ECHA
EUROPEAN CHEMICALS AGENCY

Topical Scientific Workshop on New Approach Methodologies in Regulatory Science

Helsinki, 19-20 April, 2016

Poster abstracts

Topic 1: Definite hazard assessment: improvement of read-across

Poster 1

The read-across assessment framework under REACH[1]

Niklas Andersson[2], David R. Bell[2], Ingo Bichlmaier[2], George Cartlidge[2], Karel De Raat[2], Norbert Fedtke[2], Anneli Kojo[2], Agnes Kovari[2], Tatiana Netzeva[2], Eric Stilgenbauer[2]

[2] European Chemicals Agency, Helsinki, Finland

Keywords: *In vitro* and alternatives, predictive toxicology, regulatory/policy, read-across

Grouping of substances and the read-across approach is an adaptation of the standard testing regime set out in Annex XI, Section 1.5 of the REACH Regulation (EC No 1907/2006). This adaptation allows registrants, under certain conditions, to predict the properties of a substance based on data from structurally similar substances.

To facilitate the evaluation of read-across cases encountered in the different REACH processes, ECHA has developed a read-across assessment framework (RAAF). The first version of the RAAF focuses on human health. It is a scientific framework that structures the knowledge that is relevant for evaluating a read-across case. The framework directs and guides the assessing expert by posing relevant questions and suggesting possible answers.

The RAAF defines different scenarios for different read-across approaches (e.g. two structurally similar compounds are assumed to have the same effect(s); or the prediction is based on a trend for the same effect(s) observed within a category).

The respective scenarios are selected and applied to the proposed cases. Each scenario is associated with particular aspects (assessment elements (AEs)) that are deemed crucial to the assessment (e.g. are the same effect(s) observed for the substances).

Each AE poses questions that lead an assessing expert to select pre-defined conclusions (assessment options (AOs)). The AOs reflect the strengths and weaknesses of the read-across for the aspects covered by the AEs (e.g. 'acceptable with medium confidence'; or 'not acceptable in its current form'). The outcome is a degree of confidence associated with the proposed read-across.

In summary, ECHA has developed a RAAF, which structures expert judgement so that the criteria for expert opinions on which regulatory decisions are based can be applied consistently. The RAAF increases transparency on how ECHA assesses

[1] The poster was presented also at the 55th SOT meeting in New Orleans, Louisiana, USA, 13 – 17 March 2016

read-across cases and provides registrants with a focus to assess and improve their cases.

The views expressed in this paper are solely those of the authors and the content of the paper does not represent the views or position of the European Chemicals Agency

Poster 2

SEURAT-1 Proof-of-Concept: The ab Initio safety assessment case study for daily exposure to an active ingredient in a body-lotion

Elisabet Berggren[1], Gladys Ouedraogo[2], Alicia Paini[1], Andrea Richarz[1], Andrew White[3] and Catherine Mahony[4]

[1] Joint Research Centre, European Commission
[2] L'Oreal
[3] Unilever SEAC
[4] Procter & Gamble

Keywords: Safety assessment, *ab initio*, alternative methods, SEURAT-1, ToxCast

The SEURAT-1 (http://www.seurat-1.eu/) *ab initio* case study is an attempt to structure knowledge and data in a logical workflow that would be the basis for a full risk assessment, and would be the basis for a first integrated assessment relying only on alternative methods. This will showcase that there is a feasible way forward but also pointing on weaknesses and knowledge gaps, which then will assist in shaping a more focused strategy to advance alternative assessment approaches.

Based on the SEURAT-1 conceptual framework, we have developed a general workflow to assist the risk assessment. We assume that the workflow starts from the same considerations regardless of the type of safety assessment, and only after concluding that is it not possible to apply a Threshold of Toxicological Concern (TTC) or a read-across assessment; we continue to construct the logic how to predict whether the intended exposure could be considered safe based on data solely from alternative methods. We also include uncertainty estimates for each step in the workflow.

The substance selected to illustrate the case study is piperonyl butoxide (PBO) in an imagined exposure scenario of being a new ingredient introduced in a body lotion daily applied to the skin (overall body surface). The supportive alternative data (*in vitro* and *in silico* results) were obtained from ToxCast (http://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data) or generated by using methods developed within the SEURAT-1 projects. PBO is a known hepatotoxin, even though the mechanism of action is unknown, and was therefore considered suitable as most methods developed within SEURAT-1 were focussed on hepatotoxicity, and could therefore provide relevant data for the assessment.

Poster 3

Linking LRI AMBIT Chemoinformatic system with the IUCLID Substance database to Support Read-across of Substance endpoint data and Category formation

N. Jeliazkova[1], V. Koch[2], Q. Li[2], U. Jensch[2], J. Schneider-Reigl[2], R. Kreiling[2], I. Georgiev[1], B. Hubesch[3]

[1] Ideaconsult Ltd, 4, Angel Kanchev Str., 1000 Sofia, Bulgaria
[2] Clariant Produkte (Deutschland) GmbH, Am Unisyspark 1, 65843 Sulzbach, Germany
[3] CEFIC, 4 Avenue E. Van Nieuwenhuyse, 1160, Brussels, Belgium

Keywords: read-across, category formation, workflow, database, IUCLID, substance identity, reporting, data integration

Read-across and category formation are indispensable techniques in safety assessments of chemicals. The read-across approach is used on average in 20% of the Endpoint Study Records, while (Q)SAR is used in less than 1% of the dossiers, according to European Chemical Agency reports. Although many tools are available, only a limited number is capable to provide easily accessible data on substance identity, composition together with chemical structures and high quality endpoint data.

The AMBIT software, funded initially within the CEFIC LRI programme, provides a web service and user friendly web interface to a chemical database, various chemical structure search facilities and toxicity prediction models. The AMBIT data model was further extended to support substances with complex compositions and substances experimental data which allows importing data from the International Uniform Chemical Information Database (IUCLID) as well as other sources. Currently AMBIT supports manual upload of i5z files exported from IUCLID or semi-automatic import via IUCLID Web services. The chemical structures already contained in AMBIT are automatically linked to constituents/impurities/additives of the imported substances. The flexible data storage and visualization allows for user friendly presentation of study data (physicochemical properties, environmental fate, ecotoxicological and toxicological information) and composition. Comprehensive assessment workflows are developed for read-across and category formation based on all the data available in AMBIT. The assessment workflow facilitates the search for target and source structures, generating data matrices, gap filling and generating assessment reports with predefined formats automatically. The enhanced AMBIT facilitates drafting and improves quality for read-across and category formation and will be a useful tool for experts responsible for substance assessments.

Poster 4

Exploring Uncertainty in Exposure Thresholds to help Downstream Companies in Acquisition, Analysis and Evaluation of Exposure Data to Implement adequate Activities in their Daily Work

Dr. Uwe König[1], Dr. Malte-Matthias Zimmer[1], Prof. Dr. Göran Kauermann[2]

[1] Eupoc GmbH, Memmingen, Germany
[2] Department of Statistics, Ludwig-Maximilians-University Munich, Germany

Keywords: Statistics, Datamonitoring, low doses, extrapolation outside observation, company support, SME Support

Usually, the assessment of health hazards of a chemical substance needs the combination of the toxicological information and the human situation /1/. It is commonly agreed that there is a gap between these two types of information. The main challenge is caused by the fact that toxicological tests are not done on humans in common exposition situations. The results of estimated real life exposures mostly differ from the exposure of the experimental animals.

Especially the results of examinations performed on high dose levels extrapolated to low doses are often very uncertain. During the authorization process of various substances this issue is becoming important as it is currently pointed out by the opinion of RAC and SEAC /2/:

"RAC has established a non-legally binding reference dose response relationship for carcinogenicity of hexavalent chromates for both inhalation and intestinal exposure by linear extrapolation (RAC/27/2013/07 Rev. 1). Extrapolating outside the range of observation inevitably introduces uncertainties. As the mechanistic evidence is suggestive of non-linearity, it is acknowledged that the excess risks in the low exposure range might be an overestimate."

This is supported by the analysis of the exposure thresholds for hexavalent chromium carried out by the Chair of statistics of the Ludwig-Maximilians-University Munich /3/ which is addressed by a consortium of companies using hexavalent chromium.

As a consequence, companies are often overstrained in estimating the real risk properly, even if it is done in cooperation with the authorities. It is necessary, to develop an adequately defined approach of assessing, analyzing and interpreting the available data in an adequate way.

Furthermore it is necessary, to describe the real risk based on the human situation. Commonly used calculations based on simplifying software models do not provide valuable results. It is well known that e.g. the widely used EASE model of production based airborne concentration at workplaces tends to overestimate exposure. In conjunction with the problematic extrapolation to low doses a reliable assessment is almost impossible. This is especially problematic if the results should be used to evaluate the risk situation in certain production circumstances of single companies.

