

# INTERLABORATORY LOCAL LYMPH NODE ASSAY USING CBA/J MICE TO EVALUATE DERMAL SENSITIZATION POTENTIAL OF PESTICIDE FORMULATIONS DILUTED IN PLURONIC L92 SURFACTANT AS A VEHICLE

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## Abstract

The mouse local lymph node assay (LLNA) had become broadly accepted and is requested as the primary animal test for evaluating the dermal sensitization potential of chemicals. As pesticide formulations are typically a specific complex blend of chemicals, traditional vehicles prescribed for the LLNA can be incompatible with these formulations leading to inaccurate test results and hazard identification. The objective of this study was to evaluate the effectiveness of an aqueous dilution of Pluronic® L92 block copolymer surfactant (L92) as a vehicle in the mouse LLNA amongst 5 labs using 3 chemicals with known sensitization potential, hexylcinnamaldehyde (HCA), 36.5% formaldehyde in water (FOR) and potassium dichromate (PDC). Identical LLNA protocols and test material lots were used. Female CBA/J mice were treated on three consecutive days with HCA (3-30%), FOR (1-20%) or PDC (0.02-0.5%) diluted in 1% L92. After 2 days rest, lymph nodes were harvested for evaluation of cell proliferation (<sup>3</sup>H-thymidine incorporation). All labs observed positive LLNA responses (stimulation index (SI) >3) for the three chemicals. For HCA, the range of concentrations (6.7-17.6%) calculated to elicit 3-fold increases in proliferation (EC<sub>3</sub> values) were similar with that reported (8%) using acetone-olive oil. PDC elicited EC<sub>3</sub> values (0.06-0.33%) which encompass that reported using L92 (0.17%) or DMSO (0.06-0.18%). EC<sub>3</sub> values for 36.5% FOR ranged from 3.8-12.3% (v/v). These data reproduced previous results using 1% L92 and are only slightly above the EC<sub>3</sub> measured with FOR in acetone. LLNA results using 1% L92 as a vehicle were reproducible amongst the 5 labs and support the use of 1% L92 as a vehicle in the mouse LLNA. This work was sponsored and funded by the European Crop Protection Association.

## Introduction

As certain chemicals can cause allergic reactions in humans (e.g., contact dermatitis), there remains a need for testing chemical products for the ability to cause allergy. As prediction of allergy potential can not be accurately performed using computer software or in vitro methods, the use of laboratory animals is still necessary.

Over the past several years, the mouse local lymph node assay (LLNA) has been requested by European and North American regulatory organizations as the preferred animal test to evaluate dermal sensitization potential for individual chemical components does not necessarily define the hazard of a final product. The advantages of the mouse LLNA make it very desirable to test formulated products, and offers the additional advantage of a consistent testing paradigm between components and products.

As many product formulations (particularly pesticides) are designed using mostly aqueous ingredients, and can be incompatible with traditional LLNA vehicles (e.g., acetone), the use of standard vehicles would lead to inaccurate identification of sensitization potential of the final product.

The objective of this study was to formally evaluate the predicted accuracy of the LLNA for chemical formulations while using an aqueous dilution of Pluronic® L92 block copolymer surfactant (L92) as a vehicle in the mouse LLNA.

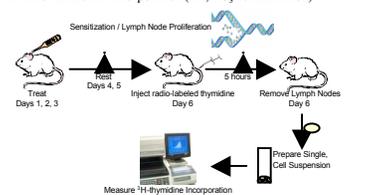
## Overview of LLNA

In the LLNA, the allergy potential of a chemical (product) is measured as a function of cell proliferation in the local lymph nodes of mice exposed topically (ears) to a chemical.

To measure cell proliferation, mice are injected (tail vein) with a radio-labeled chemical (DNA nucleotide) several days after chemical applications.

A 3-fold increase in radio-nucleotide measurement between treated and untreated mice is considered to be indicative of chemical allergy potential.

The relative potency of different products can be compared by examining the chemical dose needed to cause the 3-fold increase in radio-nucleotide incorporation (i.e., EC<sub>3</sub> concentration).



