Quantitative structure-activity relationships for green algae growth inhibition by polymer particles
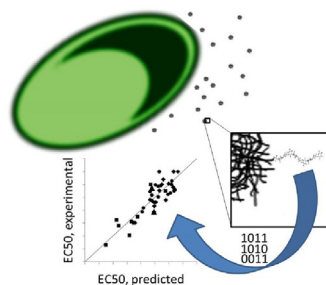
Tom M. Nolte [a, *], Willie J.G.M. Peijnenburg [b], A. Jan. Hendriks [a], Dik van de Meent [b]

[a] Department of Environmental Science, Institute for Water and Wetland Research, Radboud University Nijmegen, P.O. Box 9010, 6500 GL Nijmegen, The Netherlands
[b] National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

After use and disposal of chemical products, many types of polymer particles end up in the aquatic environment with potential toxic effects to primary producers like green algae. In this study, we have developed Quantitative Structure-Activity Relationships (QSARs) for a set of highly structural diverse polymers which are capable to estimate green algae growth inhibition (EC50). The model (N = 43, $R^2 = 0.73$, RMSE = 0.28) is a regression-based decision tree using one structural descriptor for each of three polymer classes separated based on charge. The QSAR is applicable to linear homo polymers as well as copolymers and does not require information on the size of the polymer particle or underlying core material. Highly branched polymers, non-nitrogen cationic polymers and polymeric surfactants are not included in the model and thus cannot be evaluated. The model works best for cationic and non-ionic polymers for which cellular adsorption, disruption of the cell wall and photosynthesis inhibition were the mechanisms of action. For anionic polymers, specific properties of the polymer and test characteristics need to be known for detailed assessment. The data and QSAR results for anionic polymers, when combined with molecular dynamics simulations indicated that nutrient depletion is likely the dominant mode of toxicity. Nutrient depletion in turn, is determined by the non-linear interplay between polymer charge density and backbone flexibility.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Use of chemical substances is regulated in various national and international legal frameworks. In Europe, chemicals can be marketed only if the tonnage is below a threshold of 1 tonne or after the

possibility of 'safe use' has been demonstrated in a REACH registration dossier (ECHA, 2012). 'Conventional' chemicals, such as polycyclic aromatic hydrocarbons (PAH) and other persistent organic pollutants (POPs) have been studied and evaluated extensively (Verbruggen, 2012); fate- and effect models exist to aid chemical safety analysis (Chen et al., 2006). For 'emerging pollutants', such models are available to a much lesser extent, particularly so for micro- and nano-sized particles (Braakhuis et al., 2015; Leszczynska and Shukla, 2009). While there are methods available that can estimate the effects of individual parent monomers (ECHA, 2012; Netzeva et al., 2007), the polymeric versions of the compounds are often left unevaluated. It is important to study the potential effects of nano- and micron-sized polymers because they represent a wider used class of potential pollutants. It is generally assumed that the increase in size relative to the monomer causes an overall decrease in toxicity (Congress 1983; Bergmann et al., 2009). However, this assumption was found incorrect for certain chemical species which exert higher toxicity than their individual constituents, like in the case of asbestos and cationic antimicrobial polymers (Boulanger et al., 2014; Uppu et al., 2016; Tsuji et al., 2006). Upon their release in the aquatic environment (Lambert and Wagner, 2016), nano and micro polymers have several potential detrimental effects on the ecosystem (Bergmann et al., 2009; da Costa et al., 2016). For example, bioconcentration or a loss in primary photosynthetic production by green algae may affect organisms higher up in the food chain (von Moos and Slaveykova, 2014; Khan and Arif, 2012; EFSA, 2016).

For chemicals that occur as nano- and micron-sized particles there are limited toxicological models available. Specifically, there is a lack of models for polymeric particles in the aquatic environment, with most of the models available developed for humans (Jagiello et al., 2016). Current modelling platforms like EPI Suite and ECOSAR (US EPA, 2012) include models predicting effects for algae, daphnia and fish for polymeric materials but have several important limitations. Datasets from which such models are derived are limited in size (and sometimes not publically available). Models are developed for specific polymer classes, i.e. cationics and anionics only, and can often give categorical classifications only (e.g. 'toxic' versus 'non-toxic') instead of quantitative estimations (US EPA, 2013). Due to these limitations, often no prediction is possible for a newly synthesized polymer since it does not conform the predetermined applicability domain or required input metric. For example, the only quantitative structure-activity relationship (QSAR) for algae toxicity available was developed by Boethling and Nabholz (Boethling and Nabholz, 1996), who found that the amine to polymer weight percentage (%A-N) correlated with green algae chronic toxicity. However, the equation did not apply above a certain threshold (%A-N > 3.5) for which chronic toxicity was found constant. Moreover, no statistical parameters were provided. For other polymer classes like non-ionics and anionics there exists much variance in algal toxicity data (Dědková et al., 2014; van Hoecke et al., 2013; Boethling and Nabholz, 1996) and because there still exists uncertainty on the mechanisms by which they exert effect, it has not been possible to capture the variance into a model.