The attempt presented here is based on the following issues:

1. Describing the risk of workers by real human related field data
2. Regular monitoring of the data under comparable conditions

3. Compare simulations with real data under reliable statistical conditions

4. Careful extrapolation outside the range of observation.

Assessing the applied exposure-risk model and quantifying the uncertainty if an extrapolation outside the range is necessary.

5. Improve cooperation of companies involved and authorities by reliable criteria. The confidentiality of specific data has to be considered in conformance with current data security regulations.

For quite some time the VECCO e.V. association is addressing the statistical methods for reliable and objective assessment of the available field-data, especially at small concentration levels.

The final objective is to get information about the way of collecting the data needed to get representative and significant information that can be handled by the companies to setup reliable expositions scenarios and take suitable risk reduction measures.

/1/ "Understanding the importance of assessment factors in finding safe human exposure levels", Karel de Raat, ECHA Science 1/2014, pp. 8

/2/ Authorization "Lead Chromate Pigments", Compiled RAC and SEAC opinions, December 2014

/3/ "Exploring Uncertainty in Exposure Thresholds for Hexavalent Chromium (Cr(VI) based on the Baltimore and Painesville Cohort Studies"; G. Kauermann, H. Becher, V. Maier; submitted

Poster 5

Adverse Outcome Pathway 'footprinting': an integrated read-across approach to the assessment of mixtures

Jason C. Lambert[1]

[1] United States Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH

Keywords: Read-across, Adverse Outcome Pathway, mixtures, risk assessment

Human health and ecological viability are challenged daily by mixtures of chemical and non-chemical stressors. However, the cumulative impact of mixture exposures are often exceedingly difficult to characterize due to the lack of available hazard and dose-response information for many stressors. With the advent of 21st century toxicity testing and approaches such as adverse outcome pathways (AOP), opportunities to evaluate hazards associated with exposure to 'data-poor' mixture stressors has advanced significantly. Potential health impact(s) of mixtures stressors may be informed using an integrated read-across approach that includes AOP 'footprinting' when adverse outcome data derived from traditional bioassays is lacking. AOP footprinting is the stepwise profiling and comparison of AOPs at the level of key events moving backward from the most downstream key event to the molecular initiating event. The goal is to identify the key event(s) within each AOP suspected of contributing to a

given adverse outcome at which similarity between mixture stressors can confidently be determined. These key events are identified as the 'footprint' for a given AOP. Mixture stressors are then assigned to the appropriate 'footprint' category, and the key event dose-response relationship(s) for each stressor within a category are then used to evaluate mixture additivity. When adverse outcome data is lacking, the integration of AOP footprinting with structural, physicochemical, and/or toxicokinetic information will significantly advance read-across application in mixtures risk assessment by providing a more biologically-based hazard categorization or grouping strategy, and, providing a critical alternative for dose-response assessment of environmental stressors.

The views expressed in this abstract are those of the author and do not necessarily reflect the views and policies of the U.S. EPA.

Poster 6

REACH-across - making the publicly available safety data for 9,801 substances registered under REACH (2008-2014) a resource for read-across

Thomas Luechtefeld[1], Alexandra Maertens[1], Daniel Russo[2], Hao Zhu[2,3], Costanza Rovida[4] and Thomas Hartung[1,4]

[1]Center for Alternatives to Animal Testing (CAAT), Johns Hopkins Bloomberg School of Public Health, Environmental Health Sciences, Baltimore, USA
[2] The Rutgers Center for Computational & Integrative Biology Rutgers University at Camden, USA
[3] Department of Chemistry Rutgers University at Camden, USA
[4]CAAT-Europe, University of Konstanz, Germany

Keywords: Read-across, data-mining, REACH, hazard prediction, computational toxicology

The European Chemical Agency (ECHA) warehouses the largest dataset of *in vivo* and *in vitro* toxicology tests. This data was extracted using linguistic search engines into a structured, machine readable and searchable database. The constructed database, downloaded in December 2014, contains data for 9,801 unique substances (identified by EC-Number) – including 3,609 unique study descriptions (i.e. 'Exp Key Skin irritation corrosion') and a total of 816,048 study documents for substances). This data can be used to explore toxicological data on a scale not previously seen.

The most prevalent hazards are H317 "May cause an allergic skin reaction" with 20% and H318 "Causes serious eye damage" with 17% positive substances. Such prevalences obtained for all hazards here are key for the design of integrated testing strategies. The abundance of chemicals tested multiple times allows to assess the reproducibility of the different animal tests.

Substance similarity analysis was used to determine clustering of substances with similar hazard labels. To perform similarity analysis, substances were mapped from REACH to PubChem. Similarity was measured using the Pubchem 2d conformational substructure fingerprints which gives a binary vector 'fingerprint' to every chemical. Fingerprints were then compared for similarity via the tanimoto metric. Following K-Core filtration the Blondel et al. (2008) module

recognition algorithm was used to identify chemical modules, which further shows clusters of substances in use within the chemical universe.

The large amount of valuable ECHA data not available in other chemical databases warrants an examination of database overlaps. The extracted ECHA substances cover ~20% of substances in the high throughput biological assay database Tox21 (a 1737 substance overlap) and a 917 substance overlap with the Comparitive Toxicogenomics database (making up ~7% of the substances in CTD). The biological data available in these datasets combined with *in vivo* endpoints from REACH represent an enormous modeling potential. A case is made, that REACH should systematically open regulatory data for research purposes.

Together with the currently developed Good Read-across Practice guidance (Ball et al., ALTEX 2016) and the ongoing development of a web-based tool, this will facilitate the application of high quality read-across for the REACH 2018 deadline.

Poster 7

Predicting skin sensitisation using a decision tree integrated testing strategy with an *in silico* model and *in chemico*/*in vitro* assays

Donna S. Macmillan[1], Steven J. Canipa[1], Martyn L. Chilton[1], Richard V. Williams[1] and Christopher G. Barber[1]

[1] Lhasa Limited, Granary Wharf House, 2 Canal Wharf, Leeds, LS11 5PS, UK

Keywords: Skin sensitisation, Derek Nexus, DPRA, KeratinoSens, LuSens, h-CLAT, U-SENS, Integrated Testing Strategy, *in silico* assessment

There is a pressing need for non-animal based methods for the prediction of skin sensitisation potential. This is in part a result of the implementation of EU regulation 1223/2009 prohibiting the use of animals for the safety assessment of cosmetics or cosmetic ingredients. A number of non-animal assays have been designed to assess key events in the skin sensitisation adverse outcome pathway (AOP) but test chemicals outside their applicability domains (lipophilic compounds and pre-/pro-haptens) are generally not predicted well. In principle, Derek Nexus is capable of modelling the entire AOP and skin sensitisation alerts can take lipophilicity and pre- and pro-haptens into account, as alerts are expert-derived and have a mechanism-based domain. To this end, an integrated testing strategy (ITS) using Derek Nexus and a maximum of two *in chemico*/*in vitro* assays (from DPRA, KeratinoSens, LuSens, h-CLAT and U-SENS) has been developed. A decision tree was evaluated from a 213 compound data set where, after considering the Derek Nexus prediction and if the test chemical was inside or outside the *in chemico*/*in vitro* domain, the number of *in chemico*/*in vitro* assays required to adequately assess the skin sensitisation hazard was indicated. The performance of the decision tree was compared to single assays and the following ITS: a 1 out of 2 conservative call, a 2 out of 3 weight of evidence call and a 3 out of 3 consensus call. Generally, the decision tree improved upon other ITS approaches evaluated in this study with positive and negative predictivity of 86% and 81%, respectively. Our results demonstrate that an ITS using an *in silico* model such as Derek Nexus with a maximum of two *in*

chemico/in vitro assays can predict the sensitising potential of a number of chemicals, including those outside the applicability domain of existing non-animal assays.

Poster 8

Predicting hazardous properties of substances from related substances – some case reports

Gesine Muller[1], Jonas Nygren[1], Silvia Lapenna[1], Ari Karjalainen[1], Fabrice Broeckaert[1], Chiara Perazzolo[1], Linda Spjuth[1], Alexis Nathanail[1], Ricardo Simoes[1]

[1] European Chemicals Agency, Helsinki, Finland

Keywords: Risk assessment committee, RAC, CLP, CLH, target substance, source substance, mode of action, classification

CLP allows, when justified, the use of endpoint-specific information for one or multiple substances ('source') to predict the same hazard for another substance ('target'), based on similar chemical structures and/or physico-chemical, environmental fate, toxicokinetic and/or (eco)toxicological properties.

ECHA's Committee for Risk Assessment (RAC) started its work on assessing proposals for harmonised classification in 2009 and has since developed opinions on such proposals for over 200 substances. In about 35 % of 99 proposals submitted between 2013 and 2015, data from substances other than the target substance has been used.

In some cases, data on source substances have been supportive and part of a weight of evidence approach. In others, source substances were the key data supporting the classification.

The types of considerations have been manifold. Among others, data on metabolic precursor substances, substances with physico-chemical and structural similarities as well as on substances formed and released after a chemical reaction have been used. Substances acting via identical modes of action have also been considered by RAC and have formed the basis for the classification conclusion.

