

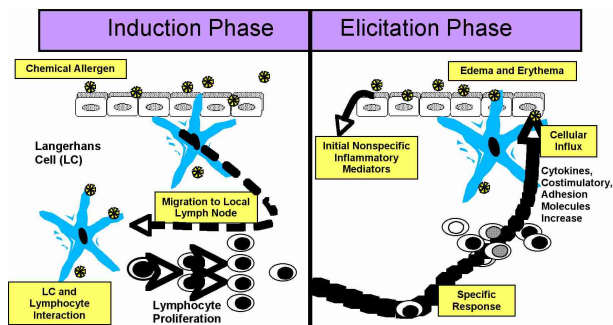


Dermal Sensitization Services



Leading the Field of *In Vitro* Toxicology

Skin sensitization is a multiple step process involving non-specific and specific mechanisms



CeeTox offers *in vitro* dermal skin sensitization testing services, which produce reliable outcomes at reasonable cost compared to *in vivo* LLNA or GPMT, especially for large numbers of compounds.

The skin sensitization system is versatile and can assess virtually any test sample. This includes finished goods or raw material, and soluble or non-soluble test articles. When solubility is high, a standard culture system of immortalized human keratinocytes (HaCaT cells) is used as the test system.

However, when finished products or chemicals with low solubility are evaluated, a 3D reconstructed human epidermis (RHE) model cultured at an air/liquid interface is used as the test system.

In Europe, sensitization testing requirements for cosmetics and chemicals are covered by the European Cosmetics Directive and REACH, respectively. *In vitro* alternatives to animal testing are needed to support the EU regulations.

US agencies which may require sensitization testing include the Environmental Protection Agency (EPA), Consumer Product Safety Commission (CPSC), Food and Drug Administration (FDA), and Occupational Safety and Health Administration (OSHA).

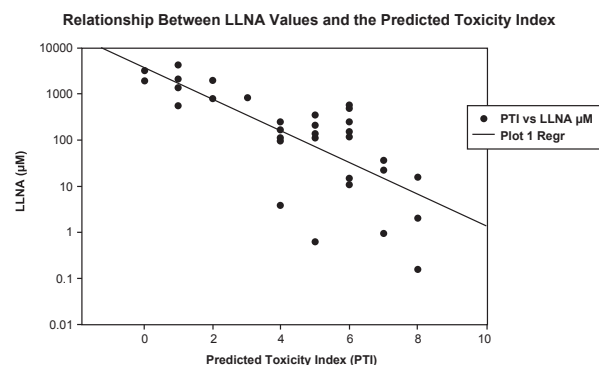
The CeeTox Skin Sensitization data provides a means of identifying hazard and expressing the data in terms of a predicted LLNA. This *in vitro* system provides a useful tool for identifying sensitizers and predicting LLNA EC3 values without the use of animals.

The Globally Harmonized System for Classification and Labeling of Chemicals (GHS) defines a skin sensitizer as "a substance that will induce an allergic response following skin contact" (UNECE, 2004, p. 151). A substance is classified as a skin sensitizer when human data show it can induce a sensitization response following skin contact "in a substantial number of persons" or when "there are positive results from an appropriate animal test" (UNECE, 2004, p. 152).



Dermal sensitization is a skin response to a hapten (a foreign, low molecular weight substance) that acts like an allergen. In some individuals, certain haptens can induce a type IV (delayed, cell-mediated) hypersensitivity response of the skin. The hapten (allergen) must be able to penetrate the skin, combine with skin proteins, and then produce an immune response (hapten-specific T cells are primed in lymph nodes by dendritic (Langerhans) cells that emigrate from the skin). The initial exposure is called the **sensitization phase** and has no clinical symptoms. The delayed skin response from a later exposure to the allergen is called the **elicitation phase**.

CeeTox' ability to correctly identify and classify chemical sensitizers is accomplished by combining chemical reactivity, gene expression profiling, and cytotoxicity. These data can also be used to estimate *in vivo* parameters, such as the mouse LLNA EC3 and human HRIPT. It may also be possible to estimate human NOAELs from this type of information.



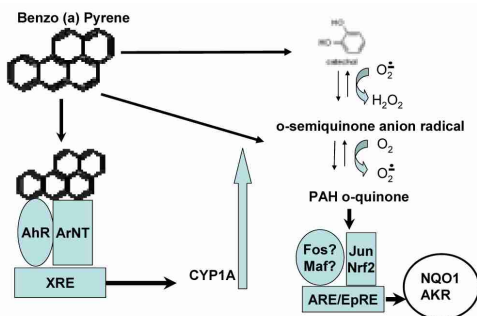
The *in vitro* test system developed by CeeTox takes advantage of these properties by evaluating chemical reactivity and ARE linked gene expression.

- Chemicals that are known to be sensitizers react with GSH based on their potency.
- Genes controlled through Nrf2 and the antioxidant response element (ARE) responded to chemical sensitizers in a concentration related manner.
- The magnitude of gene expression and the number of genes responding correlated with *in vivo* potency.
- Several human cell systems responded in a similar manner.

Large collaborative validation studies are currently underway with corporate partners.

MODEL	The EpiDerm SIT (MatTek Corporation) – or Skin Ethic EpiSkin	HaCaT cell (Human Keratinocyte) for soluble test articles
Purpose of Assay	Dermal sensitization is a skin response to a hapten (a foreign, low molecular weight substance) that acts like an allergen. In some individuals, certain haptens can induce a type IV (delayed, cell-mediated) hypersensitivity response of the skin. The hapten (allergen) must be able to penetrate the skin, combine with skin proteins, and then produce an immune response (hapten-specific T cells are primed in lymph nodes by dendritic (Langerhans) cells that emigrate from the skin). The initial exposure is called the sensitization phase and has no clinical symptoms. The delayed skin response from a later exposure to the allergen is called the elicitation phase.	
Assays Performed	Solubility Assay MTT Gene Expression (6 genes) Histology	Solubility Assay MTT Gene Expression
Controls	Solvent (vehicle), positive, blank, and no compound controls	Solvent (vehicle), positive, blank, and no compound controls
Concentrations	4	4
Number of Replicates	3	3
Number of Time Points	1	1
Standard Turn Around Time	3 weeks from sample and tissue receipt	3 weeks from sample and tissue receipt
Scientific Endorsement		
International Regulatory Acceptance		
National Regional Regulatory Acceptance		

An Intrinsic Sensor System Capable of Responding to Reactive Chemicals and Metabolites



This system is present and functional in both HaCaT and 3D Skin Models

Literature Cited:

www.alttox.org/ttrc/toxicity-tests/skin-irritation/

McKim, JM et. al. A New In Vitro Method For Identifying Skin Sensitizers and Predicting LLNA EC3 Values, Poster presented at the Society of Toxicology, March, 2009.

