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Introduction

Skin irritation is a condition caused by acute damage to keratinocytes following exposure to a chemical. Testing for skin irritation potential of medical devices has typically involved the use of laboratory animals.

In an effort to reduce the need for *in vivo* testing, alternative *in vitro* skin irritation test methods have been developed and validated [1-3]. While these methods have been shown to provide results consistent with *in vivo* data, they have involved the use of chemical solutions or spiked extracts as positive controls. In order to provide a method more applicable to medical device testing – in which devices are extracted in polar and non-polar solvents – attempts have been made to find an extractable material that will induce a positive skin irritation response in both polar and non-polar extraction vehicles.

Here we report the findings of *in vitro* skin irritation testing performed with heat-pressed polyvinyl chloride (PVC) sheets infused with Genapol X-080 at known concentrations (Y-1, Y-2, Y-3 and Y-4).

Methods

Reconstructed human epidermis (RHE) tissues were exposed to material extracts and irritation response was determined by measuring MTT reduction and IL-1 α release. *In vitro* results were compared to *in vivo* intracutaneous reactivity testing and histopathology.

Sample Extraction

- Y-1, Y-2, Y-3 and Y-4 materials, along with appropriate extraction vehicle controls were extracted for 72 h in both saline and sesame oil at 37°C and 50°C according to ISO 10993-12 [5].
- 6 cm²/mL surface area to extract volume ratio was used.

Methods (cont.)

Tissue Exposure

- RHE tissues were exposed to 100 μ L of extracts and incubated at 37°C and 5% CO₂ for 24 h.
- 100 μ L of 1% sodium dodecyl sulfate (SDS) was used as a positive control and 100 μ L of the corresponding vehicle control was used as a negative control.
- After exposure, tissues were rinsed with phosphate buffered saline.

MTT Viability Assay

- Tissues were placed in a 1 mg/mL MTT solution and incubated at 37°C and 5% CO₂ for 3 h.
- Formazan was extracted in isopropanol at room temperature on an orbital shaker for 2 h.
- Formazan extracts were mixed thoroughly and transferred in duplicate to a 96 well plate.
- OD_{570nm} was measured and percent viability of each sample was calculated relative to negative control.
- Tissue viability of \leq 50% indicates skin irritation.

Interleukin-1 α (IL-1 α) Assay

- Thermo Scientific Human IL-1 alpha ELISA kit (Catalog No. EH2IL1A) was used according to manufacturer protocol to measure IL-1 α released into culture medium during sample exposure.
- IL-1 α values >100 pg/mL indicate skin irritation.

