4–10% output loss (Murphy, 2009). By the end of the century in the northwestern US, wind energy is anticipated to decrease by up to 40% due to the reduced temperature differential between the polar regions and lower latitudes (a consequence of continued global warming) (Sailor, Smith and Hart, 2008).

A.2 Brief review on the effects of typical PAHs on the reproduction of F. candida

Table AI. Literature screening on the EC_{50} (Part A) and EC_{10} (Part B) values (mg kg^{-1} dw soil) of PAHs and N-PAHs specifically toward the reproduction of *F. candida* over 28 days.

Part A ------ EC_{50} values						
No.	**Chemicals**	**Soil**	**Log K_{ow}**	**Log K_{oc}**	**Effective conc.**	**Ref.**
PAHs						
1	Naphthalene	LUFA 2.2‡	3.47	3.47 or 2.72	31.85	c.
2	Anthracene	LUFA 2.2	4.53	4.46 or 4.32	679.77	c.
3	Phenanthrene	LUFA 2.2	4.48	4.58 or 4.22	45.81	c.
		Soil (10% OM) ‡	4.48	4.58 or 4.22	124.00	d.
4	Pyrene	LUFA 2.2	4.84	4.34 or 5.02	21.24	c.
5	Benz[a]anthracene	LUFA 2.2	5.54	5.62 or 5.12	991.92	c.
6	Benzo[a]pyrene	LUFA 2.2	6.02	6.27	931.06	c.
N-PAHs						
7	Quinoline	LUFA 2.2	2.23	2.89 or 2.52	75.04	c.
		OECD‡	2.03	1.74	230.00	e.
8	Acridine	LUFA 2.2	3.27	4.00 or 4.12	312.74	c.
		OECD	3.4	2.85	1212.00	e.
9	Phenanthridine	LUFA 2.2	3.44	4.06 or 3.82	37.28	c.
10	9(10H)-acridone	LUFA 2.2	1.69 or 2.952	2.0123[a]	437.68	f.
11	6(5H)-phenanthridinone	LUFA 2.2	2.84 or 2.702	1.3933[a]	399.42	f.
13	1,10-Phenanthroline	OECD	1.78	1.54	928.00	e.
Part B ------ EC_{10} values						
No.	**Chemicals**	**Soil**	**Log K_{ow}**	**Log K_{oc}**	**Effective conc.**	**Ref.**
PAHs						
1	Naphthalene	Contam. soil‡	3.4	3.47 or 2.72	0.17	g.
2	Anthracene	Contam. soil	4.5	4.46 or 4.32	5.60	g.
3	Phenanthrene	LUFA 2.2	4.48	4.58 or 4.22	24.95	f.
		Contam. soil	4.6	4.58 or 4.22	11	g.
4	Pyrene	LUFA 2.2	4.84	4.34 or 5.02	11.33	f.
		Contam. soil	5.2	4.34 or 5.02	11	g.
5	Benz[a]anthracene	Contam. soil	5.6	5.62 or 5.12	7	g.
6	Benzo[a]pyrene	Contam. soil	6.5	6.27	4.4	g.

Appendix

Part B ------ EC_{10} values						
No.	**Chemicals**	**Soil**	**Log K_{ow}**	**Log K_{oc}**	**Effective conc.**	**Ref.**
N-PAHs						
7	Quinoline	LUFA 2.2	2.23	2.89 or 2.52	60.58	f.
		OECD	2.03	1.74	118	e.
8	Acridine	LUFA 2.2	3.27	4.00 or 4.12	174.38	f.
		OECD	3.4	2.85	600	e.
9	Phenanthridine	LUFA 2.2	3.44	4.06 or 3.82	20.79	f.
12	Phenazine	OECD	2.84	2.4	162	e.
13	1,10-Phenanthroline	OECD	1.78	1.54	701	e.
14	Fluorene	Contam. soil	4.2	3.6274[a] or 4.0[b]	3.1	g.
15	Fluoranthene	Contam. soil	5.2	4.478[a] or 4.5[b]	17	g.
16	Acenaphtylene	Contam. soil	4.1	3.4191[a] or 3.72[b]	0.14	g.
17	Acenaphtene	Contam. soil	3.9	3.4018[a]	1.38	g.
18	Chrysene	Contam. soil	5.9	5.0421[a]	5.9	g.
19	Benzo[b]-fluoranthene	Contam. soil	6.1	5.0161[a]	4.4	g.
20	Dibenzo[ah]-anthracene	Contam. soil	6.5	5.6757[a] or 5.76[b]	0.45	g.
21	Benzo[ghi]perylene	Contam. soil	7.1	5.8145[a]	1.95	g.
22	Indeno[1,2,3-cd]-pyrene	Contam. soil	6.6	5.8145[a] or 6.3[b]	2.5	g.
23	Benzo[k]-fluoranthene	Contam. soil	6.8	5.3025[a] or 7.36[b]	2.7	g.

‡ **LUFA 2.2 soil** (Bleeker *et al.*, 2003): sandy loam soil (particle size distribution: 50–2000 µm, 75.3%; 2–50 µm, 16.6%; and < 2 µm, 8.1%) with the OC of 2.3% ± 0.2%, and $pH_{(CaCl2)}$ 5.6 ± 0.4.

‡ **OECD soil** (Kobetičová *et al.*, 2008): mixture of 70% sand, 20% kaolin clay and 10% finely ground sphagnum peat. The OC is 4.68% with $pH_{(KCl)}$ 6.0.

‡ **Contaminated soil** (Eom *et al.*, 2007): sandy loam soil constituted of 67% sand, 22% silt, and 11% clay. Sampling from a site polluted with cokery residues and PAHs.

[a] Predicted by EPI. [b] Predicted by Hazardous Substance Data Bank (U.S. National Library of Medicine)[*]. [c] (Bleeker *et al.*, 2003). [d] (Doubern, 2003). [e] (Kobetičová *et al.*, 2008). [f] (Droge *et al.*, 2006). [g] (Eom *et al.*, 2007).

[*] Hazardous Substance Data Bank: https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm.

A.3 Additional research on IONP

From April 2013 to April 2015, a parallel study on iron oxide nanoparticles was scheduled and conducted. This study sought to answer similar questions that were posed with regard to the environmental hazards of novel chemicals as what had been proposed in this thesis for the LOHCs, including the ecotoxicity, potential behavior and bioavailability of iron oxide nanoparticles. The work aimed to understand the effects and colloidal stability of DMSA (dimercaptosuccinate)-coated (DMSA-IONP) and uncoated magnetic iron oxide nanoparticles (IONP) in the aquatic environment.

This research was conducted in cooperation by the research group of Prof. Dr. Ralf Dringen (CBIB - Centre for Biomolecular Interactions Bremen, Neurobiochemistry, Faculty 2 (Biology/Chemistry), University of Bremen, Germany). The work was published in the journal "Environmental Science: Nano" in 2016.

"**Zhang, Y.-Q.**, Dringen, R., Petters, C., Rastedt, W., Köser, J., Filser, J. and Stolte, S. (2016) '**Toxicity of dimercaptosuccinate-coated and un-functionalized magnetic iron oxide nanoparticles towards aquatic organisms**', *Environ. Sci.: Nano*. Royal Society of Chemistry, 3(4), pp. 754–767. doi: 10.1039/C5EN00222B."

As a brief introduction to this research, DMSA-IONP and uncoated IONP are anthropogenic nanoparticles with promising potential for application in medical treatments and environmental remediation. However, the consequences of these substances in the aquatic environment are unknown. The hazards that these chemicals might pose must be determined, particularly with regard to the amount of IONP released through their use as adsorbents of pollutants in environmental governance. Synthesized coated and uncoated IONP were studied to investigate the following:

(i) effects of DMSA-coated and uncoated IONP (fresh and aged) on green algae (*Raphidocelis subcapitata*), duckweed (*Lemna minor*) and water fleas (*Daphnia magna*), with the data compared with the effects of free iron ions to determine the mechanisms of toxicity; and

(ii) colloidal stability of these IONPs by considering a series of factors, such as the coating, concentration, ionic strength of different test media, and pH. The speciation in test media was also considered and evaluated.

Overall, the EC_{50} values for each test organism were obtained for the three types of IONPs. Test organisms demonstrated different sensitivities to the IONP, and algae *R. subcapitata* was the most sensitive organism to IONP. Free iron ions stimulated the growth of algae over wide concentration ranges. Therefore the adverse effects were assumed to be representative of toxicity induced by the flocculation of algae cells. The accumulation of IONP was also observed in daphnids. Increased amounts of

divalent cations significantly decreased the colloidal stability of DMSA-IONP and uncoated IONP; for the latter, pH effects were also evident. Nonetheless, DMSA-IONP generally had higher colloidal stability in the different test conditions due to the coatings. By combining the data obtained from the ecotoxicity and colloidal stability measurements, the bioavailability of these IONP to the test aquatic organisms was evaluated. The different amounts of IONP used in applications should be considered to reduce the impact of these substances in the environment.

Contributions of Zhang, Y. Q.:

- Performance of all experiments concerning to the culturing of organisms, ecotoxicity, characterization of the nanoparticles in terms of colloidal stability (at different pH and in different media);
- Data processing and all statistical analysis;
- Preparation of all Figures and Tables; and
- Manuscript preparation and writing.

Dringen, R. provided advice that contributed to the development of this project. The stock suspensions of the iron oxide nanoparticles were synthesized in his research group by Petters, C. and Rastedtm W.. Petters, C. provided the structural picture “Figure 1 B”. Köser, J. conducted the theoretical calculations of the ionic strength of the three media that are presented in Table 3 and the computational simulation of the speciation of iron ions in the standard iron-free algae medium by PHREEQCi (shown in Table § 4 in the supporting information). He also provided the text on dynamic light scattering (in section 2.4.1 in the paper), zeta-potential measurements (in section 2.4.2 in the paper) and iron species calculations (the text following Table § 4 in the paper). Filser, J. provided comments and suggestions for the manuscript. Stolte, S. guided the research and development of the manuscript. Each co-author contributed to the revisions of the manuscript.

