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IN VITRO SKIN SENSITIZATION: KERATINOCYTE-BASED ARE-NRF2 LUCIFERASE REPORTER GENE TEST WITH DIFFERENT CLASSES OF CHEMICALS

Vashi K.D.[1], Mishra P.K.[1], Barad I.M.[1], Patel M.V.[1],Piccirillo, V.J.[2], Date R.D.[1]and Nagane R.M.[1]
[1]Jai Research Foundation, Vapi, Gujarat, India, [2]VPTOX, LLC, Ashburn, VA, USA

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ABSTRACT

Skin sensitization potential of agrochemical products is an important part of safety determination process. A recent trend is development of KeratinoSens™assay as an alternative for the*in vivo* tests. This assay has been shown to be predictive of sensitization potential of both pure substances as well as multi-component mixtures. The KeratinoSens™ assay uses an immortalised adherent human keratinocytes cell line that is transfected with a selectable plasmid. In this laboratory, assay based on Keratinocyte-Based ARE-Nrf2 Luciferase Reporter Gene method was conducted to evaluate skin sensitization potential of different classes of sensitizing and non-sensitizing chemicals to include extreme, strong, moderate, weak and non-sensitizers. In KeratinoSens™ assay, Nrf2-dependent luciferase induction and MTT-viability assay were performed in parallel plates. Keratinosens™ (HaCaT) cell line was seeded in 96-well plates and after 24 h incubation, cells were treated with varioustest chemicals at concentrations ranging from 2000 μM to 0.098 μM or with positive control substances with a test range of 4 to 64 μM for 48 h. Following 48 h, cells were washed with DPBS and placed in passive lysis buffer and incubated for 20 min at 37°C. The cell lysate plates were evaluated in microplate readerto measure luminescence. Cell viability of treated cells was also evaluated parallel using MTT test. I_{max}, $EC_{1.5}$ and IC_{50} values were calculated based on luciferase activity i.e. luminescence reading, while IC_{50} was calculated based on MTT results. Based on these values,test chemicals weredischriminated between sensitizers and non-sensitizers. Under the specified experimental conditions, 2, 4-Dinitrochlorobenzene, 4-Methylaminophenol sulphate, Methyldibromo glutaronitrile, 2-Mercaptobenzothiazole, Ethylene glycol dimethacrylate and Cinnamyl alcoholwere considered sensitisers, while Salicylic acid, Isopropanol, Lactic acid and Glycerol were deemed as non-sensitisers in KeratinoSens™ assay. These results validate the capability of KeratinoSens™ assay to predict hazard and potency of skin sensitizers in both qualitatively (sensitizing potential) as well as quantitatively (sensitising concentration) and shows this assay as a viable replacement for the *in vivo* sensitization tests.

OBJECTIVE

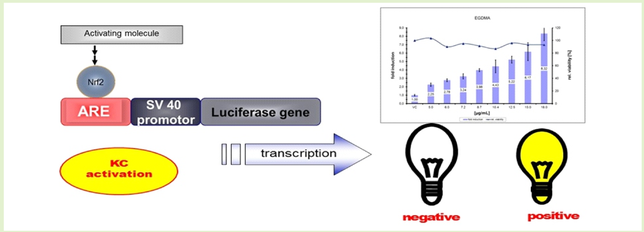
KeratinoSens assay conducted to evaluate skin sensitisation potential of proficiency chemical based on Keratinocyte-based ARE-Nrf2 Luciferase (*In Vitro* Skin Sensitization) reporter gene test method using HaCaT human keratinocytes.

TEST SYSTEM

The test system used for the in vitro KeratinoSens assay was KeratinoSens (HaCaT) cell line, an adherent cell line which contains a luciferase gene under the transcriptional control of a constitutive promoter fused with an ARE element from a gene that is known to be up-regulated by contact sensitisers. As luciferase induction and cytotoxicity are the endpoints measured, this cell line was ideal for the assay.

PRINCIPLE

Keratinocytes must be activated to produce essential signaling molecules Method(s): e.g. Antioxidant Response Element Reporter cell line based KeratinoSens assays.



MATERIALS & METHODS : (OECD Guideline 442D):

- 1× 104 cells seeded into wells of 96-well plate on day prior to assay.
- Positive control-5 concentration of trans-cinnamaldehyde (CAS-14371-10-9)
- 12-point dose series of test item
- 48 hour treatment period, then read luminescence and assess viability
- Considered positive with a > 1.5 fold statistically significant luciferase induction

RESULTS

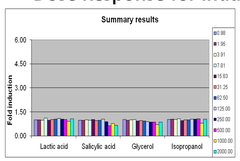
Luciferase Activity

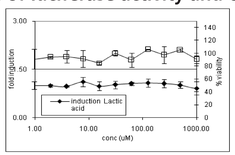
Details of the mean values of EC1.5, IC50 and Imax observed for proficiency chemicals in all the experiments are given as below:

