non-standard algae species including a recovery step represents a special challenge for the planning and conducting personnel. During the reregistration process of a PPP we had the chance to perform two such studies. We will present the data of both tests during the exposure and the recovery phases (free of growth inhibiting substance) and discuss the difficulties of such tests, beginning with the search of appropriate species, the pretesting, the development of a proper test design, the performance of the test itself and the interpretation of the test.

TU025

Evaluation of bioaccumulation potential of several pharmaceuticals based on log D/BCF data from the standard OECD 305 protocol and the OECD 305 minimised test designed

L.A. Constantine, Pfizer, Inc. / Pharmacokinetics Dynamics and Metabolism; T. Davidson, Bristol-Myers Squibb / EHS; B. Hoeger, Novartis Pharma AG / Health, Safety and Environment; K. Hutchinson, AstraZeneca Global Environment; M. Lee, Eli Lilly and Company / Environmental Risk Assessment; J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; J. Straub, F.Hoffmann-La Roche Ltd / Roche Group Safety, Health & Environmental Protection; J.G. Tell, Merck & Company, Inc. / Global Safety the Environment; I. Van Ginneken, Johnson Johnson

As per the EMA Guideline for Environmental Risk Assessment of Medicinal Products for Human Use, a fish bioconcentration study is triggered in Phase I for pharmaceuticals having a log $K_{ow} > 4.5$ to support the Persistence, Bioaccumulation and Toxicity (PBT) assessment and in Phase II, Tier A for pharmaceuticals having a log $K_{ow} > 3$. The recommended protocol for bioconcentration is OECD Test Guideline 305: Bioaccumulation in Fish, Aqueous and Dietary Exposure. Based on the standard 305 sampling schedule, approximately 200-300 fish per study may be required to determine a steady-state (BCF_{ss}) and a kinetic (BCF_k) bioconcentration factor. The current 305 guideline (2012) includes the minimised test design as described by Springer *et al* (2008). This design uses fewer sampling time points, reducing the number of animals and resources required to establish BCF values, and as per the guideline, may be used for substances in which uptake and depuration are expected to follow first order kinetics; behavior generally associated with non-ionisable organic substances. Considering the potential for fish to metabolise xenobiotics and the number of animals required for a standard BCF test, an evaluation of BCF values obtained for several pharmaceuticals was conducted to understand 1) whether existing data support the current log K_{ow} trigger of 3 for BCF testing and 2) whether the minimised test design may be considered appropriate for ionisable compounds such as pharmaceuticals. Based on the current log K_{ow} trigger and 'B' classification criteria, BCF < 1000 (US) and < 2000 (EU), the suitability of the current trigger was assessed using BCF and log K_{ow} values for approximately 45 pharmaceuticals. For those pharmaceuticals classified as 'B', a further assessment of persistence and toxicity classifications was conducted to understand the percent of pharmaceuticals within this dataset that are also classified as PBT, or vPvB. In addition, the BCF_k values obtained using standard OECD 305 test method were compared to the estimated kinetic (BCF_{km}) values determined using the reduced sampling method or Minimised Test Design, to determine if such a test design may be considered appropriate for ionisable substances. Evaluation of the data support an increase in the log K_{ow} trigger for BCF and indicate the minimised test design may be appropriate for ionisable substances such as pharmaceuticals, provided uptake and depuration follow first order kinetics.

TU026

Toxic and Activation Effects of Low-Level Radiation via Bacterial Luminescent Assay. Description in Terms of Hormesis and Threshold Models

N. Kudryasheva, Institute of Biophysics SB RAS; T.V. Rozhko, Siberian Federal University; A.S. Petrova, Insitute of Biophysics SB RAS; O.A. Guseynov, Siberian Federal University; G. Badun, Lomonosov Moscow State University

