and skin samples were collected and processed for the comet assay. DNA single strand breaks were quantified by the differences observed in comet moment, percent (%) tail DNA, and comet tail length. These parameters were significantly increased by BaP compared to vehicle in all animals. When the level of DNA damage was analyzed using comet moment to compare the distribution of the skin cells in BaP- and vehicle-treated animals, BaP treatment resulted in a significant increase in the number of cells with DNA damage. This response elicited by BaP did not increase in severity beyond day 3. These findings indicate that a net balance of DNA damage and repair is likely achieved in the skin of BaP-treated animals over the course of 10 days of exposure. Furthermore, chemically-induced DNA damage can be detected using the comet assay in a short-term exposure model.

2190 EFFECT OF METHYL SUBSTITUTION OF BENZENE ON THE PERCUTANEOUS ABSORPTION AND SKIN IRRITATION HAIRLESS RATS.

J. Ramapuram[1], E. AHAGHOTU[1] and M. SINGH[1]. *[1]Florida A&M University, Tallahassee, FL and [2]SOT, E\TEST, AL.*

To study the structure activity relationship (SAR) between the methyl substitution of benzene and 1) permeation or retention in skin layers 2) their irritation potential in hairless rats after dermal exposures. Methods: The skin permeation or retention studies were carried out on freshly excised hairless rat skin mounted on Franz diffusion cells filled with 6% Brij 98 in normal saline. Benzene, dimethyl benzene (xylene) and tetramethyl benzene (isodurene) spiked with respective 3H radiolabeled chemicals were applied to the epidermal surface. After defined exposure period, the receptor samples were collected; the exposed skin area was tape stripped for SC, and sectioned into epidermis and dermis with a cryotome. The permeation rate and retention in stratum corneum (SC), epidermis and dermis were determined by scintillation counting of the samples. The skin irritation of the chemicals was studied in hairless rats by occlusive (230 µl for 1 h using Hill top chambers®) and unocclusive (15 µl every 2 h for 8 h a day for 4 days) dermal exposures. The transepidermal water loss (TEWL) and erythema were measured at different time intervals after dermal exposures. Results: The permeation of benzene decreases with increase in methyl groups of benzene (2.5 and 80 times higher flux by xylene and isodurene). The retention of chemicals in SC showed a direct correlation between amount retained in SC and methyl substitution or log P of the chemical. The retention in epidermis and dermis of different chemicals were approximately 10-15 times higher than SC. The skin irritation (TEWL and Erythema) increases with methyl substitution of benzene (isodurene > xylene > benzene). The TEWL data correlated "\very well with erythema scores of the chemicals. Conclusion: A clear relationship between the flux or skin retention and methyl substitution of the chemical was established. The skin irritation increases with methyl substitution of benzene under the experimental conditions of present study.

2191 LONG TERM REPRODUCIBILITY OF EPIDERM™, AN EPIDERMAL MODEL FOR DERMAL TESTING AND RESEARCH.

M. Klausner, J. Kubilus, J. E. Sheasgreen and P. J. Hayden. *MatTek Corporation, Ashland, MA.*

An *in vitro* model of human epidermis, EpiDerm (EPI-200), cultured from neonatal, normal human epidermal keratinocytes has been sold commercially by MatTek Corporation since 1993. Using serum free medium, weekly lots of EpiDerm are produced and shipped for dermal irritancy, product efficacy, percutaneous absorption, pharmacological, and basic skin research studies. In 2000 and 2002, respectively, European and US regulators approved the use of EpiDerm to assess the skin corrosivity of chemicals. Validation studies utilizing EpiDerm for phototoxicity and skin irritation are currently underway. For commercial and regulatory purposes, it is crucial that the model is reproducible both within a given lot and between lots, especially over extended periods. To address tissue reproducibility, quality control testing of each EpiDerm lot involves exposure to the surfactant, 1% Triton X-100 (TX), and a negative control, ultrapure water. Using the MTT assay, which historically has been the *in vitro* endpoint of choice for European and US regulators, a dose response curve is constructed and an exposure time which reduces the tissue viability to 50% (ET-50) is interpolated. The yearly average ET-50 since 1996 has varied from 6.5 hr (2000) to 7.5 hr (1998). The coefficients of variation (CV) for the negative control have averaged under 7% for every year since 1997 and the average CV for all tissues has never exceeded 13.2%. Using light microscopy, histological H&E cross-sections show an epidermis-like morphology that is reproducible both within and between lots. Hence, over the past 10+ years of commercial production, EpiDerm has remained a highly reproducible, stable toxicological model that is ideally suited for industrial and regulatory toxicology and other skin related studies.

