

## Continuing Education Courses (CEC)

### CEC 1: Scientific and Regulatory Approaches for the Preclinical Safety Evaluation of Biologics

#### C01-01

#### Scientific and regulatory approaches for the preclinical safety evaluation of biologics

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Since the origin and development of biotechnology-derived pharmaceuticals several guidelines and points-to-consider have been issued to identify the most appropriate scientific and regulatory approaches for the preclinical safety evaluation of new biological entities (NBEs) in support to their clinical development. Regulatory standards for the preclinical safety assessment, outlined in the ICH S6 guidance are comparable among geographic Regions (EU, USA, and Japan) and a flexible science-based approach to nonclinical safety evaluation of biologics has been agreed. The selection of the most relevant models and the responsiveness of different species for toxicity testing are crucial. Best practices include *in vitro* studies in mammalian cell lines to predict *in vivo* activity and to assess the sensitivity in various species, including humans. These studies are generally designed to evaluate receptor occupancy, affinity and pharmacological effects assisting in the selection of the most appropriate models for further investigations in *in vivo* pharmacology and toxicity studies. Whenever possible, toxicity studies should be performed in two animal species relevant for the prediction of human safety. The duration of treatment, route of administration and study design should support the targeted clinical indication, and special attention should be given to the potential immunogenicity/immunotoxicity of the product and its impact on the pharmacodynamics, pharmacokinetics and toxicity.

Finally, also the criteria adopted to assess the safety of biotechnology-derived pharmaceuticals may significantly deviate from those generally used for standard chemical small molecules (i.e. MABEL vs. NOAEL).

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#### C01-02

#### Preclinical evaluation of the immunological safety of biologics

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Biologics are mostly large molecules, e.g. therapeutic proteins that are often intended to bind to specific targets expressed on the immune system, or to counteract immunopathological processes resulting in human diseases. Therefore, biologics have a high potential for immunotoxicity, which is reflected in the clinical experience accumulated so far on their adverse effects related to immunosuppression, immunostimulation, hypersensitivity (immunogenicity) and/or autoimmunity. The preclinical evaluation of the immunological safety of biologics should focus individually on each broad type of immunotoxic effects using dedicated animal models or *in vitro* assays. However, only a small number of standardized and validated procedures are available, and none for the evaluation of particular aspects, such as hypersensitivity and autoimmunity. As

biologics are either increasingly humanized or intended to bind to human-specific targets, the selection of relevant species, and the induction of specific and often neutralizing antibodies are major limitations to be overcome. Only limited guidance is offered by currently available regulatory documents. More adequate animal models and biomarkers of immunotoxicity are needed to improve the preclinical immunotoxicity evaluation of biologics and to ensure a better transition from preclinical to clinical evaluation.

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#### C01-03

#### A European perspective on nonclinical development of biologics

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The value of the nonclinical studies for proof of concept, kinetics or safety evaluation of therapeutic candidates depends on the human predictability of experimental approaches. When designing these studies, the experimental models should be carefully chosen and justified. For biopharmaceuticals the choice of *in vitro* or *in vivo* models is a difficulty as these products mostly correspond e.g. to human molecules obtained by DNA recombinant technology or to molecules (e.g. monoclonal antibodies) designed to specifically interact with human targets/receptors/epitopes. It is recognised that subtle structural differences between the human and animal equivalent molecules/targets lead to differences on the activity and their pharmacological response. However, even if the homology of human vs animal target is high, aspects like cellular cascades, their interrelated mechanisms and the type of target-related cellular responses may influence the human/animal responsiveness. These aspects, in addition to pharmacokinetics, need consideration and integration for species selection for nonclinical evaluation of (bio)candidates. Additionally, the development of neutralising antibodies by animals against the human molecules may modify their responsiveness and needs investigation. The relevance of the animal species chosen for nonclinical studies is therefore fundamental to warrant its human predictability, especially concerning the safe use in the earliest phases of clinical investigation. The CHMP/ICH/302/95 (ICH-S6) guideline discusses principles for biopharmaceuticals nonclinical testing. One addendum with clarifications/updates is being produced in ICH. Also EMEA/CHMP/SWP/28367/07 guideline for risk mitigation in first in man clinical trials is relevant in this context.

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#### C01-04

#### Regulatory approach to first-in-human trials with monoclonal antibodies

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Monoclonal antibodies (mAbs) are in many cases effective pharmaceuticals and, in general, have proved to be safe. However, on rare occasions mAbs can be highly toxic. For this reason, pre-clinical safety assessment is a crucial step in the development program especially prior to the first application in humans and should be regarded as a tool for pro-active risk identification.

Possible factors of risk are the nature of the target and the mode of action especially if they are novel. Careful consideration should be given to the extent of knowledge for these parameters.

Another important point is the relevance of the animal model. The high species specificity of mAbs makes the selection of an appropriate animal mode challenging. Nevertheless, it is paramount to choose a species in which the mAb binds to the target and elicits the same pharmacological effect as that expected in humans.

Together, data from pre-clinical safety studies and mechanistic in vitro/ex vivo investigations lead to the estimation of the first dose in humans. Different approaches such as the no observed adverse effect level (NOAEL) and the minimal anticipated biological effect level (MABEL) have to be taken into consideration.

Ultimately, the first dose as well as subsequent dose escalation has to be designed to guard the safety of subjects participating in the first-in-human trial.