Poster 9

Essential Aspects of Read-across for Repeated-Dose Toxicity Predictions

Andrea-N Richarz[1], Mark TD Cronin[2], Terry W Schultz[3]

[1] European Commission Joint Research Centre, IHCP, Ispra, Italy

[2] Liverpool John Moores University, Liverpool, United Kingdom

[3] The University of Tennessee, College of Veterinary Medicine, Knoxville, TN, USA

Keywords: read-across, repeated-dose toxicity prediction, similarity, uncertainty

Read-across (RA) based on chemical grouping approaches is often proposed as means of data gap filling for chemical safety assessments. However, the lack of agreement on how to carry out RA hampers acceptance by regulatory authorities. One of the key aspects of performing a RA is the confirmation that the source substance(s) and the target substance(s) belongs to the same category because they are toxicologically similar. In repeated-dose toxicity-related RA, similarity assessment takes the form of comparing chemical, toxicodynamic and toxicokinetic properties. The level of evidence required to accept similarity arguments is not defined and may not be quantifiable. Thus, one is left with uncertainties. While there are many areas where dissimilarity can be identified and uncertainty can be defined, there is no agreement whether the dissimilarity is toxicologically relevant or the uncertainty warrants rejection of the prediction(s).

Our work over the past several years focussed on cosmetic-related substances has revealed that while RA is conceptually simple, in practice it is difficult especially for complex health endpoints such as repeated-dose toxicity. These difficulties have led us to identify what we consider essential aspects to applying RA to repeated-dose endpoints. Namely that RA arguments must be transparent and thoroughly and adequately documented. For RA to be used for risk assessment (for repeated-dose scenarios) it must be quantitative. While it is not always possible to definitively state a mode-of-action, less uncertainty is linked directly to strong mechanism plausibility. The limitations to quantify RA include the availability of suitable *in vivo* data to be read-across and lack of toxicologically-relevant *in vitro* or alternative methods data to support the toxicodynamics. However, the most notable limitation to using RA for repeated-dose endpoints is the lack of toxicokinetics understanding and data. We propose using RA 1) in a context-dependent manner, 2) on an one-to-one (analogue) or many-to-one (category) basis even if repeated several times to cover several target derivatives within a common group, and 3) on a single endpoint (e.g., time, species, route of exposure, etc.) basis.

Poster 10

SEURAT-1 Proof-of-Concept Read-across Case Study for Repeated-Dose Toxicity

Andrea-N Richarz[1], Elisabet Berggren[1], Martina Klaric[2], Catherine Mahony[3]

[1] European Commission Joint Research Centre, IHCP, Ispra, Italy
[2] Cosmetics Europe, Brussels, Belgium
[3] Procter & Gamble, Egham, UK

Keywords: read-across, toxicity prediction, similarity, uncertainty, case studies

The SEURAT-1 Research Initiative with the long-term goal of achieving "Safety Evaluation Ultimately Replacing Animal Testing" aimed at finding alternative approaches for the safety assessment for repeated-dose toxicity. Within the framework of proof-of-concept case studies for applied safety assessment, SEURAT-1 investigated the practical application of the read-across approach for repeated-dose toxicity.

In a first step, a strategy for structuring and reporting read-across for repeated-dose toxicity predictions was developed (Schultz et al 2015), including templates to guide the user through the collection of data for building the chemical category and constructing the similarity argument and to support the transparent documentation. Emphasis was given to the description and assessment of the uncertainty regarding the different steps of the read-across exercise, in order to make an informed decision about the read-across prediction result.

Based on the outcome of a SEURAT-1 initiated workshop with external experts, case studies were conceived for four chemical similarity scenarios (Berggren et al 2015) to evaluate the practical application of the read-across strategy and uncertainty assessment, and in particular appraise the possibility of reducing uncertainty by taking into consideration "new approach" data. The case studies focussed on repeated-dose liver toxicity, where applicable. They considered similar chemicals 1) not requiring/undergoing metabolism to exert a potential adverse human health effect, 2) metabolised to the same/similar toxic metabolite, 3) with general low or no toxicity, 4) with markedly different potency or effects. They took into account available chemical, mechanistic, existing *in vivo* and non-test information, and carefully assessed the uncertainty, both for the category similarity and the overall read-across prediction. Furthermore, "new approach" data such as *in vitro* and *in silico* data from the SEURAT-1 initiative, as well as ToxCast, were evaluated for their ability to reduce the uncertainty and strengthen the read-across argumentation.

Berggren E, Amcoff P, Benigni R, Blackburn K, Carney E, Cronin M, Deluyker H, Gautier F, Judson RS, Kass GEN, Keller D, Knight D, Lilienblum W, Mahony C, Rusyn I, Schultz T, Schwarz M, Schüürman G, White A, Burton J, Lostia A, Munn S, Worth A (2015) Chemical safety assessment using read-across: Assessing the use of novel testing methods to strengthen the evidence base for decision making. Environ. Health Perspect. 123: 1232-1240

Schultz TW, Amcoff P, Berggren E, Gautier F, Klaric M, Knight DJ, Mahony C, Schwarz M, White A, Cronin MTD (2015) A strategy for structuring and reporting a read-across prediction of toxicity. Reg. Toxicol. Pharmacol. 72: 586–601

Poster 11

Practical needs to implement advanced strategies for a proper justification of the read-across/category approach

Costanza Rovida[1], Monica Locatelli[2]

[1] CAAT Europe
[2] REACH mastery

Keywords: Read-across, *in vitro* methods, mechanistic elucidation

REACH was the first Regulation accepting alternative strategies for risk assessment. This was a revolution in the area of regulatory science and therefore some time is required to fully accept and implement this new opportunity.

The EU chemical industry has been facing the REACH requirements for many years, getting through two important deadlines: registration of substances manufactured/imported in the tonnage band > 1000 t/y (30th November 2010, Annex X) plus CMRs (Carcinogenic, Mutagenic and toxic for Reproduction) Category 1, and registration of substances manufactured/imported in the tonnage band > 100 t/y (1st May 2013, Annex IX). About 13,000 registration dossiers have been submitted to ECHA and they are available in the public database. By going into the details about how toxicological concern is assessed in those dossiers, it becomes evident that the read-across is very much used, even though often with only weak scientific justification. Recently, ECHA has distributed a new document (Read-across Assessment Framework, RAAF) that explains how to present read-across justification. This includes the application of *in vitro* methods to elucidate mechanistic biological behavior that should back the similarity between two substances or within a category of substances.

There are many practical reasons behind that. First, there is still no culture on alternative methods. Most Universities are preparing professional in toxicology in a traditional way, considering *in vivo* tests the gold standard for an endpoint-by-endpoint approach rather than in a more holistic way. Now in the EU, few scientists are aware of alternative methods even though concepts like IATA (Integrated Approach for testing and Assessment) and AOP (Adverse Outcome Pathways) are getting more and more familiar among toxicologists. Probably because of that, there is the misconception that regulators are not accepting alternative methods. This is false as regulators usually rejects alternative strategies only if they are not duly explained and justified.

Another practical constraint is the lack of well-equipped CROs, or better, the fact that *in vitro* and *in vivo* methods are generally available in different facilities, with all the problems related to the shipping of the sample, following the studies in different labs and combining results written into reports with different format.

Poster 12

Read-across for 90-Day Oral Repeated-Dose Toxicity for Low or No Toxicity Substances: The Importance of Toxicokinetic Similarity

Terry W. Schultz[1], Steven P. Bradbury[2] and Mark T.D. Cronin[3]

[1] The University of Tennessee, Knoxville TN, USA
[2] Iowa State University, Ames, IA, USA;
[3] Liverpool John Moores University, Liverpool, UK

Keywords: Read-across, repeated-dose toxicity, toxicokinetic similarity

One of the key advantages of read-across is its potential to clear a large number of low or no toxicity substances for chronic endpoints, which are typically resources-demanding when evaluated by standard testing regimes. Saturated primary aliphatic alcohols are a group of low or no toxicity substances where the category approach to read-across may be used to estimate the repeated-dose endpoint.

In this example, uncertainty was initially reduced by limiting the applicability domain to intermediate size (i.e., carbon atom (C) chain length of C5 to C13) derivatives. Twenty analogues (nine n-alkanol and eleven 2-alkyl-1-alkanols) were included in the evaluation. Because this is a well-tested and well-understood group of chemicals, confidence in the weight-of-evidence associated with this category is high. Uncertainty associated with mechanistic relevance and completeness of the read-across is low.

This group of chemicals are considered nonpolar narcotics, which act via unspecific interaction with biological membrane in a manner similar to depressant anaesthetics. This read-across also assumes absorption via the gastrointestinal tract and first-pass metabolism in the liver with oxidation to CO_2 and/or eliminated as glucuronides and no significant production of stable, reactive metabolites.