2192 DETERMINING SKIN IRRITATION POTENTIAL OF INDUSTRIAL FLUIDS IN HUMANS USING TRANS-EPIDERMAL WATER LOSS (TEWL).

M. Wragg[1] and S. Signs[2]. *[1]Lubrizol Limited, Derby, United Kingdom and [2]The Lubrizol Corporation, Wickliffe, OH.*

Evaluating the skin irritation potential of industrial fluids is becoming increasingly important for downstream lubricant users. In the absence of validated alternative methods, animal models (eg. OECD method guideline 404) have been relied upon to provide a prediction of the irritant potential of a fliud in the workplace. It is suggested that evaluating the irritant potential of industrial fluids in human volunteers using trans-epidermal water loss (TEWL) and colorimetry measurements could offer a scientifically-valid alternative to established animal models, and provide greater predictive accuracy for estimating potential skin reactions in workers following occupational exposure. Several industrial fluids were evaluated for skin irritation potential using a 4-hour semi-occluded exposure in rabbits (OECD method 404). These same fluids; in addition to 0.2% SLS as a positive control, and negative control treatments including water, highly refined lubricant base oil and an untreated site; were also examined in a panel of 30 human volunteers using a 24-hour occluded exposure. Irritation was quantitatively scored using ΔTEWL, colorimetry, and estimation of erythema/edema by a trained dermatologist. RESULTS: Three of the supplied fluids produced Primary Irritation Index (PII) scores in rabbits of >3.0 (erythema and edema values met the EU criteria for a skin irritant, R38), but were found to be unsuitable for investigation in volunteers based upon initial observations of severe irritation that exceeded ethical limits for testing in humans. Other fluids produced PII = 1.0 when tested in rabbits (did not meet the EU skin irritant criteria) were found using the TEWL model to have a slightly higher potential to cause skin irritation in the workplace than that of non-additized, highly-refined mineral oil. The results show that measurement of ΔTEWL following 24-hour occluded exposure in human volunteers is sufficiently sensitive to identify industrial fluids that have the potential to cause an adverse skin reaction in the workplace under unprotected exposure conditions.

2193 EVALUATION OF HISTORICAL POSITIVE CONTROL DATA FOR RESPONSE CONSISTENCY AND REDUCTION IN ANIMAL USE IN PHOTOTOXICITY AND PHOTOALLERGY ASSESSMENTS.

D. B. Learn, C. P. Sambuco, M. Arocena, M. L. Davis, T. S. Coston, P. D. Forbes and A. M. Hoberman. *Center for Photobiology, Charles River Discovery and Development Services, Argus Division, Horsham, PA.*

In phototoxicology, positive control agents (PCA) have been included in animal studies when assessing potential adverse effects of pharmaceuticals and chemicals. Whether to use PCA groups in all such assays is a decision based on regulatory requirements, the effort to reduce animal use and confidence in the consistency of the required response. In acute phototoxicity (PT) assays performed in our laboratory, 8-methoxypsoralen (8-MOP) and lomefloxacin (LOM) are commonly used PCA as is 3, 3', 4', 5'-tetrachlorosalicylanilide (TCSA) for contact hypersensitivity (CH) and photoallergy (PA) assessments. Historical control data (HCD) were compiled from studies over the past 7 years for cutaneous PT induced by 8-MOP in hairless mice, haired (Long-Evans) rats, haired guinea pigs and hairless guinea pigs, by LOM in hairless mice and CH and PA induced by TCSA in guinea pigs. Ocular PT HCD using Long-Evans rats were also compiled. The routes of administration for PT included topical, oral (gavage) and intravenous while the topical route of administration was evaluated for CH and PA. In all studies, the light source was a filtered xenon arc solar simulator emitting environmentally-relevant ultraviolet, visible and infrared radiation. Review of the HCD revealed that erythema, edema and flaking assessed by visual observation were the most common signs of cutaneous PT, CH and PA. Corneal opacity and/or edema detected by ophthalmologic examination and substantiated by histopathological evaluation were the hallmarks of ocular PT. The incidences of the cutaneous and ocular findings were consistent and reproducible across studies and over time. Therefore, the HCD revealed that the *in vivo* phototoxicology assays are robust and these data, rather than positive control animals, can be used to demonstrate the validity of the assays and appropriately reduce the use of animals in these assessments.