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#### C01-05

##### **Biomarkers-driven early efficacy assessment for biological drugs in clinical proof-of-concept studies**

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Biological molecules, and typically monoclonal antibodies, constitute a challenge when developed as Biopharmaceuticals, especially in terms of their preclinical profiling, mainly concerning predictive ADME-PK and safety features. Whilst ADME characteristics of monoclonal antibodies are nowadays fairly well understood and PK/PD modeling approaches are often times applied successfully in extrapolating their MABEL in early human trials, the toxicity features of such molecules are sometimes underestimated and their application to man simply considered safe. Biomarkers for safety and prediction of efficacy constitute powerful tools in this respect, as they can be used to assess on-target effects in dose-escalation studies, characterize and understand the molecular mode of toxic effects and guide regimens and the optimal biological dose setting in subsequent more advanced clinical studies. We will present some applications of biomarkers for the purposes indicated above in guiding the successful development of biological molecules into efficacious drugs.

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#### **CEC 2: Risk Assessment Under REACH—How to Deal With the Read Across Approach**

##### C02-01

##### **Chemical categories: Filling data gaps by read-across and trend analysis, the OECD approach**

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A chemical category is a group of chemicals whose physico-chemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity

(or other similarity characteristic). In principle, more members are generally present in a chemical category, enabling the detection of trends across endpoints. Categories of chemicals are selected based on the hypothesis that the properties of a series of chemicals with common structural features will show coherent trends in their physicochemical properties, and more importantly, in their toxicological (human health/ecotoxicity) effects or environmental fate properties. Common behaviour or consistent trends are generally associated with a common underlying mechanism of action, or where a mechanism of action exhibits intensity changes in a consistent manner across the different members of a category.

As a result, it is possible to extend the use of measured data to similar untested chemicals, and reliable estimates that are adequate for classification and labelling and/or risk assessment can be made without further testing.

The assessment of chemicals by using a category approach differs from the approach of assessing them on an individual basis, since the effects of the individual chemicals within a category are assessed on the basis of the evaluation of the category as a whole, rather than based on measured data for any one particular substance alone.

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##### C02-02

##### **Risk assessment under REACH-Glycols ethers and read across approach**

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In Europe, the new regulation for the chemicals safety management named REACH lays down a clear obligation to carry out vertebrate testing only as a last resort. In that context, REACH promotes the use of alternative methods and all available information or approaches. One of the approaches to fulfill data gaps when registration of a chemical is proceeded (preparation of the chemical safety report) is to use the category and read across approach.

To illustrate how read across works and could be used to assess hazard for chemical category, the example of glycol ethers and in particular diethylene glycol ethers (di EGEs) was chosen. di EGE category gathers 5 chemicals (DGEE, DGEEA, DGPE, DGBEA, DGHE). Read across approach will be presented in details for toxicological hazard characterization. Results of the hazard and risk assessment show that read across has been successfully used for the di EGEs category.

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##### C02-03

##### **Alkyl sulphates, alkane sulphonates and alpha-olefin sulphonates**

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This part of the course deals with the practical application of a read-across approach for structurally and mechanistically related chemicals. The chemicals are from three related chemical classes: alkyl sulphates, alkane sulphonates, and alpha-olefin sulphonates. Chemicals of these three classes have been included in a single cat-

egory under the OECD SIDS program and were evaluated taking into account structural features, mechanisms of action, metabolic pathways, and exposure information. Advantages, opportunities and limitations of this approach for risk assessments under REACH will be discussed.

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#### C02-04

##### Read-across approach in risk assessment: Ferrochromium as an example

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In the REACH-regulation, alloys belong to the group of special preparations. In the assessment of special preparations, not only the bulk composition of the alloy, but also the way the constituent substances are bonded in the matrix, shall be noticed. The toxicological profile of an alloy may markedly be affected by the way the surface characteristics limit the release of constituent metals from the matrix, and therefore the surface and release rates should be taken into account in risk assessments of alloys.

Ferrochromium is an alloy composed mainly of chromium and iron with trace amounts of other metals, e.g. nickel. Almost no studies have been published on the health effects of ferrochromium. On the basis of its bulk composition, ferrochromium would most likely, due to the nickel content, automatically be classified as a sensitizer (according to the CLP-system). However, we decided to start the ferrochromium risk assessment by studying the surface composition and dissolution rates of its metal constituents into artificial body fluids. These studies clearly demonstrated a minimal release of nickel, and showed that ferrochromium can be likened to chromium metal, chromium(III) oxide and stainless steel. Available data on these substances, in relation to all toxicity end points needed for a REACH-compliant chemical safety assessment, were collected and analyzed. Based on this assessment, conclusions on ferrochromium health risks and suggestions for classification could be made, without any need to carry out new toxicity tests.

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#### C02-05

##### How valid are old repeated dose toxicity studies for chemical risk assessment?

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An essential endpoint for assessing human health effects also within the REACH process is the repeated toxicity in rodents. In many cases experimental studies are already available. However, these studies often have not been performed according to (current) guidelines, or without GLP.

The question is, if the NOAELs and LOAELs derived from these old studies are valid, or if the studies must be repeated. To answer this question the RepDose database on repeated dose toxicity in rodents (Bitsch et al., 2006) created by Fraunhofer ITEM with funding from Cefic LRI was analysed.

As expected, the extensive examination in recent Guideline studies usually results in effects in several organs/targets already at the LOAEL of a study. However, the number of organs affected is not crucial for the validity of the NOAEL or LOAEL, as long as one of the organs, which determines the LOAEL, has been examined. The most frequently affected targets at the LOAEL of high quality studies are liver, kidney, haematology, clinical chemistry and clinical symptoms, or body weight is reduced. In inhalation studies nose and lung are also affected quite often. If only one target organ triggers the LOAEL, in >80% of the cases it is one of the frequently affected target organs.