Test Item Name	Expected Range* (Imax)	Actual Observation (Imax)	Expected Range* ($EC_{1.5}$)	Actual Observation ($EC_{1.5}$)	Expected Range* (IC_{50})	Actual Observation (IC_{50})	In Vivo Prediction*	KeratinoSens™ Prediction *
Isopropanol	<1.5	1.15	>1000	>1000	>1000	1124.33	Non-sensitiser	Negative
Salicylic acid	<1.5	1.06	>1000	>1000	>1000	1153.63	Non-sensitiser	Negative
Lactic Acid	<1.5	1.19	>1000	>1000	>1000	1161.02	Non-sensitiser	Negative
Glycerol	<1.5	1.05	>1000	>1000	>1000	1081.95	Non-sensitiser	Negative
2-Mercaptobenzothiazole	>1.5	3.59	25 - 250	134.99	>500	689.65	Moderate Sensitiser	Positive
Methyl dibromo glutaronitrile	>1.5	1.48	< 20	12.48	20-100	35.26	Strong Sensitiser	Positive
4-Methyl amino phenol sulfate	>1.5	9.02	< 12.5	2.14	20-200	24.87	Strong Sensitiser	Positive
2, 4 Dinitro-Chlorobenzene	>1.5	7.65	< 12.5	2.30	5-20	8.18	Extreme Sensitiser	Positive
Ethylene Glycol Dimethacrylate	>1.5	106.84	5-125	3	>500	681.18	Weak Sensitiser	Positive
Cinnamyl Alcohol	>1.5	2.15	25-175	457.86	>1000	>2000	Weak Sensitiser	Positive

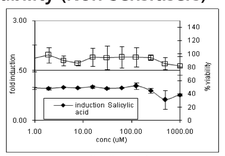
*= Expected Range and Predictions criteria were obtained from OECD Guideline 442D.

Dose Response for induction of luciferase activity and cell viability (Non-Sensitisers)

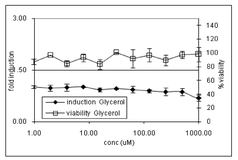




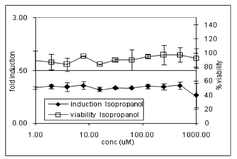
Lactic Acid



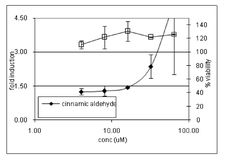
Salicylic acid



Glycerol

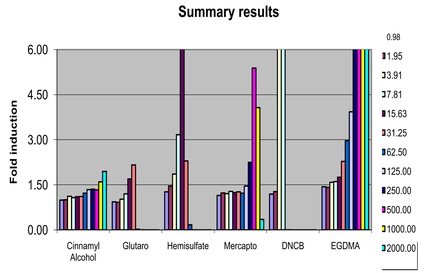


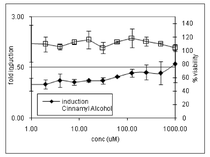
Isopropanol



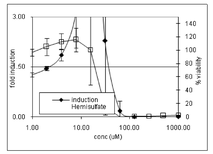
Trans Cinnamaldehyde

Dose Response for induction of luciferase activity and cell viability (Sensitisers)

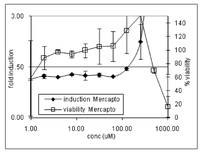




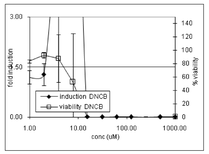
Cinnamyl Alcohol



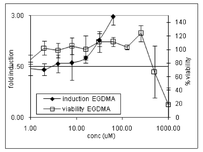
4-Methyl amino phenol sulfate



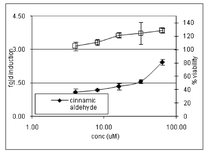
2-Mercaptobenzothiazole



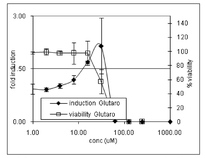
2, 4 Dinitro-Chlorobenzene



Ethylene Glycol Dimethacrylate



Trans Cinnamaldehyde



Methyl dibromo glutaronitrile

CONCLUSION

- From the results of this study, under the specified experimental conditions, 2, 4 Dinitro-clorobenzene, 4-Methylaminophenol sulphate, Methyldibromo glutaronitrile and 2-Mercaptobenzothiazole were concluded as sensitisers and Salicylic acid, Isopropanol, Lactic acid and Glycerol were concluded as non- sensitisers in KeratinoSens assay.

REFERENCES

- OECD, 2015: The Organisation for Economic Co operation and Development (OECD) Guidelines for the Testing of Chemicals, TG 442D, In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method, adopted by the Council on February 2015.
- DB-ALM (INVITTOX) (2013) Protocol 155: KeratinoSensTM., 17pp.

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