Marie Curie Initial Training Network

Environmental Chemoinformatics (ECO)

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Report for the reviewers

Oral uptake of hydrophilic and hydrophobic chemicals: the role of passive diffusion, transport proteins and marine debris.

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1 Overview on the scientific work completed during the long term fellowship

1.1 Background

In the EU, REACH requires the assessment of compounds regarding their potential to accumulate and induce toxic effects in organisms and in the environment. Since it is not possible to test all chemicals, models are needed to predict bioaccumulation and toxicity of these untested compounds. Mechanistic bioaccumulation models often include the following processes: uptake and elimination by absorption through gills/lungs/skin, by food through the gastro intestinal tract (GIT), and elimination by transformation and growth (see e.g. [1], [2]). During uptake processes, the chemical has to permeate through several lipid and aqueous layers, which are considered as barriers with respective resistances [1, 3].

During the first year, we focused on membrane permeation of hydrophilic compounds and how to improve uptake estimation (→ see first milestone). However, beside passive diffusion, chemicals can also be transported by transport proteins [4] which might lead to higher uptake than expected from passive diffusion solely. Therefore, the aim of the second mile stone was to find general patterns or trends in the transport kinetics, and relate the kinetic parameters to chemical descriptors. The aim of the third mile stone was to compare the kinetics of carrier mediated transport to the uptake by passive diffusion, and evaluate the relevance of both pathways. This aim was achieved by incorporating carrier mediated transport into the model developed in milestone 1.

1.2 Outcome of Milestone 1: Uptake by passive diffusion

The work of milestone 1 was published under the title “Predicting the oral uptake efficiency of chemicals in mammals: Combining the hydrophilic and lipophilic range” in Toxicology and Applied Pharmacology (Volume 266, Issue 1, 1. January 2013, Pages 150-156).

Summary of the article: Environmental risk assessment requires models for estimating the bioaccumulation of untested compounds. So far, bioaccumulation models have focused on lipophilic compounds, and only a few have included hydrophilic compounds. Our aim was to extend an existing bioaccumulation model to estimate the oral uptake efficiency of pollutants in mammals for compounds over a wide K_{ow} range with an emphasis on hydrophilic compounds, i.e. compounds in the lower K_{ow} range. Usually, most models use octanol as a single surrogate for the membrane and thus neglect the bilayer structure of the membrane. However, compounds with polar groups can have different affinities for the different membrane regions. Therefore, an existing bioaccumulation model was extended by dividing the diffusion resistance through the membrane into an outer and inner membrane resistance, where the solvents octanol and heptane were used as surrogates for these membrane regions, respectively. The model was calibrated with uptake efficiencies of environmental pollutants measured in different mammals during feeding studies combined with human oral uptake efficiencies of pharmaceuticals. The new model estimated the uptake efficiency of neutral (RMSE=14.6) and dissociating (RMSE=19.5) compounds with $\log K_{ow}$ ranging from −10 to +8. The inclusion of the K_{hw} improved uptake estimation for 33% of the hydrophilic compounds ($\log K_{ow}<0$) (r^2=0.51, RMSE=22.8) compared with the model based on K_{ow} only (r^2=0.05,

RMSE=34.9), while hydrophobic compounds ($logK_{ow}$>0) were estimated equally by both model versions with RMSE=15.2 (K_{ow}&K_{hw}) and RMSE=15.7 (K_{ow} only). The model can be used to estimate the oral uptake efficiency for both hydrophilic and hydrophobic compounds.

1.3 Outcome of Milestone 2: Kinetics of carrier mediated transport

The work of milestone 2 was submitted under the title "Relating kinetic parameters of human intestinal influx transporter to molecular properties of pharmaceuticals and environmental contaminants". The article is currently under review.

Summary of the article: Most toxicokinetic and bioaccumulation models include passive diffusion as the only uptake mechanism. However, xenobiotics can act as substrates for membrane transport proteins. The aim of this study was to analyse the carrier-mediated uptake by identifying molecular descriptors that account for differences in uptake kinetics between substances. We collected data for the Michaelis-Menten constant K_M and the maximum uptake rate v_{MAX} of 22 pharmaceuticals, 21 endobiotics/genous, 3 environmental contaminants, 1 toxin and 2 other chemicals, i.e. indican and bromosulfophthalein. Kinetic parameters were measured in *in vitro* studies using transport proteins expressed in the apical membrane of the human intestinal enterocytes. We found that $logK_M$ and $logv_{MAX}$ values of the different transport proteins and chemicals were highly correlated (r^2=0.76, $RMSE_{FIT}$=0.53), while the clearance rate $log(v_{MAX}/K_M)$ of most chemicals deviated less than one order of magnitude from the mean of 0.96 $pmol/mg_{protein}/min$. We were able to relate $logK_M$ to descriptors accounting for maximal projection radius, positive charge, solvent accessible positively charged surface area, hydrogen bond acceptor count and aromatic atom count (r^2=0.56, $RMSE_{FIT}$=0.89). The prediction for $logv_{MAX}$ included the maximal projection radius only (r^2=0.39, $RMSE_{FIT}$=0.86). Our study provides a first insight into descriptors relevant for carrier-mediated intestinal uptake kinetics of xenobiotics.