Effects of alpha- and beta-emitting radionuclides (americium-241 and tritium), and gamma radiation (^{137}Cs-containing particles) on luminous marine bacteria were studied under conditions of chronic low-dose irradiation (< 0.2 Gy) in aqueous media; bioluminescent intensity was used as a tested physiological parameter. The luminous bacterium is a proper tool for study the low level exposures due to simplicity and high rates of assay procedure, providing a lot of samplings under comparable conditions and, hence, a proper statistical treatment. Non-linear dose-effect dependencies were demonstrated. Three successive stages in the bioluminescent response to alpha- and beta-emitting radionuclides were found: 1 - absence of effects (stress recognition or threshold effect), 2 - activation (adaptive response), and 3 - inhibition (suppression of physiological function, i.e. radiation toxicity). Gamma irradiation demonstrated only stages 1 and 3, while the bioluminescence activation stage (2) was not found. The bacterial response was found to be independent on activity concentrations of radionuclides or dose rates of gamma-radiation. The nonlinear dose-effect dependencies of ionizing radiation with activation phenomenon included (stage 2), were ascribed to the "hormesis" phenomenon. The effects of gamma-radiation were described in terms of "threshold" toxicity model. Experiments with tritiated water and tritium-labeled polyethylene films (liquid and solid courses of beta-particles, respectively) showed that activation of the intracellular bioluminescence process can take place without penetration of tritium into the cells. Sequence analysis did not reveal mutations in bacterial DNA under conditions of the experiments. The results give preference to a "non-genomic" mechanism of bioluminescence activation. Probably, the activation effects result from ionization of aqueous media followed by the intensification of cellular membrane processes. Biological role of reactive oxygen species, secondary products of radioactive decay, is discussed. Key words: bacterial luminescent assay, low-dose radiation toxicity, hormesis model, threshold model.

TU027

Use of an integrated testing strategy to fill data gaps for environmental risk assessment of iso-alcohols

G.E. Bragin, ExxonMobil Biomedical Sciences, Inc. / Environmental Sciences Division; C. Sutherland, ExxonMobil Biomedical Sciences, Inc. / Toxicology and Environmental Science; B. Hedgpeth, B. Kelley, D. Letinski, ExxonMobil Biomedical Sciences Inc; J. Butler, ExxonMobil Biomedical Sciences, Inc / Environmental Toxicology and Chemistry Laboratory; M. Lampi, ExxonMobil Biomedical Sciences, Inc.

Environmental risk assessments require quality data to provide defensible environmental quality benchmarks. Quantitative Structure Activity Relationship (QSAR) endpoint estimates are often appropriate for alcohols with a very strong correlation to aquatic toxicity test data. However, QSAR estimates require comprehensive justification to demonstrate applicability, and still may not fully meet regulatory requirements, leading to extensive long-term toxicity testing. Here, limited, strategic environmental testing was used to support QSAR predictions, thereby reducing animal testing while still meeting regulatory requirements. Aquatic toxicity testing with algae, daphnids and fathead minnows (OECD 201, ISO 20665 and OECD 210) was performed with isooctanol and isoundecanol. The study objective was to employ a testing program consisting of long-term fish (limit test), invertebrate and algal toxicity tests to demonstrate that QSAR estimations accurately predict aquatic effects from long-term continuous exposure to these substances, further supporting the use of QSAR models across a range of iso-alcohols. The data demonstrate that the QSAR model employed accurately characterized the hazard of iso-alcohols and is protective of these endpoints. Moreover, this combined information, by demonstrating a regular and predictable pattern of toxicity amongst these substances, further justifies read-across between substances for other endpoints (such as bioaccumulation) and supports efficient use of data for general purpose risk assessments.

TU028

OECD 201 - Comparison of Algae Growth Inhibition Reference Studies Conducted in a Conventional and Closed System

S. Höger, Innovative Environmental Services (IES) Ltd / Environmental Toxicology; H. Eckenstein, A. Dupont, J. Schreitmüller, Innovative Environmental Services IES Ltd

