

*False Negative Substances in the Bovine Corneal Opacity and Permeability (BCOP) Test Method.*

As described in Section 6.3.6 of the draft ICCVAM BRD (<http://iccvam.niehs.nih.gov/restrict/ocupanel/mildmod/BCOP-BRD.pdf>), there were eight EPA Category III ocular irritants that were classified as Category IV using the BCOP test method (i.e., were false negatives, see **Table 1**). Among the eight false negatives for the EPA system, 100% (8/8) were EPA Category III substances based on Draize data. For 38% (3/8) of these substances, the categorization was based on at least one rabbit with a corneal opacity score of one that was not resolved until day three of the study. Another substance was categorized based on all six rabbits with a conjunctival redness score of three that was not resolved until day seven of the study. Among the seven false negative substances for which chemical class and/or physical properties could be assigned, 71% (5/7) were from "problematic" classes that have previously been identified for the BCOP (i.e., either ketones or solids, see also ICCVAM [2006]).

**Table 1 BCOP False Negative Substances Using the EPA Classification System.**

Substance	Discordant Class?	IVIS <sup>2</sup>	In Vivo Scores		
			N <sup>3</sup>	Corneal Opacity: score (day cleared) <sup>4</sup>	Conjunctival Redness: score (day cleared) <sup>4</sup>
Dimethylbiquanide	Yes (solid)	0, 3, 1, 3, 1, 5, 3, 1, 2, 5, 8	3	N=1 1(2) N=1 1(3)	N=2 2(3)
EDTA	Yes (solid)	-1, 0, -8, 2, 2, 2, 1, -6, 1, -1, 3, 1	3	N=1 1(3)	N=3 2(2)
Iminodibenzyl	Yes (solid)	0, 1, 6, 0, 4, 0, 1, 12, 0, 6, -4	3	N=3 1(2)	-
Magnesium Carbonate	Yes (solid)	3, 6, 3, 3, 0, 1, 7, 3, 1, 0, 6	3	N=1 1(2) N=1 1(3)	-
Methylcyclopentane	Yes (ketone)	3.8, 4.6, 1.4, 1.71, 2.7	6	-	N=1 2(3)
Polyalkenylsuccinate ester/amine salt	No	2.3	6	N=2 1(2)	N=4 3(2) N=2 3(7)
Tween 20	No	-0.6, -1, -1.6, 2.711, 0.4, 0.3, 0.0, 0.4, 0.4	4	-	N=2 2(2)
Compound I	Unknown	0.6	6	N=1 1(2)	-

<sup>1</sup> False negative compounds are those that test as nonirritants in vitro but are mild, moderate, or severe ocular irritants/corrosive in vivo i.e. EPA category I, II, and III; GHS 1, 2A, and 2B; and EU R41 and R36.

<sup>2</sup> IVIS: In Vitro Irritancy Score = mean opacity value + (15 x mean OD490 value).

<sup>3</sup> N: Number of Animals.

<sup>4</sup> For the purposes of this evaluation, clearing is defined in the EPA Hazard Category system as opacity or iritis scores = 0 or redness or chemosis scores = 1.