## Materials & Methods

- Female CBA mice
- 9-12 weeks of age
- 5 mice/group

Animal Welfare: In accordance with the U.S. Department of Agriculture's rules on animal welfare, 9 CFR Parts 1-4, the Animal Care and Use Activities required for the conduct of these studies were reviewed and approved by the Institutional Animal Care and Use Committee.

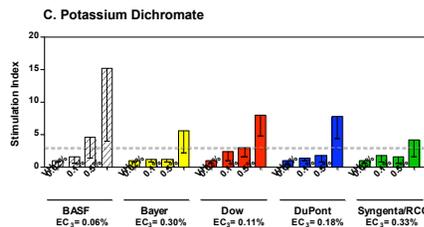
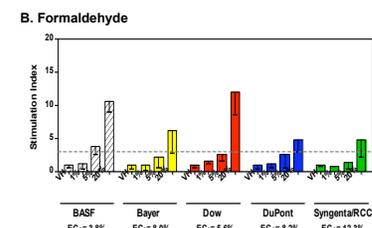
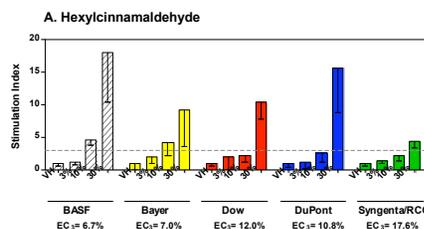
### Test Materials

- Hexylcinnamaldehyde (HCA); Sigma-Aldrich; 95% minimum purity
  - Formaldehyde (FOR); Sigma-Aldrich; 36.5-38% purity
  - Potassium dichromate (PDC); Sigma-Aldrich; 99% minimum purity
  - Oxyfluorfen EC; Dow AgroSciences LLC, purity not available
  - Quinoxifen/Cyproconazole SC; Dow AgroSciences LLC, purity not available
  - Dinocap EC; Dow AgroSciences LLC, purity not available
  - Trifluralin EC; Dow AgroSciences LLC, purity not available
- (EC = emulsion concentrate; SC = suspension concentrate)

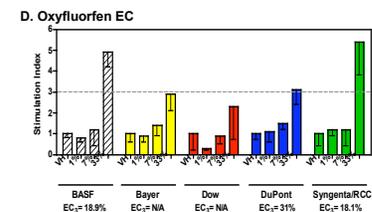
### Previous Toxicity Information

Chemical	Human
Known sensitizer	

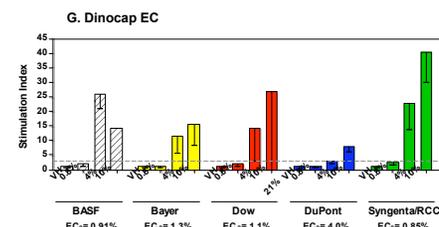
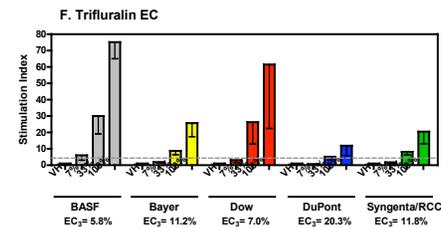
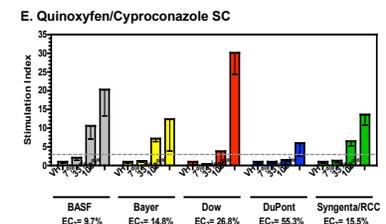
## Results – Positive Controls



## Results – Pesticide Products



## Results – Pesticide Products



## Conclusions

- These results support the use of the mouse LLNA for testing formulated, pesticide products using 1% L92 as an alternative, aqueous-based vehicle.
- Evaluation of known chemical allergens (HCA, FOR, & PDC) produced positive results using 1% L92 as an alternative, aqueous-based vehicle. Results were reproducible among the five laboratories and demonstrated consistent, relative potencies for the three positive controls. Results indicate that a concurrent positive control is not required when using this vehicle.
- Pesticide products demonstrated reproducible results using the LLNA amongst five laboratories. Results were largely consistent with that generated previously using guinea pigs or from human experience.
- LLNA provides both scientific and animal welfare advantages when compared to guinea pig allergy tests. Replacement of guinea pig models presents both a refinement and a reduction in animal usage.