of GEN protection of diabetes development in female NOD mice is complex, and more studies are needed to identify the immune components that are targeted by GEN (Supported in part by NO1-ES05454).

PS 1514 ACETAMINOPHEN REVEALS IMMUNE-SENSITIZATION IN ORAL EXPOSURE MOUSE MODEL.

I. S. Ludwig, L. Kwast, D. Fiechter and R. Pieters. *Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands.*

Many drugs are known to induce adverse immune reactions in susceptible individuals and may result in clinical diseases. The reporter antigen popliteal lymph node assay (RA-PLNA) is assessing this immune-sensitization capacity of low molecular weight compounds. However this assay only assesses local responses and does not include oral exposure. Therefore systemic effects are missed and furthermore, when metabolism is required compounds may test false negative in this assay. Preclinical systemic exposure models with a predictive value do not exist.
In this study the immune-sensitization of acetaminophen (APAP) was investigated when orally administered to mice. Trinitrophenyl-ovalbumin (TNP-OVA) was used as a reporter antigen (RA) to assess the capacity of the drugs to stimulate systemic immune responses to a bystander antigen. Female C3H/HeOuJ mice were exposed to 30, 100 or 300 mg/kg APAP daily for 7 days and co-exposed to TNP-OVA at day 1. 15 days after the start of the exposure delayed type hypersensitivity was determined by a challenge with the RA. A vigorous response to the RA was detected in animals exposed to APAP. RA-specific IgG1 and IgG2a antibodies could be detected in the serum at day 21. Furthermore, isolated LN cells from APAP treated animals displayed a higher IFN-γ, IL-4 and TNF-α production than cells from control animals upon restimulation in vitro. In conclusion, APAP, with a known capacity to induce immune mediated adverse reactions in patients, displays in this oral exposure mouse model a mixed TH1/Th2 response. Combined with results from other studies in which ofloxazine, diclofenac and D-penicillamine, all known immune-sensitizing drugs and induced antibody responses in this mouse model, this experimental set up could be useful for preclinical assessment of sensitizing potential of new chemical entities.

PS 1515 EXAMINATION OF POTENTIAL CHEMICAL INTERACTIONS IN SKIN SENSITIZATION : ACTIVITY OF CLOVE BUD OIL ADMIXED WITH OXAZOLONE IN THE LOCAL LYMPH NODE ASSAY.

J. Lalko[1], I. Kimber[2], R. J. Dearman[2], G. Gerberick[3] and A. Api[1]. *[1]Research Institute for Fragrance Materials Inc., Woodcliff Lake, NJ, [2]The University of Manchester, Manchester, United Kingdom and [3]Procter & Gamble Co., Cincinnati, OH.*

The potential for interaction between contact allergens during the induction phase of skin sensitization has rarely been investigated. Understanding of the potential for additive and/or synergistic effects might prove relevant and inform risk assessment strategies. In order to explore the potential for interactions between chemicals during the acquisition of skin sensitization a series of local lymph node assays (LLNA) were conducted with a well-characterized sample of clove bud oil - an essential oil containing multiple contact sensitizers – alone, and in combination with a constant amount of the strong sensitizer oxazolone. Clove bud oil was tested at five concentrations ranging from 1.0% to 25% w/v in a vehicle of 1:3 ethanol:diethyl phthalate (EtOH:DEP), both with and without 0.005% oxazolone. Two vehicle controls were included, one containing 0.005% oxazolone and one without. The concentration of oxazolone was selected based on a known EC3 value of <0.001% in EtOH:DEP. Stimulation indices (SI) were calculated for each dose level with an SI ≥ 3 compared with the relevant vehicle control being considered positive. Theoretical dose response curves based upon summation of the individual responses to clove oil and the oxazalone containing vehicle controls were also generated. Comparison of the theoretical with experimentally derived dose responses revealed that the combination of clove bud oil and oxazolone had a slightly greater than additive effect, particularly at higher doses. These results are consistent with other reports of additive effects in the LLNA. Further work is needed to determine if these effects translate into increased levels of sensitization to the individual materials.

PS 1516 INDUCTION OF SENSITIZATION AND CYTOTOXICITY TO TRICHLOROETHYLENE IN MICE: ROLE OF METABOLITES.

D. Yufei, L. Haishan, L. Qing, S. Yaofeng, N. Yong, B. Ping and Z. Yuxin. *Key laboratory, National institute for occupational health and poison control, Chinese center for disease control and prevention, Beijing, China.* Sponsor: Z. Yuxin.

