1288 A PROTYPE *IN VITRO* NEUROTOXICITY DATABASE.

A. D. Weissman. *NovaScreen Biosciences Corp, Hanover, MD.* Sponsor: J. Sina.

There is a current need to develop credible *in vitro* endpoints that parallel the toxic effects of chemical compounds in humans. This information, incorporated into a relational database. would be useful for the virtual screening of compounds at an early stage in their development. Improving the predictive capabilities of current *in vitro* testing requires a battery of endpoints that include mechanism-based assays as well as general cxytotoxicity in cells appropriate to the target organ of interest. Towards this end we have tested a library of 204 compounds thought to be relevant to neurotoxicity in both a proliferative and differentiated human neuronal cell line. We examined seven measures of cellular toxicity with these compounds at six concentrations in duplicate across seven days of treatment. This has resulted in an initial *in vitro* toxicity database annotated with chemical data and LD50 values from *in vivo* studies. The database has shown internal consistency between *in vitro* toxicity measures, as well significant correlations with animal and human toxicity data.

1289 LYSIS OF ADHERENT HUMAN EPIDERMAL KERATINOCYTES IN SITU BY A MISONIX TISSUE CULTURE PLATE SONICATOR.

C. L. Gross, O. E. Clark, E. W. Nealley, M. T. Nipwoda and W. J. Smith. *USAMRICD, APG-EA, MD.* Sponsor: A. Sciuto.

Human epidermal keratinocytes (HEK) are used extensively in our laboratories as an *in vitro* model of human epidermis to study the vesicant action of sulfur mustard (HD). These adherent cells are released from their tissue culture vessels by proteolytic treatment that detaches the HEK from their plastic supports. However, this common procedure may compromise cells that are struggling to survive and influence the interpretation of damage from these moribund cells. Biomarkers of damage are isolated by lysing cells using specialized buffers or by subjecting the cellular pellet to sonication or other means of disruption. It is especially critical to be able to lyse cells in the desired buffer in situ to minimize losses of biological material used in analysis. Using a Misonix tissue culture plate sonicator, both 24- and 96-well plates of HEK were subjected to 30-sec bursts in a 4oC tray horn ultrasonic bath at the maximum setting of 10. There was 1 min of cooling between bursts for total sonication times of 2 to 5 minutes. The plates were inspected by microscopy, and wells were photographed to give a rough measurement of HEK lysis. Sonicated plates were also checked for the number of *via*ble cells remaining on the plate or in the supernatant by an MTS-PMS chromogenic *via*bility assay. The supernatant fluid was removed from the well and wells were again checked for the percentage of remaining adherent cells by microscopy. Glutathione levels in the supernatant fluid by both sonicated HEK and acid-extracted HEK were measured and appeared identical. From these results, it appears that the sonication technique may be a useful alternative for the proteolytic method commonly used for the isolation of specific biochemical markers.

1290 DIFFERENTIATION OF THE ABSORPTION KINETICS OF JET FUEL HYDROCARBONS WITH AN ETHANOL/WATER SYSTEM AND A MEMBRANE-COATED FIBER TECHNIQUE.

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After dermal exposure to jet fuel, hydrocarbon components will penetrate the stratum corneum, transport and deposition through the epidermis and dermis into the vascular system. The distribution and transport mechanisms of different hydrocarbons through the lipophilic and aqueous routes are unknown. We have used an ethanol/water system to simulate the lipophilic and aqueous routes and studied their absorption kinetics with a membrane-coated fiber technique. The major aromatic and aliphatic components in jet fuel were selected as study compounds. The equilibrium absorption amounts of the aromatics in log scale decreased linearly with increasing ethanol/water ratio, while their absorption rates among different aromatics were not significantly different. Aliphatics showed quite complicated absorption kinetics as all of the jet fuel aliphatics are hydrophobic compounds with very small water solubility. The absorption rate of the aliphatics in water could be linearly predicted with their carbon number. When the carbon number was larger than 14 (tetradecane), the water solubility was so low that the absorption rate approached zero. Increasing the ethanol/water ratio will increase the hydrocarbon solubility. At a given ethanol/water ratio, the absorption rates were higher for smaller aliphatics, while the equilibrium absorption amounts were higher for larger aliphatics. When the ethanol/water ratio reached 50%, all of the aliphatics reached their highest absorption rates and could reach absorption equilibrium within 200 min. Further increasing the ethanol/water ratio, the equilibrium absorption amounts were decreased, while the absorption rates did not change significantly. These experimental results could aid in the understanding of the absorption and transport mechanisms of hydrocarbons in a human body after jet fuel exposure.