Acute oral toxicity values are available for eight of nine n-alkanols and four of eleven 2-alkyl-1-alkanols. Acute oral toxicity (NOAEL) is very low, ranging from >1500 to <5000 mg/kg bw with an average value of ≈3000 mg/kg bw. High throughput screening assay (i.e., ToxCast) results indicate primary alkanols at the concentrations tested did not elicit responses in most of the assays. Only 104 of 4412 (2.4%) ToxCast test results based on up to 700 assays showed any activity and none of the positive responses were associated with a particular pathway or specific bioactivity. These results do not contradict an assumption of non-polar narcosis.

Perfused rat liver toxicity data suggest that 2-alkyl-1-alkanols may not be in the same read-across category as other primary alkanols. Five n-alkanol analogues have been experimentally evaluated for 90-day oral repeated-dose toxicity. These 90-day findings are quantitatively consistent (NOAELs ≥ 1000 mg/kg bw/d). Repeated-dose toxicity test results exhibit qualitative consistency- only mild changes consistent with low-grade effects (typically including decreased body weight, slightly increased liver weight which in some cases is accompanied

by clinical chemical and haematological changes but generally without concurrent histopathological effects). Two 2-alkyl-1-alknaols analogues have been experimentally evaluated for 90-day oral repeated-dose toxicity. These 90-day findings are quantitatively consistent (NOAEL ≥ 125 mg/kg bw/d). Although these compounds are more toxic than primary alkanols, the repeated-dose toxicity test results exhibit qualitative consistency- again only mild changes consistent with low-grade effects.

Further consideration of metabolism is considered critical in reducing uncertainty associated with assessing toxicokinetic similarity. In the case of n-alkanols, metabolism leads to two-step oxidation in the liver, resulting in the formation of the corresponding aldehydes and carboxylic acids which subsequently undergo mitochondrial β-oxidation to CO_2. A minor amount of glucuronidation with subsequent elimination in the urine is noted. In the case of 2-alkyl-1-alkanols, metabolism, while highly efficient, involves processes that are more complex than with n-alkanol metabolism. Experimental data reveals the major pathways of metabolism and fate of 2-alkyl-1-alkanols include: 1) conjugation of the alcohol group with glucuronic acid, 2) oxidation of the alcohol group, 3) side-chain oxidation yielding additional polar metabolites, which may be subsequently conjugated and excreted or 4) further oxidized and excreted.

To assure confidence in chemical similarity assumptions, potential differences in the toxicodynamics and toxicokinetics of these compounds need to be addressed. Consideration of these factors indicates primary alkanols and 2-alky-1-alkanols should be subcategorized prior to read-across for repeated-dose endpoints. With reasonable certainties, a 90-day oral repeated-dose NOAEL value of 1000 mg/kg bw/d can be read-across to fill the data gaps of untested n-alkanols and a NOAEL value of 125 mg/kg bw/d can be read-across to fill the data gaps of untested 2-alkyl-1-alkanols.

Poster 13

How to deal with uncertainties regarding the occupational exposure to antineoplastic mixtures – Additive effect should always be considered?

C. Ladeira[1,2,3], S. Viegas[1,3], A. Costa-Veiga[1]

[1] Environment and Health Research Group, Escola Superior de Tecnologia da Saúde de Lisboa – IPL, Portugal.
[2] Grupo de Investigação em Genética e Metabolismo, Escola Superior de Tecnologia da Saúde de Lisboa – IPL, Portugal.
[3] Centro de Investigação e Estudos em Saúde Pública, Escola Nacional de Saúde Pública, ENSP, Universidade Nova de Lisboa

Keywords: antineoplastic agents, occupational exposure, mixtures, additive effect, risk assessment approach

The concept that "safe levels of exposure" for humans can be identified for individual chemicals is central to the risk assessment of compounds with known toxicological profiles.

The more common approach to chemotherapy involves administration of multiple agents to target as many types of cells in the tumour as possible. Usually, the antineoplastic treatment is made by a combination of two or more drugs. Selection of agents for combination chemotherapy regimens involves minimize overlapping of mechanisms of action, antitumor activity and toxicity profile.

Although the toxicological profile and mechanism of action of each individual drug is well characterized, the toxicological interactions between drugs are likely, but poorly established at occupational exposure context.

The synergistic nature of interactions may help in understanding the adverse health effects observed in healthcare workers, where exposure situations are characterized by complex mixtures of chemical agents, and the levels of individual exposing agents are often not sufficiently high to explain the health complaints. However, if a substance is a genotoxic carcinogen, this would be the "lead effect"; normally, no OEL based on a NOEL would be derived and the level would be set so low that it would be unlikely that other effects would be expected.

Antineoplastic agents are genotoxic agents, meaning that exposure to them is considered an unacceptable risk, which is considered intolerable, whatever the benefit. However, and due to the high need of these drugs for cancer treatment their use is unavoidable. Therefore, between the acceptable and unacceptable risks we have to consider the tolerable risk, where a balance has to be found between risk and benefit. Tolerance of risk is strengthened by control of risks, such that they are as low as reasonably practical (ALARP).

ACGIH® adopts the approach that the combined effect of a mixture of two or more hazardous substances, which act on the same organ, should be given primary consideration, rather than the effects of each substance individually. In the absence of information of the contrary, the effects of the different hazards should be considered as additive.

Beside the knowledge that occupational exposure to antineoplastic drugs should be virtually null, studies from occupational settings verified that workers are exposed to these drugs. Considering that nowadays the combined therapy with two or more agents is increasing, it is important to understand that, although the drugs dose is lower, the effects of the mixtures should be investigated as additives, and further studies are required in this field.

These studies are of particular interest to healthcare professionals who has the potential to come in contact with these drugs (pharmacists, pharmacy technicians, nursing personnel, physicians, operating room personnel, shipping and receiving personnel, waste handlers, maintenance workers, workers in veterinary practices, and health and safety personnel).

Countries like Portugal, which witnessed an increase in hospital production in the field of oncological diseases since 2007, resulting in an intensification of occupational exposure to antineoplastic drugs of health care workers in hospital cytotoxic circuit, consequence partly by the NHS hospital centralization process, should be aware to this problem.

Poster 14

In silico and Read-across Mutagenicity and Carcinogenicity Assessment to Close Data Gaps for the Pharmaceutical Intermediate Trans-1,4-dibromobut-2-ene

Gian C. Winkler[1], Phil Bentley[2], Susanne Glowienke[3], Ester Lovsin Barle[4]

[1] Novartis Institutes for Biomedical Research, Preclinical Safety, Basel, Switzerland
[2] Toxicodynamix International LLC, Hendersonville, NC, USA
[3] Novartis Institutes for Biomedical Research, Preclinical Safety, Basel, Switzerland
[4] Novartis Pharma AG, BC & HSE, Basel, Switzerland

Keywords: In silico, read-across, mutagenicity, genotoxicity, carcinogenicity, pharmaceutical intermediate, alkylating compound, trans-1,4-dibromobut-2-ene, trans-1,4-dichlorobut-2-ene

Pharmaceutical companies regularly generate and receive requests for assessment of potential hazards of drugs and intermediates. It is a part of many regulations for pharmaceutical development and good manufacturing practices designed to protect workers from occupational exposure.

Trans-1,4-dibromobut-2-ene [CAS no. 821-06-7; EC number 212-472-7] is a commercially available pharmaceutical intermediate (IM). It is pre-registered by ECHA. Under REACH/GHS regulations pharmaceutical IMs are considered equal to other industrial chemicals. For IMs, health hazard assessment has been traditionally done by animal experiments and laboratory tests. This is increasingly replaced by alternative methods considering animal welfare and resources. It would be especially attractive, if some of the health endpoints could be predicted by grouping and read-across. Since IMs are usually chemically reactive manufacturing components, they may pose a pronounced occupational risk for manufacturing workers.

The objective of this poster is to describe the criteria applied for the IM testing read-across approach and explain how they can be used to fill the data gaps in the trans-1,4-dibromobut-2-ene dossier.

We started on the basis of a previously received read-across study using trans-1,4-dichlorobut-2-ene [CAS no. 110-57-6; EC number 203-779-7] as the analogue substance. It is pre-registered by ECHA and is labeled carcinogenic (Carcinogen 1B H350: May cause cancer) and reproductive toxicant by inhalation. We followed closely the chemical safety assessment steps using read-across as described by Berggren et al., in EHP 123 (12) 2015 for repeated dose toxicity.

As a first step, we built up the read-across case based on physico-chemical and molecular properties and functional groups. We analysed existing toxicological data available in the ECHA pre-registration documents. In a second step, we conducted *in silico* predictions for mutagenicity using knowledge based and statistical systems, i.e. Derek (v. 4.1.0) and Sarah (v.1.2.0) Nexus, for trans-1,4-dibromobut-2-ene and its analogue substance trans-1,4-dichlorobut-2-ene. Both structures were predicted mutagenic in each of the systems used. Finally, we examined the mode of action for mutagenicity and carcinogenicity (of trans-

1,4-dichlorobut-2-ene) and the likelihood of read-across results for the carcinogenicity endpoint for trans-1,4-dibromobut-2-ene. In the case of trans-1,4-dibromobut-2-ene, it appears to be a reasonable assumption that chronic irritation of this corrosive IM in conjunction with direct DNA interaction provides the basis for chemical carcinogenesis.