2194 QUANTIFICATION OF MIXTURE INTERACTIONS ON DERMAL PERMEABILITY–A SOLVATOCHROMATIC APPROACH.

R. E. Baynes, B. M. Barlow, X. R. Xia, J. L. Yeatts, J. D. Brooks and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

The influences of mixture–mixture interactions, differing solvent systems, and surfactants on permeability and dermal absorption have not been well studied. Past research has focused on the toxicity of a single solute dosed in an organic solvent; unfortunately, these conditions do not generally represent real world scenarios of

occupational use. The scope of our study is to identify and quantify physicochemical interactions that drive dermal permeability using Linear Solvation Energy Relationships (Log permeability = $c + r^*R_2 + s^*\pi_2^H + a^*\Sigma\alpha_2^H + b^*\Sigma\beta_2^H + v^*v_x$) during complex mixture exposure. Seven solutes (propylbenzene, biphenyl, naphthalene, methyl benzoate, 4-nitrotoluene, phenol, and 3-methyl phenol) with a wide range of known physiochemical properties (Log p, solubility, molecular weights, etc.) and LSER solvatochromatic parameters (R_2, π_2^H, α_2^H, β_2^H, v_x) were prepared in various mixtures (0-100% aqueous or ethanol solutions and/or 0.1%, 1% and 10% SLS), and applied to a porcine skin flow thru diffusion cell system. Dermal permeability was then calculated using the resultant perfusate and SPME GC/MS or GC/FID detection. The LSER system coefficients (r, s, a, b, v) were compared to derive an interaction coefficient value (Δr, Δs, Δa, Δb, Δv). Preliminarily results indicate that ethanol at 50% strength decreased both hydrogen bond acceptor activity (Δr-2.010) and McGowan molecular volume (Δv -0.729), while the presence of surfactants increased both hydrogen bond acceptor activity (Δb1.979), and McGowan molecular volume (Δv1.305). These results indicated that system basicity and lipophilicity play a significant role in dermal permeability of solutes after exposure to complex solvent and surfactant mixtures. Further studies are aimed at discerning quantitatively the biological and chemical contributions of these interactions. Supported by NIOSH Grant R01-OH-03669

2195 PREDICTING HUMAN SKIN ABSORPTION OF CHEMICALS: DEVELOPMENT OF A NOVEL QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP.

W. Luo[1], S. Medrek[2], H. Nguyen[1] and W. Fung[1]. *[1]Toxicology, L'Oreal USA, Clark, NJ and [2]Department of Chemical Engineering, Princeton University, Princeton, NJ.*

The objective of this study was to construct and validate a simple model using over 340 chemicals of diverse structure for the quantitative prediction of percutaneous absorption based on their physicochemical properties. Such a model is a valuable tool for screening and prioritization in safety evaluation and risk assessment of chemicals. A number of models will be presented. Of particular interest is a simple three-parameter QSAR model to predict the permeability coefficient, Kp, with a training set of 306 chemicals and their physicochemical descriptors. In addition to the experimental Kow values, over 300 2D and 3D atomic and molecular descriptors were analyzed using MDL's QSAR computer program (MDL Information Systems, Inc., San Leandro, CA 94577). All Kp values used in training and validation were experimental data obtained from various sources. Using the stepwise regression analysis, three molecular descriptors were determined to have significant statistical correlation with Kp (R2=0.8225): logKow, x0 (quantification of both molecular size and the degree of skeletal branching), and SsssCH (count of aromatic carbon groups). Subsequently, the model was validated using both internal (leave-one-out) and external validation (90/10 random split) procedures. The R2 from internal validation is 0.8108, and that from external validation is 0.5829, Log Kp (cm/hr) = 0.5892*logKow - 0.1006*x0 - 0.1617*SsssCH - 2.62353 (R2= 0.8225) In conclusion, an algorithm to estimate human skin absorption was derived based on physicochemical properties of over 300 compounds. Compared to other skin absorption QSAR models in the literature, our model has incorporated more chemicals and explored more descriptors in addition to the commonly used MW and Kow. Additionally, our model is reasonably predictive and has stood up to both internal and external statistical validations.

2196 A NOVEL SYSTEM COEFFICIENT APPROACH FOR QUANTITATIVE ASSESSMENT OF DERMAL ABSORPTION FROM CHEMICAL MIXTURES BY USING THE MEMBRANE-COATED FIBER TECHNIQUE.

