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

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\textbf{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, antihistamines 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 for a short interval using an innovative testing platform based on reconstructed oral tissues. A total of 7 cough/cold products were tested using a screening approach in which the tissues were applied topically to the surface of reconstructed oral tissues (EpiOral\textsuperscript{™}, MatTek Corporation, Ashland, MA) for 2 hours, followed by evaluation of tissue viability (by MTT reduction method) and assessment of inflammatory cytokines IL-10 and IL-12. The compositions tested were finished products, in liquid or tablet form, 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 inflammation or irritation 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 demonstrate the relevancy of such platforms to human exposure while providing, biologically relevant safety data.

\textbf{Introduction}

Over-the-counter (OTC) products designed to alleviate cough and cold symptoms are comprised of several types of active ingredients such as decongestants, antihistamines, 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 flavor. 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 salvable. This approach protects consumers, but it also supports alternatives to animal testing and provides valuable internal analysis.

\textbf{Materials & Methods}

\textbf{Tissue Preparing & Tissue Viability} (The tissues are rinsed with sterile saline and transferred to 96 well plates containing fresh hypothermic buffer (HTAB) and incubated for 30 min/15 min/5 min. at 37°C).

\textbf{Tissue Refraining} (All tissues are maintained on moist gauze and the media are saturated with saline for cytokine analysis).

\textbf{MTT Extraction} (The tissues incubated with MTT solution for 2 hours).

\textbf{MTT Reduction} (All OTC products are suspended in plates containing tissue culture free media).

\textbf{Figure 4a: Flavor Change Effect on Oral Tissue Viability} (The tissues are rinsed with sterile saline and transferred to 96 well plates containing fresh hypothermic buffer (HTAB) and incubated for 30 min/15 min/5 min. at 37°C).

\textbf{IL-1α Immunoassay} (In-10 cytokine analysis is performed on the positive media sample using an IL-1α kit).

\textbf{IL-1β Immunoassay} (In-10 cytokine analysis is performed on the positive media sample using an IL-1β kit).

\textbf{Solid test article preparation for testing} (A single tablet of the monographed OTC cough syrup is crushed and used media are gently mixed and the media pipetted into well plates and read with a spectrophotometer at 570nm).

\textbf{RESULTS}

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

\textbf{Monographed OTC Cough/Cold Medicine: Tissue Viability}

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

\textbf{References}

3. Flavor demists 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 for the directions of use. At the 5 minute exposure time, which is more reflective of the proper use, the tissue viability results were comparable between formulations and to the relevant benchmarks. Our study also demonstrates the importance of selecting 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. 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 salvable.