However, literature references on the structure-activity relationship of genotoxicity of alkylating compounds points towards a good correlation between mutagenicity and alkylating activity for substituted allyl chlorides. The alkylating activity of the bromide leaving group is substantially stronger than the one of the chloride leaving group. Hence, the mutagenic activity (measured as the number of revertant colonies in the Ames test) was much larger as well. The mode of action has been attributed to alkylation by nucleophilic substitution following a SN-1 binding mechanism (Boerth et al. 1991). Metabolic activation decreased the number of revertant colonies almost completely (Eder et al. 1986).

Topic 2: Screening and priority setting

Poster 15

Integrating the threshold of toxicological concern (TTC) with high throughput exposure assessment for risk-based screening of several thousand commodity chemicals

R.A. Becker[1], J. Wambaugh[2], G. Patlewicz[2], S. Felter[3], T.W. Simon[4]

[1] American Chemistry Council
[2] US EPA National Center for Computational Toxicology
[3] Procter & Gamble
[4] Ted Simon LLC

Keywords: TTC, high throughput exposure, risk-based screening

Although progress has been made with HTS (high throughput screening) in profiling biological activity (e.g., EPA's ToxCast™), challenges arise interpreting HTS results in the context of adversity & converting HTS assay concentrations to equivalent human doses for the broad domain of commodity chemicals. Here, we propose using TTC as a risk screening method to evaluate exposure ranges derived from NHANES for 7968 chemicals. Because the well-established TTC approach uses hazard values derived from *in vivo* toxicity data, relevance to adverse effects is robust. We compared the conservative TTC (non-cancer) value of 90 ug/day (1.5ug/kg/day) (Kroes et al., Fd Chem Toxicol, 2004) to quantitative exposure predictions of the upper 95% credible interval (UCI) of median daily exposures for 7968 chemicals in 10 different demographic groups (Wambaugh et al., Environ Sci Technol. 48:12760-7, 2014). Results indicate: (1) none of the median values of credible interval of exposure for any chemical in any demographic group was above the TTC; & (2) fewer than 5% of chemicals had an UCI that exceeded the TTC for any group. However, these median exposure predictions do not cover highly exposed (e.g., occupational) populations. Additionally, we propose an expanded risk-based screening workflow that comprises a TTC decision tree that includes screening compounds for structural alerts for DNA reactivity, OPs & carbamates as well as a comparison with bioactivity-based margins of exposure (Wetmore et al., Toxicol. Sci., 2015). This TTC risk-based screening approach may be useful in a modernized TSCA as the first step in risk-based prioritization. Subsequent steps for substances not deprioritized by this TTC method could include analysis using HTS bioactivity/mechanistic screening, read-across as part of integrated testing or exposure refinement.
This abstract does not necessarily reflect U.S. EPA policy.

Poster 16

Leveraging the power of high-dimensional data for integrated screening and prioritization decisions

David M. Reif[1]

[1] Bioinformatics Research Center, Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA

Keywords: integration, HTS, computational toxicology

The emergence of high-dimensional toxicological datasets that cover diverse areas of chemical and biological space can be used to inform estimates of quality, uncertainty, and potency for integrated hazard assessment. These new data, generated in a highly systematic manner across partially-related sets of assay endpoints using *in silico*, *in vitro*, and *in vivo* systems, offer context for individual dose-response assessments. Specifically, global patterns of responses across diverse chemicals and assays can be used to inform assessments of quality (What is the probability that a given dose-response is real, given data on similar chemicals and assays?), uncertainty (What were the distributions of responses in related experiments?), and potency (How do characteristic activity concentrations compare across test systems?). Globally, this contextual approach leverages the power of high-dimensional data to integrate complementary information sources to aid empirical evaluation of screening and prioritization decisions.

Poster 17

Adaptation of Human Cell Based Safety Tests to Animal Product Free Conditions

Dr Carol Treasure[1], Dr Nathalie Belot[1], Bushra Sim[1]

[1] XCellr8 Ltd

Keywords: Cell culture, Human, Animal-free, KeratinoSens, Sensitisation, Bluescreen, Genotoxicity, LD50, Acute Toxicity

Much progress has been made in terms of the regulatory acceptance of human cell based methods for the assessment of human safety. However, the majority of these methods still use animal-derived components in the cell culture systems, such as serum and liver extract. Therefore, such methods cannot be considered truly animal-free. Many stakeholders in the cosmetics industry have both scientific and ethical concerns relating to this issue and have stated a strong preference for fully human *in vitro* test systems. XCellR8 has adapted two key human cell based safety tests to animal-free conditions: the regulatory KeratinoSens test for skin sensitisation, and the non-regulatory BlueScreen test for genotoxicity. In addition, we have developed a new pre-screen for acute toxicity using human cells in animal-free culture. Our in-house validation of these methods has shown equivalence - and improvements - to the fully validated published methods. Here we present an overview of this work so far

as well as next steps to seek regulatory acceptance for the animal-free methods and encouraging the scientific community to use animal-free cell cultures from the outset, when developing new tests.

Poster 18

Metabolomics: a tool for mechanistic toxicology

Mark R. Viant[1,2,3]

[1] Environmental Metabolomics Research Laboratory
[2] Phenome Centre – Birmingham
[3] NERC Biomolecular Analysis Facility - Birmingham; all at School of Biosciences, University of Birmingham, Birmingham, B15 2TT, U.K.

Keywords: Metabolomics, systems biology, mass spectrometry, bioinformatics, AOP, key event, mechanistic toxicology

Metabolomics – the study of low molecular weight metabolites and metabolic pathways within living organisms – began more than a decade ago with pioneering investigations of the biochemical responses of mammals, invertebrates, plants and microbes to toxicants. Since those early studies, the field has blossomed into an active international research community working across a wide range of applications, with thousands of research papers published annually, an international Metabolomics Society established, and active research programs in academia, industry and government laboratories. Metabolomics shows considerable promise as a tool to screen and more deeply investigate the metabolic responses of organisms to chemical stress, complementing the related tools of genomics and gene expression profiling. A unique strength of metabolomics is that it measures the functional status of an organism, i.e. the manifestation of the interaction of the genome and the environment. The methodology has been shown to discover metabolic markers that are predictive of whole organism apical endpoints that remain of considerable interest to the regulatory community. As such, metabolomics is anticipated to play an important role in the discovery of molecular key events in the AOP framework. The University of Birmingham is a world-leader in this methodology, hosting the UK's national environmental metabolomics facility and the new £8m Phenome Centre – Birmingham, which investigates the impacts of stressors on human health. The facilities include sixteen NMR and mass spectrometers dedicated to metabolomics research and for service to the community. This poster introduces metabolomics, describes its applications in toxicology, and highlights the facilities at the University of Birmingham.

Poster 19

New free Danish online (Q)SAR predictions database with >600,000 substances

Eva B. Wedebye[1], Marianne Dybdahl[1], Trine K. Reffstrup[1], Sine A. Rosenberg[1], Nikolai G. Nikolov[1]

[1] Technical University of Denmark (DTU), National Food Institute, Moerkhoej Bygade 19, 2860 Soeborg, Denmark

Keywords: (Q)SAR predictions, free online database, physical-chemical properties, environmental fate, aquatic toxicity, ADME, acute rodent toxicity, sensitisation, irritation, endocrine activity, genotoxicity, carcinogenicity, reproductive toxicity

Since 2005 the Danish (Q)SAR Database has been freely available on the Internet. It is a tool that allows single chemical substance profiling and screenings based on predicted hazard information. The database is also included in the OECD (Q)SAR Application Toolbox which is used worldwide by regulators and industry. A lot of progress in (Q)SAR model development, application and documentation has been made since the publication in 2005.

A new and completely rebuild online (Q)SAR predictions database was therefore published in November 2015 at http://qsar.food.dtu.dk. The number of chemicals in the database has been expanded from 185,000 to >600,000. As far as possible all organic single constituent substances that were pre-registered under REACH have been included in the new structure set. The new Danish (Q)SAR Database includes estimates from more than 200 (Q)SARs covering a wide range of hazardous properties relevant for human health and the environment such as acute toxicity to rat, mouse, fish, daphnia and algae, as well as many physical-chemical and environmental fate properties, skin irritation, sensitization, genotoxicity, cancer, endocrine activity and reproductive toxicity. In agreement with software vendors, (Q)SAR predictions for 600,000 substances from commercial and free software (CASE Ultra, Leadscope PDM, SciQSAR, ACD/Tox Suite and EPI Suite) are included in the database.

