

#2753 Special stain for detection of corneal histopathological changes in BCOP (Bovine Corneal Opacity and Permeability) assay

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Background

In the Bovine Corneal Opacity and Permeability (BCOP) assay for evaluating eye irritation potential, when no histopathological changes or changes which are limited in the squamous cell layer of epithelium of bovine cornea exposed to test substance are observed, these substances are considered to be non-ocular-irritants. In the case of in vitro irritancy score (IVIS) ≤ 6 , no changes were observed in the cornea except for the squamous cell layer in our study. We therefore suggested in SOT 2016 that the test substance with IVIS ≤ 6 would be defined as non-ocular-irritants. On the other hand, no changes were observed in the corneal epithelium exposed to n-butanal (butyraldehyde) defined as a severe irritant. We prepared some special stain specimens of cornea, which were exposed to distilled water (negative control) or n-butanal and investigated histopathologically. In addition, we investigated corneas exposed to aldehydes (isobutyraldehyde, valeraldehyde, hexylaldehyde and propionaldehyde) similarly.

Materials and Methods

This study was conducted in accordance with the OECD Test Guideline 437. Eyes were obtained from slaughtered beef cows, and isolated bovine cornea were exposed to distilled water (negative control), isobutyraldehyde (Fig. 1), valeraldehyde (Fig. 2), hexylaldehyde (Fig 3), propionaldehyde (Fig. 4) or n-butanal (Fig. 5). After measurement of opacity and permeability of exposed corneas, IVIS was calculated. After fixation, the exposed corneas were embedded in paraffin, and sectioned, and stained with hematoxylin-eosin (HE), alcian blue (AB), toluidine blue (TB) and periodic acid schiff (PAS). These corneal specimens were histopathologically examined.

Results and Discussion

Table 1 shows IVIS values of each test substance. Photo. 1 is control corneal epithelium of HE stain.

Although IVIS of n-butanal was 72.2 which was categorized as severe irritants, HE stain revealed no changes in the cornea exposed to n-butanal (Photo. 2). PAS stain showed positive reaction to the epithelial cells of the control cornea (Photo. 3) and PAS stain-positive substance was digested by alpha-amylase (diastase). These indicated that this substance was glycogen. PAS stain showed no positive reaction to the epithelial cells of the cornea exposed to n-butanal (Photo. 4). These suggested that the glycogen in the corneal epithelium was lost by n-butanal treatment. AB and TB stains showed no remarkable changes.

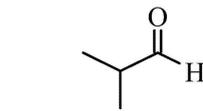


Fig. 1 isobutyraldehyde

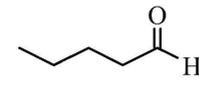


Fig. 2 valeraldehyde

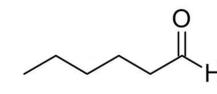


Fig. 3 hexylaldehyde

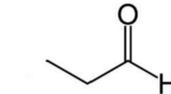


Fig. 4 propionaldehyde

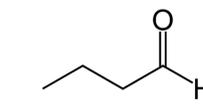


Fig. 5 n-butanal (butyraldehyde)

Table 1 IVIS values of each test substance

Test substance	Opacity	Permeability	IVIS
isobutyraldehyde	24.4	0.980	39.1
valeraldehyde	20.7	1.596	44.6
hexylaldehyde	23.7	2.414	59.9
n-butanal	36.6	2.372	72.2
propionaldehyde	98.0	1.449	119.7

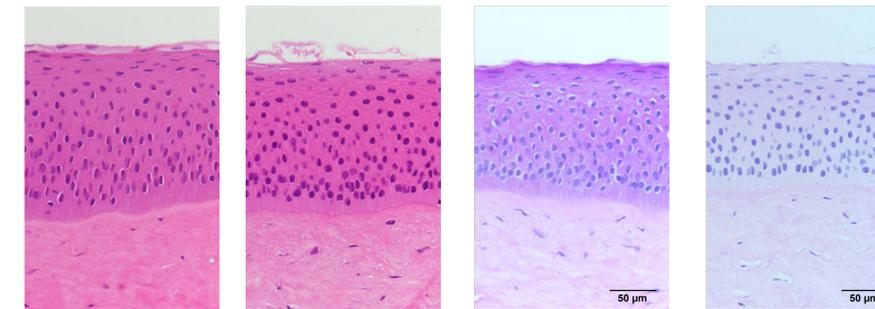


Photo. 1 Photo. 2 Photo. 3 Photo. 4

Photo. 1: Corneal epithelium exposed to distilled water (H.E. stain). No morphological changes observed.