Based on these findings it can be concluded that in most cases also older studies can be used to derive a reliable NOAEL or LOAEL, if the frequently affected target organs of the respective guideline study have been investigated. A repetition of studies should be considered carefully.

#### Reference

Bitsch, et al., 2006. REPDOSE: a database on repeated dose toxicity studies of commercial chemicals—a multifunctional tool. *Regul. Toxicol. Pharmacol.* 46 (3), 202–210.

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#### C02-06

##### Ten proposals to improve testing and risk assessment

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Within REACH considerable resources will be spent on testing and risk assessment of industrial chemicals. These efforts are instrumental in the work towards a sustainable use of chemicals. Therefore, systematic evaluations aiming at constantly improving these activities are motivated. Based on previous research, ten suggestions for improving the risk assessment process are proposed and briefly discussed in this contribution. The tentative proposals are:

- (1) Improve the scientific basis of risk assessments for industrial chemicals with the aim to increase the robustness of the process.
- (2) Always use a data set that is as complete as scientifically motivated and reasonably achievable.
- (3) Both standardised and non-standardised data should be accepted for risk assessment purposes. A general standard for data reporting should be considered.
- (4) The scientific quality (reliability) and the assessed relevance (validity) of individual studies should be clearly evaluated as two distinct aspects.
- (5) The predictive power of individual studies should be reported when relevant.
- (6) Defined criteria for using mechanistic data for risk assessment and extrapolation purposes should be aimed at.
- (7) Any extrapolation of data should be transparently accounted for with relevant references.
- (8) The risk assessment conclusions should be presented in standardized manners, e.g. as follows: (i) conclusions based on animal data, (ii) conclusions based on epidemiology, (iii) overall assessment of human risk and (iv) inherent uncertainties.
- (9) If assessment factors are used they should be applied systematically and motivated explicitly.

- (10) A risk assessment includes both scientific and policy-related matters but these should be transparently separated.

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## C02-07

### Qualitative versus quantitative assessment

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The decision on a quantitative or qualitative read-across approach is based on the intended use. For classification purposes, a qualitative read-across approach may be sufficient as indicating that a property is absent or present.

The similarity for the read-across may exist in a common functional group which is of importance for the effect in question. A second mechanism may be a common precursor and/or breakdown product (metabolic pathway similarity). The most simple case is to group different salts or esters hydrolysing to the same active moiety.

Building of subcategories may be helpful: If a change of physical form from liquid to solid results in a reduced uptake a subcategory for liquid and solid members of the category can be proposed. Different approaches exist for quantitative assessment: The closest chemical analogue may be used as a surrogate and its value may be carried forward which may apply for different salts of the same chemical. Internal QSAR may be performed and the relationship between structure/molecular weight/physico-chemical property and numerical values of an endpoint may be established. Averaging the values of several compounds or taking the most representative value or the most conservative value are other possibilities to derive a numerical value in a read-across approach.

Examples are existing in aquatic toxicity. At present, there is no experience with the quantitative read-across to derive DNEL in human health risk assessment.

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## CEC 3: The Use of QSAR in the Screening of Carcinogens

### C03-01

#### Application of (Q)SAR and in vitro testing within the framework of REACH

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Information requirements, which concern, e.g. the intrinsic toxic and ecotoxic properties of chemicals, have been set in the REACH Regulation. According to article 13 of the Regulation, this information may also be generated by means other than animal tests, provided that the conditions of Annex XI are met. For (Q)SAR these conditions are:

- the QSAR model is scientifically valid,
- the substance under consideration is within the applicability domain of the QSAR model,
- adequate and reliable documentation of the applied method is provided, and
- results are adequate for C&L and/or risk assessment.

Positive in vitro test results can be used instead of in vivo data, when the method is sufficiently well developed, e.g. the “ECVAM criteria for entry into prevalidation process” are met. Depending on the potential risk confirmation by in vivo testing may be necessary. If results of in vitro studies are negative, the relevant (in vivo) tests shall be carried out to confirm the negative result. Such confirmation may not be necessary, if

- the method has been scientifically validated, according to internationally agreed validation principles,
- results are adequate for the purpose of classification, and
- adequate and reliable documentation of the method is provided.

Although ECHA may check the compliance and acceptability of (Q)SAR and in vitro data, it is emphasized that manufacturing and importing industry is responsible for providing data that meets the conditions given above.

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## C03-02

### Structure–Activity models for chemical carcinogens

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The Structure–Activity Relationships paradigm provides a wide range of tools, with different degrees of uncertainty, and that apply to different scopes.

On one side, there are coarse-grain approaches such as the Structural Alerts (SA). Beside being a repository of the science on chemical biological interactions, the SAs have a crucial role in risk assessment, for: (a) description of sets of chemicals; (b) preliminary hazard characterization; (c) formation of categories; (d) generation of subsets of congeneric chemicals to be analyzed subsequently with Quantitative Structure–Activity Relationships (QSAR) methods; (e) priority setting.

On the other side, there are fine-tuned QSARs for congeneric classes of chemicals. A range of good quality, local QSARs for mutagenicity and carcinogenicity have been assessed in our laboratory, and challenged for their predictivity in respect to real external test sets. The QSARs for potency generated predictions 30–70% correct, whereas the QSARs for discriminating between active and inactive chemicals were 70–100% correct in their external predictions. The same study showed that internal, statistical validation methods – often assumed to be good diagnostics for predictivity – did not correlate well with external predictivity.