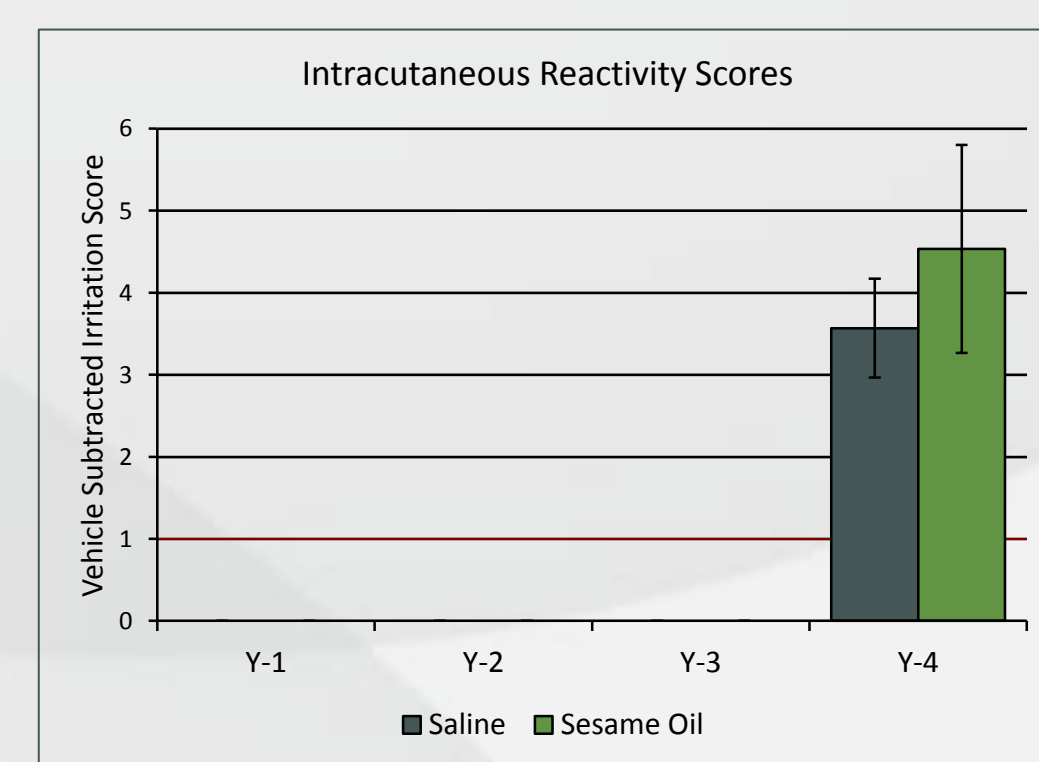
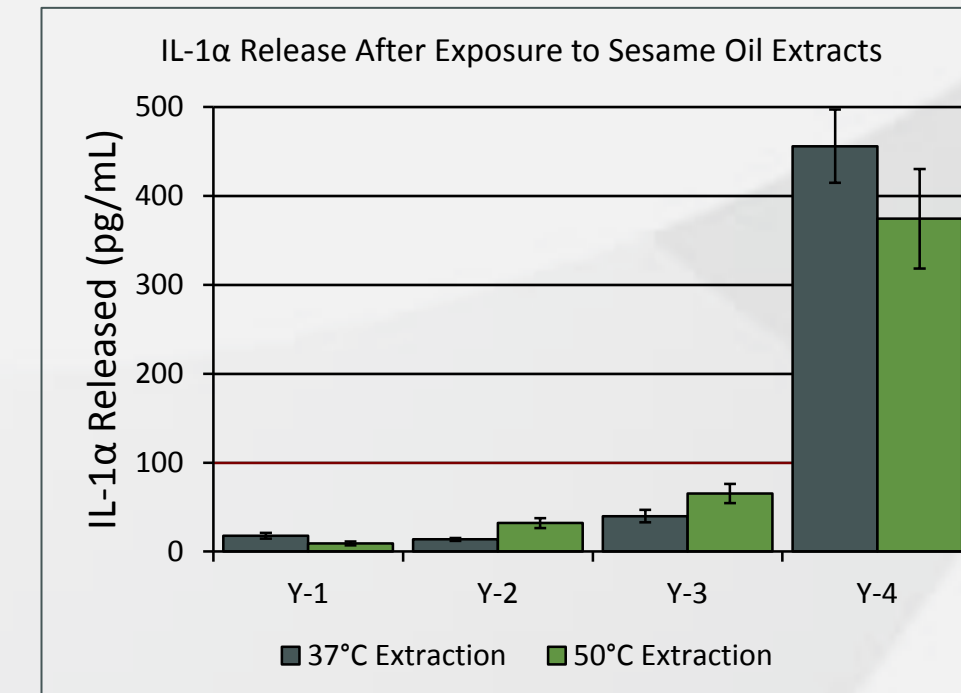
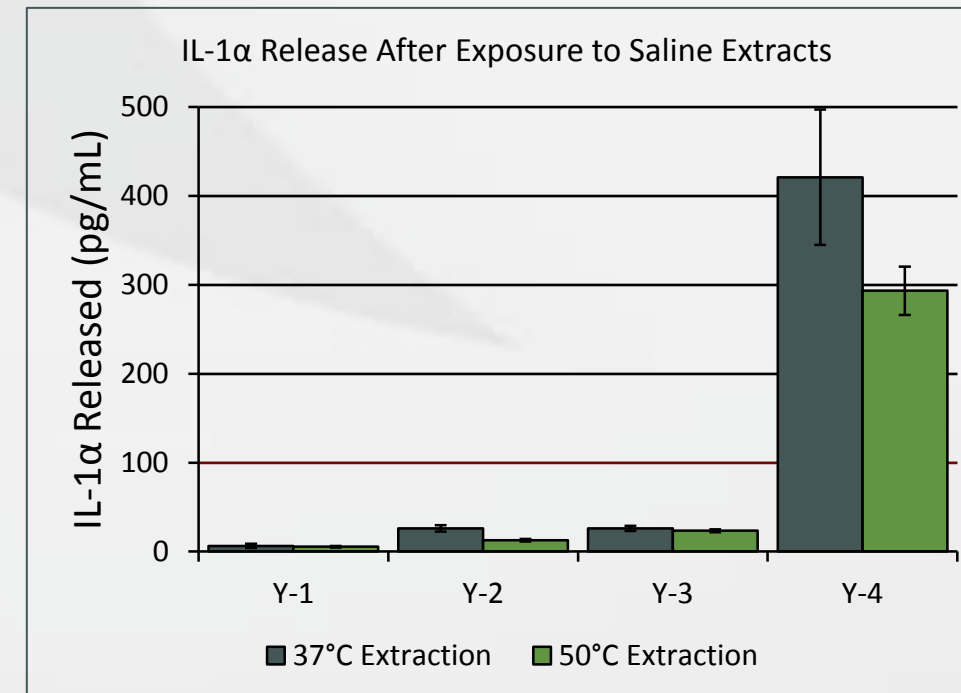