A.4 Narcosis and state-of-the-art of research

Narcosis refers to the changes in the properties and disruptions of the functions in biological membranes induced by chemically inert compounds (Schwarzenbach, Gschwend and Imboden, 2003). This process most likely depends on the space that the present compounds occupy in the membrane rather than the chemical structures; therefore, it is considered to have nonspecific toxicity (Schwarzenbach, Gschwend and Imboden, 2003). The consequent toxicity would cause the deterioration of associated processes, such as photosynthesis, membrane-mediated transport, energy transduction, enzyme activities and nerve impulse transmission (Schwarzenbach, Gschwend and Imboden, 2003).

The specific extrapolation of the narcosis of LOHCs based on experimental-derived QSARs with respect to K_{ow} or log D is preferable. Although many studies have focused on the QSARs for PAHs and N-PAHs, which share similar chemical structures with LOHCs, the results were not applicable for this thesis. For example, survival or reproduction inhibition was investigated with other soil organisms than *F. candida* (28 days), such as with *Folsomia fimetaria* (21 days) (Sverdrup, Nielsen and Krogh, 2002), *Eisenia veneta* (28 days) (Sverdrup *et al.*, 2002) or *E. crypticus* (28 days) (Droge *et al.*, 2006) (Kobetičová *et al.*, 2011). Moreover, the log K_{ow} values applied for certain studies covered only values higher than 3, signifying that indoles and quinaldines are excluded from the prediction capacity of the proposed models. In previous studies conducted by (Bleeker *et al.*, 2003) and (Droge *et al.*, 2006), the narcotic effect of PAHs and N-PAHs on the reproduction of *F. candida* (28 days) was established and presented log K_{ow} values between 2 and 6. However, because specific equations were not shown, the data could not be applied to specific calculations. The same problem was also found in studies by (Giesen, Jonker and van Gestel, 2012) and (Kobetičová *et al.*, 2011). In addition, the narcotic effect on *F. candida* proposed by (Bleeker *et al.*, 2003) was derived from the aquatic organism *Chironomus riparius*; therefore, this correlation may be a speculative, although the authors indicated a good fit for the narcosis between aquatic and soil organisms in their study. One of the most important problems associated with these proposed narcosis models is that the number of compounds (usually from 8 to 11) used to establish the QSARs was often too limited to produce statistically reliable correlations. Therefore, an information gap is observed regarding the known relationship between the narcotic mode of action and hydrophobicity of PAHs or N-PAHs, or more specifically, LOHCs.

VIII References

Abas, N., Kalair, A. and Khan, N. (2015) 'Review of fossil fuels and future energy technologies', *Futures*. Elsevier Ltd, 69, pp. 31–49. doi: 10.1016/j.futures.2015.03.003.

Ahtiainen, J. (2002) *Microbiological tests and measurements in the assessment of harmful substances and pollution*, *Monographs of the boreal environment reasearch*.

Akella, A. K., Saini, R. P. and Sharma, M. P. (2009) 'Social, economical and environmental impacts of renewable energy systems', *Renewable Energy*. Elsevier Ltd, 34(2), pp. 390–396. doi: 10.1016/j.renene.2008.05.002.

Amende, M., Gleichweit, C., Schernich, S., Höfert, O., Lorenz, M. P. A., Zhao, W., Koch, M., Obesser, K., Papp, C., Wasserscheid, P., Steinrück, H. P. and Libuda, J. (2014) 'Size and structure effects controlling the stability of the liquid organic hydrogen carrier dodecahydro-N-ethylcarbazole during dehydrogenation over Pt model catalysts', *Journal of Physical Chemistry Letters*, 5(8), pp. 1498–1504. doi: 10.1021/jz500157r.

Andrews, J. and Shabani, B. (2012) 'Re-envisioning the role of hydrogen in a sustainable energy economy', *International Journal of Hydrogen Energy*. Elsevier Ltd, 37(2), pp. 1184–1203. doi: 10.1016/j.ijhydene.2011.09.137.

Anyanwu, I. N. and Semple, K. T. (2015a) 'Fate and behaviour of nitrogen-containing polycyclic aromatic hydrocarbons in soil', *Environmental Technology and Innovation*. Elsevier B.V., 3, pp. 108–120. doi: 10.1016/j.eti.2015.02.006.

Anyanwu, I. N. and Semple, K. T. (2015b) 'Phytotoxicity of phenanthrene and its nitrogen polycyclic aromatic hydrocarbon analogues in ageing soil', *Water, Air, and Soil Pollution*, 226(10). doi: 10.1007/s11270-015-2589-x.

Anyanwu, I. N. and Semple, K. T. (2016) 'Effects of phenanthrene and its nitrogen-heterocyclic analogues aged in soil on the earthworm *Eisenia fetida*', *Applied Soil Ecology*. Elsevier B.V., 105, pp. 151–159. doi: 10.1016/j.apsoil.2016.04.014.

B. Maliszewska-Kordybach (2005) 'Dissipation of polycyclic aromatic hydrocarbons in freshly contaminated soils – the effect of soil', *Water, Air and Soil Pollution*, 168(1–4), pp. 113–128. Available at: https://link.springer.com/article/10.1007%2Fs11270-005-0940-3?LI=true.

Bandow, C., Coors, A., Karau, N. and Römbke, J. (2014) 'Interactive effects of lambda-cyhalothrin, soil moisture, and temperature on *Folsomia candida* and *Sinella curviseta* (Collembola)', *Environmental Toxicology and Chemistry*, 33(3), pp. 654–661. doi: 10.1002/etc.2479.

Baumann, J., Sakka, Y., Bertrand, C., Köser, J. and Filser, J. (2014) 'Adaptation of the *Daphnia sp.* acute toxicity test: miniaturization and prolongation for the testing of nanomaterials.', *Environmental science and pollution research*, 21(3), pp. 2201–13. doi: 10.1007/s11356-013-2094-y.

Bi, E., Schmidt, T. C. and Haderlein, S. B. (2007) 'Environmental factors influencing sorption of heterocyclic aromatic compounds to soil', *Environ Sci Technol*, 41(9), pp. 3172–3178. doi: Doi 10.1021/Es0623764.

Biniwale, R. B., Rayalu, S., Devotta, S. and Ichikawa, M. (2008) 'Chemical hydrides: a solution to high capacity hydrogen storage and supply', *International Journal of Hydrogen Energy*, 33(1), pp. 360–365. doi: 10.1016/j.ijhydene.2007.07.028.

Bleeker, E. A. J., Wlegman, S., Droge, S. T. J., Kraak, M. H. S. and Van Gestel, C. A. M. (2003) 'Towards an improvement of the risk assessment of polycyclic (hetero)aromatic hydrocarbons', *Report*, (1), pp. 27–32.

Boethling, R., Fenner, K., Howard, P., Klečka, G., Madsen, T., Snape, J. R. and Whelan, M. J. (2009) 'Environmental persistence of organic pollutants: guidance for development and review of POP risk profiles', *Integrated Environmental Assessment and Management*, 5(4), pp. 539–556. doi: 10.1897/IEAM_2008-090.1.

Chiou, C. T., McGroddy, S. E. and Kile, D. E. (1998) 'Partition characteristics of polycyclic aromatic hydrocarbons on soils and sediments', *Environ. Sci. Technol.*, 32(2), pp. 264– 269. doi: 10.1021/es970614c.

Clot, E., Eisenstein, O. and Crabtree, R. H. (2007) 'Computational structure–activity relationships in H_2 storage: how placement of N atoms affects release temperatures in organic liquid storage materials', *Chem. Commun.*, (22), pp. 2231–2233. doi: 10.1039/B705037B.

Coleman, D. C., Crossley, D. A. and Hendrix, P. F. (2004) *Fundamentals of Soil Ecology*. second, *Fundamentals of Soil Ecology*. second. Edited by David Cella. San Diego, California: Elsevier Academic press. doi: 10.1016/B978-012179726-3/50009-5.

Covert, T., Greenstone, M. and Knittel, C. R. (2016) 'Will we ever stop using fossil fuels?', *The Journal of Economic Perspectives*, 30(1), pp. 117–137. doi: 10.2139/ssrn.2720633.

Crabtree, R. H. (2008) 'Hydrogen storage in liquid organic heterocycles', *Energy & Environmental Science*, 1(1), p. 134. doi: 10.1039/b805644g.

Crabtree, R. H. (2017) 'Nitrogen-containing liquid organic hydrogen carriers: progress and prospects', *ACS Sustainable Chemistry and Engineering*, 5(6), pp. 4491–4498. doi: 10.1021/acssuschemeng.7b00983.

Cristhy, A., Carina, J., Elizabeth, M., Correia, F. and Silva-filho, E. V. (2016) 'Ecotoxicity of mercury to *Folsomia candida* and *Proisotoma minuta* (Collembola : Isotomidae) in tropical soils : baseline for ecological risk assessment', *Ecotoxicology and Environmental Safety*. Elsevier, 127, pp. 22–29. doi: 10.1016/j.ecoenv.2016.01.009.

Crotogino, F. and Hamelmann, R. (2007) 'Wasserstoff-Speicherung in Salzkavernen zur Glättung des Windstromangebots', in *Symposium zur Nutzung regenerativer Energiequellen und Waqsserstofftechnik*.

Crouau, Y., Chenon, P. and Gisclard, C. (1999) 'The use of *Folsomia candida* (Collembola, Isotomidae) for the bioassay of xenobiotic substances and soil pollutants', *Applied Soil Ecology*, 12(2), pp. 103–111. doi: 10.1016/S0929-1393(99)00002-5.

