Safety assessment of monographed OTC cold/cough medicine using an *in vitro* testing platform based on human reconstructed oral tissues

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Abstract

Over-the-counter (OTC) products are available to alleviate concurrent symptoms of colds and flu. They are primarily based on a combination of decongestants, antitussive and alpha adrenergic agonists, which are well-established pharmaceutical agents covered by U.S. monographs. Many of the active components of the OTC cough/cold drugs are bitter and must be masked using flavoring agents. Bayer internally employed a stringent safety testing program for OTC cough/cold medicine line extensions that require the products to be held in the mouth for a short period using an innovative testing platform based on reconstructed oral tissues. A total of 7 OTC cough/cold products were tested using a screening approach in which the products were applied topically to the surface of reconstructed oral tissues (EpiOral™, MatTek Corporation, Ashland, MA, USA) for 2 hours, followed by evaluation of tissue viability (by MTT reduction method) and assessment of inflammatory cytokines IL-1α and IL-1β. The compositions tested were finished products, in liquid or tablet forms, and designed for children and adult use. Our tests confirmed that the products were safe to use based on the endpoints investigated that indicated no induction of irritation or inflammation up to 2 hours. The adoption of this *in vitro* testing platform attests the applicability and reliability of the modern technologies that not only support industry's due diligence and reduction in animal testing, but also demonstrate the relevancy of such platforms to human exposure while providing fast, biologically relevant safety data.

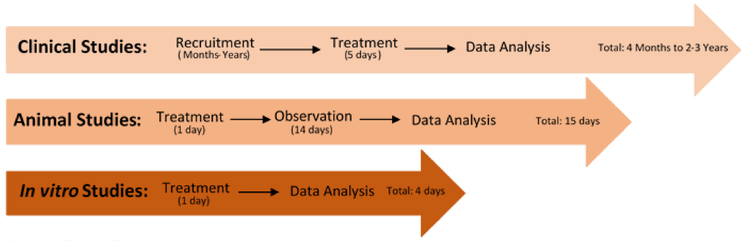


Figure 1: Testing Timelines
Timelines of available methods that can be used to test the oral toxicity and irritation of new OTC oral medicine formulations. The times displayed represent relative averages based on available public records.

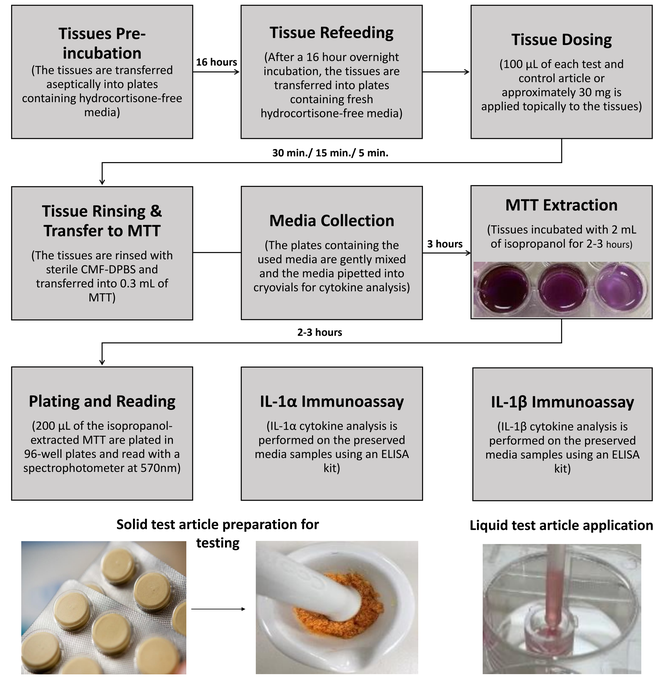
Introduction

Over-the-counter (OTC) products designed to alleviate cough and cold symptoms are comprised of several types of **active ingredients** such as decongestants, antitussives, and alpha adrenergic agonists as well as many **inactive ingredients** like stabilizers, thickeners, sweeteners, food-grade dyes, and flavors. Generally, these individual ingredients are approved for use in specific concentrations in OTC products as monographs and ingredients based in food/pharmaceutical industry without requiring further safety testing of each combination. For example, when a monographed OTC cough syrup flavor changes, there is no regulatory obligation to perform any safety testing.

The monographs establish conditions for the safe use of the **active ingredients** such as the dosage level, the combination of active ingredients, labeled indications, warnings, and directions for use. However, when selecting an OTC medicine, the active ingredients often take a back seat to the flavors. For products like the ones tested in our study, consumers are likely to choose based on the flavor. Since flavor formulations are typically proprietary to the manufacturer, a more conservative approach should be taken to test for any possible synergistic effects in new monographed OTC formulations that are not immediately swallowed.

Bayer provides a good example of due diligence for consumer safety by testing newly flavored monographed OTC products that are not immediately swallowed to ensure no synergistic effects are observed. In collaboration with IIVS, Bayer conducted safety testing using an *in vitro* platform based on the EpiOral™ reconstructed tissue model and on tissue viability and cytokine expression endpoints. Not only does this approach protect consumers, but it also supports alternatives to animal testing and provides valuable internal analysis.