The crucial role of mechanistic knowledge when applying Structure–Activity to risk assessment should be strongly emphasized. Mechanistic knowledge provides a ground for interaction between modelers, toxicologists and regulators, and permits the integration of the (Q)SAR results into a wider regulatory framework, where different types of evidence concur as a basis for making decisions and taking actions.

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**C03-03****Prediction of PAH and Nitro-PAH mutagenicity and PAC genotoxicity by QSAR modeling**

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Polycyclic Aromatic Compounds (PACs), particularly Polycyclic Aromatic Hydrocarbons (PAH) and Nitro-PAHs are ubiquitous environmental pollutants, that are recognized mutagens and carcinogens. QSAR modeling is a tool for the screening of chemicals with potential adverse effects, starting only from the chemical structure information. Different sets of mutagenicity or genotoxicity data have been modeled and used for predicting data, by using theoretical molecular descriptors in both regression or classification models. The models were developed according to the OECD principles for the validation of QSAR models for REACH.

In particular, a set of mutagenicity data (TA100) for 48 Nitro-PAHs was modeled by regression method. The proposed Multiple Linear Regression (MLR) models are based on topological molecular descriptors, selected by Genetic Algorithm. The applicability domain was verified by the leverage approach.

Human B-lymphoblastoid mutagenicity data of a set of 70 oxygenated, nitrated and unsubstituted PAHs, sorbed in urban aerosol, were successfully modeled by QSAR classification using the k-NN (k-Nearest Neighbors) and CART (Classification and Regression Tree) methods.

The genotoxicity values, obtained on the basis of compounds' IMAx (maximal SOS induction factor), of 276 chemicals were also modeled by CART and k-NN classification methods, based on theoretical molecular descriptors.

All the developed models were validated for predictivity by both internal and external validation. For the external validation, three different splitting approaches, D-optimal Experimental Design, Self Organizing Maps (SOM) and Random Selection by activity sampling, were applied to the original data sets for methodology comparison and selection of the best predictive model, independently of the splitting.

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**C03-04****Prediction of mutagenicity based on empirical physicochemical descriptors**

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Empirical descriptors of molecular reactivity (physicochemical properties of bonds) were investigated for their ability to predict mutagenicity in *Salmonella* (Ames assay). The bond properties are easily calculated from the molecular structure and can be quickly generated for large data sets of compounds (<http://www2.chemie.uni-erlangen.de/software/petra>). Our approach tries to account for the known relationship between chemical reactivity and mutagenicity (namely via covalent binding to DNA). Properties of chemical bonds are expected to strongly influence chemical reactivity.

In this presentation we will show the application of machine learning techniques to estimate mutagenicity on the basis of bond properties.

In order to use the information concerning several properties of bonds for an entire molecule, and at the same time to keep its representation within a reasonable fixed length, MOLMAP molecular descriptors were implemented. They encode the types of bonds available in the structure—a fingerprint of reactivity features.

In one study (Zhang and Aires-de-Sousa, 2007), global molecular descriptors and MOLMAP descriptors of bond physicochemical properties were used to train Random Forests to predict mutagenicity. Error percentages as low as 15% and 16% were achieved for an external test set with 472 compounds, and in the internal validation with the 4083 structures of the training set, respectively. High sensitivity and specificity were observed. Random Forests were able to associate meaningful probabilities to the predictions, and to explain predictions in terms of similarities between query structures and compounds in the training set.

**Reference**Zhang, Q.-Y., Aires-de-Sousa, J., 2007. *J. Chem. Inf. Model.* 47 (1), 1–8.

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**CEC 4: Safety Pharmacology in the Pharmaceutical Industry****C04-01****Introduction to safety pharmacology**

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Functional side effects remain one of the main reasons for adverse drug reactions as well as drug discontinuation during the discovery and development of pharmaceuticals. Safety Pharmacology (SP) is an evolving discipline combining the principles of physiology, pharmacology, toxicology, as well as other basic sciences. SP aims to predict functional side effects to human volunteers and ultimately patients by assessing drug effects on key physiological functions in relation to exposure in the therapeutic range and above; therefore the outcome of SP studies could significantly contribute to reducing the incidence and impact of ADRs and safety related attrition. The objectives of SP studies are (i) *to identify* undesirable pharmacodynamic properties of a substance that may have relevance to its human safety; (ii) *to evaluate* adverse pharmacodynamic and/or pathophysiological effects of a substance observed in toxicology and/or clinical studies; (iii) *to investigate* the mechanism of the adverse pharmacodynamic effects observed and/or suspected. To achieve these objectives, the discipline uses *in silico* approaches, *in vitro* assays and *in vivo* models. The impact of SP studies in relation to these objectives refer primarily to (i) *hazard identification and elimination* during the early discovery phases, (ii) *risk assessment*, primarily prior to first administration to man and (iii) *risk management and mitigation*, primarily during clinical development and life cycle management, respectively. The introductory lecture on SP will (i) highlight the importance of SP in the drug discovery and development process; (ii) present how and why SP has evolved so rapidly over the last decade; (iii) review the generic aspects dictating the design, conduct and reporting of SP studies;

(iv) show how SP data are used to form an integrated risk assessment to support decision-making at all stages of drug discovery and development and finally (v) briefly consider the predictability of SP assays towards the clinical outcome.

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#### **C04-02 Cardiovascular safety pharmacology (including QT interval)**

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This section will focus on the testing for drug-induced effects on the cardiovascular system including systemic hemodynamics and the electrocardiogram. The requirements as specified by regulatory guidances will be reviewed but more importance will be placed upon designing a testing approach that detects a wider spectrum of potential effects that could be of importance for the ultimate utility of a given compound, even if not posing a danger to first in man trials.