The database is one of the most comprehensive freely available (Q)SAR tools for substance evaluations and large-scale screenings. The online interface to the database allows for advanced combination of searches as well as sorting functions on chemical similarity. Negotiations are underway with the OECD to integrate the new database with the OECD (Q)SAR Application Toolbox. The database was developed by the DTU National Food Institute in cooperation and with financial support from the Danish Environmental Protection Agency, the Nordic Council of Ministers and the European Chemicals Agency (ECHA).

Topic 3: Prospects for regulatory science

Poster 20

Developing a US National Strategy and Roadmap for the Replacement of Animal-Based Toxicity Testing

Warren Casey[1], PhD, DABT
Director, ICCVAM

[1] US National Institute of Environmental Health Sciences

Keywords: ICCVAM, 3Rs, Alternative Methods, IVIVE, Endocrine, US, Strategy

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established by the US Congress to promote the development, validation, and regulatory acceptance of test methods that replace, reduce, or refine the use of animals in regulatory testing. In 2013, ICCVAM underwent major changes to its operating paradigm in order to increase the speed and efficiency of regulatory approval and industry adoption of 3Rs testing methods within the United States and internationally. One outcome of this new operating model was the recognition by ICCVAM in 2015 that the US needs to develop a National Strategy and Roadmap for moving away from animal-based toxicity testing. ICCVAM is committed to making the development of this Roadmap a transparent and collaborative process. Accordingly, progress in the development of the Roadmap will be conveyed via publications and presentations at national and international meetings. The information presented in this poster will provide the most recent update on US National Strategy and Roadmap, with particular emphasis on endocrine disruptor screening, acute systemic toxicity (oral, dermal, inhalation), skin sensitization, dermal irritation and corrosion, and *in vitro* to *in vivo* extrapolation (IVIVE).

Poster 21

Using 21st Century tools to identify point of departure for safety assessment of genotoxic compounds

Rebecca A. Clewell[1], Bin Sun[1], Salil Pendse[1], Patrick D. McMullen[1], Yeyejide Adeleye[2], Paul L. Carmichael[2], Melvin E. Andersen[1]

[1] ScitoVation, Research Triangle Park, NC USA
[2] Unilever, SEAC, Colworth, Bedfordshire, UK

Keywords: risk assessment, *in vitro* alternatives, genotoxicity DNA damage, dose-response

Identifying a point of departure for safety assessments *in vitro* requires an understanding of the relationship between early cellular events and phenotypic outcome. Homeostasis by cellular stress response pathways involves negative feedback acting through a series of steps. Many stress response pathways have rapid response, post-translational signaling and slower signaling through

transcriptional upregulation. We examined multiple biological read-outs in a human cell line (HT1080) treated with several DNA-damaging compounds across wide dose ranges. The readouts included dose and time-dependent whole genome gene expression, DNA repair center (DRC) formation through high content imaging of pH2AX and p5BP1, as well as key proteins in the p53 pathway and MN. Transcriptional upregulation only occurred at concentrations with clear increases in micronucleus (MN) formation. Instead, post-translational DNA-repair processes appear to be the main contributor to regulation of DNA-damage at lower doses. We have developed a computational pathway model to describe the relationship between DRCs and MN formation for a potent double strand break inducer - a gamma irradiation mimic, neocarzinostatin, that has a threshold-shaped MN response curve. This model provides a quantitative framework for assessing the key processes governing MN prevention at low chemical doses. Ultimately, these models will support decisions for *in vitro* only risk assessments, by providing a quantitative description of how low dose threshold behavior in mutation response may be achieved, and by helping to define concentrations leading to cellular adaption and potential adversity.

Poster 22

PhyloToxicology: exploiting evolutionary concepts to improve toxicity testing

John Colbourne, Mark Viant[1]

[1] School of Biosciences, University of Birmingham

Keywords: Chemical safety testing, High-throughput, Multi-omics, Computational biology, Species read-across, Adverse Outcome Pathways (AOPs), Alternative model species, Responsible innovation, Predictive toxicology, Phylogeny, Environment and human health protection

The Consortium for Environmental Omics and Toxicology (CEOT) is a unique assemblage of researchers that spans four continents and is reaching out to chemical, cosmetic, consumer products, agricultural and pharmaceutical industry sectors, and the chemical testing agencies of the EU, the US, China and Australia. CEOT is moving towards an international initiative to re-invent (eco)toxicology for animal systems. Its projects are designed to deliver an expandable, mechanistically-based platform for chemical and animal species read-across that ultimately impact the prioritization of chemicals for both human and environmental safety, and reduce the need for traditional animal testing.

PhyloToxicology (PhyloTox) is CEOT's multi-OMICS comparative (pilot) study of how genes and their products interact in multiple organisms, by repeatedly perturbing their normal functions using relevant concentrations of chemical compounds to altogether reveal the phylogenetic origins of networks that include toxicological pathways that are predictive of adverse outcomes in most animals, including humans.

This poster introduces the Consortium, its PhyloTox project and explores, for discussion, how accumulated data obtained by cost-effective and high-

throughput testing of the effects of chemicals across a panel of model species can positively impact current and future regulatory practices and laws.

Poster 23

Removing Blockers to the Acceptance of New Methodology in Regulatory Science

Dr John Doe[1]

[1] Parker Doe Partnership LLP, Cheshire, UK

Keywords: In vitro methods, Hazard classification, Risk assessment, Regulatory Acceptance

The current paradigm for testing chemicals for potential human health effects is expensive and relies heavily on experimental animals. Although millions of Euros have been invested in new technology for assessing the safety of chemicals progress is very slow. We are making advances in addressing the difficult task of assessing repeat dose toxicity based on new biological and computational techniques, but their acceptance and use remains difficult to foresee.

ECHA has developed criteria for the acceptance of non-standard data: adequate for classification; adequate for risk assessment; key parameters from standard study addressed; thorough scientific justification. These criteria seem to be based on the premise that the new methods should aim to predict the results of standard studies which should then be used in chemical safety assessment in the same way as before.

Is this premise blocking progress in the use of new methods to assess more chemicals and use fewer animals? The poster will explore different approaches which could help to overcome this blocker by:

- Exploring the current hazard classification system to derive a revised framework for assessing hazard that could allow new methodology to be used.
- Using "Fit for Concern" criteria for the use of new methodology in risk assessment.
- Describing a stepwise approach to the adoption of new methodology for definitive regulatory decision making.

Poster 24

A 21st Century Roadmap for Human Health Risk Assessment

Alan Boobis[1], John Doe[2], Angelo Moretto[3], Timothy Pastoor[4], Michelle Embry[5]

[1] Imperial College London, London, UK
[2] Parker Doe Partnership LLP, Cheshire, UK
[3] University of Milan, Milan, ITALY
[4] Pastoor Science Communications, LLC, Greensboro, NC, USA
[5] ILSI Health and Environmental Sciences Institute, Washington, DC, USA

Keywords: Risk Assessment, Hazard Characterization, Exposure Assessment, Decision Framework, Integrated Strategy

The ILSI Health and Environmental Sciences Institute (HESI) Risk Assessment in the 21st Century (RISK21) project was initiated to develop a scientific, transparent, and efficient approach to the evolving world of human health risk assessment. RISK21 developed a framework that reconsiders the way chemical risk assessment information is obtained and used. It is a problem formulation-based, exposure driven, tiered data acquisition approach that allows an informed decision on human health safety to be made when sufficient evidence is available. The RISK21 approach maximizes the ability to inform decisions and optimize resource usage. Two case studies were developed to illustrate these principles. The first example identified testing needs for a new 'nth' in class pesticide to be used in mosquito netting for malaria prevention, and illustrated how existing information from other pesticides in the same chemical class and knowledge of use patterns can inform data needs and decision making. In the second example, a large number of chemicals which might be present in drinking water were prioritized and evaluated to determine which are of highest potential concern for human health risk assessment. Both case studies identified key issues and possible approaches to address cumulative risk and established the utility of the RISK21 framework in assessing the value of available information and making decisions about what, if any, additional information is needed to inform a decision.

Poster 25

Integration into risk assessment of open source human omics data from *in vitro* studies

Lucian Farcal[1], Thomas Exner[1], Barry Hardy[1]

[1] Douglas Connect GmbH, Switzerland

Keywords: risk assessment, transcriptomics, human *in vitro* data, adverse outcome of pathways

Background. A milestone in toxicity research was the emergence of toxicogenomics, resulting from the application of knowledge gained from genomics science into conventional toxicology. Toxicogenomics specifically tackles the complex interactions between toxic effects and the structure and

activity of the genome. Thus, predictive toxicology is undergoing a paradigm shift, from phenomenological to mechanistic (e.g. -omics data based) models that may represent an important alternative to the classical *in vivo* approach applied in chronic and systemic toxicity testing. Along those lines, numerous international projects have recently generated and gathered valuable data and created open data warehouses that are required to build these predictive models.