Some of the substances which have to be registered under REACh are volatile. The design of ecotoxicological studies such as the fish toxicity test (OECD 203), Daphnia immobilisation test (OECD 202) and Algae growth inhibition test (OECD 201) is different from standard cases as indicated in the OECD 23 and ISO 14442 Guidance documents on aquatic toxicity testing of difficult substances and mixtures. Especially for the algae growth inhibition test following the OECD 201 the differences are obvious: without the CO_2 – supply from the surrounding air the algae cannot grow. Therefore these algae need other carbon sources, e.g. $NaHCO_3$. Additionally to keep the pH of the test media as constant as possible, HEPES-buffer has to be added to the test water. The consequences of these special conditions for the algal growth have to be checked by performing controls. Not much literature is available which compares the growth of algae under open and closed conditions or compares the possible differences in sensitivity of the algae to substances between algae which grow under normal condition and in a closed system. To have a reliable data set we conducted studies with the same algae strain and under conventional and closed system conditions to investigate these differences. The results show that there are differences in sensitivity of the algae when the growth inhibition between the studies conducted in the closed (ECr_{50} = around 0.7 mg potassium dichromate/L) and the open (ECr_{50} = around 0.9 mg potassium dichromate /L) system are compared, but the differences are, in most cases, negligible for the risk assessment. In certain cases, when the toxicity EC_{50} values are closed the trigger values, e.g. 1.0 mg/L, the difference can be critical for the classification of the substance.

TU029

Development of a zebrafish embryo test for environmental risk assessment of pharmaceuticals with different endocrine disrupting modes of actions

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 are obligated to perform an environmental risk assessment for each pharmaceutical that is launched on the market. The current tests for potential endocrine disrupting compounds are 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 the European regulation on the use of laboratory animals, and which is capable of detecting and discriminating among 5 endocrine disrupting modes of action (MOAs). The MOAs that are studied

are estrogen receptor (ER) agonism and antagonism, androgen receptor (AR) agonism and antagonism and aromatase inhibition. As a first stage of test development, one pharmaceutical per MOA was investigated to map differences among the 5 compounds in zebrafish embryos on morphological, physiological and molecular levels. 17α-ethinylestradiol (EE2) was chosen as ER agonist, fulvestrant as antagonist. 17β-trenbolone and flutamide were respectively chosen as the androgen receptor (AR) agonist and antagonist and letrozole was the aromatase inhibitor. Zebrafish embryos were exposed to one of the pharmaceuticals or to dimethyl sulfoxide (vehicle control) and monitored until 120 hours post fertilization (hpf). During these 5 days multiple endpoints were evaluated. Survival was scored daily, heart rate at 24 hpf, hatching from 48 hpf and morphological endpoints and swimming activity at 120 hpf. Both agonists showed multiple morphological and physiological effects, the antagonists showed no effects or only mortality and the aromatase inhibitor only showed physiological effects. The differentiation potential of all those endpoints was however not high enough to differentiate the 5 MoAs. To increase this potential a transgenic zebrafish line (*5xERE-GFP*, obtained from the Carnegie Institution of Washington) was used. It expresses GFP upon ER activation and fluorescent intensity was measured at 120 hpf. Even at low concentrations embryos exposed to EE2 caused a significantly higher fluorescent signal compared to controls. Embryos exposed to the non-aromatizable androgen, 17β-trenbolone also gave a significant signal at very high concentrations, suggesting that another pathway is triggered to elevate estrogen concentrations. The other 3 compounds caused no significant signals as expected. In the future other endpoints will be added in order to find a combination of endpoints that can be used to discriminate the MoAs.

TU030

Development and need of a new marine cyanobacteria test for the ecotoxicological risk assessment of antibiotics applied in the marine environment

J.H. Heseding, C. Floeter, Hamburg University of Applied Sciences / Environmental Engineering