Trichloroethylene (TCE) is an important industrial chemical and is widely used. The severe allergic skin damage induced by TCE has been described. The aim of the study was to explore role of metabolites of TCE in the process of allergic skin damage in mice using a combination of *in vivo* and *in vitro* endpoints. Female BALB/c mices pretreated with ethanol or phenobarbital (PB) were administered TCE for 6 weeks, followed by challenge onto the ears to evaluate delayed-type hypersensitivity (DTH) response. After the mice were sacrificed, activities of aspartate aminotransferase and alanine aminotransferase in serum were measured as markers of hepatic damage. The TCE-specific lymphocytes proliferation and cytotoxicity of TCE and its metabolites were determined in cultured spleen lymphocytes exposed to TCE and SKF-525A (CYP450 inhibitor) or aminooxyacetic acid (AOAA, cysteine conjugate β-lyase inhibitor) *in vitro*. The results showed that no DTH response could be observed in 3 groups of mice treated by TCE, TCE+PB and TCE+ethanol. However, a significant proliferation of spleen cells from TCE-treated mice was evidenced following incubation with TCE *in vitro*, it was interesting that this proliferation disappeared when AOAA was added and cytotoxicity of TCE decreased when SKF-525A was added. In mice treated by TCE+PB and TCE+ethanol, no significant proliferation of spleen cells was found in culture with TCE *in vitro*, but hepatoxicity of TCE *in vivo* was promoted by PB and ethanol through increasing the CYP450-dependent oxidation metabolic flux. This study provides an indirect evidence that Glutathion-dependent pathway might play an important role in allergic reaction induced by TCE, and dichlorovnyl thiol-metabolite catalyzed by cysteine conjugate β-lyase is responsible for proliferation of hapten- specific lymphocytes in TCE-sensitized mice. The CYP- dependent oxidation cause cytotoxicity of spleen cells *in vitro*, and hepatotoxicity *in vivo* as well.

PS 1517 INCREASED CELL PROLIFERATION IN SPLEEN AND LYMPH NODES PERIPHERAL TO CONTACT ALLERGEN APPLICATION SITE.

I. Chipinda, S. E. Anderson, L. F. Butterworth, D. H. Beezhold and P. D. Siegel. *HELD/ACIB, CDC/NIOSH, Morgantown, WV.*

The local lymph node assay (LLNA) is widely used to identify chemicals that are contact sensitizers. The assay involves dosing mice with the chemical on both ears and pooling the cervical lymph nodes for assessment of lymphocyte proliferation as a marker of sensitization. The present study explored potential reduction in animal usage by dosing one ear with the allergen and the other with vehicle only. The respective draining lymph nodes were processed separately for quantification of cell proliferation. Cell proliferation in axillary and renal nodes, and in the spleen was also assessed. Cross contamination of the chemicals from the dosed ears to other parts of the body via preening was prevented by dosing restrained animals and washing off the residual chemical with saline after 4 hours. Dosing the left ear with 0.02% oxazolone (OX) on unrestrained animals resulted in marked cell proliferation in its draining lymph node (Stimulation index, SI = 12.8) and in the lymph node draining the contra-lateral vehicle dosed ear (SI = 6), and the axillary lymph nodes (SI = 3.3). Increased tritiated thymidine (^{3}H-TdR) incorporation was not observed in the renal lymph nodes (SI = 1.1). Similar stimulation of cells was observed in the lymph node draining the ear contra-lateral to the allergen dosed ear when 30% hexylcinnamaldehyde (HCA) was applied. Increased proliferative activity was observed in non-directly draining lymph nodes of restrained mice demonstrating that these results can not be attributed to cross-contamination of adjacent skin. A significant increase in proliferation of splenocytes was observed. It is concluded that epicutaneous application of a contact allergen, as exemplified by OX and HCA, may induce cell proliferation in the neighboring lymph nodes and spleen indicative of hapten and/or haptenated proteins diffusing through the skin to peripheral nodes and the blood to produce systemic sensitization. Thus the node draining the contra-lateral ear can not be used as a control for application of contact allergen to a single ear in a modified LLNA.

PS 1518 COMPARISON OF GLOVE CONTACT ALLERGEN CONTENT AND CLINICAL PATCH TEST.

