812 THE LOCAL LYMPH NODE ASSAY: FURTHER COMPARISONS WITH GUINEA PIG AND HUMAN SENSITIZATION DATA.

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The local lymph node assay (LLNA) is an alternative to guinea pig assays for identification of skin sensitizing chemicals. It has been endorsed as a stand alone method for the determination of contact allergenic hazard by the FDA, the EPA and others following approval by the Interagency Co-ordinating Committee for the Validation of Alternative Methods (ICCVAM). The LLNA has also received formal approval from the European Centre for the Validation of Alternative Methods (ECVAM) and been submitted as an Organization for Economic Co-operation and Development (OECD) guideline by the UK. In the standard LLNA protocol, CBA strain mice receive 3 consecutive daily topical applications of test substance (at 3 dose levels). Five days after the initiation of exposure, proliferative responses in draining lymph nodes were measured by radiolabelled thymidine incorporation. Chemicals which induce proliferative responses greater than 3x than those in vehicle-treated control animals are considered to be skin sensitizers. Approximately 230 chemicals have been evaluated by 3 independent laboratories in standard LLNAs and results compared with guinea pig and/or human data. The data demonstrate an approximately 90% concordance between guinea pig and mouse results (based on 134 points of comparison). In terms of the prediction of human data, the guinea pig was 88% accurate (based on 74 points of comparison), while the LLNA had an accuracy of 86% (based on 98 points of comparison). The data confirm that the LLNA is as reliable as traditional guinea pig methods for the identification of significant human skin sensitizers. Furthermore, the LLNA confers significant animal welfare benefits in terms of reduction and refinement of animal usage compared with standard guinea pig tests.

813 CONTACT ALLERGENIC POTENCY: CORRELATION OF HUMAN AND LOCAL LYMPH NODE ASSAY DATA.

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Effective toxicological evaluation of skin sensitization demands that potential contact allergens are identified and the likely risk of sensitization among exposed populations is assessed. Sensitization hazard is not an all or nothing phenomenon; dose response relationships can be discerned and thresholds identified for the induction and elicitation of contact allergy. These parameters, under the heading of potency, are vital for the risk assessment process. The murine local lymph node assay (LLNA) is an accepted method for the identification of sensitization hazard. We compared potency assessments derived from LLNA data with clinical determinations of relative potency based on human data. No effect levels (NOELs) for 21 chemicals were determined from human repeat patch test studies reported in the literature. These levels were compared with LLNA EC3 values, the estimated concentration required to produce a 3-fold increase (positive response) in lymph node cell proliferation. Using human data together with expert judgment, the compounds were classified as strong, moderate, weak, extremely weak or non-sensitizers. Also, the potency of each chemical was classified independently based on its LLNA EC3 value. Overall, the potency ranking of the 15 allergens was very similar using EC3 values or reported human NOELs along with clinical experience. A good correlation existed across all potency classifications. The 6 chemicals evaluated and classified as non-sensitizers for humans were classified identically in the LLNA. This investigation demonstrates that the LLNA can provide quantitative estimates of relative skin sensitizing potency which correlate closely with human NOELs. These results support strongly the use of EC3 values for estimating skin sensitization potency and the use of that information in risk assessment.

814 RESTRAINT STRESS MODIFIES LANGERHANS CELL MIGRATION AND LYMPH NODE CELL PROLIFERATION.

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Previously we have demonstrated that restraint applied to a naive mouse prior to sensitization decreases ear swelling and restraint applied prior to challenge increased ear swelling. We hypothesize that these dichotomous effects of restraint would be reflected in changes in LC trafficking from the epidermis or alteration of their antigen presenting capabilities to T cells in the draining lymph nodes. To assess the effect of restraint on T cell proliferation in vivo, male BALB/c mice were exposed on the dorsum of both ears on days 1, 2 and 3 with 0.25% di-nitrofluorobenzene (DNFB; n = 5) or vehicle only, and restrained for 2 hours prior to chemical application on day 1. On day 5, T lymphocyte proliferation was examined using the local lymph node assay. To evaluate the effects of restraint on LC, mice were sensitized with 0.25% DNFB immediately after 2 hour restraint. LC morphology and migration were examined in epidermal sheets by counting the number of FITC-conjugated Ia stained cells/mm2 at 0, 2 & 18 hours after chemical application. We found that restraint significantly decreased DNFB-induced cellular proliferation in lymph nodes by 50%. We also determined that LC from restrained mice appeared to be rounder with fewer dendritic processes than observed in non restrained mice. In addition, DNFB caused a 50% reduction of the number of LC in the epidermis of non-restrained mice at 18 h, but only a 39% reduction in LC for restrained mice. These data suggest that restraint-induced decrease in T cell proliferation may be due, in part, to decreased LC migration to the local lymph nodes.