X. R. Xia, R. E. Baynes, N. A. Monteiro-Riviere and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Chemical exposure in most environmental and occupational settings involves complicated chemical mixtures, not individual chemicals. However, current health risk assessment of dermal absorption is based on permeation data for individual chemicals due to lack of available quantitative methods or experimental data from chemical mixtures. In the system coefficient approach, a set of solute descriptors, [R, π, α, β, V], represents the molecular interaction strengths of a chemical of interest, while a set of system coefficients, [r, s, a, b, v], represents those of the skin/medium system. The permeation coefficient or partition coefficient (logK) is correlated with the system coefficients and the solute descriptors via Abraham's linear free energy relationship. The major components of the mixture determine the system coefficients. When the major components change in composition or proportion, the system coefficients will be changed. Therefore, the changes in the system coefficients can be used to study the changes of the chemical mixtures: $\log K = c + (r+\delta r)R + (s+\delta s)\pi + (a+\delta a)\alpha + (b+\delta b)\beta + (v+\delta v)V$. When 25% of ethanol was added to the water solution of the 32 calibration compounds, the system coefficient changes of the polyacrylate/water system [δr, δs, δa, δb, δv] were [-0.12, 0.17, 0.23, 0.33, -0.42], respectively. When 1% of sodium lauryl sulfate was added to the water solution, the system coefficient changes [δr, δs, δa, δb, δv] were [-0.41, 0.34, 0.23, 0.74, -1.53], respectively. In practical risk assessments, the changes in system coefficients can be treated as sufficiently similar mixtures. If the system coefficients of the mixture of concern and the system coefficients of the sufficiently similar mixtures are determined, the permeability or partition coefficient of any chemical can be obtained by using the system coefficient approach. Supported by NIOSH R01-OH-07555 and OH-03369 and AFOSR F49620-01-0080

2197 PREDICTION OF DERMAL ABSORPTION OF CHEMICAL MIXTURES USING BOTH PENETRANT AND MIXTURE COMPONENT PROPERTIES IN A HYBRID QUANTITATIVE STRUCTURE PERMEABILITY RELATIONSHIP (QSPR).

J. E. Riviere and J. D. Brooks. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Occupational and environmental exposure to topical chemicals is usually in the form of complex chemical mixtures, yet risk assessment is based on experimentally derived data from individual chemical exposures or computed physiochemical properties. We present an approach using modified QSPR models where absorption through two systems (porcine skin flow-through diffusion cells-PSFT and isolated perfused porcine skin flaps-IPPSF) are well predicted using a QSPR model describing the individual penetrants, coupled with a mixture/vehicle factor (MF) that accounts for physicochemical properties of the vehicle/mixture components. The baseline equation is $\log Kp = c + mMF + rR + s\pi + a\alpha + b\beta + vV$ where R is molar refractivity, π is polarizability constant, α is H-bonding acidity, β is H-bonding basicity and V the McGowan molecular volume of the penetrants of interest; c, m, r, s, a, b and v are strength coefficients coupling these descriptors to skin permeability (Kp) in PSFT (12 penetrants in 24 mixtures) or to AUC of the absorption flux profile in IPPSF (8 penetrants in 4 mixtures). Mixtures consisted of different combinations of vehicles (water, ethanol, propylene glycol) and additives (sodium lauryl sulfate, methyl nicotinate). Across all exposures with no MF, R2 for absorption was 0.68 in IPPSF and 0.58 in PSFT. With the MF, correlations increased to 0.74 for IPPSF and 0.85 for PSFT. The initial MF is a function of the Henry Constant for the mixture components, although other factors are presently being investigated. The importance of these findings is that there is an approach whereby the effects of a mixture on absorption of a penetrant of interest can be quantitated in a standard QSPR model if physicochemical properties of the mixture are also taken into account. The good correlations obtained in the IPPSF, which has previously shown to be predictive of *in vivo* human absorption, suggests that this approach merits attention. Supported by NIOSH R01-OH-07555

2198 COMPUTER-CONTROLLED SYSTEM FOR GENERATION OF CHEMICAL VAPORS IN *IN VITRO* DERMAL UPTAKE STUDIES.

M. Rauma and G. Johanson. *Work Environment Toxicology, Karolinska Institutet, Stockholm, Sweden.*

Recent work in our laboratory shows that dermal uptake of chemicals may be assessed *in vitro* by thermogravimetric analysis (TGA), i.e. by passing chemical vapor over a piece of skin while recording the weight increase under carefully controlled temperature and humidity conditions. This technique requires (1) a high-precision TGA balance and (2) a stable generation and supply of water and chemical vapors at known concentrations. For the latter purpose, we have developed a high precision computer-controlled exposure system. Clean, dry air flows at controlled rates (45 ml/min) through stainless steel gas impinger bottles containing the chemicals of interest. The impingers reside in a water bath maintained at exactly 25°C. To prevent retention and condensation of chemical vapor downstream, all tubing and connectors are made of stainless steel and insulated and kept at 30°C with a heat cable. Two parallel impinger systems with separate mass flow regulators allow for different exposure sequences, for example: 50% water + 50% chemical A for 1 hour, followed by 50% water only for 3 hours then 50% water + 50% chemical B etc. The airflow is redirected to the impingers by computer-controlled magnetic valves. A dedicated computer software allows execution of exposure sequences with millisecond precision. Measurements of dry air leaving an impinger containing cyclohexanone showed that the concentration in outlet air (measured by photoionization detection) rapidly reached a plateau (90% response in approx. 8 sec) and remained stable at a level corresponding to the theoretical saturation concentration. Cyclohexanone has a low vapor pressure compared to many other solvents, and we expect that chemicals with a higher vapor pressure behave similarly. In conclusion, this computerized system with automated, high-precision generation of sequences