Materials & Methods



Results

Positive Control (1% Triton X-100): Tissue Viability & IL-1α Concentration

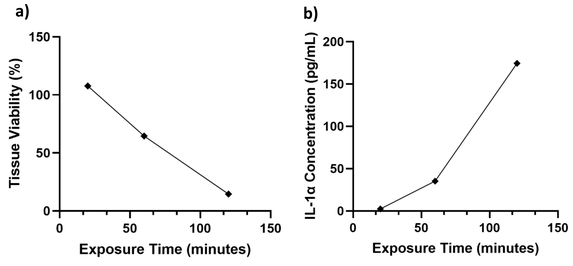


Figure 2: Positive Control Performance
Representative data sets for viability (MTT assay) and cytokine expression (IL-1α assay) endpoints pertaining to positive control-treated tissues and showing expected performance of the test system. The two endpoints indicate a correlative inverse time response of the tissues as a result of exposure to the assay positive control.

Monographed OTC Cough/Cold Medicine: Tissue Viability

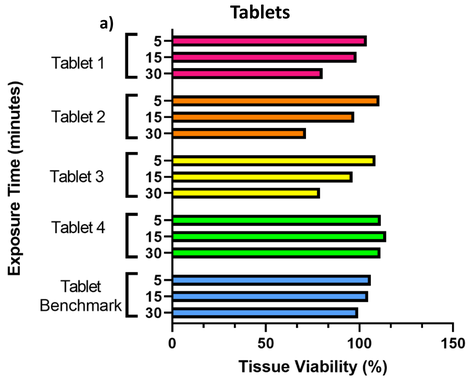


Figure 3a: Tissue Viability – Tablets
The tablets tested contained different flavors. The addition of a relevant Tablet Benchmark provided the possibility to rank order the products based on their oral toxicity induction potential.

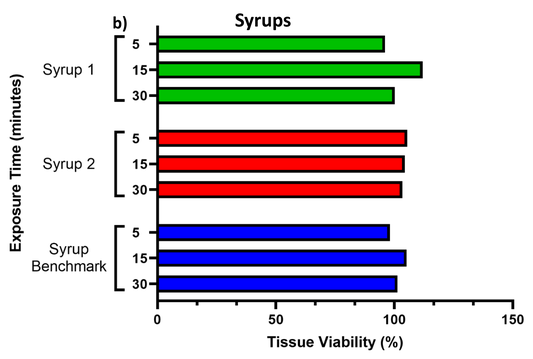


Figure 3b: Tissue Viability – Syrups
The oral tissues treated with the two OTC cough syrups along with the benchmark were consistently viable throughout all exposure times. This is an example of a good practice for ensuring consumer safety by testing that flavors are not irritating as they are held in the mouth.

Flavor Change for Monographed OTC Cough/Cold Syrups: Tissue Viability & IL-1α Concentration

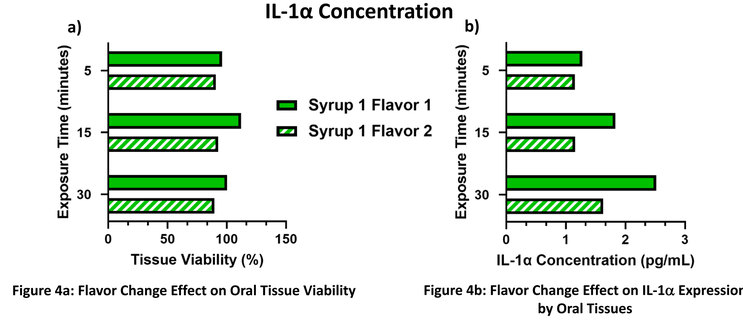


Figure 4a: Flavor Change Effect on Oral Tissue Viability

Figure 4b: Flavor Change Effect on IL-1α Expression by Oral Tissues

The tissue viability and IL-1α expression results indicate that the flavor change in a base formulation for Syrup 1 did not change the safety profile of the monographed OTC cough/cold syrup.

Conclusions

- Minor adjustments of formulations such as flavor changes are needed to adapt to an ever-changing, marketing-driven child and adult market for monographed OTC cough/cold medicines that are not immediately swallowed. The challenge lies in the fact that the full flavor formulas are not typically disclosed to the OTC manufacturer due to proprietary nature. In order to protect the consumers, testing upon each flavor change to products not immediately shallowed is important to maintain good safety practices. As shown in **Figure 1**, *in vitro* testing has its merits over animal testing or clinical trials. It is particularly advantageous for testing these subtle flavor changes in formulations, as the results are generally achieved in a much shorter time frame and are generated using a relevant human-based complex tissue model.
- Flavor diversity is typical to various forms of cough and cold monographed OTC medications, including tablets and syrups. Our results showed that the tested tablet and syrup formulations containing a variety of flavors induced a range of irritation as assessed by the tissue viability over time, particularly at the 30 minute exposure time **(Figure 3).** However, this exposure time is exaggerated compared to the directions for use of the products. At the 5 minute exposure time, which is more representative of the proper use, the tissue viability results were comparable between the formulations and to the relevant benchmarks. Our studies also demonstrate the importance of using benchmark materials for relevant and reasonable comparison with the prototypes, especially when using *in vitro* methodologies that are not validated for regulatory purposes.
- Our results (**Figure 4**) showed that a simple flavor change in a base formulation for Syrup 1 did not change the safety profile of the monographed OTC cough/cold syrup based on the tissue viability and IL-1α expression. This testing strategy demonstrates the use of good scientific practices that confirm that a flavor change does not affect the safety profile of the formulation that is not immediately swallowed.

References

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