1.4 Outcome of Milestone 3: Comparing the contribution of carrier mediated transport and passive diffusion to the overall uptake

The work of this milestone will be submitted soon under the title "Theoretical framework to include carrier-mediated transport into toxicokinetic models".

Summary of the article: Most mechanistic bioaccumulation models consider passive diffusion as the only uptake route. However, xenobiotics, such as pharmaceuticals, toxins and environmental pollutants, have been shown to act as substrates for membrane transport proteins, which might increase their overall uptake. Yet, it remains unclear how important the contribution is of carrier-mediated transport to overall uptake, and opinions range from all is carrier-mediated to the coexistence of both routes. In this study, we evaluated the importance of both mechanisms for the ingestion of pollutants by incorporating carrier-mediated transport into an uptake model originally developed for passive diffusion only. The model was validated using oral absorption efficiencies of nutrients and pharmaceuticals. This theoretical framework suggests that carrier-mediated transport is always important for hydrophilic chemicals ($logK_{ow}$<-2.5), and probably unimportant for lipophilic chemicals (e.g. $logK_{ow}$>0 and no hydrogen bond donors). For chemicals with intermediate lipophilicity ($logK_{ow}$ ranging from -2.5 to +2 depending on the hydrogen bond donor), the relative contribution of carrier-

mediated uptake depends on the transport kinetics, substrate concentrations and transport protein concentrations. Research needs to further advance the modelling approach are discussed as well.

1.5 Side projects

In addition to the three projects described above, I have also been working on two side projects, both related to the problematic of plastic debris in the marine environment. In the first article, I reviewed plastic-water partition coefficients for a diverse set of organic chemicals and different types of plastic and evaluated whether there are differences between the various plastic types. The second article was produced in corporation with another research group (Plymouth, UK), where I will be 2nd author. I performed the model calculations in order to evaluate whether the ingestion of marine plastic debris containing organic contaminants such as PCBs will increase the contaminant load of three model species, i.e. lugworm, fish and seabird.

1.6 Overview of articles published/submitted/in preparation

- O'Connor, I. A., Huijbregts, M. A. J., Ragas, A. M. J. and Hendriks, A. J. (2013) 'Predicting the oral uptake efficiency of chemicals in mammals: Combining the hydrophilic and lipophilic range', *Toxicology and Applied Pharmacology,* 266(1), 150-156.
- O'Connor, I. A., Veltman, K., Huijbregts, M. A. J., Ragas, A. M. J., Russel F.G., and Hendriks, A. J. 'Relating kinetic parameters of human intestinal influx transporters to molecular properties of pharmaceuticals and environmental contaminants'. *Under review.*
- O'Connor, I. A., Veltman, K., Huijbregts, M. A. J., Ragas, A. M. J., Russel F.G., and Hendriks, A. J. 'Theoretical framework to include carrier-mediated transport into toxicokinetic models '. *Manuscript in preparation.*
- O'Connor, I. A., Golsteijn, L., Hendriks, A. J. 'Comparing the partitioning of organic contaminants into different plastics'. *Manuscript in preparation.*
- Bakir, A., O`Connor, I.A., Stadnicka, J., Rowland, S.J., Hendricks, A.J., and Thompson, R.C. 'Predicting the uptake of persistent organic pollutants desorbed from microplastics for a benthic feeder, a fish and a seabird'. *Manuscript in preparation.*

2 Additional activities

2.1 Secondment

I spent a month at Knoell Consult GmbH in Leverkusen (1-31 July 2013). During my stay at the Reach group (Environmental dossiers), they showed me the methods and models they use in order to determine ecotoxicological endpoints of chemicals, perform the corresponding exposure and the risk assessment. During my stay at Knoell there was another visiting employee starting up a business unit abroad. I was allowed to join her for her introduction week in Leverkusen such that I had a short visit at also other groups of the company (project/consortium managers, evaluation of biocides, chemical properties and QSAR).

I really enjoyed the time at Knoell Consult, and it gave me many insights. First, I think it is very useful for researchers developing models that they are aware what people use in practice, and what the needs are. The insights I obtained during my stay will help me to guide my own future research in a direction where I can focus on topics, models, etc. that are relevant for the society and hopefully will be applied at some point. Besides this aspect, it was also very nice just to see the type of work they do in a consulting company.

