Investigation of Ocular Irritation Threshold of Leachables and Impurities in Pharmaceutical Products

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Introduction:

Impurities are a common issue in ophthalmic drug formulations. A common source of impurities is extractables and leachables from the container closure system. As analytical chemistry methods have improved, impurities are now detected down to increasingly minute concentrations. Although there are guidelines dictating thresholds to address impurities in packaging solutions, these guidelines are targeted toward systemic exposure rather than a specific organ [ICH Q3(BR2)].

Ocular irritation is frequently a concern with ophthalmic drug formulations. Impurities that have been identified must be addressed for ocular irritation. Regarding extractables and leachables, the US FDA has presented a strategy whereby 1 ppm is the reporting threshold, 10 ppm the identification thre shold, and 20 ppm the qualification threshold. Data supporting the 20 ppm qualification threshold is not available in the public domain. To better understand this threshold, and perhaps broaden the applicability to all impurities, we are exploring the potential of low levels of impurities in ophthalmic products to cause chemically-induced ocular irritation to support this threshold. To do this, we have identified relevant chemical classes, and from each class, selected the chemical(s) which has demonstrated the most irritation potential. The identified chemicals were then tested in rabbits at 20 ppm and 100 ppm.

Selection of test compounds:

The compounds selected are described in Table 1. Nine chemical classes were identified as likely to be found as an impurity, as well as having representatives of the class known to be severe ocular irritants. Out of these classes the most severely irritating representative, or the representative demonstrating the highest irritation potential at the lowest concentration, was chosen for evaluation. Where possible an established Draize score was used as the criteria to determine severity; however other information was taken into account as appropriate.

Materials and Methods Overview:

Each chemical for test was formulated to 20 ppm and 100 ppm concentrations. Where appropriate the vehicle was phosphate buffered saline (PBS). For chemicals insoluble in PBS, cottonseed oil was used as the vehicle. In each case, the vehicle was also used as the control solution in the contralateral eye of the rabbit. Each chemical was evaluated first at 20 ppm, and then proceeded only to 100 ppm if there was no evidence of irritation.

All rabbit studies were conducted by the Moog Medical Device Group (Rush, NY). Adult New Zealand White rabbits with clinically normal eyes were weighed prior to treatment initiation on Day 1 and Day 3. The rabbits' eyes were examined by the McDonald-Shadduck method using a slit lamp and fluorescein stain before study initiation. Eyes were also macroscopically examined and scored before study initiation using the Draize Method.

A preliminary study was conducted to evaluate the tolerability of cottonseed oil in the rabbit eye (data not shown). It was determined that cottonseed oil resulted in mild redness to the palpebral conjunctiva when dosed six times daily. As a result of this, all chemicals which used cottonseed oil as a vehicle (2-chloroethyl acrylate, p-toluenesulfonyl chloride, 2,4-di-tert-butylphenol) were dosed only four times daily in order to eliminate the inherent irritation to the palpebral conjunctiva caused by this particular vehicle. The remaining chemicals were dosed six times daily.

On Days 1 - 3 of the studies, six instillations of 50 µl of test solution were administered to the right (test) eyes of each animal at approximately 1.5 hour intervals. The test solution was placed into the inferior ocular cul de sac. The left (control) eyes of each animal received 50 µl of the control solution in the same manner.

The rabbits' eyes were examined daily macroscopically by Draize Method prior to the 1st dose and a minimum of ten (10) minutes after the 6th dose. One additional Draize observation was made 20 minutes after the first dose on Day 1 only. The animals were also examined microscopically via the McDonald-Shadduck method using a slit lamp. This microscopic examination was conducted on Day 3 following the final Draize evaluation.