Cui, Y., Kwok, S., Bucholtz, A., Davis, B., Whitney, R. A. and Jessop, P. G. (2008) 'The effect of substitution on the utility of piperidines and octahydroindoles for reversible hydrogen storage', *New Journal of Chemistry*, 32(6), pp. 1027–1037. doi: Doi 10.1039/B718209k.

Čvančarová, M., Křesinová, Z. and Cajthaml, T. (2013) 'Influence of the bioaccessible fraction of polycyclic aromatic hydrocarbons on the ecotoxicity of historically contaminated soils', *Journal of Hazardous Materials*, 254–255(1), pp. 116–124. doi: 10.1016/j.jhazmat.2013.03.060.

Dalebrook, A. F., Gan, W., Grasemann, M., Moret, S. and Laurenczy, G. (2013) 'Hydrogen storage: beyond conventional methods', *Chemical Communications*, 49(78), p. 8735. doi: 10.1039/c3cc43836h.

Dean, D., Davis, B. and Jessop, P. G. (2011) 'The effect of temperature, catalyst and sterics on the

rate of N-heterocycle dehydrogenation for hydrogen storage', *New Journal of Chemistry*, 35, pp. 417–422. doi: 10.1039/c0nj00511h.

DIN (2002) 'German standard methods for the examination of water, waste water and sludge bio-assays (group L) — Toxicity test with *Arthrobacter globiformis* for contaminated solids (L48). DIN 38412 L48.', *Deutsches Institut für Normung*. Berlin.

Doelman, P., Nieboer, G., Schrooten, J. and Visser, M. (1984) 'Antagonistic and synergistic toxic effects of Pb and Cd in a simple foodchain: nematodes feeding on bacteria or fungi', *Bulletin of Environmental Contamination and Toxicology*, 32(1), pp. 717–723. Available at: https://link.springer.com/article/10.1007%2FBF01607562?LI=true.

Domene, X., Alcañiz, J. M. and Andrés, P. (2007) 'Ecotoxicological assessment of organic wastes using the soil collembolan *Folsomia candida*', *Applied Soil Ecology*, 35(3), pp. 461–472. doi: 10.1016/j.apsoil.2006.10.004.

Dong, Y., Yang, M., Yang, Z., Ke, H. and Cheng, H. (2015) 'Catalytic hydrogenation and dehydrogenation of N-ethylindole as a new heteroaromatic liquid organic hydrogen carrier', *International Journal of Hydrogen Energy*. Elsevier Ltd, 40(34), pp. 10918–10922. doi: 10.1016/j.ijhydene.2015.05.196.

Doubern, P. E. T. (2003) *PAHs: An ecotoxicological Perspective*, *John Wiley & Sons*. Chichester. doi: 10.1002/0470867132.ch16.

Droge, S. T. J., Paumen, M. L., Bleeker, E. A. J., Kraak, M. H. S. and Van Gestel, C. A. M. (2006) 'Chronic toxicity of polycyclic aromatic compounds to the springtail *Folsomia candida* and the enchytraeid *Enchytraeus crypticus*', *Environmental toxicology and chemistry*, 25(9), pp. 2423–2431. doi: 10.1897/05-628R.1.

Dufour, J., Serrano, D. P., Gálvez, J. L., Moreno, J. and García, C. (2009) 'Life cycle assessment of processes for hydrogen production. Environmental feasibility and reduction of greenhouse gases emissions', *International Journal of Hydrogen Energy*, 34(3), pp. 1370–1376. doi: 10.1016/j.ijhydene.2008.11.053.

Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K. H. and Stackebrandt, E. (2006) 'The Prokaryotes. A handbook on the biology of bacteria', *Springer, New York*.

e Silva, C. de L., Brennan, N., Brouwer, J. M., Commandeur, D., Verweij, R. A. and Gestel, C. A. M. Van (2017) 'Comparative toxicity of imidacloprid and thiacloprid to different species of soil invertebrates', *Ecotoxicology*. Springer US, 26(4), pp. 555–564. doi: 10.1007/s10646-017-1790-7.

Eberle, U., Felderhoff, M. and Schüth, F. (2009) 'Chemical and physical solutions for hydrogen storage', *Angewandte Chemie - International Edition*, 48(36), pp. 6608–6630. doi: 10.1002/anie.200806293.

Eberle, U., Müller, B. and von Helmolt, R. (2012) 'Fuel cell electric vehicles and hydrogen infrastructure: status 2012', *Energy & Environmental Science*, 5(10), pp. 8780–8798. doi: 10.1039/c2ee22596d.

Eblagon, K. M., Rentsch, D., Friedrichs, O., Remhof, A., Zuettel, A., Ramirez-Cuesta, A. J. and Tsang, S. C. (2010) 'Hydrogenation of 9-ethylcarbazole as a prototype of a liquid hydrogen carrier', *International Journal of Hydrogen Energy*. Elsevier Ltd, 35(20), pp. 11609–11621. doi: 10.1016/j.ijhydene.2010.03.068.

ECETOC (1990) 'Technical Report 040 – Hazard Assessment of Chemical Contaminants in Soil'. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals, pp. 1–136.

ECETOC (1992) 'Technical Report No.50 – Estimating Environmental Concentrations of Chemicals using Fate and Exposure'. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals, pp. 1–90.

ECETOC (1993) 'Technical Report No. 51 – Environmental Hazard Assessment of Substances'. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals, pp. 1–103.

Eisentraeger, A., Brinkmann, C., Hollert, H., Sagner, A., Tiehm, A. and Neuwoehner, J. (2008) 'Heterocyclic compounds: toxic effects using algae, daphnids, and the *Salmonella*/microsome test taking methodical quantitative aspects into account.', *Environmental toxicology and chemistry*, 27(7), pp. 1590–1596. doi: 10.1897/07-201.1.

Engelke, M., Köser, J., Hackmann, S., Zhang, H., Mädler, L. and Filser, J. (2014) 'A miniaturized solid contact test with *Arthrobacter globiformis* for the assessment of the environmental impact of silver nanoparticles', *Environmental Toxicology and Chemistry*, 33(5), pp. 1142–1147. doi: 10.1002/etc.2542.

Eom, I. C., Rast, C., Veber, A. M. and Vasseur, P. (2007) 'Ecotoxicity of a polycyclic aromatic hydrocarbon (PAH)-contaminated soil', *Ecotoxicology and Environmental Safety*, 67(2), pp. 190–205. doi: 10.1016/j.ecoenv.2006.12.020.

European Chemicals Agency (2008) 'Guidance on information requirements and chmical safety assessment. Chapter R.10: characterisation of dose [concentration]-response for environment', *European Chemicals Agency*, (May), pp. 1–65.

European Chemicals Agency (2011) 'Guidance on information requirements and chemical safety assessment Part B: Hazard assessment', (December), pp. 1–68. Available at: http://echa.europa.eu/documents/10162/13643/information_requirements_part_b_en.pdf .

European Chemicals Agency (2012) *Guidance on Information Requirements and Chemical Safety Assessment - Part E: Risk Characterisation*, *European Chemicals Agency*.

European Chemicals Agency (2015) 'Introductory Guidance on the CLP Regulation', *European Chemicals Agency*, (August), pp. 1–102.

European Chemicals Agency (2016) 'Guidance on information requirements and chemical safety assessment Chapter R. 16: Environmental exposure assessment', *European Chemicals Agency*, (February), pp. 1–24.

European Commission (2004) 'Review report for the active substance alpha-cypermethrin.SAN-CO/4335/2000 Final Report.' Brussels.

Evans, A., Strezov, V. and Evans, T. J. (2009) 'Assessment of sustainability indicators for renewable energy technologies', *Renewable and Sustainable Energy Reviews*, 13(5), pp. 1082–1088. doi: 10.1016/j.rser.2008.03.008.

Feldmannová, M., Hilscherová, K., Maršálek, B. and Bláha, L. (2006) 'Effects of N-heterocyclic polyaromatic hydrocarbons on survival, reproduction, and biochemical parameters in *Daphnia magna*', *Environmental toxicology*, 21(4), pp. 425–431. doi: 10.1002/tox.20198.

Fikrt, A., Brehmer, R., Milella, V. O., Müller, K., Bösmann, A., Preuster, P., Alt, N., Schlücker, E., Wasserscheid, P. and Arlt, W. (2017) 'Dynamic power supply by hydrogen bound to a liquid organic hydrogen carrier', *Applied Energy*, 194, pp. 1–8. doi: 10.1016/j.apenergy.2017.02.070.

Filser, J., Wiegmann, S. and Schröder, B. (2014) 'Collembola in ecotoxicology – any news or just boring routine?', *Applied Soil Ecology*. Elsevier B.V., 83, pp. 193–199. doi:

10.1016/j.apsoil.2013.07.007.

Fountain, M. T. and Hopkin, S. P. (2005) '*Folsomia candida* (Collembola): a "standard" soil arthropod', *Annual Review of Entomology*, 50(1), pp. 201–222. doi: 10.1146/annurev.ento.50.071803.130331.

Geburtig, D., Preuster, P., Bösmann, A., Müller, K. and Wasserscheid, P. (2016) 'Chemical utilization of hydrogen from fluctuating energy sources – catalytic transfer hydrogenation from charged Liquid Organic Hydrogen Carrier systems', *International Journal of Hydrogen Energy*, 41(2), pp. 1010–1017. doi: 10.1016/j.ijhydene.2015.10.013.

Geisler, A., Oemisch, L., Endo, S. and Goss, K. U. (2015) 'Predicting storage-lipid water partitioning of organic solutes from molecular structure', *Environmental Science and Technology*, 49(9), pp. 5538–5545. doi: 10.1021/es506336m.

