descriptor. We observed a pH dependent toxicity for ionisable APIs, which suggests that the neutral species is the driver of toxicity. This supports the use of the D_{lipw} (pH) as physicochemical descriptor in baseline-toxicity QSARs for other bioassays to identify APIs with specific mode of action indicated by a TR>10.

MO189

Factors influencing freshwater pharmaceutical uptake using in vitro gill cell culture

E. Chang, Kings College London; C. Hogstrand, Kings College London / Division of Diabetes and Nutritional Sciences; S.F. Owen, AstraZeneca / Safety Health Environment; N. Bury, University of Suffolk / Division of Diabetes and Nutritional Sciences

Pharmaceuticals are becoming increasingly prevalent in the environment and regulations to assess the environmental risks requires the use of fish in animal testing. A primary fish gill cell culture system has shown significant promise as an *in vitro* replacement model system for whole fish compound uptake studies. The current study assessed the uptake of 8 pharmaceuticals with a range of chemical parameters from the apical freshwater compartment of the primary gill cell culture system. An HPLC method was developed to extract and analyze compounds simultaneously from the OECD water matrix (warfarin, ibuprofen, beclomethasone, carbamazepine, diclofenac, ketoprofen, norethindrone, gemfibrozil). The relationship between the uptake of the compounds and the various chemical parameters were analysed using linear regression. There was no correlation to Log K_{ow} (R=0.002), weaker correlation to Log S (R^2=0.24) and molecular weight (R^2=0.23), and a stronger correlation to Log D (R^2=0.57), and pKa (R^2=0.56). The strength of correlation of Log S (R^2=0.83) and pKa (R^2=0.91) were greatly improved with carbamazepine and diclofenac excluded, respectively. Uptake of the compounds was found to be a function of cell permeability and overall findings suggest that ionizable pharmaceuticals are able to interact and enter fish gill cells.

MO190

Development of a zebrafish embryo test for the evaluation of endocrine disrupting pharmaceuticals with nano-injection as an alternative exposure route

E.D. Michiels, L. Vergauwen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; S.J. Van Cruchten, University of Antwerp / Applied Veterinary Morphology, Dept Veterinary Sciences; D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences

Pharmaceutical companies have to perform an environmental risk assessment for every new drug. The tests for potential endocrine disrupting (ED) compounds require a lot of time and test animals, which is not consistent with the 3R principle. Therefore, the goal of this study is to develop a zebrafish embryo test, which is not considered an animal test according to European regulations. However, it is often difficult to expose fish aquatically to ED pharmaceuticals because of their lipophilicity. Nano-injection is therefore proposed as an alternative exposure route because the yolk of zebrafish embryos contains many lipids, and this route mimics maternal transfer. To use nano-injection as an alternative it needs to be characterised and compared to the classical exposure route via water, because it is known that toxicity can depend on the exposure route. In this part of the study 17α-ethinylestradiol (EE2, estrogen receptor (ER) agonist) was chosen to compare malformations, swimming activity, ER activation and uptake dynamics between both routes. Transgenic (*5xERE-GFP*, obtained from the Carnegie Institution of Washington) zebrafish embryos, expressing GFP upon ER activation or wildtype embryos were aquatically exposed or injected and observed until 120 hours post fertilization (hpf). 0.5 nl of EE2 dissolved in dimethyl sulfoxide (DMSO) was injected. DMSO was also used as vehicle control. Each day, fluorescent intensity was measured to compare the time-dependent dynamics of ER activation in both routes and malformations were scored. Growth was monitored daily from 54 hpf and swimming activity from 72 hpf. We observed different physiological effects and malformations between both routes. During the 5 days, fluorescent signals were in the same order of magnitude for both routes. This indicates that EE2 was still active 5 days after injection. The fluorescent intensity increased every day in both exposure scenarios, similar to the increase of *esr2b* mRNA levels during normal development. However, the relative contributions of EE2 accumulation and ER expression to the signal are not known yet. The dynamics of ER activation are similar between both routes, suggesting that other mechanisms (e.g. uptake in different organs) are responsible for the morphological and physiological differences. This future knowledge will be essential to determine the applicability of nano-injection as an exposure route for lipophilic compounds.