815 WATER SOLUBLE MATERIALS IN THE LOCAL LYMPH NODE ASSAY: VEHICLE SELECTION.

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The murine local lymph node assay (LLNA) is a validated method for identifying skin sensitization hazard which can also provide information on relative potency. It has been shown that the choice of vehicle can influence the sensitization potential of haptens in the LLNA and in humans. The selection of a relevant and appropriate vehicle is important. Organic solvents and organic-aqueous mixtures have been suggested as vehicles for use in the LLNA. However, this can be a problem in testing aqueous soluble materials as water is not recommended as a vehicle. The aim of this study was to identify a water-based vehicle which provides better skin wetting and to assess its performance in the LLNA relative to other solvents using the water soluble hapten, dinitrobenzene sulfonic acid (DNBS). The nonionic surfactant Pluronic L92 (L92) was selected as the wetting agent. When tested in the LLNA, L92 was negative at up to 50%. 1% aqueous L92 was selected for use. Three doses of DNBS, were evaluated in each of four vehicles; water, 1% L92, dimethylsulfoxide (DMSO) or dimethylformamide (DMF). An estimate of the sensitizing potency in each vehicle was determined by calculation of an EC3 value (the estimated concentration required to induce a positive response). With water, only 20% DNBS was positive with an EC3 of 16.0%. In 1% L92, DNBS produced positive responses at 10 and 20%, giving an EC3 value of 6.4%. DNBS in DMSO was also positive at 10 and 20 % and had an EC3 value of 2.0%. When tested in DMF, DNBS was positive at all concentrations tested. As such, an EC3 could not be calculated but would be below 1%. While DNBS was identified as a contact allergen in all four vehicles, its relative potency varied, with the lowest EC3 value in water. The addition of the wetting agent resulted in a >2-fold increase in potency. These data demonstrate that 1% L92 may provide a better alternative to water alone for the analysis of highly hydrophilic materials in the LLNA.

816 EVALUATION OF THE DERMAL SENSITIZATION POTENTIAL OF 3-AMINO-5-MERCAPTO-1,2,4-TRIAZOLE AND N-(2,6-DIFLUOROPHENYL)-5-METHYL)1,2,4) TRIAZOLO-(1,5A)-PYRIMIDINE-2-SULFONAMIDE.

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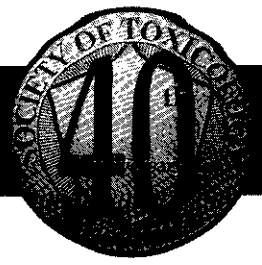
3-Amino-5-mercapto-1,2,4-triazole (AMT) is an intermediate used in the production of the herbicide, N-(2,6-Difluorophenyl)-5-methyl)1,2,4) triazolo-(1,5a)-pyrimidine-2-sulfonamide (DE498), and has been listed by the EPA as a chemical needing further toxicity testing. These studies focus on evaluating the immunomodulatory effects of AMT and DE498 using a murine model. Female BALB/c mice (N=5-8 per group) were used for all studies. Initial evaluation of irritancy/sensitizing potential was accomplished using a modified Local Lymph Node Assay (LLNA) with a 3 day dosing scheme. The Mouse Ear Swelling Test (MEST) was used to further evaluate contact hypersensitivity potential and the IgE inducing potential was assessed by measurement of total serum IgE levels and phenotypic analysis of IgE+/ B220+ cells in the draining lymph nodes. Airway hyperreactivity following chemical exposure was evaluated by methacholine challenge using whole body plethysmography. No signs of systemic toxicity or irritancy were observed with either chemical at concentrations up to 25% AMT and 40% DE498. DE498 was negative in the LLNA. A dose dependent increase in lymphocyte proliferation



Society of Toxicology

40th Annual Meeting

An Official Journal of the
Society of Toxicology
Supplement

TOXICOLOGICAL SCIENCES
Formerly Fundamental and Applied Toxicology

The Toxicologist

Abstracts of the 40th Annual Meeting

Oxford University Press

Volume 60, Number 1, March 2001

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

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The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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