Van Gestel, C. A. M. (1997) 'Scientific basis for extrapolating results from soil ecotoxicity tests to field conditions and the use of bioassays', in *Ecological risk assessment of contaminants in soil*. Springer, pp. 25–50. Available at: https://link.springer.com/chapter/10.1007/978-1-4615-6361-7_2.

Gevao, B., Mordaunt, C., Semple, K. T., Piearce, T. G. and Jones, K. C. (2001) 'Bioavailability of nonextractable (bound) pesticide residues to earthworms', *Environmental Science and Technology*, 35(3), pp. 501–507. doi: 10.1021/es000144d.

GHS (2011) 'Globally harmonized system of classification and labelling of chemicals (GHS) (4th Edition)', *United Nations*. New York.

Giesen, D. and Van Gestel, C. A. M. (2013) 'QSAR development and bioavailability determination: the toxicity of chloroanilines to the soil dwelling springtail *Folsomia candida*', *Chemosphere*, 90(11), pp. 2667–2673. doi: 10.1016/j.chemosphere.2012.11.045.

Giesen, D., Jonker, M. T. O. and van Gestel, C. A. M. (2012) 'Development of QSARs for the toxicity of chlorobenzenes to the soil dwelling springtail *Folsomia candida*', *Environmental Toxicology and Chemistry*, 31(5), pp. 1136–1142. doi: 10.1002/etc.1805.

Giordano, R., Weber, E., Waite, J., Bencivenga, N., Krogh, P. H. and Soto-Adames, F. (2010) 'Effect of a high dose of three antibiotics on the reproduction of a parthenogenetic strain of *Folsomia candida* (Isotomidae: Collembola)', *Environmental Entomology*, 39(4), pp. 1170–1177. doi: 10.1603/EN10027.

Grasemann, M. and Laurenczy, G. (2012) 'Formic acid as a hydrogen source – recent developments and future trends', *Energy & Environmental Science*, 5(8), pp. 8171–8181. doi: 10.1039/c2ee21928j.

Greenland, D. J. (1980) 'Soil organic matter', *Field Crops Research*, 3, pp. 97–98. doi: 10.1016/0378-4290(80)90012-X.

Grünenfelder, N. F. and Schucan, T. H. (1989) 'Seasonal storage of hydrogen in liquid organic hydrides: description of the second prototype vehicle', *International journal of hydrogen energy*. Elsevier, 14(8), pp. 579–586. Available at: http://www.sciencedirect.com/science/article/pii/0360319989901171.

Hartmann, N. B., Stefania, G. and Sokull-Klüttgen, B. (2014) *Review of available criteria for non-aquatic organisms within PBT/vPvB frameworks Part II : Toxicity assessment*. Luxembourg: European Union, 2014. doi: 10.2788/989.

Hartnik, T., Sverdrup, L. E. and Jensen, J. (2008) 'Toxicity of the pesticide alpha-cypermethrin to four soil nontarget invertebrates and implications for risk assessment', *Environmental*

Toxicology and Chemistry, 27(6), pp. 1408–1415. Available at: http://onlinelibrary.wiley.com/doi/10.1897/07-385.1/full.

Häussinger, P., Lohmüller, R. and Watson, A. M. (2000) 'Hydrogen', *Ullmann's encyclopedia of industrial chemistry*. Wiley Online Library. Available at: http://onlinelibrary.wiley.com/doi/10.1002/14356007.a13_297/full.

Hentati, O., Lachhab, R., Ayadi, M. and Ksibi, M. (2013) 'Toxicity assessment for petroleum-contaminated soil using terrestrial invertebrates and plant bioassays', *Environmental Monitoring and Assessment*, 185(4), pp. 2989–2998. doi: 10.1007/s10661-012-2766-y.

Hodoshima, S., Arai, H., Takaiwa, S. and Saito, Y. (2003) 'Catalytic decalin dehydrogenation/naphthalene hydrogenation pair as a hydrogen source for fuel-cell vehicle', *International Journal of Hydrogen Energy*, 28(11), pp. 1255–1262. doi: 10.1016/S0360-3199(02)00250-1.

Hodoshima, S., Takaiwa, S., Shono, A., Satoh, K. and Saito, Y. (2005) 'Hydrogen storage by decalin/naphthalene pair and hydrogen supply to fuel cells by use of superheated liquid-film-type catalysis', *Applied Catalysis A: General*, 283(1–2), pp. 235–242. doi: 10.1016/j.apcata.2005.01.010.

Houx, N. W. H., Dekker, A., Van Kammen-Polman, A. M. M. and Ronday, R. (1996) 'Acute toxicity test for terrestrial hazard assessment with exposure of *Folsomia candida* to pesticides in an aqueous medium', *Archives of Environmental Contamination and Toxicology*, 30(1), pp. 9–14. doi: 10.1007/s002449900002.

Hu, P., Fogler, E., Diskin-Posner, Y., Iron, M. A. and Milstein, D. (2015) 'A novel liquid organic hydrogen carrier system based on catalytic peptide formation and hydrogenation', *Nature Communications*. Nature Publishing Group, 6, p. 6859. doi: 10.1038/ncomms7859.

Ibrahim, H., Ilinca, A. and Perron, J. (2008) 'Energy storage systems-characteristics and comparisons', *Renewable and Sustainable Energy Reviews*, 12(5), pp. 1221–1250. doi: 10.1016/j.rser.2007.01.023.

IEA (2007) *World Energy Outlook 2007: China and India Insights*, *IEA Publications*. Available at: www.iea.org/Textbase/%5Cnhttp://www.iea.org/publications/freepublications/publication/weo_2007.pdf.

IEA (2010) 'World Energy Outlook 2010', *Oraginasiton for Economic Co-operation and Development*, (November), p. 23. Available at: http://www.oecd.org/berlin/46389140.pdf.

Islam, M. R., Mekhilef, S. and Saidur, R. (2013) 'Progress and recent trends of wind energy technology', *Renewable and Sustainable Energy Reviews*. Elsevier, 21, pp. 456–468. doi: 10.1016/j.rser.2013.01.007.

Jones, G. A. and Warner, K. J. (2016) 'The 21st century population-energy-climate nexus', *Energy Policy*. Elsevier, 93, pp. 206–212. doi: 10.1016/j.enpol.2016.02.044.

Jungnickel, C., Mrozik, W., Markiewicz, M. and Luczak, J. (2011) 'Fate of ionic liquids in soils and sediments', *Current Organic Chemistry*, 15(12), pp. 1928–1945. doi: 10.2174/138527211795703702.

Juvonen, R., Martikainen, E., Schultz, E., Joutti, A., Ahtiainen, J. and Lehtokari, M. (2000) 'A battery of toxicity tests as indicators of decontamination in composting oily waste', *Ecotoxicology and Environmental Safety*, 47(2), pp. 156–166. doi: 10.1006/eesa.2000.1943.

Kalogirou, S. A. (2009) 'Solar energy engineering: processes and systems', *Solar Energy*

Engineering: Processes and Systems: Second Edition.

Kamoun, I. J., Jegede, O. O., Owojori, O. J., Bouzid, J., Gargouri, R. and Römbke, J. (2017) 'Effects of deltamethrin , dimethoate, and chlorpyrifos on survival and reproduction of the Collembolan *Folsomia candida* and the predatory mite *Hypoaspis aculeifer* in two African and two European soils', *Integrated Environmental Assessment and Management*, pp. 1–13. doi: 10.1002/ieam.1966.

Kariya, N., Fukuoka, A. and Ichikawa, M. (2002) 'Efficient evolution of hydrogen from liquid cycloalkanes over Pt-containing catalysts supported on active carbons under "wet-dry multiphase conditions"', *Applied Catalysis A: General*, 233(1–2), pp. 91–102. doi: 10.1016/S0926-860X(02)00139-4.

Kariya, N., Fukuoka, A. and Ichikawa, M. (2003) 'Zero-CO_2 emission and low-crossover "rechargeable" PEM fuel cells using cyclohexane as an organic hydrogen reservoir', *Chemical Communications*. Royal Society of Chemistry, (6), pp. 690–691. Available at: http://pubs.rsc.org/en/content/articlehtml/2003/cc/b211685e.

Klamt, A., Eckert, F., Reinisch, J. and Wichmann, K. (2016) 'Prediction of cyclohexane-water distribution coefficients with COSMO-RS on the SAMPL5 data set', *Journal of Computer-Aided Molecular Design*, 30(11), pp. 959–967. doi: 10.1007/s10822-016-9927-y.

Klamt, A., Huniar, U., Spycher, S. and Keldenich, J. (2008) 'COSMOmic: a mechanistic approach to the calculation of membrane−water partition coefficients and internal distributions within membranes and micelles', *J. Phys. Chem. B*, 112(38), pp. 12148–12157. doi: 10.1021/jp801736k.

Kobetičová, K., Bezchlebová, J., Lána, J., Sochová, I. and Hofman, J. (2008) 'Toxicity of four nitrogen-heterocyclic polyaromatic hydrocarbons (NPAHs) to soil organisms', *Ecotoxicology and Environmental Safety*, 71(3), pp. 650–660. doi: 10.1016/j.ecoenv.2008.01.019.

Kobetičová, K., Šimek, Z., Brezovský, J. and Hofman, J. (2011) 'Toxic effects of nine polycyclic aromatic compounds on *Enchytraeus crypticus* in artificial soil in relation to their properties', *Ecotoxicology and Environmental Safety*, 74(6), pp. 1727–1733. doi: 10.1016/j.ecoenv.2011.04.013.