In vitro studies investigate the potential for a compound to block repolarizing potassium current (I<sub>Kr</sub>) through the so-called hERG channel. These can be done using high(er) throughput systems rather early in research to avoid this unwanted activity. Further in vitro profiling of the electrophysiological properties of a compound can also be done should the sponsor want a more thorough description of electrophysiological properties.

Hemodynamic effects have been typically investigated *in vivo* using models ranging from small, acute animal (e.g. mouse, rat, guinea pig) models to large animals (e.g. dog, pig, non-human primate) instrumented for the telemetric collection of data. A review of possible models, together with their advantages and disadvantages will be provided.

Cardiovascular endpoints can also be integrated into toxicological studies. This includes the arterial blood pressure and heart rate, as well as the ECG. The limitations of such data will be discussed. Newer approaches available that reduce endpoint variability and thereby improving test sensitivity will be discussed.

The participant should gain insight into the factors to consider when designing an effective test strategy for investigating cardiovascular liabilities of new drugs using state-of-the-art non-clinical studies.

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#### **C04-03 CNS safety pharmacology (including drug dependency)**

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CNS safety pharmacology is a well established discipline guided by ICH S7A. This universally accepted guidance describes a core battery of studies of which contains a CNS assessment. Motor activity, behavioural changes, coordination, sensory/motor reflex responses and body temperature should be evaluated. A functional observation battery (FOB) or modified Irwin's assessment in rodents are most often performed in the pre-clinical Safety Assessments

prior to First in Human administration a novel chemical entity. Follow up assessments based upon findings or other causes for concern can include behavioural pharmacology, learning and memory, ligand-specific binding, neurochemistry, visual, auditory and/or electrophysiology examinations, etc.

Drug dependency or abuse liability, although not a novel research area, is emerging as a key area of scientific and regulatory interest. Pre-clinical models can be used at any stage of drug development and can guide the clinical abuse liability trials.

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#### **C04-04 Respiratory/GI/renal/immune safety pharmacology**

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The Safety Pharmacology ICH S7A guideline distinguishes between core battery studies and supplemental studies: The core battery functions i.e., the cardiovascular, respiratory, and central (and/or peripheral) nervous functions were selected for the safety pharmacology core battery (mandatory) based on the rationale that an acute failure of one of these organ systems would represent a major cause of concern for human safety. The examination of additional organ systems may also be appropriate based on a cause of concern for human safety. According to the ICH S7A guideline, supplemental studies are meant to evaluate potential adverse pharmacodynamic effects on organ system functions not addressed by the core battery or repeated dose toxicity studies when there is a cause for concern. This notion of non-vital organ system is including renal, gastrointestinal or metabolism functions, but the list is not intended to be exhaustive, and other organ systems like immune, endocrine or skeletal muscle functions can be investigated.

The objective of this lecture is to provide a basic, broad overview of the approaches and methodologies used in Safety Pharmacology for assessing the drug safety risk in the field of respiratory, renal/GI and immune functions.

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#### **C04-05 Emerging trends and approaches in safety pharmacology**

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The future of safety pharmacology (SP) will depend, in part, upon the scientific and technological advances and regulatory challenges that envelop the discovery and development of pharmaceuticals for human use as well as the availability of trained scientists to design, conduct, interpret and report studies. The presentation will aim at reviewing these challenges with a focus on how:

- (i) to develop and refine strategies to assess the safety of pharmaceuticals that act at novel molecular sites (e.g., cell membrane, intracellular, intra-nuclear) and new therapeutic approaches (e.g., gene therapy, biopharmaceuticals products, combinations products);

- (ii) to keep pace with science to improve our ability to detect, predict and ideally eliminate human safety liabilities from novel molecular entities (e.g., PK/PD relationship, adult human stems cells, QT shortening); to adapt and to incorporate new technologies using *in silico* approaches (e.g., cardiac action potential modelling), *in vitro* assays (e.g., optical action potential measurement) and *in vivo* models (e.g., Zebrafish larvae). To facilitate this, SP should primarily focus on areas of highest impact and/or incidence such as the safety liabilities associated with the cardiovascular, gastrointestinal and nervous systems including special senses.
- (iii) to shape the legal regulatory framework of any SP related issues by actively promoting a dialogue between key opinion leaders and recognised experts from industry, contract research organisations, academia and regulatory agencies to foster the integration of suggestions from these partners to develop and adopt optimised solutions to recognised safety issues. For instance, scaling down the usage of animals according to the 3Rs (Reduction, Replacement, Refinement) could profit from such collaboration.
- (iv) to attract, train, and certify investigators in integrative approaches to physiology and pharmacology to ensure the future of the discipline.

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#### **C04-06 Special considerations in safety pharmacology: Biologicals**

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Safety pharmacology studies are a critical component of preclinical drug development, and the execution of these studies is described by the ICH S7A and S7B guidelines. While these guidelines have shaped the evaluation of small molecule therapeutics, the application of these studies to the safety assessment of protein therapeutics, or biologicals, is not straightforward and questionable. Like small molecules, biologicals are being developed as important new medicines for the treatment of human disease. From a drug safety perspective, biologicals require a unique approach to safety pharmacology and toxicology evaluation, especially in the area of cardiovascular safety evaluation. This presentation will provide some perspectives and approaches to consider in the safety pharmacology testing of biological therapeutics.