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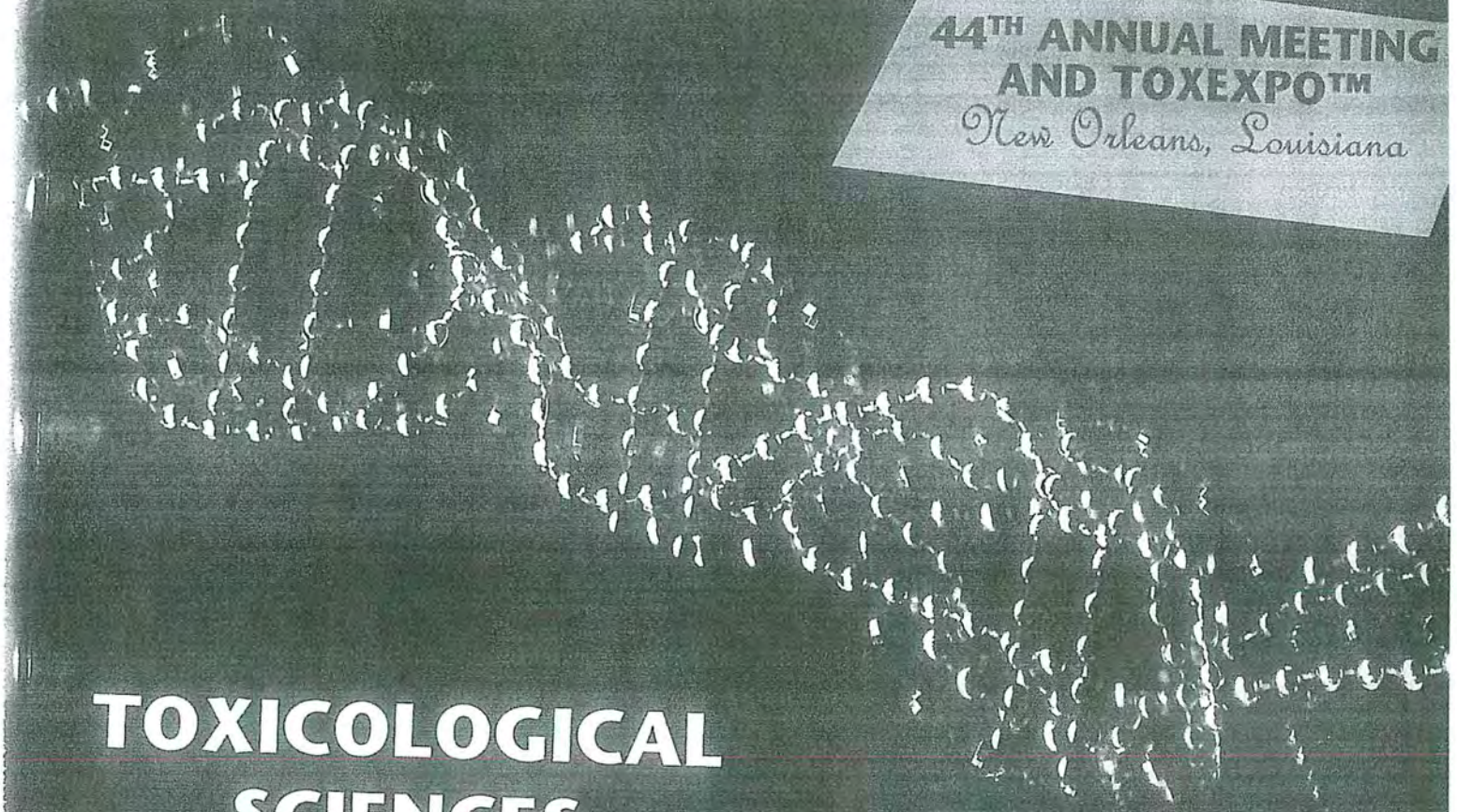

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