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Dr. William Stokes
Director, NICEATM
Executive Director, ICCVAM
NIEHS
P.O. Box 12233, EC-17
Research Triangle Park, NC 27709

Dear Dr. Stokes,

I would like to nominate a new electrophilic contact allergen identification screening assay to the Interagency Coordination Committee on the Validation of Alternative Methods (ICCVAM). This assay is an *in chemico* low molecular weight chemical probe assay for the identification of electrophilic allergic contact dermatological (ACD) hazards. Two probes are employed in this assay; 4-nitrobenzenethiol (NBT), a “soft” nucleophile and pyridoxylamine (PDA) a “hard” nucleophile. NBT and PDA effectively replace the cysteine (thiol) and lysine (ϵ -amine) containing model peptides in the Direct Peptide Reactivity Assay (DPRA - presently under evaluation by ICCVAM), respectively. These probes are very water soluble; have high absorbance coefficients (and fluorescence) and are readily commercially available with nominal cost. Covalent binding of an electrophilic allergen to the amine or thiol on these probes produces a shift in the absorbance and/or fluorescence (for PDA) that is independent of the species bound. The shift (loss) of absorbance/fluorescence is related to the allergens chemical reactivity and can be directly monitored continuously or through end-point measures. This eliminates the requirement for physical separation of free from bound probe (i.e. need for chromatographic separation). This *in chemico* assay is designed as a preliminary screening method as it will not detect prohaptens (those requiring metabolic or chemical oxidation to be allergenic) or other non-electrophilic species such as metal allergens.

Initial assessment of the NBT probe for use in allergen screening was published in 2010 (Chipinda *et al.* Chem Res Toxicol 23(5):918-925). A strong correlation between the murine local lymph node assay EC3 and the rate of electrophilic allergen reaction to NBT was observed. We have since added the PDA probe that can detect the hard electrophilic, non-thiol reactive allergens (Chipinda *et al.* abstract in The Toxicologist, San Francisco, CA, March 11-15, 2011). The advantages of this assay over DPRA include: (1) much lower chemical concentrations are needed and thus most solubility problems are avoided; (2) the assays require only simple spectrophotometer and spectrofluorometer instrumentation; (3) total assay cost is very low; and (4)

total assay time requires < 1 day. All 10 non-allergens tested, to date, have tested negative in this *in chemico* assay suggesting that the test has very high specificity.

I respectfully request that you accept this nomination to ICCVAM for consideration as a screening assay for identification of contact allergens and propose that we work with NICEATM to conduct validation studies and the most appropriate cut-off values to maximize assay of the *in chemico* assay.

Sincerely,

/s/

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