An increasing amount of pharmaceutical products in waterbodies all over the world is been detected. Antibiotics play a special role here. In addition to the high amount of medication, antibiotics are as well an ecologically important pharmaceutical group due to the specific activity against prokaryotes and a possible resistance formation in the environment. Especially in oligotrophic marine waters, phototrophic prokaryotes together with small eukaryotic algae make up a large percentage of the total primary production. The gramnegative phototrophic prokaryotes including the cyanobacteria and thus have prokaryotic target structures of antibiotics. For the approval of pharmaceutical products, no prokaryotic test system in the marine field of application is considered in the risk assessment so far. The potential effects of antibiotics especially for aqua cultural use on the primary production and the Nitrogen fixation are unknown. To investigate the effects on marine cyanobacteria a marine cyanobacteria test was developed. As test organisms two marine species *Anabaena spec.* and *Synechocystis spec.* were selected and cultivated in the laboratory. The cultivation method was transferred to 24-well microtiter plates and optimized. The test was carried out on a 24-well microtiter plate. In the test the percent inhibition of the growth rate (cell number) compared to the negative control is determined over a period of 72 hours. Marine cyanobacteria react more sensitive to the tested antibiotics than the marine eukaryotic algae *Phaeodactylum tricornutum* (Bacillariophyceae, DIN EN ISO 10253). The results show effects on non-target organisms in relevant environmental concentrations (µg L^{-1}). The investigations are carried out within the PharmCycle project with the overall aim to reduce pharmaceuticals in surface water, ground water and drinking water. PharmCycle has the goal to optimize the ecotoxicological risk assessment, to reduce pharmaceuticals in waste water treatment and to develop sustainable biotechnological and chemical produced antibiotics (green pharmacy). The project is conducted in several cycles, in which priority antibiotics and sustainable antibiotics are examined.

TU031

Evaluation of skin sensitisation potential of 4-tert-butylphenol and 4-(benzyloxy)phenol using in vitro skin sensitisation assays

P. Mishra, N. Patel, Jai Research Foundation JRF / R&D; R. Nagane, Jai Research Foundation JRF; R. Date, Jai Research Foundation (JRF) Global / R&D

Contact dermatitis is an inflammation of the skin characterised by redness, itching, blistering and in chronic cases, flaking of scales of skin, resulting from exposure of the skin to substances in the environment. Various *in vitro* assays like Direct Peptide Reactivity Assay (DPRA), Amino acid Derivative Reactivity Assay (ADRA), KeratinoSen, USens, hClat have been developed and accepted for the prediction of skin sensitisation potential. p-tertiary-butylphenol formaldehyde resins (PTBP-FR) are widely used as binders in industry and in numerous materials of everyday use, such as glues, adhesives, or inks. The adhesive property of these resins is also exploited for preparation of '**bindi'** adhesives (adhesives used at the back of decorative patch, usually put on the middle of the forehead by girls/ women in the sub-Indian continent). The objective of the present study was to determine the sensitizing potential of 4-tert-butylphenol & 4-(Benzyloxy)phenol (constituents of PTBP-Formaldehyde resins) using *in chemico*, *in vitro, and in silico* skin sensitisation assays. The *in chemico* skin sensitisation assays employed were DPRA & ADRA. The mean percent depletion of cysteine and lysine for 4-tert-butyl phenol and 4-(Benzyloxy)phenol were 2.40 and 18.85 respectively in DPRA assay. While, the mean percent depletion for 4-tert-butyl phenol and 4-(Benzyloxy)phenol were 1.36 and 34.53 respectively in ADRA assay. From the results of the *in chemico* assays, 4-tert-butylphenol was found to be a non-sensitiser while, 4-(Benzyloxy)phenol was identified as a sensitiser. In ARE-Nrf2 Luciferase Test method (i.e, KeratinoSens assay), calculated $EC_{1.5}$ values of 4-tert-butylphenol & 4-(Benzyloxy)phenol were found to be 3 μM & 24 μM respectively. Thus, from the results of KeratinoSens assay, both 4-tert-butylphenol & 4-(Benzyloxy)phenol were found to be sensitisers. In U-Sens assay, the endpoint is the calculation of Simulation Index (SI) which if found to be > 1.5 is considered as a sensitiser. Both the chemicals showed SI > 1.5. Hence, these chemicals were predicted as sensitisers. Results of QSAR also suggest these chemicals to be sensitisers. Thus, on the basis of results of all assays and using the '3 of 5' weight of evidence approach, 4-tert-butylphenol & 4-(Benzyloxy)phenol were concluded as sensitisers.