In theory, polymeric particles can exert toxicity in various ways. Polymeric materials >20 nm are unlikely to pass the cell wall of algae, but larger sized polymer particles can disrupt the cell wall, and cause photosynthesis inhibition (Navarro et al., 2008; Boethling and Nabholz, 1996; Bhattacharya et al., 2010). This is especially relevant in the case of cationic polymers since they electrostatically adsorb to cell walls and are subsequently able to react as nucleophiles in displacement reactions with various electrophilic moieties. In comparison, neutral and anionic polymers are usually less toxic and subtle effects could be explained by indirect shading (i.e. photosynthesis inhibition) or nutrient depletion through chelation (Nolte et al., 2016, Bhattacharya et al., 2010). For anionic polymers, chelation of cationic nutrients can be determined by measuring e.g. stability constants or complexation capacity (Wilson and Nicholson, 1993; Amjad, 2007), which are both highly dependent on chemical functionality. Since these potential modes of toxicity are driven for a large part by surface chemistry of the polymers, rather than surface area (Depan, 2016), we hypothesized that algae toxicity may be largely independent of particle size or underlying non-polymeric material. Therefore, in this study the aim was to develop mechanistically interpretable QSAR models for growth inhibition of green algae by a heterogeneous set of polymeric materials and polymeric coatings. Because the mode of action for anionic polymers is relatively uncertain, we also investigated the potential involvement of micro-nutrient depletion from the testing media. This was done by studying the conformational behaviour of anionic polymers in response to Mg^{2+} and Ca^{2+} ions using molecular dynamics. Subsequently, the information was used to explain some of the variance in the growth inhibition data.

2. Methods

2.1. Toxicity data

Data was collected from public literature using Google Scholar and Web of Science. Toxicity data were mostly 72-h growth inhibition EC50 values for freshwater green algae. However, since data was limited, marine algae species, 96-h growth-, and photosynthesis inhibition data were included as well. If both 96-h and 72-h EC50 data were available, the latter was used. Units were standardized to g/L and transformed logarithmically. No distinction was made between polymers of different sizes since this was not within our aim. The final data set contained algal toxicity data both for particles coated with polymers and pure polymer particles. If available, selection of compounds was limited to experiments using the actual exposure concentration (i.e. the suspended polymer) instead of the nominal concentration, thereby excluding highly hydrophobic polymers. Most growth inhibition tests were performed using OECD201 guidelines (OECD, 2011; van Hoecke et al., 2013; ECHA, 2012). For further details on the dataset see SI.

2.2. QSAR development

Custom 1D and 2D molecular descriptors were generated with RDKit using smiles input (RDKit, 2016). Polymer structures were drawn using Chemsketch (ACD/ChemSketch, 2013) and appropriate physiological (pH~7.4) charges attributed to each functional group. Unless mentioned otherwise, for each polymer eight repeating units were drawn and hydroxyl groups were added as termini, assuming radical polymerisation in aqueous media. In case of esterification and condensation, carboxylates and carbonyls were included as termini, respectively. Boethling and Nabholz (Boethling and Nabholz, 1996) did not provide structural representations of their polymers. Therefore, we estimated structural elements based on their detailed textual description (see SI). All the topological descriptors calculated were normalized using molecular weight. Correlation-based feature selection was used to extract relevant features from the pool of molecular descriptors. Subsequent feature selection was performed for the three distinct datasets separated based on the charge of the polymer. Charge carrying groups were amine and amidine (cationic), and carboxylic and sulfonic acid (anionic) groups. Pearson's correlation (R^2) coefficients, root mean square errors (RMSE) and p-values (at CI = 0.05) were determined using standard Python and Microsoft Excel statistical analysis packages (Anaconda, 2016).