1291 THE PERFORMANCE OF *IN VITRO* TEST BATTERIES AS PRE-SCREENS TO *IN VIVO* SKIN AND EYE IRRITATION TESTS.

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Our approach to rabbit skin and eye irritation tests has been refined to include elective *in vitro* pre-screens. For the detection of severe eye irritants, a simple cytotoxicity test is used followed, where necessary by the isolated rabbit eye test. The outcome of this test strategy is used to guide subsequent *in vivo* testing, and represents a refinement and potential reduction in the use of animals. Materials predicted to be severe irritants are initially tested at low volume in a single animal. Only if less that severe *in vivo* results are obtained is the full group size of animals completed. Test battery performance over a number of years shows an 86% ability of this approach to correctly predict materials that are less than severe ocular irritants *in vivo*. The ability of this battery of tests to correctly predict severe eye irritants is lower at 67%. For the assessment of skin corrosion, the Transcutaneous Electrical Resistance (TER) test followed by a dye binding step is used as the pre-screen. Materials identified as potentially corrosive *in vitro* may not need to be tested in the live animal. The ability to correctly predict materials as non-corrosive *in vivo* by this approach is high at 94%. The ability to correctly predict materials as corrosive is lower at 40%. However, this outcome may be influenced by the low prevalence of corrosive materials assessed. Data from over 300 tests covering a wide range of chemical classes are presented in this analysis.

1292 UPTAKE KINETICS OF JET FUEL AROMATIC HYDROCARBONS FROM AQUEOUS SOLUTIONS STUDIED BY A MEMBRANE-COATED FIBER TECHNIQUE.

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The absorption of aromatic hydrocarbons from aqueous media is a critical step involved in their transport and deposition in a human body after jet fuel exposure. We have developed a membrane-coated fiber (MCF) technique to study this process, in which a polymer membrane is coated on an inert fiber as a permeation membrane. A polydimethylsiloxane (PDMS) membrane intending to simulate human stratum corneum and a polyacylate (PA) membrane intending to simulate cell membrane were used in this study. The uptake kinetics of the major aromatic components in jet fuel were studied in a flow system, which provided a constant concentration for the prolonged permeation experiments. The absorption profiles of the aromatic compounds were regressed with a mathematical model of the MCF technique. The equilibrium absorption amount and a kinetic parameter that determined the absorption kinetics were obtained from the regression for each compound. The uptake and elimination rate constants of 6 benzene derivatives and 3 naphthalene derivatives were determined with PDMS and PA MCFs. An equilibration method and a regression method were developed to determine membrane/water partition coefficients. The PDMS/water partition coefficients of the benzene and naphthalene derivatives were linearly correlated with their octanol/water partition coefficients (LogKpdms/w=0.871LogKo/w-0.241, R2=0.995). The PA/water partition coefficients of the benzene derivatives and the naphthalene derivatives were correlated differently with their octanol/water partition coefficients. The correlation equations for benzene and naphthalene derivatives were LogKpa/w=0.865LogKo/w+0.0045, R2=0.997 and LogKpa/w=0.763LogKo/w+0.911, R2=1.00, respectively. This shows that the MCF technique can detect the subtle differences in intermolecular interactions of the two group derivatives between the two-membrane/water systems.

1293 DEVELOPMENTAL TOXICITY OF TRIETHYLENE GLYCOL, TRIETHYLENE GLYCOL MONOMETHYL ETHER AND TRIETHYLENE GLYCOL DIMETHYL ETHER IN INTACT *DROSOPHILA MELANOGASTER*.

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To further characterize the *Drosophila*-based prescreen to detect developmental toxicants, triethylene glycol (TEG), triethylene glycol monomethyl ether (TEGMME) and triethylene glycol dimethyl ether (TEGDME) were evaluated using our published protocol (*Teratogenesis, Carcinogenesis, and Mutagenesis* 11:147-173, 1991). One or two experiments, each employing 10 equimolar concentrations (.0013-1.64mM/*via*l) of test chemical and including a concurrent control, were conducted per chemical. *Drosophila* were exposed throughout development (egg through third instar larva) in culture *via*ls to medium containing the test chemical. Each *via*l contained 1g of powdered medium and 5ml of distilled deionized water or a solution of test chemical in water. A mated, untreated, Oregon-R wild-type female (Mid-American Drosophila Stock Center, BGSU, Ohio) was added to each culture *via*l