Kördel, W., Bernhardt, C., Derz, K., Hund-Rinke, K., Harmsen, J., Peijnenburg, W., Comans, R. and Terytze, K. (2013) 'Incorporating availability/bioavailability in risk assessment and decision making of polluted sites, using Germany as an example', *Journal of Hazardous Materials*. Elsevier B.V., 261, pp. 854–862. doi: 10.1016/j.jhazmat.2013.05.017.

Kuśmierz, M., Oleszczuk, P., Kraska, P., Pałys, E. and Andruszczak, S. (2016) 'Persistence of polycyclic aromatic hydrocarbons (PAHs) in biochar-amended soil', *Chemosphere*, 146, pp. 272–279. doi: 10.1016/j.chemosphere.2015.12.010.

Lemmon, E. W., Huber, M. L. and McLinden, M. O. (2013) 'NIST Stand. Ref. Database 23 Ref', *Fluid Thermodyn. Transp. Prop. REFPROP*.

Li, S., Turaga, U., Shrestha, B., Anderson, T. A., Ramkumar, S. S., Green, M. J., Das, S. and Cañas-Carrell, J. E. (2013) 'Mobility of polyaromatic hydrocarbons (PAHs) in soil in the presence of carbon nanotubes', *Ecotoxicology and Environmental Safety*. Elsevier, 96, pp. 168–174. doi: 10.1016/j.ecoenv.2013.07.005.

Liu, C., Li, F., Ma, L.-P. and Cheng, H.-M. (2010) 'Advanced materials for energy storage', *Advanced Materials*, 22(8), pp. E28–E62. doi: 10.1002/adma.200903328.

Lopes, T. J. and Furlong, E. T. (2001) 'Occurrence and potential adverse effects of semivolatile

organic compounds in streambed sediment, United States, 1992–1995', *Environmental Toxicology and Chemistry*, 20(4), pp. 727–737. Available at: http://onlinelibrary.wiley.com/doi/10.1002/etc.5620200406/full.

Ma, J., Wang, S., Wang, P., Ma, L., Chen, X. and Xu, R. (2006) 'Toxicity assessment of 40 herbicides to the green alga *Raphidocelis subcapitata*', *Ecotoxicology and Environmental Safety*, 63(3), pp. 456–462. doi: 10.1016/j.ecoenv.2004.12.001.

Manas, M. G., Sharninghausen, L. S., Lin, E. and Crabtree, R. H. (2015) 'Iridium catalyzed reversible dehydrogenation-hydrogenation of quinoline derivatives under mild conditions', *Journal of Organometallic Chemistry*. Elsevier B.V, 792, pp. 184–189. doi: 10.1016/j.jorganchem.2015.04.015.

Marchal, R., Penet, S., Solano-Serena, F. and Vandecasteele, J. P. (2003) 'Gasoline and diesel oil biodegradation', *Oil & Gas Science and Technology – Rev. IFP*, 58(4), pp. 441–448. doi: 10.2516/ogst:2003027.

Markiewicz, M., Jungnickel, C., Cho, C.-W. and Stolte, S. (2015) 'Mobility and biodegradability of an imidazolium based ionic liquid in soil and soil amended with waste sewage sludge', *Environ. Sci.: Processes Impacts*. Royal Society of Chemistry, 17(8), pp. 1462–1469. doi: 10.1039/C5EM00209E.

Markiewicz, M., Zhang, Y. Q., Bösmann, A., Brückner, N., Thöming, J., Wasserscheid, P. and Stolte, S. (2015) 'Environmental and health impact assessment of Liquid Organic Hydrogen Carrier (LOHC) systems – challenges and preliminary results', *Energy Environ. Sci.*, 8(3), pp. 1035–1045. doi: 10.1039/C4EE03528C.

Mazloomi, K. and Gomes, C. (2012) 'Hydrogen as an energy carrier: prospects and challenges', *Renewable and Sustainable Energy Reviews*. Elsevier Ltd, 16(5), pp. 3024–3033. doi: 10.1016/j.rser.2012.02.028.

McCall, P. J., Swann, R. L., Laskowski, D. A., Unger, S. M., Vrona, S. A. and Dishburger, H. J. (1980) 'Estimation of chemical mobility in soil from liquid chromatographic retention times', *Bulletin of Environmental Contamination and Toxicology*, 24(1), pp. 190–195. doi: 10.1007/BF01608096.

Møller, K. T., Fogh, A. S., Paskevicius, M., Skibsted, J. and Jensen, T. R. (2016) 'Metal borohydride formation from aluminium boride and metal hydrides', *Phys. Chem. Chem. Phys.*, 18(39), pp. 27545–27553. doi: 10.1039/C6CP05391B.

Møller, K. T., Jensen, T. R., Akiba, E. and Li, H. W. (2017) 'Hydrogen – a sustainable energy carrier', *Progress in Natural Science: Materials International*. Elsevier B.V., 27(1), pp. 34–40. doi: 10.1016/j.pnsc.2016.12.014.

Moriarty, P. and Honnery, D. (2012) 'What is the global potential for renewable energy?', *Renewable and Sustainable Energy Reviews*. Elsevier Ltd, 16(1), pp. 244–252. doi: 10.1016/j.rser.2011.07.151.

Müller, K., Geng, J. and Arlt, W. (2013) 'Reversible vs. irreversible conversion of hydrogen: how to store energy efficiently?', *Energy Technology*, 1(1), pp. 42–47. doi: 10.1002/ente.201200022.

Müller, K., Stark, K., Emel'yanenko, V. N., Varfolomeev, M. A., Zaitsau, D. H., Shoifet, E., Schick, C., Verevkin, S. P. and Arlt, W. (2015) 'Liquid Organic Hydrogen Carriers: thermophysical and thermochemical studies of benzyl- and dibenzyl-toluene derivatives', *Industrial and Engineering Chemistry Research*, 54(32), pp. 7967–7976. doi: 10.1021/acs.iecr.5b01840.

References

Murphy, D. M. (2009) 'Effect of stratospheric aerosols on direct sunlight and implications for concentrating solar power', *Environmental Science and Technology*, 43(8), pp. 2784–2786. doi: 10.1021/es802206b.

Neumann-Hensel, H. and Melbye, K. (2006) 'Optimisation of the solid-contact test with *Artbrobacter globiformis*', *Journal of Soils and Sediments*, 6(4), pp. 201–207. doi: DOI 10.1065/jss2006.10.188.

Nickerl, J., Tsurkan, M., Hensel, R., Neinhuis, C. and Werner, C. (2014) 'The multi-layered protective cuticle of Collembola: a chemical analysis', *Journal of The Royal Society Interface*, 11(99), p. 20140619. doi: 10.1098/rsif.2014.0619.

Nielsen, M., Alberico, E., Baumann, W., Drexler, H.-J., Junge, H., Gladiali, S. and Beller, M. (2013) 'Low-temperature aqueous-phase methanol dehydrogenation to hydrogen and carbon dioxide', *Nature*. Nature Publishing Group, 495(7439), pp. 85–89. doi: 10.1038/nature11891.

OECD (2000) 'Test No. 106: Adsorption-desorption using a batch equilibrium method', *OECD Guidelines for Testing of Chemicals*, (January), pp. 1–44. doi: 10.1787/9789264069602-en.

OECD (2001) 'Test No. 121: Estimation of the adsorption coefficient (Koc) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC)', *OECD Guidelines for Testing of Chemicals*, (January), pp. 1–11. doi: 10.1787/9789264069909-en.

OECD (2004a) 'Test No. 202: *Daphnia sp.* acute immobilisation test', *OECD Guideline for the Testing og Chemicals, Section 2*, (April), pp. 1–12. doi: 10.1787/9789264069947-en.

OECD (2004b) 'Test No. 312: Leaching in soil columns', *OECD Guidelines for the Testing of Chemicals*, (April), pp. 1–15.

OECD (2006) 'Test No. 221: *Lemna sp.* growth inhabition test', *OECD Guidelines for Testing of Chemicals*, (March), pp. 1–22. Available at: http://www.oecd-ilibrary.org/environment/test-no-221-lemna-sp-growth-inhabition-test_9789264016194-en.

OECD (2009a) *Environment directorate, Joint meeting of the chemicals committee and the working party on chemicals-the 2007 OECD list of high production volume chemicals,No.112*. doi: ENV/JM/MONO(2007)10.

OECD (2009b) 'Test No.232: Collembolan reproduction test in soil', *OECD Guidelines for Testing of Chemicals*, (September), pp. 1–19. doi: 10.1787/9789264076273-en.

OECD (2011) 'Test No. 201: Freshwater alga and cyanobacteria, growth inhibition test', *OECD Guidelines for Testing of Chemicals*, (July), pp. 1–25. Available at: http://www.oecd-ilibrary.org/environment/test-no-201-alga-growth-inhibition-test_9789264069923-en.

Orecchio, S. and Mannino, M. R. (2010) 'Chemical speciation of polycyclic aromatic hydrocarbons in sediments: partitioning and extraction of humic substances', *Marine Pollution Bulletin*. Elsevier Ltd, 60(8), pp. 1175–1181. doi: 10.1016/j.marpolbul.2010.03.036.

Pablo-Romero, M. del P., Román, R., Sánchez-Braza, A. and Yñiguez, R. (2016) 'Renewable energy, emissions, and health', in Cao, W. and Hu, Y. (eds) *Renewable Energy - Utilisation and System Integration*. Rijeka: InTech. doi: 10.5772/61717.

Papp, C., Wasserscheid, P., Libuda, J. and Steinrück, H. P. (2014) 'Liquid organic hydrogen carriers: surface carbazole derivatives', *The Chemical Record*, 14(5), pp. 879–896. doi: 10.1002/tcr.201402014.

Patt, A., Pfenninger, S. and Lilliestam, J. (2010) 'Vulnerability of solar energy infrastructure and output to extreme events: climate change implications', in *Joint ICTP/IAEA Workshop on Vulnerability of Energy Systems to Climate Changes and Extreme Events*.