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#### **CEC 5: The TTC Concept: Past, Present And Future Developments**

##### **C05-01 The scientific basis and application of the threshold of toxicological concern (TTC)**

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The threshold of toxicological concern (TTC) is a pragmatic form of risk characterisation that can be applied to chemicals with limited or no chemical-specific toxicity data, but to which humans are

exposed at low levels. TTC values are levels of human intake predicted to be without significant adverse effects based on the toxicity of structurally related compounds. The TTC approach means that safety advice can be provided without the need for unnecessary animal testing.

A tiered TTC decision-tree has been developed that incorporates a very low value for potentially genotoxic carcinogens (but excluding the three most potent groups), based on linear extrapolation to a one in a million risk from the TD50s for known carcinogens. For compounds without structural alerts for possible genotoxic carcinogenicity a series of TTC values were derived based on the application of a 100-fold uncertainty factor to the 5th percentile of the distribution of NOAELs from chronic studies of compounds in three broad classes that share structural characteristics.

The approach originated for the evaluation of packaging migrants, and has been refined and used extensively for flavouring substances. Recently the TTC approach has been extended to allow the assessment of cosmetic ingredients and potentially genotoxic impurities in therapeutic drugs. The approach has the potential for application to the vast array of low-molecular-weight environmental compounds to which humans are exposed at low levels.

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##### **C05-02 Application of the TTC concept on food packaging materials**

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The Threshold of Toxicological Concern (TTC) concept can be of help for various day-to-day challenges in safety assessment. One of them is the evaluation of materials intended to come into contact with food and which may release certain quantities of some substances into food.

Real life situations will be presented in which safety assessors may consider to apply the TTC concept to certain substances in food-contact materials. Those will in most cases concern low levels of non-regulated substances or of non-intentionally added substances stemming from impurities or reaction by-products.

Practical examples of food-contact material safety assessments will be used to demonstrate in which cases and how the TTC concept can be applied.

Prerequisites for a safety assessment applying the TTC concept are:

- characterisation, approximate quantification and estimation of the exposure to the substances to be assessed,
- exclusion of the cohort of concern, evaluation of any concern for genotoxicity and the assignment of the Cramer class.

Challenges in the application of the TTC concept to food-contact materials will be discussed, such as the characterisation of substances migrating from food-contact materials in the trace amounts relevant for the application of the TTC concept, the sheer number of substances which has to be dealt with as well as the exclusion of certain classes of substances from the TTC concept.

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### C05-03 The Threshold of Toxicological Concern (TTC) and its application in the safety evaluation of flavouring substances

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Flavourings are agents that are intentionally added to food with the sole intention of imparting flavour. Whilst nature is the biggest producer of flavourings (far more than 6500 different volatile substances have been detected in foods) more than 3000 of these substances are intentionally added to food on a global basis. The majority of the flavouring substances are used at very low use levels having a simple chemical structure and a long history of safe use. Given the large number of flavouring substances requiring a safety evaluation and the fact that for the majority of flavouring substances human intakes are low and self-limiting it was recognised by various authorities that a standard toxicological evaluation is not feasible and therefore a different approach should be followed. In 1995, a new procedure for the evaluation of flavouring substances was considered by the JECFA (FAO/WHO Joint Expert Committee on Food Additives) at its 44th meeting which was subsequently applied in an adjusted version to the evaluation of more than 1800 chemically defined flavouring substances.

This procedure is based on the TTC concept that integrates a series of thresholds of toxicological concern, structure-activity relationships, information on intake, metabolic and toxicity information.

In 1999, the same procedure was also acknowledged by the Scientific Committee on Food as the most up-dated and systematic procedure for the evaluation of flavourings substances and is applied since 2000 for the safety evaluation of flavouring substances in Europe.

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### C05-04 Evaluation of the TTC concept with the database RepDose

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The Thresholds of Toxicological Concern (TTC) are generic human exposure threshold values derived for oral uptake for groups of chemicals below which no appreciable risk to human health is assumed. To reduce animal tests under REACH, in ANNEX XI of the REACH Guidance exposure based waiving is described for complex endpoints like repeated dose- or repro-toxicity, where no/limited alternative testing methods are available. The thresholds of the TTC concept could be used to define a structure based safe level of exposure for chemicals below which no further testing is needed. So far, the TTC concept has already been applied for contaminants in food and pharmaceuticals and for flavouring substances. These applications are characterised by very low exposure levels of the substances.

We evaluated, whether the TTC concept is applicable to existing chemicals relevant for REACH. The database RepDose (Bitsch et al., 2006), which focuses on repeated dose toxicity studies of existing chemicals, was compared to the Munro database, which was originally used for deriving the TTCs. The distributions of NOELs and LOELs in both databases are very similar, although only 100 of

about 600 chemicals are common in both databases. The TTC concept therefore may be applied to existing chemicals. The analysis of both databases also showed that the Cramer Classes do not discriminate adequately toxic from non-toxic chemicals, indicating a need to improve the Cramer decision tree. The analysis further revealed that local effects trigger low NOELs and LOELs in inhalation studies. Thresholds for inhalation exposure are derived.

### Reference

Bitsch, et al., 2006. REPDOSE: a database on repeated dose toxicity studies of commercial chemicals—A multifunctional tool. *Regul. Toxicol. Pharmacol.* 46 (3), 202–210.