TU032

Chemoassay Profiling to Characterize the Skin Sensitization Potential and Potency of Organic Electrophiles

A. Böhme, UFZ - Helmholtz Centre for Environmental Research / Ecological chemistry; J. Moldrickx, Helmholtz centre for environmental research - UFZ / Ecological chemistry; G. Schuurmann, Helmholtz centre for environmental research - UFZ / Institute for Organic Chemistry

Allergic contact dermatitis is a worldwide health disease and is initiated by sensitizing the immune system through dermal contact with a skin protein covalently bound to an electrophilic xenobiotic. Possible sources of respective organic electrophiles include care products, cosmetics and industrial products. At present, REACH recommends the murine local lymph node assay (LLNA) as method of choice for evaluating the skin sensitization potential of chemical substances, calling for viable alternatives to replace this animal test. In this context, chemoassays have been recognizes as promissing non-animal alternatives to identify potential skin sensitizers through characterizing electrophilic reactivity. So far, only full-kinetic and mechanism specific approaches inform about the sensitization potency. For volatile electrophiles, chemoassays tend to overpredict their LLNA potency because these test compounds significantly evaporate from the murine skin, and thus are less available in vivo to react with nucleophiles of skin proteins (as compared to chemoassay conditions). These volatile compounds are typically excluded from respecitve reactivity-based prediction models. Moreover, for imine formers as aldehydes no chemoassay-based models for predicting the LLNA-type sensitization potency are available. The present communication demonstrates for Michael-acceptor carbonyls how LLNA potency of volatile electrophiles can be predicted through combining the chemoassay thiol reactivity[1] with the concept of chemoavailability.[2] The latter enables one to specifiy the conditions under which a given electrophilic reactivity triggers a reactive toxicity pathway in the physiological context of interest. Moreover, a chemoassay-based model to predict the LLNA-type sensitization potency of imine formers is presented, and thus extends chemoassays toward this class of potential sensitizers.

The authors thank the EU-funded project OSIRIS (contract no. GOCE-CT-2007-037017) and the BMBF-funded project ProHapTox (FKZ 031A422A and 031A422B) for financial support. [1] Böhme A, Thaens D, Paschke A, Schüürmann G 2009. Kinetic glutathione chemoassay to quantify thiol reactivity of organic electrophiles-application to \nα,β-unsaturated ketones, acrylates, and propiolates. *Chem. Res. Tox.* 22: 742-750. [2] Böhme A, Laqua A, Schüürmann G 2016. Chemoavailability of organic electrophiles – Impact of hydrophobicity and reactivity on their aquatic excess toxicity. *Chem. Res. Toxicol.* 29: 952-962.

TU033

Using a Database of Historical Control Avian Reproductive Performance to Enhance Evaluation of Agrichemical Product Toxicity

D.L. Temple, EAG Laboratories / Avian; J. Ross, T. Springer, K. Hammett, H. Krueger, EAG Laboratories

Avian reproduction studies with northern bobwhite *(Colinus virginianus)* and mallard *(Anas platyrhynchos)* have been conducted for nearly 40 years. The information collected from these gamebird species has been a primary part of the OECD and EPA regulatory program in evaluating agrichemical products and making environmental risk assessments. Control data from these two species have been collected and maintained since the late 1970's by Wildlife International (now operating as a division of EAG Laboratories). The databases compile values for reproductive parameters measured from birds used in each control group as part of each reproduction study conducted at the lab in Easton, Maryland, USA. The database summarizes mean numbers of eggs laid, fertility of the eggs, normal development including viability and survival of the embryos, hatchability, offspring survival, egg shell thickness as well as weights of offspring and 14-day old survivors. Data compiled in these records are becoming more critical in supporting evaluation of reproduction study results. There is complexity in making meaningful decisions about the toxicity of agrichemical products. When 'wild type' birds are used we can expect inherent differences in performance and that occasional anomalies will occur. Rather than repeating multiple tests or increasing the number of birds tested, databases, such as this one, can help risk assessors interpret the