Paumen, M. L., Steenbergen, E., Kraak, M. H. S., Van Straalen, N. M. and Van Gestel, C. A. M. (2008) 'Multigeneration exposure of the springtail *Folsomia candida* to phenanthrene: from dose-response relationships to threshold concentrations', *Environmental Science and Technology*, 42(18), pp. 6985–6990. doi: 10.1021/es8007744.

Peijnenburg, W., Capri, E., Kula, C., Liess, M., Luttik, R., Montforts, M., Nienstedt, K., Römbke, J., Sousa, J. P. and Jensen, J. (2012) 'Evaluation of exposure metrics for effect assessment of soil invertebrates', *Critical Reviews in Environmental Science and Technology*, 42(17), pp. 1862–1893. doi: 10.1080/10643389.2011.574100.

Pez, G. P., Scott, A. R., Cooper, A. C. and Cheng, H. (2006) 'US patent, 7101530'. September.

Pez, G. P., Scott, A. R., Cooper, A. C., Cheng, H., Wilhelm, F. C. and Abdourazak, A. H. (2008) 'Hydrogen storage by reversible hydrogenation of pi-conjugated substrates'. Available at: https://www.google.com/patents/US7351395.

Pradhan, A. U., Shukla, A., Pande, J. V., Karmarkar, S. and Biniwale, R. B. (2011) 'A feasibility analysis of hydrogen delivery system using liquid organic hydrides', *International Journal of Hydrogen Energy*. Elsevier Ltd, 36(1), pp. 680–688. doi: 10.1016/j.ijhydene.2010.09.054.

Preuster, P., Papp, C. and Wasserscheid, P. (2017) 'Liquid Organic Hydrogen Carriers (LOHCs): toward a hydrogen-free hydrogen economy', *Accounts of Chemical Research*, 50(1), pp. 74–85. doi: 10.1021/acs.accounts.6b00474.

Pukazhselvan, D., Kumar, V. and Singh, S. K. (2012) 'High capacity hydrogen storage: basic aspects, new developments and milestones', *Nano Energy*. Elsevier, 1(4), pp. 566–589. doi: 10.1016/j.nanoen.2012.05.004.

Radić, S., Stipaničev, D., Cvjetko, P., Marijanović Rajčić, M., Širac, S., Pevalek-Kozlina, B. and Pavlica, M. (2011) 'Duckweed *Lemna minor* as a tool for testing toxicity and genotoxicity of surface waters', *Ecotoxicology and Environmental Safety*, 74(2), pp. 182–187. doi: 10.1016/j.ecoenv.2010.06.011.

Reinecke, A. J., van Wyk, M. and Reinecke, S. A. (2016) 'The influence of soil characteristics on the toxicity of oil refinery waste for the springtail *Folsomia candida* (Collembola)', *Bulletin of Environmental Contamination and Toxicology*. Springer US, 96(6), pp. 804–809. doi: 10.1007/s00128-016-1792-9.

Revitt, D. M., Balogh, T. and Jones, H. (2014) 'Soil mobility of surface applied polyaromatic hydrocarbons in response to simulated rainfall', *Environmental Science and Pollution Research*, 21(6), pp. 4209–4219. doi: 10.1007/s11356-013-2231-7.

Rusek, J. (1998) 'Biodiversity of Collembola and their functional role in the ecosystem', *Biodiversity and Conservation*, 7(9), pp. 1207–1219. doi: 10.1023/A:1008887817883.

Sailor, D. J., Smith, M. and Hart, M. (2008) 'Climate change implications for wind power resources in the northwest United States', *Renewable Energy*, 33(11), pp. 2393–2406. doi: 10.1016/j.renene.2008.01.007.

Santorufo, L., Van Gestel, C. A. M., Rocco, A. and Maisto, G. (2012) 'Soil invertebrates as bioindicators of urban soil quality', *Environmental Pollution*. Elsevier Ltd, 161, pp. 57–63. doi: 10.1016/j.envpol.2011.09.042.

Saxena, S., Kumar, S. and Drozd, V. (2011) 'A modified steam-methane-reformation reaction for

hydrogen production', *International Journal of Hydrogen Energy*. Elsevier Ltd, 36(7), pp. 4366–4369. doi: 10.1016/j.ijhydene.2010.12.133.

Schaffer, M., Börnick, H., Nödler, K., Licha, T. and Worch, E. (2012) 'Role of cation exchange processes on the sorption influenced transport of cationic β-blockers in aquifer sediments', *Water Research*, 46(17), pp. 5472–5482. doi: 10.1016/j.watres.2012.07.013.

Scherer, G. W. H., Newson, E. and Wokaun, A. (1999) 'Economic analysis of the seasonal storage of electricity with liquid organic hydrides', *International Journal of Hydrogen Energy*, 24(12), pp. 1157–1169. doi: 10.1016/S0360-3199(98)00177-3.

Schildhauer, T., Newson, E. and Uller, S. (2001) 'The equilibrium constant for the methylcyclohexane-toluene system', *Journal of Catalysis*, 198(2), pp. 355–358. doi: 10.1006.

Schlanges, I., Meyer, D., Palm, W. U. and Ruck, W. (2008) 'Identification, quantification and distribution of PAC-metabolites, heterocyclic PAC and substituted PAC in groundwater samples of tar-contaminated sites from Germany', *Polycyclic Aromatic Compounds*, 28(4–5), pp. 320–338. doi: 10.1080/10406630802377807.

Schmidt, S. N., Smith, K. E. C., Holmstrup, M. and Mayer, P. (2013) 'Uptake and toxicity of polycyclic aromatic hydrocarbons in terrestrial springtails-studying bioconcentration kinetics and linking toxicity to chemical activity', *Environmental Toxicology and Chemistry*, 32(2), pp. 361–369. doi: 10.1002/etc.2051.

Schüürmann, G., Ebert, R.-U. and Kühne, R. (2006) 'Prediction of the sorption of organic compounds into soil organic matter from molecular structure', *Environmental science & technology*, 40(22), pp. 7005–7011. doi: 10.1021/es060152f.

Schwarzenbach, R. P., Gschwend, P. M. and Imboden, D. M. (2003) *Environmental Organic Chemistry (2nd Edition)*, *Jonh Wiley & Sons, Inc.* New Jersey: Wiley-Interscience.

Semple, K. T., Doick, K. J., Jones, K. C., Burauel, P., Craven, A. and Harms, H. (2004) 'Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated', *Environmental Science & Technology*, 38(12), p. 228A–231A. doi: 10.1021/es040548w.

Shafiee, S. and Topal, E. (2009) 'When will fossil fuel reserves be diminished?', *Energy Policy*, 37(1), pp. 181–189. doi: 10.1016/j.enpol.2008.08.016.

Solano-Serena, F., Marchal, R., Ropars, M., Lebeault, J. M. and Vandecasteele, J. P. (1999) 'Biodegradation of gasoline: kinetics, mass balance and fate of individual hydrocarbons', *Journal of Applied Microbiology*, 86(6), pp. 1008–1016. doi: 10.1046/j.1365-2672.1999.00782.x.

Srogi, K. (2007) 'Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review', *Environmental Chemistry Letters*, 5(4), pp. 169–195. doi: 10.1007/s10311-007-0095-0.

Stark, K., Keil, P., Schug, S., Müller, K., Wasserscheid, P. and Arlt, W. (2016) 'Melting points of potential liquid organic hydrogen carrier systems consisting of N-alkylcarbazoles', *Journal of Chemical and Engineering Data*, 61(4), pp. 1441–1448. doi: 10.1021/acs.jced.5b00679.

Stepnowski, P., Mrozik, W. and Nichthauser, J. (2007) 'Adsorption of alkylimidazolium and alkylpyridinium ionic liquids onto natural soils', *Environmental Science and Technology*, 41(2), pp. 511–516. doi: 10.1021/es062014w.

Stokes, J. D., Paton, G. I. and Semple, K. T. (2005) 'Behaviour and assessment of bioavailability of organic contaminants in soil: relevance for risk assessment and remediation', *Soil Use and Management*, 21(s2), pp. 475–486. doi: 10.1079/SUM2005347.

Sverdrup, L. E., Kelley, A. E., Krogh, P. H., Nielsen, T., Jensen, J., Scott-Fordsmand, J. J. and Stenersen, J. (2001) 'Effects of eight polycyclic aromatic compounds on the survival and reproduction of the springtail *Folsomia fimetaria L.* (Collembola, Isotomidae).', *Environmental Toxicology and Chemistry*, 20(6), pp. 1332–1338. doi: 10.1002/etc.5620200623.

Sverdrup, L. E., Krogh, P. H., Nielsen, T. and Stenersen, J. (2002) 'Relative sensitivity of three terrestrial invertebrate tests to polycyclic aromatic compounds', *Environmental Toxicology and Chemistry*, 21(9), pp. 1927–1933. doi: 10.1002/etc.5620210921.

Sverdrup, L. E., Nielsen, T. and Krogh, P. H. (2002) 'Soil ecotoxicity of polycyclic aromatic hydrocarbons in relation to soil sorption, lipophilicity, and water solubility', *Environmental Science and Technology*, 36(11), pp. 2429–2435. doi: 10.1021/es010180s.

Taube, M., Rippin, D., Knecht, W., Hakimifard, D., Milisavljevic, B. and Gruenenfelder, N. (1985) 'A prototype truck powered by hydrogen from organic liquid hydrides', *International Journal of Hydrogen Energy*. Elsevier, 10(9), pp. 595–599. Available at: http://www.sciencedirect.com/science/article/pii/0360319985900357.