2.2 Platform and Poster Presentations at Conferences

- SETAC Europe 21st Annual Meeting, Milan (Italy), 15-19. May 2011.
  Poster: O`Connor IA, Huijbregts MAJ, Ragas AMJ, Hendriks A, "Predicting the uptake efficiency via ingestion of neutral and polar chemicals".
- SETAC 6th World Congress, Berlin (Germany), 20-24. May 2012.
  Poster: O`Connor IA, Huijbregts MAJ, Ragas AMJ, Hendriks AJ, "Predicting the oral uptake efficiency of chemicals in mammals: combining the hydrophilic and lipophilic range"
  Poster: O`Connor IA, Veltman K, Huijbregts MAJ, Ragas AMJ, Russel FG, Hendriks AJ "Is carrier mediated uptake relevant for the overall dietary uptake of environmental pollutants?"
- CADASTER Workshop on the development and application of QSAR models with respect to REACH guidelines, Munich (Germany), 7.-9. Sep 2012.
  Poster: O`Connor IA, Huijbregts MAJ, Ragas AMJ, Hendriks AJ, "Predicting the oral uptake efficiency of chemicals in mammals: combining the hydrophilic and lipophilic range"
- SETAC North America 33rd Annual Meeting, Long Beach (USA), 11-15. Nov 2012.
  Platform presentation: O`Connor IA, Huijbregts MAJ, Ragas AMJ, Hendriks AJ "Predicting the oral uptake efficiency of chemicals in mammals: combining the hydrophilic and lipophilic range"
- CEMEPE/SECOTOX 4th International Conference, Mykonos (Greece), 24-28 June 2013.
  Platform presentation: O`Connor IA, Veltman K, Huijbregts MAJ, Ragas AMJ, Russel FG, Hendriks AJ ,"Carrier mediated transport over the intestinal membrane - Analysis of the kinetics"
- SETAC GLB 18.Jahrestagung, Essen (Germany), 23-26. Sep 2013.
  Platform presentation: O`Connor IA, Veltman K, Huijbregts MAJ, Ragas AMJ, Russel FG, Hendriks AJ ,"Theoretical framework to include carrier-mediated transport into toxicokinetic models"

2.3 Schools / Courses

2.3.1 ITN Eco Summer and Winter Schools

- 18-22 Oct 2010: Summer School organized by Helmholtz Zentrum München, Munich (Germany)
- 21-25 Feb 2011: Winter School organized by Hochschule Fresenius, Idstein (Germany)
- 19-30 Sep 2011, Summer School organized by Universiteit Leiden, Leiden (Netherlands)

- 27 Feb - 2 Mar 2012: Winter School organized by Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid (Spain)
- 11-15 June 2012: Summer School organized by University of Milano-Bicocca, Milan, held in Verona (Italy) in conjunction with the annual meeting of the International Academy of Mathematical Chemistry (IAMC).
- 25 Feb – 1 Mar 2013: Winter School organized by Linnaeus University, Kalmar (Sweden)

2.3.2 Other Courses related to ITN Eco

- 5-9. Mar 2012: Variable Selection by the LASSO method. Internal training Action, Environmental Chemoinformatics organized by University of Milano-Bicocca, Milano (Italy)

2.3.3 Courses offered by the Radboud University Nijmegen, Nijmegen (Netherlands)

- Autumn 2011: Scientific Writing
- Spring 2012: Management voor Promovendi
- Autumn 2013: Presentation skills

2.3.4 Language courses

- Spring 2011: Nederlands als tweede taal (NT2) niveau A2
- Autumn 2011: Nederlands als tweede taal (NT2) niveau B1

3 General remarks and acknowledgements

I started my long term fellowship on the 1st of September 2010, and my contract with ITN Eco finishes on the 31st of August 2013. I have an additional contract until 31st December 2013 which will allow me to finish my thesis according to the Dutch standards. I would also like to use this moment to thank the Marie Curie Actions through the ITN Eco project for the funding of my time as a PhD student here at the Radboud University Nijmegen, Netherlands, and for the financial support to do all the winter and summer schools, visiting the conferences and the secondment at Knoell Consult GmbH. I learnt a lot during the last three years and this is true for all aspects; regarding extending my scientific knowledge and scientific working approaches, as well as gaining experiences from working abroad and meeting other researchers.

4 Literature

1. Hendriks, A.J., et al., *The power of size. 1. Rate constants and equilibrium ratios for accumulation of organic substances related to octanol-water partition ratio and species weight.* Environmental Toxicology and Chemistry, 2001. **20**(7): p. 1399-1420.
2. Arnot, J.A. and F.A.P.C. Gobas, *A food web bioaccumulation model for organic chemicals in aquatic ecosystems.* Environmental Toxicology and Chemistry, 2004. **23**(10): p. 2343-2355.
3. Arnot, J.A., et al., *Molecular size cutoff criteria for screening bioaccumulation potential: Fact or fiction?* Integrated Environmental Assessment and Management, 2010. **6**(2): p. 210-224.
4. Sugano, K., et al., *Coexistence of passive and carrier-mediated processes in drug transport.* Nat Rev Drug Discov, 2010. **9**(8): p. 597-614.