Objective. Within the SEURAT-1 - ToxBank project, case studies have been proposed in which the toxicity of different compounds is assessed using only available human *in vitro* data. In one of these, *ab initio* risk assessment is modelled, i.e. it is assumed that no information on the toxicity of the compound is available. An approach using the combination of omics data with information extracted from adverse outcome pathways (AOPs) to identify areas of concern and support an evidence-driven risk assessment was proposed and performed on piperonyl butoxide (PBO).

Methodology. To quickly identify areas of concern, omics data represents a good starting point, since they show general adaptations of the cell to the exposure. We have identified and used a set of transcriptomics measurements performed on three different human liver *in vitro* models (HepaRG, HepG2 and hES-DE-Hep). The methodology applied included (i) identification of relevant pathways (the transcriptomics data was analyzed using the program InCroMAP), (ii) correlation between pathways and diseases (the top pathways were selected and analyzed further using the services of the Comparative Toxicogenomics Database), and (iii) verification of adverse effects versus specific AOP Key Events (using the information from the Adverse Outcome Pathway Knowledge Base).

Results. We showed that transcriptomics data is able to identify fibrosis as one major adverse outcome of treatment with PBO and that HepaRG was the most appropriate cell model to be used for testing this specific adverse effect. Using information from AOPs, we were able to verify key events and, in this way, strengthening the evidence for this specific adverse effect. Finally, we proposed some additional testing on some key events for which we could not identify available data.

Poster 26

Integrated Approaches to Testing and Assessment (IATA) can facilitate acceptance and regulatory use of non-animal methods

Jarlath Hynes[1], Kate Willett[1]

[1] Humane Society International

Keywords: Adverse Outcome Pathway (AOP), OECD IATA, Regulatory acceptance, Skin sensitisation

Advances in molecular biology have allowed the consideration of dramatically different approaches to understanding disease and toxicology than those

traditionally practiced. Increasingly, collections or batteries of non-testing and non-animal test methods are being used to replace apical animal tests.

Our greater understanding of the underlying mechanisms of toxicity, for example in the form of Adverse Outcome Pathways (AOPs), can now form the logical basis of Integrated Approaches to Testing and Assessment (IATA). Within an assessment framework that considers all relevant data, *in chemico* and *in vitro* assays can be targeted at key molecular and cellular events that lead to a toxic endpoint.

These testing strategies can improve the efficiency of hazard assessment whilst providing higher-quality data, including the potential to define accurate dose-response curves.
There is real potential for the development of high-throughput systems to assess mixtures. In turn, this will better inform human and environmental risk assessments.

In light of number and variety of new methods and types of information that are replacing traditional tests, new approaches are needed to support both assay validation and harmonized regulatory use.

The Organization for Economic Cooperation and Development (OECD) Environment Directorate has made a significant investment in development of AOPs, and is currently developing guidance for IATA. This combined guidance, in the form of IATA supported by AOPs, along with streamlined assay validation, has the potential to facilitate increased harmonized use and acceptance of these new methods.

This poster will discuss how the OECD guidance on IATA could facilitate timely regulatory acceptance of new assessment methods, including the underlying *in chemico* and *in vitro* assays, and using skin sensitisation as example.

Poster 27

The scientific background for identification of selected substances

Kaja Ilmarinen[1], Merike Nugin[1], Riina Aav[2], Dzmitry Kananovich[2], Sandra Kaabel[2], Maria Fomitšenko[2], Arvo Mere[2]

[1] Health Board, Department of Chemical Safety
[2] Tallinn University of Technology

Keywords: Identification, comparison of analytical methods, NMR, XRD, BPR, REACH

Development in scientific and technical fields require chemicals policy to take into account new approaches of analysis to identify substances for REACH1, BPR2 and CLP3 purposes. A substance is defined in REACH by Article 31 and in CLP by Article 23.

Organic and inorganic substances that have an action on or against harmful organisms are active substances by meaning of the BPR2. The identification

approach for active substances is based on BPR Article 62 and the dossier includes the core data set (CDS), which should, in principle, be provided as a minimum set required for all active substances, to justify their properties.

The quality assurance for the identification of substances is essential for the health and environmental assessment. Currently nuclear magnetic resonance (NMR) spectroscopy and X-ray photoelectron spectroscopy (XPS) or X-ray diffraction analysis (XRD) are underrepresented in the determination of substances under scope of BPR. Nevertheless, according to scientific literature, more specific and contemporary methods are beneficial for analysis of mixtures of substances and also nanoparticles. For example, XPS provides quantitative elemental and functional group analysis which elucidated the changes in surface chemistry with formulation procedure for PLGA nanospheres stabilised with PVA and PEO-PPO copolymer surfactants.4 The detection and characterisation of nanoparticles and their properties can be appropriate for risk assessment.5 Recently, a new quality control parameter, Qp-score has been introduced for harmonization of fingerprinting protocols of quantitative NMR analysis of mixtures of organic compounds, allowing to select NMR-laboratories that can produce reliable data.6

We will present scientific background and some examples of analysis of organic compounds by NMR, single-crystal diffraction and powder diffraction analysis. Our scientific interests include analysis of organic7 and inorganic compounds and their structure characterisation. Listed methods could provide suitable analytical data for the information requirements set in the scope of BPR.

References

1. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18
December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of
Chemicals (REACH).
2. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products (BPR).
3. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures (CLP).
4. P.D.Scholes, A.G.A.Coombes, L.Illum, S.S.Davis, J.F.Watts, C.Ustariz, M.Vert, M.C.Davies J.Control. Release 1999, 59, 261-278.
5. K.Tiede, A.B.A.Boxall, S.P.Tear, J.Lewis, H.David, M.Hassellöv Food Additives & Contaminants: Part A 2008, 25(7), 795-821.
6. V.Gallo, N.Intini, P.Mastrorilli, M.Latronico, et al Anal.Chem. 2015, 87,6709-6717.
7. a) Dzmitry G. Kananovich, Alli Reino, Kaja Ilmarinen, Marko Rõõmusoks, Mati Karelson, Margus Lopp Org. Biomol. Chem., 2014, 12, 5634-5644; b) E. Prigorchenko, M. Öeren, S. Kaabel, M. Fomitšenko, I. Reile, I. Järving, T. Tamm, F. Topic´, K. Rissanen, R. Aav, Chem. Commun., 2015, 51, 10921-10924.

Poster 28

QSAR Toolbox as read-across/category building platform suitable for combining in-vivo experimental results with mechanistic data and expert knowledge

O. Mekenyan[1], S. Dimitrov[1], T. Pavlov[1], C. Kuseva[1], T. Sobanski[2], D. Hirmann[2], T. Netzeva[2], A. Gissi[2], A. Martin Aparicio[2]

[1] Laboratory of Mathematical Chemistry, Bourgas "Prof. As. Zlatarov" University "Yakimov" St. #1, 8010 Bourgas, Bulgaria
[2] Computational Assessment and Dissimination Unit, European Chemicals Agency, Helsinki, Finland

Keywords: read-across, chemical category, substance profiling

The OECD QSAR Toolbox is a computerised system for the hazard assessment ofchemicals based on the category approach. The system incorporates theoretical knowledge, experimental data and computational tools organised in a logical workflow.

The knowledge is implemented in the form of profilers, which are used to define and mechanistically justify the analogues for the target chemicals. Next, the system checks if there are experimental data available for the target chemical and query endpoint.

If there is no experimental data available, the workflow continues with the data gap filling step. The system provides various methods for filling data gaps; among them the read-across is most commonly used.

Read-across use relies on the data available for analogues to fill data gaps for the target chemical. Other data gap filling tools are trend analysis and the use of external (Q)SARs.

Thus, the QSAR Toolbox provides an opportunity to collect weight-of-evidences by combining different approaches and/or models and use the predictions from them as supporting data.

In addition, the QSAR Toolbox also incorporates additional functionalities such as: metabolism simulators, query searching, endpoint vs endpoint correlation and data filters. These functionalities might be used to support the read-across.

The obtained predictions are reported accordingly in a format similar to QPRF. The reports accommodate the information which is produced by the QSAR Toolbox.

The QSAR Toolbox is freely available software, with approximately 7 000 registered users. The next phase of the development started recently by the OECD and ECHA will improve the usability, streamline prediction workflows and significantly improve system performance.

Poster 29

Driving Risk Decisions Through Information Integration and Visualization Using Systems Biology, Ontologies and the AOPXplorer

Edward Perkins[1], Natalia Garcia-Reyero[1], Kurt Gust[1], Mitchell Wilbanks[1], Natalie Barker[1], Lyle Burgoon[1]

[1] US Army Engineer Research and Development Center, Vicksburg, MS, USA

Keywords: AOP, *in vitro* tests, biological pathway, high throughput

Risk managers and risk assessors will soon be flooded with molecular and phenotypic data coming from omics and high throughput screening technologies. Although the Adverse Outcome Pathway (AOP) framework provides a means to begin to anchor, interpret, and understand these data, biology does not occur in single pathways – it is networks of pathways interacting with each other that ultimately lead to the complexity of adverse outcomes. Currently there is a dearth of tools available to integrate and make sense of all of this systems biology data. We built the AOPXplorer to address this need, allowing us to integrate, explore, and visualize the data in ways that will help us understand what the molecular responses mean within a larger biological context, and to facilitate the automated screening of hazards and perform dose-response assessments in a more rapid fashion. The AOPXplorer uses the AOP Ontology to model and integrate the data, allowing the computer to quickly perform hazard identification on chemical data by comparing activated key events against the set key events that are sufficient to infer that an adverse outcome is likely to occur. This allows users to quickly scan through the list of potential adverse outcomes, and see visually how the computer made these inferences by overlaying the molecular data onto AOPs. The AOPXplorer will also facilitate the creation of analysis pipelines, such as automated point of departure calculations. We will show examples of the capabilities by using data from zebrafish fish embryo toxicity (zFET) and high throughput screening assays.