Taube, M., Rippin, D. W. T., Cresswell, D. L. and Knecht, W. (1983) 'A system of hydrogen-powered vehicles with liquid organic hydrides', *International Journal of Hydrogen Energy*. Elsevier, 8(3), pp. 213–225. Available at: http://www.sciencedirect.com/science/article/pii/0360319983900678.

Taube, M. and Taube, P. (1979) *A liquid organic carrier of hydrogen as a fuel for automobiles: nuclear power as a motive power for cars*. Eidg. Institut für Reaktorforschung Würenlingen.

Teichmann, D., Arlt, W. and Wasserscheid, P. (2012a) 'Flüssige Wasserstoffträger für ein Nachhaltiges Energiesystem', *ATZ-Automobiltechnische Zeitschrift*. Springer, 114(5), pp. 430–434.

Teichmann, D., Arlt, W. and Wasserscheid, P. (2012b) 'Liquid organic hydrogen carriers as an efficient vector for the transport and storage of renewable energy', *International Journal of Hydrogen Energy*. Elsevier Ltd, 37(23), pp. 18118–18132. doi: 10.1016/j.ijhydene.2012.08.066.

Teichmann, D., Arlt, W., Wasserscheid, P. and Freymann, R. (2011) 'A future energy supply based on Liquid Organic Hydrogen Carriers (LOHC)', *Energy & Environmental Science*, 4(8), pp. 2767–2773. doi: 10.1039/C1EE01454D.

Teichmann, D., Stark, K., Müller, K., Zöttl, G., Wasserscheid, P. and Arlt, W. (2012) 'Energy storage in residential and commercial buildings via Liquid Organic Hydrogen Carriers (LOHC)', *Energy & Environmental Science*, 5(10), pp. 9044–9054. doi: 10.1039/C2EE22070A.

Thomsen, A. B., Henriksen, K., Grøn, C. and Møldrup, P. (1999) 'Sorption, transport, and degradation of quinoline in unsaturated soil', *Environmental Science and Technology*, 33(17), pp. 2891–2898. doi: 10.1021/es981065t.

Thomsom, K., Liu, D. and Kaiser, K. L. E. (1986) 'A direct resazurin test for measuring chemical toxicity', *Toxicity Assessment*, 1(4), pp. 407–418. doi: 10.1002/tox.2540010403.

Tietze, V., Luhr, S. and Stolten, D. (2016) 'Bulk storage vessels for compressed and liquid hydrogen', *Hydrogen Science and Engineering: Materials, Processes, Systems and Technology*. Wiley Online Library, pp. 659–690. Available at: http://onlinelibrary.wiley.com/doi/10.1002/9783527674268.ch27/summary.

Tsibart, A. S. and Gennadiev, A. N. (2013) 'Polycyclic aromatic hydrocarbons in soils: sources,

behavior, and indication significance (a review)', *Eurasian Soil Science*, 46(7), pp. 728–741. doi: 10.1134/S1064229313070090.

Twidell, J. and Weir, T. (1986) *Renewable energy resources*. doi: 10.4324/9780203478721.

U. S. Environmental Protection Agency (2007) 'EPA Method 3550C: Ultrasonic extraction', *U. S. Environmental Protection Agency*, (February), pp. 1–17. doi: 10.1017/CBO9781107415324.004.

Vancampenhout, K., Wouters, K., De Vos, B., Buurman, P., Swennen, R. and Deckers, J. (2009) 'Differences in chemical composition of soil organic matter in natural ecosystems from different climatic regions – a pyrolysis-GC/MS study', *Soil Biology and Biochemistry*. Elsevier Ltd, 41(3), pp. 568–579. doi: 10.1016/j.soilbio.2008.12.023.

Wassenberg, D. M., Nerlinger, A. L., Battle, L. P. and Di Giulio, R. T. (2005) 'Effects of the polycyclic aromatic hydrocarbon heterocycles, carbazole and dibenzothiophene, on *in vivo* and *in vitro* cypia activity and polycyclic aromatic hydrocarbon-derived embryonic deformities.', *Environmental toxicology and chemistry*, 24(10), pp. 2526–2532. doi: 10.1897/04-440r1.1.

Weissenfels, W. D., Klewer, H. J. and Langhoff, J. (1992) 'Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity.', *Applied microbiology and biotechnology*, 36(5), pp. 689–696. doi: 10.1007/BF00183251.

von Wild, J., Friedrich, T., Cooper, A., Toseland, B., Muraro, G., TeGrotenhuis, W., Wang, Y., Humble, P. and Karim, A. (2010) 'Liquid Organic Hydrogen Carriers (LOHC): an auspicious alternative to conventional hydrogen storage technologies', *18th World Hydrogen Energy Conference*, 78, pp. 189–197.

Xu, J., Ke, X., Krogh, P. H., Wang, Y., Luo, Y. M. and Song, J. (2009) 'Evaluation of growth and reproduction as indicators of soil metal toxicity to the Collembolan, *Sinella curviseta*', *Insect Science*, 16(1), pp. 57–63. doi: 10.1111/j.1744-7917.2009.00254.x.

Yamada, K., Suzuki, T., Kohara, A., Hayashi, M., Mizutani, T. and Saeki, K. I. (2004) '*In vivo* mutagenicity of benzo[f]quinoline, benzo[h]quinoline, and 1,7-phenanthroline using the lacZ transgenic mice', *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 559(1–2), pp. 83–95. doi: 10.1016/j.mrgentox.2003.12.012.

Yamaguchi, R., Ikeda, C., Takahashi, Y. and Fujita, K. (2009) 'Homogeneous catalytic system for reversible dehydrogenation-hydrogenation reactions of nitrogen heterocycles with reversible interconversion of catalytic species', *J. Am. Chem. Soc.*, 131(24), pp. 8410–8412. doi: 10.1021/ja9022623.

Yang, L., Jin, M., Tong, C. and Xie, S. (2013) 'Study of dynamic sorption and desorption of polycyclic aromatic hydrocarbons in silty-clay soil', *Journal of Hazardous Materials*. Elsevier B.V., 244–245, pp. 77–85. doi: 10.1016/j.jhazmat.2012.11.029.

Yang, M., Dong, Y., Fei, S., Ke, H. and Cheng, H. (2014) 'A comparative study of catalytic dehydrogenation of perhydro-N-ethylcarbazole over noble metal catalysts', *International Journal of Hydrogen Energy*. Elsevier Ltd, 39(33), pp. 18976–18983. doi: 10.1016/j.ijhydene.2014.09.123.

Yang, M., Dong, Y., Fei, S., Pan, Q., Ni, G., Han, C., Ke, H., Fang, Q. and Cheng, H. (2013) 'Hydrogenation of N-propylcarbazole over supported ruthenium as a new prototype of liquid organic hydrogen carriers (LOHC)', *RSC Advances*, 3(47), p. 24877. doi: 10.1039/c3ra44760j.

Zhang, J. and Fan, S. kai (2016) 'Influence of PAH speciation in soils on vegetative uptake of PAHs using successive extraction', *Journal of Hazardous Materials*. Elsevier B.V., 320(30), pp. 114–122. doi: 10.1016/j.jhazmat.2016.08.024.

Zhang, Y.-Q., Dringen, R., Petters, C., Rastedt, W., Köser, J., Filser, J. and Stolte, S. (2016) 'Toxicity of dimercaptosuccinate-coated and un-functionalized magnetic iron oxide nanoparticles towards aquatic organisms', *Environ. Sci.: Nano*. Royal Society of Chemistry, 3(4), pp. 754–767. doi: 10.1039/C5EN00222B.

Zhu, Q. L. and Xu, Q. (2015) 'Liquid organic and inorganic chemical hydrides for high-capacity hydrogen storage', *Energy & Environmental Science*. Royal Society of Chemistry, 8, pp. 478–512. doi: 10.1039/c4ee03690e.

Acknowledgements

I would like to thank Dr. habil. Stefan Stolte, who accepted and supported me to do the Ph.D. study in his research group, an interdisciplinary research team with multiple diffusions of the knowledge from biology, chemistry and engineering technology and is full of diligent and friendly atmosphere. Thank you for the valuble advice and discussions on experiments and writings. Your idea and comments enlightened me and inspired me to carry on. Thank you for giving me the freedom every time for making my own decisions in each research. I am also very grateful for your supports on my living of stay in Bremen and for all the encouragement during my study.

I would like to say thank you to Prof. Dr. Juliane Filser, who accepted as one supervisor of my Ph.D. study. Thank you for the helpful advice regarding to ecotoxicity tests and for all encouragement during my study; thanks for all the backups, patience and helpful comments in my manuscripts. Your expertise has made me learned a lot.

I would like to express my gratitude to Dr. Marta Markiewicz for the enlightening discussion and suggestions for experiments and writings concerning to the LOHC research, which have benefited me a great deal. I am also very grateful for the professional and helpful introductions on the related chemical and instrumental analysis.

My sincere thanks also go to Prof. Dr. Ralf Dringen for the numerous times' of helpful discussions on the research project of the IONP. Your profound insights expanded my mind and taught me many so that to allow the work to grow.

Many thanks will also go to Dr. Jan Köser for the valuable discussions about IONPs and other nanoparticles. Thank you for the professional suggestions on the related experiments and paper's writing as well as the proofreading of part of the thesis. It was very interesting and informative for me to learn simulation software for chemicals under your guidance.

I would like to give my gratitude to all the colleagues from the research group of Stolte, Filser and Thöming at the UFT for every support during my Ph.D. work. Each help that goes for my experiments, work, study and the daily life in Bremen will be forever memory bearing in my mind. Especially, thank you Alica for her aids on the AAS, HPLC and leaching experiment; Ulrike, Iris and Ute for the supports in the laboratory whenever and whatever I would need. I would also like to say thank you to Andrea and Maria for the guidance in the *Arthrobacter* test; Prof. Dr. Hartmut H. Koehler and Elaheh for the instruction and aid for microscopes; Xin and Moira for the help discussions on the Collembola tests, and thanks Moira for the proofreading of part of the thesis; Steve for the co-management of our group seminars and regular routines of our research group as well as the proofreading of part of the thesis; Ruth

and Janiene for all the document supports while my stay. It is glad to have been working with you.