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### C05-05 Computer tools for Threshold of Toxicological Concern elucidation

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The Threshold of Toxicological Concern (TTC) concept is based on the assumption that similar compounds exhibit similar activities. TTC methods aim to derive safe exposure levels through analysis of toxicological data. Computational tools which facilitate the use of this method have been developed. *Ambit* is open source software for chemoinformatics data management, allowing storage of large number of chemical structures and toxicological data and providing flexible means for exploration of structural and similarity spaces. *Toxtree* implements several classification schemes. The Cramer rules, which are the core of the TTC assessment procedure, assign chemicals into three classes with an increasing level of toxic hazard. They rely primarily on chemical structure, metabolic pathways and lists of substances which are usual ingredients of food and/or human body. The Verhaar scheme divides the chemicals into five groups and is widely used to predict the mode of action for aquatic toxicity. Two modules, for estimating the skin irritation and corrosion potential, and eye irritation and corrosion potential, are based on published ranges of physico-chemical properties and structural alerts. The Benigni/Bossa rulebase for mutagenicity and carcinogenicity prediction assigns labels to chemicals for potential mutagenicity and is able to recognize genotoxic and non-genotoxic carcinogenicity by structural alerts, and QSARs for specific chemical classes. Several new TTC-relevant *Toxtree* modules are under development and in beta testing phase.

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### C05-06 Applying the TTC concept to skin sensitisation and inhalation toxicity

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Scientific, animal welfare and regulatory drivers have been instrumental in increasing the interest in development of non-animal alternatives for toxicological hazard characterisation. In addition, improvements in the way that we conduct risk assessments

can also help to reduce the need for animal based hazard assessments.

One of the tools which has been available for characterizing the risk of human exposure to chemicals is the Threshold of Toxicological Concern (TTC). This approach is based upon a human exposure threshold below which there is no appreciable risk to health, even when the toxicological profile of the chemical under evaluation is unknown. To date this approach has only been applied to systemic toxicology end-points.

Methodologies have now been developed to apply the principles of TTC to the hazard endpoints of skin sensitisation and inhalation toxicity. In both cases, probabilistic analysis of existing data has been conducted and, in the case of skin sensitisation, an estimate of the percentage of chemicals that are sensitizers. Using these data exposure levels for which no appreciable risk of skin sensitisation or inhalation toxicity exist have been determined.

In scenarios where exposure to a given material is sufficiently low, a TTC approach for safety evaluation/risk assessment of skin sensitisation and inhalation toxicity would preclude the need for testing. It could also provide another risk assessment tool for use in conjunction with *in vitro/in silico* measures of these hazard endpoints.

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#### C05-07

##### Applicability domain of TTC (Threshold of Toxicological Concern) schemes—A conceptual approach

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The TTC concept is based on the assumption that for a wide range of chemical substances there can be defined a threshold level of exposure below which there is no significant risk of adverse effects to human health. Originally proposed for the regulation of food-packing materials, a TTC of 1.5 mg/day had been established in 1995 by the US FDA for food additives expected to be non-carcinogenic. Subsequently, statistical analysis of oral rodent and rabbit NOELs for 613 compounds (Munro database) lead to TTC values for three general structural classes with different inherent toxicity potentials (Cramer scheme) of 1800, 540 and 90 mg/day. Extension to organophosphate neurotoxicity and potential genotoxicity yielded respective additional TTCs of 18 and 0.15 mg/day. An ongoing study using the RepDose database investigates the possibility of deriving TTC values for the inhalation pathway.

With regard to TTC applications for the risk evaluation of industrial substances under REACH, an important question is how the compound class coverage of the TTC database relates to the chemistry of REACH substances. In the present communication, we show how the ACF (atom-centered fragment) methodology can be used for identifying and characterizing the structural domain of chemical substances. Application to both the Munro and RepDose databases as well as to the EINECS list of ca. 70.000 organic compounds provides a first characterization of their overlaps with respect to the structural domain. Moreover, examples are taken to demonstrate

the integration of the ACF-based applicability domain characterization into the TTC approach.

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#### CEC 6: Characterizing and Communicating Human exposure for chemical Risk Assessment

##### C06-01

##### Introduction to World Health Organization globally harmonized risk assessment methodology

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This presentation in the Continuing Education Course Characterizing and Communicating Human Exposure for Chemical Risk Assessment provides an overview of the WHO/IPCS Project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals (Harmonization Project), which produced the guidance that is the subject of the present course. This project aims to improve and harmonize global approaches to risk assessment by: increasing understanding and agreement on basic risk assessment principles; developing international guidance documents on specific issues.

The Project enables risk assessments to be performed using internationally accepted methods and these assessments can then be shared to avoid duplication of effort. It translates advances in scientific knowledge into new harmonized methods, promotes transparency in risk assessment, and reduces unnecessary testing of chemicals. The project benefits all those involved in chemical hazard/risk assessment (chemical assessment authorities and other risk assessment bodies, professionals, and researchers).

The presentation will describe major outcomes of the project in a number of areas, such as cancer and non-cancer mode of action assessment, risk and exposure assessment terminology, development of chemical-specific adjustment factors, and principles for characterizing exposure models. Current and planned future work on assessment of combined exposures, PBPK models, and other topics will be outlined.