and allowed to oviposit for 20 hours, then removed. Emerging offspring were collected over 10 days and examined microscopically (25x) for bent humeral bristles - a morphological defect shown to occur with an increased incidence in fruit flies exposed to developmental toxicants. The incidence of bent bristles was statistically increased compared to concurrent controls (chi-square) at the two highest TEG concentrations - 1.32mM, 38/181, p<0.001 and 1.64mM, 29/99, p<0.001. For TEGMME, the incidence of bent bristles was statistically increased at the four highest concentrations - 0.66mM, 8/155, p<0.01; 0.99mM, 12/134, p<0.001; 1.32mM, 11/75, p<0.001; and 1.64mM, 3/5, p<0.001. For TEGDME, the incidence of bent bristles was statistically increased at three concentrations - 0.34mM, 10/159, p<0.01; 0.66mM, 28/171, p<0.001; and 0.99mM, 6/25, p<0.001) - no flies emerged at 1.32 and 1.64mM. Based on these results, TEGDME > TEGMME > TEG in terms of developmental toxicity. Results with TEG, TEGMME and TEGDME parallel the developmental toxicity seen in mammals and support further utilization of this *Drosophila*-based assay as a prescreen for developmental toxicants.

1294 THE IMPACT OF ETHANOL ON THE *IN VITRO* SKIN PENETRATION RATES OF CAFFEINE IN ENGINEERED SKIN CONSTRUCTS.

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The reference material, caffeine (prepared in ethanol), was evaluated in 3 *in vitro* models to compare the rates of skin penetration in each of the models. The models were human donor skin, an engineered skin construct (MatTek Corporation, Model EPI-606X) and slaughterhouse-derived pig skin. The tissues were mounted in flow-through diffusion cells (PermeGear, Inc., 0.64 cm^2 surface area), qualified for barrier function by $^3H_20$ passage, followed by the application of a 9 µL dose of ^{14}C-caffeine in ethanol (~4 µg/cm^2). The study duration was 24 hours. Total recovery of caffeine was acceptable (typically 95 to 100%) in each model. In human and pig skin, the rates of skin penetration were uniform and continuous throughout the 24-hour period. The mean amounts of caffeine that had been absorbed after 24 hours were 11% and 15% of applied dose, respectively (n=2 trials each). However, in the engineered skin model, the rate of penetration was remarkably high in the first 3 hours, followed by an abrupt decrease in the penetration rate thereafter. The amount absorbed after 3 hours was approximately 60% of applied dose. After 24 hours, the amount had increased to only 62% of applied dose. Subsequent experiments were conducted to evaluate the impact of the vehicle on caffeine's penetration rate in engineered skin. In the first experiment, caffeine prepared in water was tested in parallel with caffeine prepared in ethanol. A notable penetration rate lag phase was observed in the water-based preparation as compared to the ethanol-based preparation, but totals absorbed were 87% vs 66% of the applied dose, respectively. In the second experiment, engineered skin was pre-treated with ethanol followed by topical application of caffeine. Caffeine penetration rates and total caffeine absorption were similar in ethanol pre-treated and non-treated engineered skin. These results suggest that ethanol may have enhanced skin penetration upon initial exposure, but the solvent effect may have been rapidly modulated.

1295 PRECISION-CUT TISSUE CHIPS AS A TOXICOLOGICAL TOOL.

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The premise of these studies is to use precision-cut tissue chips from multiple organs from mice to simultaneously examine the *in vitro* toxicity of chemical agents. The use of the smaller tissue chips allows more samples since the small organs from the mouse only provide a limited amount of slices while 4-10 fold more tissue chips can be prepared from an organ. Tissue chips are produced by using a biopsy punch to make multiple 4 mm tissue chips from one 8 mm, 250 micron-thick tissue slice. Additionally, larger-sized diameter coring tools were explored to expedite and maximize chip production and quantity. Alterations to the Vitron Tissue Slicer have allowed more consistent slices, while some modifications allow the potential to generate multiple tissue slices at once. Tissue chips are incubated in 24 well plates, placed into a rotating 37 C shaking incubator and oxygenated with 95:5 O2:CO2. Due to the smaller amount of biomass in the tissue chip, fluorescent biomarkers have been used to assess *via*bility. TMRE, Mitotracker Far Red, 6-carboxy fluorescein, and NBD-TMA have been used for their permeability and detection characteristics, and for the assessment of cellular processes. Neutral red and MTT assays have been employed to expedite processing of tissue chips, and have been compared to our previous, more time-consuming *via*bility indicators (e.g. intracellular K+ content/DNA). Iodoacetemide (IAM) treatment (0-1000 uM), a fast-acting alkylator, has shown increases in TMRE fluorescence (500 uM IAM, 45 min), indicating increased mitochondrial activity, with a subsequent decrease in *via*bility in liver and kidney tissue chips as determined by both neutral red uptake and MTT assays. IAM treatment of 10 uM in liver and kidney tissue chips showed more than 50% decrease in *via*bility with a 12 h incubation. Kidney chips exhibit decreases in *via*bility in 2 h incubations with 1000 uM IAM treatment. This system will allow for a rapid examination of toxic effects in multiple target tissues to expedite toxicity profiling of potential new agents. (NIH R33 CA97449)

