PS 1833 ADOPTING TESTING CONDITIONS OF THE VALIDATED CFU-GM ASSAY FOR HEMATOTOXICITY TO LIQUID CULTURES OF CD34+ HEMATOPOIETIC STEM CELLS IN A MICROWELL FORMAT.

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PS 1834 EVALUATING *IN VITRO* MODELS FOR THE PREDICTION OF CNS EXPOSURE TO CHEMICAL ENTITIES.

A. Stevens[1], C. Cantrill[2], P. Mistry[1], J. Wright[1] and J. Penny[2]. *[1]Syngenta, Bracknell, United Kingdom and [2]University of Manchester, Manchester, United Kingdom.* Sponsor: R. peffer.

In chemical discovery, an important early phase is the investigation of central nervous system (CNS) exposure and hence potential neurotoxicity of new chemical entities. *In vitro* models can be used to predict the exposure of compounds in the CNS. The characteristics of the model will influence the degree of predictivity. An optimum model should be representative of the *in vivo* blood brain barrier (BBB) and possess key physiological characteristics such as tight junction formation and functional activity of key transporter proteins such as P-glycoprotein (P-gp). Culturing brain endothelial cells has proved challenging and as a consequent often surrogate models such as Madin Darby canine kidney (MDCK) cells transfected with human P-gp are utilized as they offer a high throughput solution. The limitations of the MDCK model include the specificity of the overexpressed protein to measure the interaction of chemical entities with human P-gp. This study explores the utility of MDCK cells and an advanced primary porcine *in vitro* BBB model to aid the prediction of CNS exposure. The distribution of compounds into the CNS is influenced by plasma and tissue protein binding as well as passive and active transport processes across the BBB. An integrated approach involving the measure of free plasma and tissue concentration by equilibrium dialysis and permeability data generated from the *in vitro* BBB model was utilised to identify improved *in vivo* predictions. *In vitro* Kp (Kp,pred) was determined for a set of eleven test compounds with a range of physicochemical properties. Kp,pred obtained from the porcine BBB model showed 55% of compounds within 3-fold of observed *in vivo* data compared to 25% generated from MDCK cells. In particular, MDCK cells underpredicted the distribution of P-gp substrates, namely Amprenavir, Risperidone and Saquinavir whereas the porcine BBB model showed improved predictions of observed Kp for these compounds indicating that this model may provide a better prediction of CNS exposure possibly owing to the more relevant physiology of the system.

PS 1835 PARACRINE SIGNALING IN A NOVEL MODEL FOR HEPATOCYTE-PREADIPOCYTE COCULTURES.

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Obesity is a serious public health problem and a major risk factor for cardiovascular disease, certain types of cancer, and type2 diabetes. Hence there is a need for physiologically relevant cellular models that can mimic adipogenesis. Currently, most cellular models utilize single cell type cultures of 3T3-L1 preadipocytes that are induced to undergo adipogenesis. However the effect of other cell types on preadipocyte growth and differentiation has not yet been elucidated¬¬. Since secreted factors from the liver are distributed throughout the body, as are products of drug metabolism, the effects of such paracrine signals and/or drug metabolites on adipocyte function is relevant. In this study we have used a proprietary technology developed in our laboratory, termed Integrated Discrete Multi Organ Co-culture (IdMOCTM) for the co-culture of 3T3-L1 preadipocytes and hepatocyes. Using Rosiglitazone to induce adipogenesis along with quantitative PCR for the amplification of AP2, an early marker for adipogenic differentiation, we show that adipogenesis can be induced reproducibly within 48 hours. We have optimized this assay using various concentrations of MatrigelTM for cell adherence and media for concomitant culture of the two cell types. In this co-culture mode, we demonstrate the presence of hepatic factors that positively influence preadipocyte proliferation and adipogenic differentiation. To determine the specificity of Rosiglitazne action, which occurs presumably via its metabolites, N-desmethyl rosiglitazone and ρ-hydroxy rosiglitazone, an inhibitor of the major metabolizing enzyme-CYP2C8, namely trimethoprim was used. A decrease in adipogenesis in the presence of trimethoprim indicates the role of metabolism in driving drug-induced differentiation. Such a model is physiologically relevant in testing drugs that affect adipogenesis as it provides the paracrine signals and xenobiotic metabolizing capacity of hepatocytes in simultaneous culture with preadipocytes.

PS 1836 DEVELOPMENT OF AN *IN VITRO* ACUTE TOXICITY PANEL TO ASSESS THE TOXICITY OF EXTRACTS FROM SINGLE-USE BIOPHARMACEUTICAL PROCESSING PRODUCTS.

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Assessing the toxicity of extractables from biopharmaceutical processing products is required for the safety assessment of these products. A standard protocol for this type of evaluation does not currently exist. As a result, an *in vivo* method described in the USP <88> guidelines for Biological Reactivity Tests is often used as a default for evaluating potential toxicity. This protocol requires the intravenous administration of large volumes (50 ml/kg) of extracts to mice. The resulting effects on hemodynamics are a common cause of failure for materials undergoing USP testing. In addition, the current test requires significant animal use for raw material and new product screening. In order to improve testing speed and reliability and reduce requirements for animal testing, this study presents and evaluates an *in vitro* screening method that could more accurately identify a risk for *in vivo* toxicity. Rat (H4IIE) hepatoma cells were used to assess the toxicity of various dilutions of extracts generated per USP criteria (0.9% saline, 1 hour at 121 degrees C) from different biopharmaceutical purification product chemistries and raw material solutions. Cytotoxicity was determined by measuring several critical markers of cell health, which included membrane integrity (alpha-GST) and cell mass (propidium iodide) following 24 hour exposures. The *in vitro* TC50 values were derived and compared to results from *in vivo* exposure of similar dilutions. In all cases, the TC50 of the extracts predicted the dilution needed to successfully pass the *in vivo* acute toxicity test, displaying excellent accuracy and sensitivity. It is concluded that the *in vitro* screening of extracts and raw material solutions is a useful tool to assess potential toxicity issues associated with biopharmaceutical processing products, and the inclusion of these assays can provide important toxicity information, while minimizing animal testing, particularly during the product development phase.

PS 1837 EFFECT OF OXYGEN ON LIVER SLICES VIABILITY.

E. Szalowska, G. Stoopen, S. Wang, J. Rijk, M. Groot, J. Ossenkoppele and A. Peijnenburg. *RIKILT/University of Wageningen, Wageningen, Netherlands.* Sponsor: S. Rangarajan.

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