P. D. Siegel[1], F. J. Storrs[2], D. Sasseville[3], M. Pratt[4], T. A. Bledsoe[1], B. F. Law[1], D. H. Beezhold[1] and J. F. Fowler[5]. *[1]NIOSH/CDC, Morgantown, WV, [2]Oregon Health Sciences University, Portland, OR, [3]McGill University, Montreal, QC, Canada, [4]Ottawa Hospital, Ottawa, ON, Canada and [5]University of Louisville, Louisville, KY.*

Irritant dermatitis, allergic contact dermatitis (ACD) and urticarial reactions have been associated with chemical rubber accelerators present in latex and nitrile/synthetic rubber gloves. In this study, patients reporting to a dermatology clinic with glove associated ACD were allergy patch tested with the North American Contact Dermatitis Group standard rubber allergen series, medical history taken and asked to identify and supply "problem gloves" which they associated with their ACD and if possible "non-problem gloves". Medical exam, surgical and industrial-type rubber and nitrile gloves from patients were assessed for chemical content including: zinc dithiocarbamates, thiurams, mercaptobenzothiazole (MBT) and its disulfide, dimorpholine, diphenylguanidine, thiourea, and phenylene diamine. Only zinc di-

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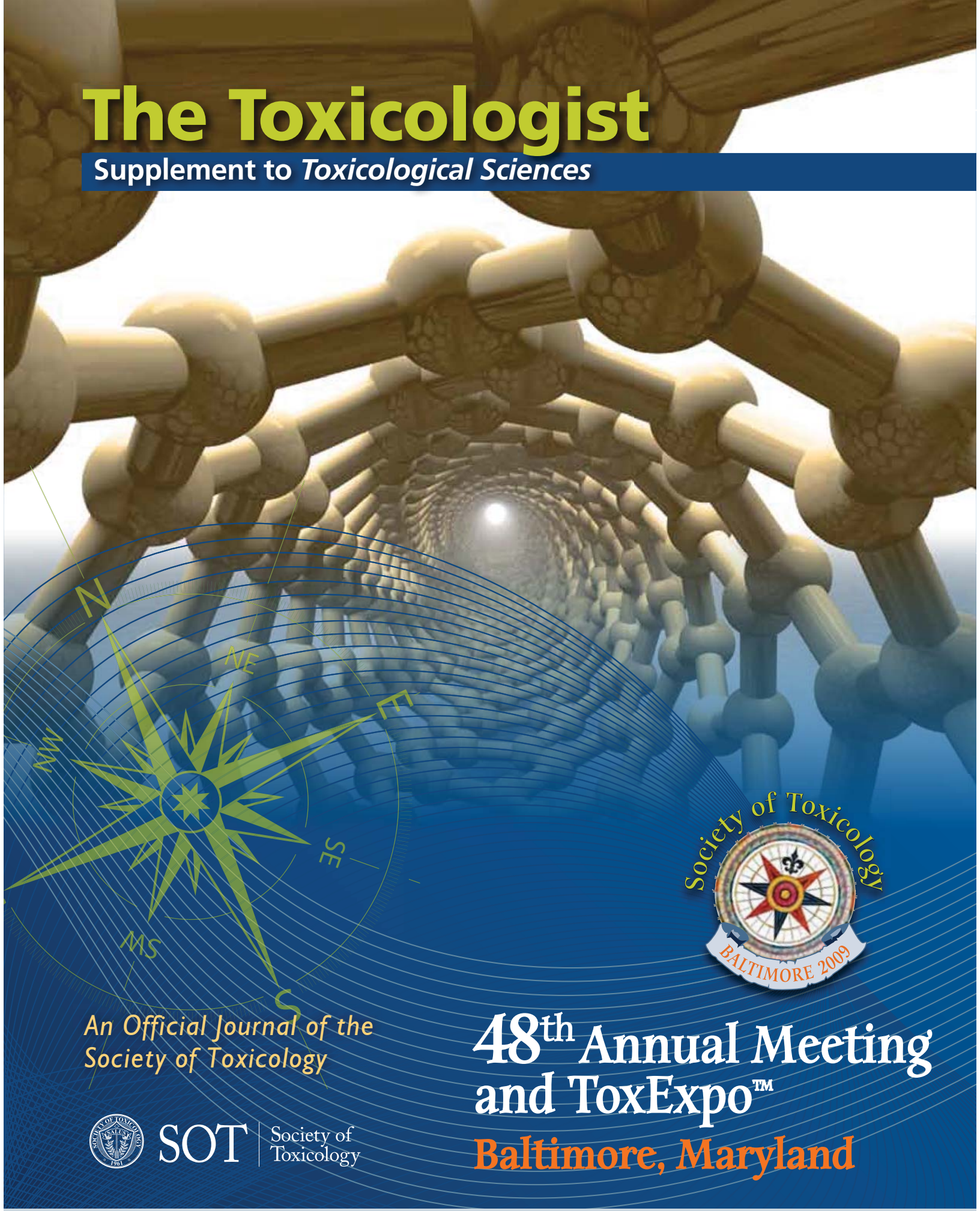

An Official Journal of the Society of Toxicology

SOT | Society of Toxicology

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Baltimore, Maryland

www.toxsci.oxfordjournals.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 108, Number 1, March 2009