MO191

Calcium channel blockers and antihistamines: specific and non-specific toxicity in Daphnia magna

A. Falkenhain, ECT Oekotoxikologie GmbH; M. Scheurer, DVGW Water Technology Center / Analysis and Water Quality; A. Coors, ECT Oekotoxicologie GmbH

Human pharmaceuticals that were authorized for a specific indication before 2006 are exempt from the requirement of a formal environmental risk assessment. Yet, various approaches have been proposed to prioritize these biologically active legacy compounds for an assessment of their potential effects in the environment. Within the project "iPiE" (*Intelligence-led Assessment of Pharmaceuticals in the Environment*, supported by Europe's Innovative Medicines Initiative (IMI)) such a prioritization scheme will be developed that utilizes also model-predicted properties for compounds without available data. Yet, only few data on human pharmaceuticals are available for developing models for acute and chronic *Daphnia* toxicity. Therefore, a series of acute and chronic *Daphnia magna* tests were conducted according to OECD Guidelines 202 and 211 with pharmaceuticals from two therapeutic groups (calcium channel blockers and antihistamines). The individual test compounds covered a range of physico-chemical properties, particularly with regard to lipophilicity and ionization. Correlations between physico-chemical properties and observed effects on survival, reproduction and growth of *Daphnia magna* will help to identify key descriptors for models of non-specific toxicity and patterns that point at specific modes of toxicity in *Daphnia*.

MO192

Impact of pharmaceuticals on aquatic invertebrates: an indoor study

I. Roessink, Alterra / Environmental Risk Assessment Team; E. Peeters, Wageningen University / Aquatic Ecology and Water Quality Management

Nowadays pharmaceuticals are measured frequently in surface water monitoring schemes. These substances reach surface waters via waste water treatment plants (WWTP) and are distributed throughout the receiving water system. The effect these substances might have on the aquatic environment, however, is in most cases not known. To study the potential effects of pharmaceuticals, several laboratory experiments were conducted varying from single species behavioural assays to indoor microcosm (using a small ecosystem) testing. In the laboratory, 16 microcosms were installed containing a small aquatic community comprising *Elodea* sp. (plant), *Lumbriculus variegatus* (worm), *Physella* sp. (snail), *Asellus aquaticus* (macrocrustacean) and *Daphnia magna* (cladoceran). Four microcosms were filled with 12L effluent, four with 12L control water and four were filled with 12L control water and received a spike of a mix of selected pharmaceuticals. The mix comprised metformin, guanylurea, metoprolol, sotalol, atenolol, irbersartan, hydrochlorothiazide, diclofenac and carbamazepine. In addition, single species behavioural assays were conducted using the Multispecies Freshwater Biomonitor (MFB) exposing *Gammarus pulex* (macrocrustacean) to fluoxetine, ibuprofen, carbamazepine and CTAB. In the microcosms the exposure to pharmaceuticals at effluent relevant concentrations did not cause effects on the survival on the tested populations of *Lumbriculus*, *Physella*, *Asellus* and *Daphnia*. The excess of nutrients from the effluent seems actually to favour the populations, resulting in increases in abundance compared to the control. In contrast, MFB assays with *Gammarus*, another macrocrustacean, showed that at low concentrations effect on behaviour were observed which again disappeared at higher test concentrations. These results indicate that pharmaceuticals do not follow the standard (eco)toxicological rules where an increase on concentration shows and increase in effects but have much more subtle effect windows. Such effects, however, will be difficult to observe in model ecosystems where only a part of the aquatic community is present.

MO193

Fate of pharmaceuticals in surface waters; a case study of the river Dommel, The Netherlands

J. de Klein, E.P. van den Brande, Wageningen University / Aquatic Ecology and Water Quality Management Group; I. Roessink, Alterra / Environmental Risk Assessment Team

Nowadays pharmaceuticals are measured frequently in surface water monitoring schemes. Their distribution in water and sediment depends on various parameters, e.g., substance characteristics, degradation and sorption kinetics. These parameters, however, are usually not known frustrating predictions on their environmental fate. In this project a DUFLOW model was parameterized to model the distribution of diclofenac, metoprolol, carbamazepine and sulfamethoxazole (SMX) in the river Dommel, The Netherlands. The model was validated using available monitoring data. Parameterization of the model was performed using tailored laboratory experiments, surface water and sediment monitoring data. In the laboratory set-up the impact of temperature, photolysis and microbial activity on compound degradation was tested. The data on surface water concentrations was obtained from regional surface water quality surveys, while sediment data was obtained by sampling and analysing sediment from different locations in the Dommel system. In the lab study, diclofenac degraded fastest (DT_{50}=2.8d), followed by SMX (DT_{50}=8.3d), metoprolol (DT_{50}=10.6d) and carbamazepine (DT_{50}=22.2d). Photolysis, increased temperature, and microbial activity all enhanced compound degradation. Although modelled dynamics of carbamazepine and metoprolol fitted the measured concentrations well, this was not the case for diclofenac. Since SMX was not included in the analysis of the monitoring surveys, the modelled dynamics could not be validated against measured concentrations in water and sediment. The DUFLOW model predicted the dynamics of metoprolol and carbamazepine well but did not so for diclofenac which was the most degradable compound tested. A sensitivity analysis on the different model parameters, i.e., degradation, resuspension, sedimentation rate and partitioning coefficients, revealed that model sensitivity was high towards degradation rates of the compounds. Since the laboratory study showed that degradation was driven for a large extend by