Photo. 2: Corneal epithelium exposed to n-butanal (H.E. stain). IVIS is 72.2. No morphological changes observed.

Photo. 3: Corneal epithelium exposed to distilled water (PAS stain). PAS stain-positive substance (pink-violet) was detected.

Photo. 4: Corneal epithelium exposed to n-butanal (PAS stain). PAS stain-positive substance was lost.

Corneal epithelium exposed to isobutyraldehyde (IVIS 39.1), valeraldehyde (IVIS 44.6), hexylaldehyde (IVIS 59.9) and propionaldehyde (IVIS 119.7) showed no alternation of nuclear, cellular or tissue structure but showed hypo-chromic staining of all layers of epithelium (Photo. 5 to 8). In PAS stain, these corneas had no PAS stain-positive substance (glycogen) in corneal epithelium (Photo. 9 to 12).

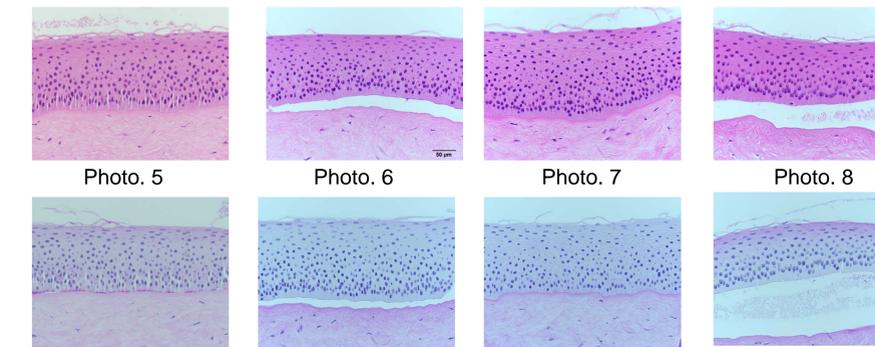


Photo. 5 Photo. 6 Photo. 7 Photo. 8
Photo. 9 Photo. 10 Photo. 11 Photo. 12

Photo. 5 to 8.: Corneal epithelium exposed to aldehydes showed hypo-chromic staining (HE stain).
Photo. 5; isobutyraldehyde. Photo. 6; valeraldehyde, Photo. 7; hexylaldehyde, Photo 8; propionaldehyde

Photo. 9 to 12: Corneal epithelium exposed to aldehydes had no PAS-positive substance (PAS stain).
Photo. 5; isobutyraldehyde. Photo. 6; valeraldehyde, Photo. 7; hexylaldehyde, Photo 8; propionaldehyde

Since these staining alternations extended to basal cell layer would indicate irreversible change, n-butanal was considered not to be false negative but to be severe irritants.

Conclusion

These results led to the conclusion that the staining alternation of the corneal epithelium could be detected by PAS stain, even if no histopathological changes were detected by HE stain. Thus when hypo-chromic staining is suspected in H.E. stain specimen, it would be required investigation using PAS stain specimen.

Reference

- 1) OECD Guidelines for the testing of chemicals 437. Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye. July 26, 2013.
- 2) Cenedello RJ and Fleschner CR. Kinetics of Corneal Epithelium Turnover *in vivo*. IOVS. 31: 1957-1962. 1990.
- 3) Hanna C, Bicknell DS, and O'Brien JE. Cell Turnover in The Adult Human Eye. Arch Ophthal. 65: 695-698. 1961.