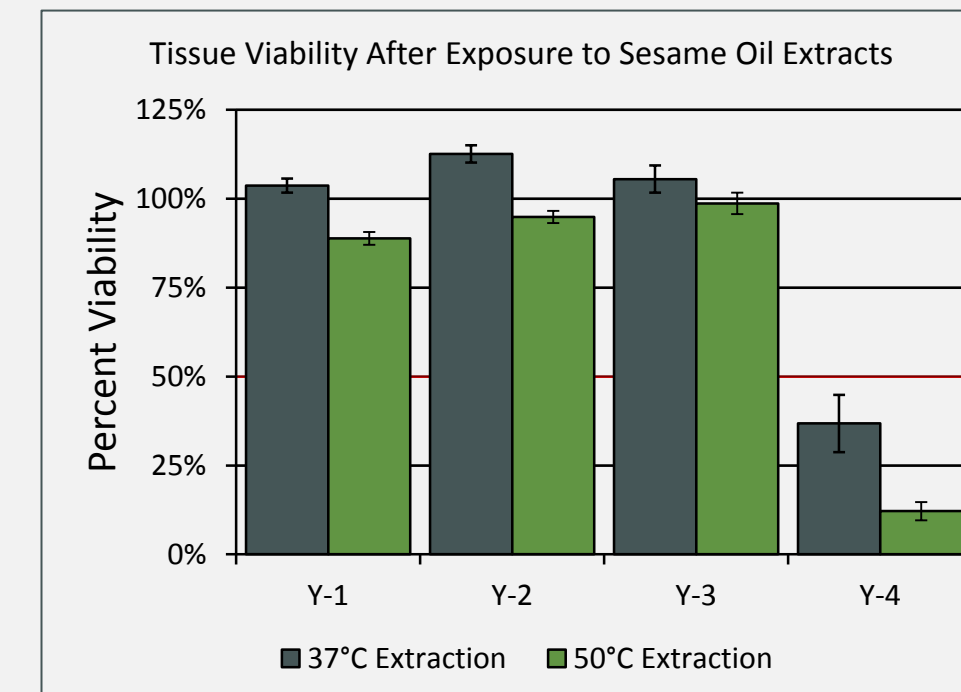
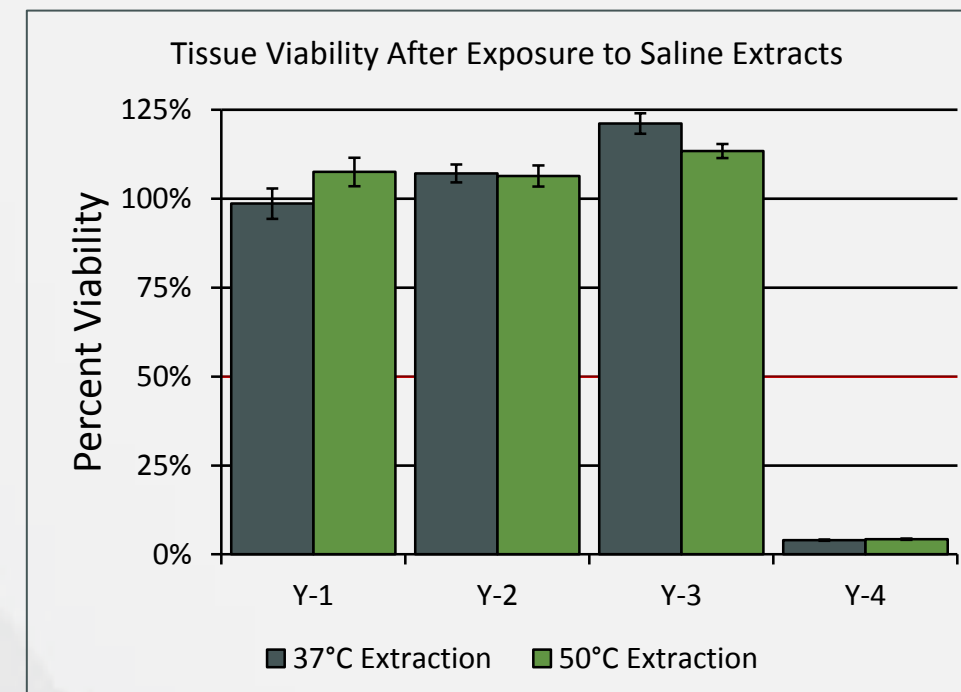
Intracutaneous Reactivity Test