Table 1: Chemical classes and representative selection

Selected Class of Chemical	Reason for selection	Chemical Representative	Known irritation level
Acids	Hydrolysis products; corrosive effects	Lactic Acid	Corneal irritation (neat) = 80/80 Draize score
Acrylates	Major source of extractables/leachables; Common in curing and resins	2- chloroethyl acrylate	Corneal irritation (neat) = 9/10 (Smyth et al 1951)
Acyl Halides	Highly reactive intermediates used in chemical synthesis; known lachrymators	p-toluenesulfonyl chloride	Serious eye damage (Category 1 as per GHS)
Alcohols	Solvents; Common degradation products	1-Hexanol 2,4-di-tert-butylphenol	Modified Maximum Average Score (MMAS) Draize = 64.8 Irritant R36 (EEC)
Aldehydes	Common degradation products	Methacrylaldehyde	Corneal irritation (neat) = 9/10 (Smyth et al 1951)
Alkalis	Used for cleaning and pH control	Sodium Hydroxide	Draize score at 10% = 108/110; Draize score at 1% = 25.8/110
Amines / Non-Alcohol solvents	Provides depth for solvents; prevalent in manufacturing processes including dyes and pharmaceuticals	Diethylamine	103% corneal swelling at a 2% concentration in solution
Surfactants (Anionic)	Detergents; Foaming agents	Sodium Lauryl Sulfate	MMAS Draize = 58.0 (10%)
Surfactants (Cationic)	Commonly used as antistatic agents, softeners, disinfection agents; Preservatives	Benzethonium Chloride Cetyltrimethylammonium Bromide (CTAB)	Maximum Average Score (MAS) = 76.3 (10%) MAS = 69.0 (10%)

Materials and Methods Overview (continued):

The rabbits were also evaluated daily for any signs of systemic toxicity. This evaluation included any respiratory changes, increase or decrease in spontaneous motor activities, convulsion, lacrimation, excessive salivation, piloerection, gastrointestinal signs, skin reactions, and death.

Results:

The results of the ocular irritation tests conducted for each chemical species are summarized in Table 2. Each chemical species was evaluated at 20 ppm and then, 100 ppm by both macroscopic and microscopic examination.

There was no evidence of irritation caused by any of the chemical species when macroscopically examined and scored using the Draize Method. The conjunctiva, iris, and cornea all appeared clinically normal at each time point during the study. Following the final Draize evaluation, each animal was also examined microscopically via McDonald-Shadduck method using a slit lamp. There was no evidence of irritation using this means of evaluation as well. There was no evidence of systemic toxicity under the conditions as well. All animals appeared clinically normal throughout the duration of the study for each chemical species evaluated.

Final Conclusion:

Our findings provide strong support for the proposed ocular irritation threshold of 20 ppm. Each chemical under evaluation is known to be a severe ocular irritant with the potential to cause serious damage to the eye at concentrations much higher than is relevant for expected levels of impurities in ophthalmic formulations. That these chemicals showed no evidence of irritation in the rabbit eye at five times the proposed threshold indicates it is unlikely that any chemical would be irritating to the tissues of the eye at a concentration of 20 ppm. As such we support a threshold of 20 ppm under which chemical impurities need not be qualified for their potential to cause ocular irritation for topical ophthalmic products.

Table 2: Test results

Chemical Tested	Concentration	Draize Score (Avg.)	Slit Lamp Score (Avg.)
Lactic Acid	20 ppm	0	0
	100 ppm	0	0
2- chloroethyl acrylate	20 ppm	0	0
	100 ppm	0	0
p-toluenesulfonyl chloride	20 ppm	0	0
	100 ppm	0	0
1-Hexanol	20 ppm	0	0
	100 ppm	0	0
2,4-di-tert-butylphenol	20 ppm	0	0
	100 ppm	0	0
Methacrylaldehyde	20 ppm	0	0
	100 ppm	0	0
Sodium Hydroxide	20 ppm	0	0
	100 ppm	0	0
Diethylamine	20 ppm	0	0
	100 ppm	0	0
Sodium Lauryl Sulfate	20 ppm	0	0
	100 ppm	0	0
Benzethonium Chloride	20 ppm	0	0
	100 ppm	0	0
Cetyltrimethylammonium Bromide (CTAB)	20 ppm	0	0
	100 ppm	0	0

References:

- ICH Harmonised Tripartite Guideline "Impurities in New Drug Products Q3B(R2)" 2 June 2006
- Draize JH, Woodard G, Calvery HO. J Pharmacol Exp Ther 1944;7.
- McDonald T, Shadduck J. Eye irritation. In:Advances in Modern Toxicology. Volume 4: Dermatotoxicology and Pharmacology. Washington, DC: Hemisphere Publishing Corp; 1977. p. 579-582.

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