1296 EVALUATION OF THE BCOP ASSAY AS A PREDICTOR OF OCULAR IRRITATION OF PETROCHEMICAL PRODUCTS.

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Many initiatives have been undertaken in recent decades to reduce, refine, and replace the use of animals in evaluating toxicity, however, relatively few alternative studies have been conducted with petrochemical products. Therefore, a program was implemented to facilitate the development and validation of alternative *in vitro* test methodologies that could predict ocular irritation and acute systemic toxicity of petrochemicals. The Bovine Corneal Opacity and Permeability (BCOP) assay, a test that measures opacity and epithelial integrity to corneas, was used to evaluate 16 petrochemical products (e.g., lubricant additives, cutting fluids, solvents). Based on *in vivo* ocular irritation data and risk phrases, test articles were subdivided into the following three main categories: Category A (risk of serious damage to eyes), Category B (irritating to eyes), and Category C (non-irritating). Test article treated corneas were incubated at 32±1°C for two exposure times of 3- and 10-minutes. BCOP assay correctly classified the products in >95% of cases with the 10-minute exposure data, suggesting that it is valid for certain petrochemicals as a screening tool before *in vivo* testing (refinement) and as an alternative stand alone assay (replacement) for assessing hazard. The same 16 test articles were examined in the 3T3 Neutral Red Uptake (NRU) Bioassay as a predictor of dose selection and for assessing potential acute systemic toxicity. Poor predictability (<60%)was observed in the NRU assay, which was likely associated with their low solubility in water and the inability of cells being exposed to concentrations below desired concentrations. Although poor predictability was observed, results did indicate the possible use of the NRU assay as a refinement-screening tool for specific chemicals. Based on this body of data, these results could facilitate the validation and acceptance of alternative testing methodologies that will ultimately benefit animal welfare.

1297 IMPROVED 'ACCOUNTABILITY' OF *IN VITRO* METHODS USED FOR ASSESSING DERMAL ABSORPTION OF ENVIRONMENTAL CONTAMINANTS.

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As part of our research to examine *in vitro* methods for quantifying dermal absorption, we have refined the method used to evaluate this process. Since both the amount and persistence of chemical remaining in the skin and of that more completely absorbed into the receivor blood simulant solution is important to measure for hazard risk assessment, the method used must provide complete 'accountability' of the chemical dose used. Human skin permeation data for the lipophilic compound, nonyl phenol, obtained using our in-house developed Automated *In vitro* Dermal Absorption (AIDA) method will be presented to show the importance of having a complete mass balance for such 'accountability' purposes. This will include simple considerations such as loss of the applied test 14C-radiolabeled chemical to the test apparatus including the plastic pump tubing used to circulate the donor chemical treatment solution. We have previously reported very significant loss of this lipophilic compound (Log octanol/water partition coefficient = 6) to peristaltic pump tubing (Akram et al., 2003). We expand this here to show 14C-nonyl phenol data for several soap water and solvent apparatus washes obtained from human skin absorption tests. The results showed loss of as much as 60 percent of the applied radiolabel test chemical and that most of this loss was due to adsorption into the test apparatus, particularly the peristaltic pump tubing used. In order to provide a possible solution to this problem, tests with 1 percent soap added to the donor solution were conducted. Although tests with 1 percent soap added prevented adsorption to pump tubing and full mass balance was obtained, further tests are needed to determine the suitability of this 'quick-fix' since the presence of soap in the donor solution, even at low concentration, could affect skin permeability and/or chemical depot storage/bioavailability and so adversely influence the data used for regulatory toxicology risk assessment.

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Preface

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