Poster 30

Potency ranking of skin sensitizers using the Reconstituted Human Epidermis (RhE) IL-18 test and the Genomic Allergen Rapid Detection (GARD) test

Erwin L Roggen[1], Els Adriaens[2], Eric Andres[3], Fanny Boisleve[4], Sun-A Cho[5], Emanuela Corsini[6], George DeGeorge[7], Horst W Fuchs[8], Pierre-Jacques Ferret[9], Sue Gibbs[10], Patrick Hayden[11], Henrik Johansson[12], Dagmar Lehmeier[13], Silvia Letasiova[14], Jean-Philippe Therrien[15]

[1] 3Rs Management and Consulting
[2] Adriaens Consulting
[3] Oroxcell
[4] Yves Rocher
[5] Amorepacific
[6] University of Milan

[7] MB Research Laboratories
[8] CellSystems
[9] Pierre-Fabre
[10] Vrije Universiteit Medical Center
[11] MatTek Cooperation
[12] SenzaGen
[13] Eurofins
[14] MatTek IVSL
[15] Stiefel

Keywords: sensitization; IL-18; GARD; potency; legislation

Background:

- IL-18 production by keratinocytes is a potentially useful endpoint for determination of contact sensitization potential of low molecular weight chemicals (Corsini et al., 2009). Potency classification of skin sensitizers relates to the irritant potential of the chemical in a reconstituted human epidermis (RHE) model (dos Santos et al., 2011). Gibbs et al. (2013) successfully integrated the IL-18 endpoint in various established RHE models, currently used to assess chemical substances for their potential to trigger irritation and corrosion (sensitivity: 91%; specificity: 90%; accuracy: 95%). This test addresses key event (KE) 2 of the AOP for skin sensitization.
- Johansson et al. (2011) identified a signature of genes, which are differentially regulated in the human myeloid cell-line MUTZ-3 when stimulated with sensitizing compounds compared to non-sensitizing compounds. The 200 genes of the GARD Prediction Signature (GPS) participate in signalling pathways known to lead to transcription of cytoprotective enzymes and dendritic cell maturation (Johansson et al., 2013). A proof of concept for the functionality of the GARD test was provided using 37 test substances, including 11 'difficult' substances (sensitivity: 89%; specificity: 88%; accuracy: 89%) (Johansson et al., 2014). This test addresses KE 3 of the AOP for skin sensitization.

Current status:

- Following topical exposure of the epiCS® model for 24 h to 17 contact allergens and 13 non-sensitizers a robust increase in IL-18 release was observed only after exposure to contact allergens. A generic protocol was developed using different 3D-epidermal models including the in house VUMC model, epiCS®, MatTek EpiDerm™ and SkinEthic™ RHE. In the process, critical issues were identified that need to be controlled to improve the comparability of quantitative data (e.g. for relative potency determination) from different skin models.

The available data indicate that potency is assessable on the basis of the amount of substance required to result in (i) 50% cytotoxicity (EC50) or (ii) an IL-18 response exceeding the cut-off stimulation index. Correlation of the EC50 with human and animal data showed a superior correlation with human DSA05(1) (µg/cm2) data (Spearman r = 0.8500; P value (two-tailed) = 0.0061) compared to LLNA data (Spearman r = 0.5968; P value (two-tailed) = 0.0542). A good correlation was also observed for release of IL-18 (SI-2) into culture supernatants with human DSA05 data (Spearman r = 0.8333; P value (two-tailed) = 0.0154).

(1) DSA05: the induction dose per skin area that produces a positive response in 5% of the tested population.

- By transcriptional profiling of chemically stimulated MUTZ-3 cells, 33 canonical pathways involved in sensitization to chemical substances were

identified. Pathways involved in metabolic processes, cell cycling and oxidative stress responses were identified as the key events activated during skin sensitization. These functions were found to be engaged differently depending on the chemical reactivity mechanism of the sensitizing agent. Furthermore, chemical reactivity groups seem to gradually engage more pathways and more molecules in each pathway with increasing sensitizing potency of the chemical used for stimulation. Also, a switch in gene regulation from up to down regulation, with increasing potency, was seen both in genes involved in metabolic functions and cell cycling. These observed pathway patterns were clearly reflected in the regulatory elements identified to drive these processes, where 33 regulatory elements were subjected to further analysis. Currently, a new GPS specific for potency assessment is being evaluated.

Prospectives:
The available data suggest a potential use of both test methods in the identification of skin sensitizers and their classification according to potency. For the chemicals for which human data are available, the potency information acquired *in vitro* is much more reliable than the data acquired *in vivo*. In combination with the high accuracy to correctly identify skin sensitizers, both methods are currently being validated, with strong involvement of industry.
The RhE IL-18 potency test and skinGARD, as stand-alone or integrated in a testing and assessment strategy, are to meet the regulatory needs and data requirements related to skin sensitization assessment in the context of the REACH legislation (Regulation 1907/2006/EC), the EU Cosmetics Regulation (1223/2009), the EU Regulation on classification, labelling and packaging of substances and mixtures (1272/2008), the EU Legislation on Plant Protection Products (1107/2009) and the EU Regulation on Biocidal Products (528/2012).

Poster 31

The need to ADAPT to new methodologies

Taylor, K.[1]

[1] ECEAE/CrueltyFreeInternational

Keywords: regulatory, acceptance, alternative method, validation

Our experience with the chemicals, medicines and cosmetics sectors over the last 20 years has highlighted the complexity of the 'path to acceptance' and the many hurdles that are placed in the path of new methodologies that replace animal tests.

Even simple, like- for –like replacements have struggled to gain acceptance and full implementation. Time scales from validation to adoption and replacement have been in excess of 10 years and for many are still not complete. This is due in part to a failing of regulatory authorities to take responsibility for identifying new methods, to assess the suitability for their sector and to then clearly notify industry of their decision. This has an impact on harmonisation worldwide if it is not even clear whether a region has accepted a new method or not.
Cruelty Free International has created the ADAPT principles help regulatory bodies identify where changes in their policies and processes are needed to ensure the more rapid implementation of alternatives. The principles are:

Assessment -does the body have a proactive mandate to assess the suitability of new methods for their sector? Decision -who takes responsibility for deciding whether an alternative method is suitable? Acceptance -have all the bureaucratic steps to acceptance such as the need to revise guidance and/or legislative text been identified? Policing -are there mechanisms in place to monitor the use of alternatives and will action be taken if animal tests are done unnecessarily? Transparency -does the authority inform all stakeholders of their actions at each stage?

It is vital that regulatory authorities take up the ADAPT principles now so that, as these new methodologies come into play, the framework is in place to rapidly evaluate and accept -or reject- them.

Poster 32

Evidence-Based Toxicology – the missing link between the advancements of science and the confidence of regulatory decisions

Katya Tsaioun[1]

[1] EBTC at the Johns Hopkins Bloomberg School of Public Health

Keywords: regulatory decisions, evidence-based toxicology, *in vitro*, safety test methods, toxicity testing, collaboration, EBTC

Most experts agree that currently mandated safety test methods are not adequate and not economically sustainable to ensure the safety of human subjects exposed to chemicals. On the other hand, major advancements in our understanding of mechanisms of human toxicology have resulted in a number of technologies that claim that they can now predict various mechanisms of human toxicity. To assess the true predictive value of these technologies and define their place in making regulatory decisions, major efforts are underway to "validate" a variety of human biology-based models. However, less progress has been made in agreement and standardization of these validation efforts, and their acceptance by the international regulatory authorities, without which the transition from the scientifically outdated, ethically questionable and resource-intensive tests to more accurate, faster, environmentally friendly and cost-efficient models will continue to be slow. It is obvious that there is a missing link between the advancements in the science of toxicology and the regulatory science. We make the case that this missing link is Evidence-Based Toxicology (EBT). EBT is set out to assemble, assess, integrate, analyze and summarize different streams of evidence (animal, human (where available), *in vitro*, *in silico*) in a transparent and objective manner. This approach holds great promise to end the "validation dilemma" and to become a standard that informs confident regulatory decisions. The theory and principles of evidence-based methodologies will be described and an example of an application of this approach to safety test methods will be described.