Thank you to all the friends from different countries that I have met in Bremen. I have had a great and happy time with you. With your companion in the free time made me really relax and enabled me to trim everything in good status thus to be back to study and work.

Last deep gratefulness will go to my parents, for their love, understanding, patience, encouragement and always being behind me with never ending supports, and without which, I would never pursue my study in Germany and finish the Ph.D. thesis.

Curriculum vitae

Personal Information

Name	Ya-Qi Zhang
Date/Place of Birth	13th December, 1983 Kunming, Yunnan, China
E-mail	s_25ydc2@uni-bremen.de; or yqzhang08@gmail.com

Education Background

12/2012 – 12/2017	Doctoral degree and thesis "Hazard assessment of Liquid Organic Hydrogen Carriers (LOHCs) in terrestrial environment." *Department Sustainable Chemistry, Centre for Environmental Research and Sustainable Technology (UFT), University of Bremen, Bremen, Germany*
04/2013 – 04/2015	Research project during Ph.D. study "Toxicity of dimercaptosuccinate-coated and unfunctionalized magnetic iron oxide nanoparticles towards aquatic organisms." *Department Sustainable Chemistry, Centre for Environmental Research and Sustainable Technology (UFT), University of Bremen, Bremen, Germany*
10/2010 – 10/2012	Master degree and thesis "Impact of natural organic matter (NOM) on the ecotoxicity of silver sulfide (Ag_2S) in aquatic environment." *Department of General and Theoretical Ecology, Centre for Environmental Research and Sustainable Technology (UFT), University of Bremen, Bremen, Germany*

04/2010 – 07/2010	Full-time German school
11/2009 – 01/2010	English school at *the Robert's English Center, Beijing, China*
06/2007 – 04/2008	Full-time English school
08/2005 – 07/2006	Bachelor thesis "The research on the expression of the HPV16-E7 polypeptide inserted into the *Salmonella typhimurium* LT2 fimbriae by recombining with a rebuilt plasmid – tentative research of Human Papilloma Virus (HPV16-E7) tumor vaccine." *School of Life Sciences, Capital Normal University, Beijing, China*; *&* *Department of Immunology, School of Basic Medical Science, Peking University, Beijing, China*
05/2005 – 05/2007	Research internship *Department of Immunology, School of Basic Medical Science, Peking University, Beijing, China*
05/2005 – 08/2005	Research and teaching assistant Vaccine research on the expression and display strategy of tumor antigen on bacterial fimbriae, and issued with the Job Qualification Training Certificate of Medical Immunology's Experimental Teaching. *Department of Immunology, School of Basic Medical Science, Peking University, Beijing, China*
09/2002 – 07/2006	Bachelor degree major in Biosciences, *School of Life Sciences, Capital Normal University, Beijing, China*
09/1996 – 07/2002	Junior High and High School *Beijing National Day School, Beijing ,China*

Publications

- **Scientific articles**

2017

Markiewicz, M.; Zhang, Y. Q.; Empl, M. T.; Lykaki, M.; Thöming, J.; Wasserscheid, P.; Bösmann, A.; Steinberg, P.; Stolte, S. (2017) 'Preliminary hazard assessment of quinaldine and alkylcarbazole based liquid organic hydrogen carriers.' Prepared manuscript for submission to Energy & Environmental Science.

Zhang, Y. Q.; Stolte, S.; Alptekin, G.; Rother, A.; Diedenhofen, M.; Filser, J.; Markiewicz, M. (2017) 'Mobility and adsorption of liquid organic hydrogen carriers (LOHCs) in soil.' Submitted manuscript to Environmental Science & Technology.

Zhang, Y. Q.; Markiewicz, M.; Filser, J.; Stolte, S. (2017) "Toxicity of a quinaldine-based Liquid Organic Hydrogen Carrier (LOHC) system toward soil organisms Arthrobacter globiformis and Folsomia candida." Submitted manuscript to Environmental Science & Technology, considered for publication after major revisions.

2016

Zhang, Y.-Q.; Dringen, R.; Petters, C.; Rastedt, W.; Köser, J.; Filser, J.; Stolte, S. (2016) "Toxicity of dimercaptosuccinate-coated and un-functionalized magnetic iron oxide nanoparticles towards aquatic organisms." Environmental Science: Nano, 3 (4), 754–767.

2015

Markiewicz, M.; Zhang, Y. Q.; Bösmann, A.; Brückner, N.; Thöming, J.; Wasserscheid, P.; Stolte, S. (2015) "Environmental and health impact assessment of Liquid Organic Hydrogen Carrier (LOHC) systems - challenges and preliminary results." Energy & Environmental Science, 8 (3), 1035–1045.

2007

Huang, H.; Li, R.-H.; Zhang, Y.-Q.; Liu, S.-L.; Wang, Y.-D. (2007) "Expression of foreign epitopes on *Samonella* Fimbrins." Progress in Modern Biomedicine, 7 (2), 189–192.

- **Presentations at scientific meetings**

Posters Presentations

2014

Zhang, Y.-Q.; Petters, C.; Dringen, R.; Gogolin M.; Köser, J.; Stolte, S. (2014) "Effects of dimercaptosuccinate-functionalized magnetic iron oxide nanoparticles on aquatic organisms." SETAC Europe 24th Annual Meeting 2014, Basel, Switzerland.

2013

Zhang, Y.-Q.; Köser, J.; Filser, J. (2013) "Ecotoxicity potential of silver sulfide in aquatic environment." 10th International Particle Toxicology Conference (IPTC) 2013, Düsseldorf, Germany.

Universität Bremen

Ort, Datum: Bremen, 08.11.2017

Versicherung an Eides Statt

Ich, Yaqi, Zhang, Gröpelinger Heerstr. 69, 28237 Bremen, Matr.-Nr. 2472032 (Vorname, Name, Anschrift, Matr.-Nr.)

versichere an Eides Statt durch meine Unterschrift, dass ich die vorstehende Arbeit selbständig und ohne fremde Hilfe angefertigt und alle Stellen, die ich wörtlich dem Sinne nach aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe, mich auch keiner anderen als der angegebenen Literatur oder sonstiger Hilfsmittel bedient habe.

Ich versichere an Eides Statt, dass ich die vorgenannten Angaben nach bestem Wissen und Gewissen gemacht habe und dass die Angaben der Wahrheit entsprechen und ich nichts verschwiegen habe.

Die Strafbarkeit einer falschen eidesstattlichen Versicherung ist mir bekannt, namentlich die Strafandrohung gemäß § 156 StGB bis zu drei Jahren Freiheitsstrafe oder Geldstrafe bei vorsätzlicher Begehung der Tat bzw. gemäß § 161 Abs. 1 StGB bis zu einem Jahr Freiheitsstrafe oder Geldstrafe bei fahrlässiger Begehung.

Bremen, 08.11.2017, Yaqi Zhang

Ort, Datum Unterschrift

Declaration on the contribution of the candidate to a multi-author article/manuscript which is included as a chapter in the submitted doctoral thesis

Chapter: 3.1 Environmental and health impact assessment of Liquid Organic Hydrogen Carrier (LOHC) systems – challenges and preliminary results.

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. _20_%
Experimental work and/or acquisition of (experimental) data:	ca. _20_%
Data analysis and interpretation:	ca. _20_%
Preparation of Figures and Tables:	ca. _20_%
Drafting of the manuscript:	ca. _30_%

Chapter: 3.2 Mobility and adsorption of liquid organic hydrogen carriers (LOHCs) in soil.

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. _80_%
Experimental work and/or acquisition of (experimental) data:	ca. _70_%
Data analysis and interpretation:	ca. _80_%
Preparation of Figures and Tables:	ca. _90_%
Drafting of the manuscript:	ca. _80_%

Chapter: 3.3 Toxicity of a quinaldine-based Liquid Organic Hydrogen Carrier (LOHC) system toward soil organisms *Arthrobacter globiformis* and *Folsomia candida*.

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. _80_%
Experimental work and/or acquisition of (experimental) data:	ca. _90_%
Data analysis and interpretation:	ca. _80_%
Preparation of Figures and Tables:	ca. _90_%
Drafting of the manuscript:	ca. _80_%

Date: 08 November 2017

Signatures: Yaqi Zhang

Declaration on the contribution of the candidate to a multi-author article/manuscript which is included as a chapter in the submitted doctoral thesis

Chapter: 3.4 Preliminary hazard assessment of quinaldine and alkylcarbazole based liquid organic hydrogen carriers.

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. _50_%
Experimental work and/or acquisition of (experimental) data:	ca. _30_%
Data analysis and interpretation:	ca. _40_%
Preparation of Figures and Tables:	ca. _20_%
Drafting of the manuscript:	ca. _40_%

Chapter: 3.5 Toxicity of dimercaptosuccinate-coated and un-functionalized magnetic iron oxide nanoparticles towards aquatic organisms.

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. _80_%
Experimental work and/or acquisition of (experimental) data:	ca. _80_%
Data analysis and interpretation:	ca. _80_%
Preparation of Figures and Tables:	ca. _80_%
Drafting of the manuscript:	ca. _80_%

Chapter:

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. __%
Experimental work and/or acquisition of (experimental) data:	ca. __%
Data analysis and interpretation:	ca. __%
Preparation of Figures and Tables:	ca. __%
Drafting of the manuscript:	ca. __%

Date: 08 November 2017

Signatures: Yaqi Zhang