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##### C06-02

##### Primer on human exposure assessment for chemical risk assessment

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Exposure assessment is the process of measuring and/or modeling the magnitude, frequency, and duration of contact between a potentially harmful chemical and a target population, including the size and characteristics of that population. For risk assessments, exposure assessment should characterize the sources, routes, pathways, and the attendant uncertainties linking a source of pollution to the dose in a receptor population. This presentation provides an introductory overview on how both models and measurements are

used to translate pollutant emissions into metrics of human exposure. Humans contact pollutants through three routes—inhalation, ingestion, and dermal uptake. Inhalation occurs in both outdoor environments and indoor environments where most people spend the majority of their time. Ingestion includes both water and food, as well as soil and dust uptake due to hand-to-mouth activity. Dermal uptake occurs through contacts with consumer products; indoor and outdoor surfaces; the water supply during washing or bathing; ambient surface waters during swimming or boating; soil during activities such as work, gardening, and play; and, to a lesser extent, from the air that surrounds us. An exposure pathway is the course that a pollutant takes from an ambient environmental medium (air, soil, water, biota, etc.), to an exposure medium (indoor air, food, tap water, etc.) and to an exposed individual. Exposure scenarios are used to define plausible pathways for human contact. Recognition of the multiple pathways possible for exposure highlights the importance of a multimedia, multi-pathway exposure framework. The magnitude and variation of exposures to environmental contaminants depend largely on (a) the concentrations of contaminants in exposure media and (b) the exposure factors of the target population. Human exposure factors include all behavioral, sociological, and physiological characteristics of a population that determine their contact rates with food, air, water, soils, etc. When an ambient pollutant is inhaled, ingested, or absorbed, only a portion of the contaminant remains in the body and is delivered to target cells or organs to result in a toxic effect. So a chemical risk assessment requires the conversion of an exposure to a resulting dose and its associated response.

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#### C06-03

##### **Understanding and Applying Human Exposure Models: WHO guidance, with demonstration/case study of an exposure model**

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Human exposure models are used to estimate quantitatively the exposure to substances from media by inhalation, skin contact or oral intake. Models are part of concepts that include the description of exposure scenarios and its translation into mathematical algorithms. Descriptors of exposure scenario are will be transferred into model variables. Sometimes, computer tools are called models, as well as conceptual descriptions of exposure situations.

This presentation will cover the “*Principles of characterizing and applying human exposure models*” WHO/IPCS (2008), to give support for developing, applying and interpreting exposure models, and which will be explained in the first part of the presentation. Exposure modeling can be performed by a tiered approach, starting with simple estimations and increasing to complex models.

The second part of this presentation reviews the types of exposure modeling approaches used in either research or regulatory setting. Specific examples will be demonstrated how tiered modeling methodologies may be applied for assessing human exposures. A variety of exposure models, ranging from simple deterministic formulations to more complex mechanistic and probabilistic models, will be demonstrated. Typically, a number of technical and operational challenges have to be dealt with during the selection and use of these models. These issues will be examined during the discussion of two broadly applicable examples, one representing

an air pollution (particulate matter) and the other a multimedia pollution (pesticides) exposure problem. The use of enhanced probabilistic exposure simulation modeling approaches for studying the more complex aggregate and cumulative exposure and risk assessment problems will be discussed.

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#### C06-04

##### **Principles of uncertainty analysis in exposure assessment: Characterizing and communicating uncertainty—WHO guidance**

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Uncertainty analysis is inclusive of both qualitative and quantitative techniques for characterizing the uncertainties in an exposure assessment. An exposure assessment should begin with a definition of the assessment objective. From this, a scenario and its conceptual exposure model are developed, upon which parameters needed and analytical model development are predicated. The conceptual model establishes exposure pathways and the relative magnitude of uptake or intake by different exposure routes. Typically the main sources of uncertainties are first characterized qualitatively and then quantified using a tiered approach. Exposure uncertainty analyses should account for several key sources of uncertainties that include the scenario, model, and model parameters. Probabilistic exposure assessment deals with inter-individual variability, arising from the certainty that different individuals will have different exposures, as well as uncertainty, arising from lack of knowledge regarding the true exposure for any particular individual at any particular time. Sensitivity analysis is a key component of uncertainty analysis that helps answer a number of key questions, such as: (1) what are the key controllable sources of variability?; and (2) what are the key contributors to the output uncertainty? WHO has developed 10 guiding principles for how to perform uncertainty analysis in exposure assessment that address: its role in exposure assessment; level of detail; identification of courses of uncertainty and variability; appropriately dealing with dependencies; use of data, expert judgment, or both; use of sensitivity analysis; documentation; evaluation such as peer review; iterative refinement of the assessment; and communication of results to stakeholders.

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#### C06-05

##### **Quantitative uncertainty analysis: Model case study**

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Overall uncertainty in an exposure assessment depends on the quantity, quality, and relevance of input data; the reliability and relevance of models used to fill data gaps or replicate known results; and the assumptions, scenarios, and decision options used in applying the assessment. This presentation provides a case study to explore advantages and limitations of different tiers of sophistication for quantitatively addressing uncertainty in exposure assessments. The case study is based on human exposures to a persistent and bioaccumulating substance called PBLx through

fish consumption. We use this case study to illustrate the problem of representing uncertainty in exposure assessment. The overall process involves (a) description of exposure assessment context and question, (b) exposure scenario definitions, (c) the proposed exposure model, (d) the parameters and data used, (e) sensitivity analysis, (f) output variance propagation, and (g) uncertainty importance evaluation. We apply quantitative uncertainty analyses by first making use of bounding sensitivity analyses (Tier 2) and then by making use of probabilistic assessments with outcome distributions reflecting uncertainty and variability (Tier 3).

We compare the insight provided by these different tiers and also illustrate differences among alternate variance propagation methods used in the Tier-3 assessment. The results illustrate that understanding the sources and magnitude uncertainty in exposure assessment is necessary to refine and target the assessment process. The tier of uncertainty analysis selected for an exposure assessment is dependent on the assessment context and question.

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