- Samples were extracted in saline and sesame oil at 50°C for 72 h.
- Performed according to ISO 10993-10 [4].
- Reactivity scores >1 indicate skin irritation.

Histopathology

- Skin with dermal layer was collected and fixed in 10% neutral buffered formalin.
- Paraffin-embedded sections were prepared and stained using hematoxylin and eosin staining.
- Three sites were examined for Y-1, Y-2 and Y-3 samples. Six sites were examined for Y-4 samples.

Results



Intracutaneous Reactivity

- Y-1, Y-2 and Y-3 samples showed no irritation response (reactivity score <1). Y-4 was positive for irritation (reactivity score >1) in both saline and sesame oil.
- Bar height represents the mean of 3 animals tested and error bars indicate the standard deviation. Cutoff for irritant classification is shown in red.

	Saline			
	Y-1	Y-2	Y-3	Y-4
Test Extract				
	Significant effect was not recognized.	Significant effect was not recognized.	Significant effect was not recognized.	Necrosis was widely recognized in epidermal tissue.
Vehicle Control				
	Significant effect was not recognized.	Significant effect was not recognized.	Significant effect was not recognized.	Infiltration of inflammatory cells was slightly observed in subcutaneous tissue.

Histopathology

- Y-1, Y-2 and Y-3 samples showed no difference from vehicle controls in both saline and sesame oil. Necrosis was widely recognized in Y-4 samples in both saline and sesame oil.
- Pictures shown are representative of results seen for all sites examined.

	Sesame Oil			
	Y-1	Y-2	Y-3	Y-4
Test Extract				
	Fissures were observed in subcutaneous tissue that may have originated from accumulation of sesame oil.	Fissures were observed in subcutaneous tissue that may have originated from accumulation of sesame oil.	Fissures were observed in subcutaneous tissue that may have originated from accumulation of sesame oil.	Necrosis, infiltration of inflammatory cells, and fissures were observed in subcutaneous tissue.
Vehicle Control				
	Fissures were observed in subcutaneous tissue that may have originated from accumulation of sesame oil.	Slight infiltration of inflammatory cells and fissures observed in subcutaneous tissue that may have originated from accumulation of sesame oil.	Fissures were observed in subcutaneous tissue that may have originated from accumulation of sesame oil.	Fissures were observed in subcutaneous tissue that may have originated from accumulation of sesame oil.

In Vitro Testing

MTT Viability Assay

- Y-1, Y-2 and Y-3 samples showed no irritation response (percent viability >50%). Y-4 was positive for irritation (percent viability <50%) in both saline and sesame oil at both extraction temperatures.
- Bar height represents the mean and error bars indicate the standard error of 8 tissues tested over 3 independent experiments. Cutoff for irritant classification is shown in red.

IL-1 α Assay

- Y-1, Y-2 and Y-3 samples showed no irritation response (<100 pg/mL). Y-4 was positive for irritation (>100 pg/mL) in both saline and sesame oil at both extraction temperatures.
- Bar height represents the mean and error bars indicate the standard error of 8 tissues tested over 3 independent experiments. Cutoff for irritant classification is shown in red.

In Vivo Testing

Conclusions

- Y-1, Y-2 and Y-3 do not induce a skin irritation response *in vitro* as measured by either tissue viability or IL-1 α release.
- Y-4 consistently results in a positive skin irritation response *in vitro* under all extraction conditions tested.
- In vitro* results are in line with *in vivo* results.
- Y-4 should be considered for use as an extractable positive control material for future *in vitro* skin irritation testing of medical device extracts.

References

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