

REVISED Correction to the ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products (NIH Publication No. 10-7553)¹

BCOP False Negative Substances for the EPA Classification System

One additional false negative substance, DL-glutamic acid, was inadvertently omitted from the ICCVAM evaluation of the BCOP for its accuracy for identifying substances not classified as eye irritants. Accordingly, this document serves to correct the applicable sections and tables in the ICCVAM Test Method Evaluation Report (ICCVAM 2010). The table below replaces Table 2-1 (page 5) and Table 6-10, Appendix C (page C-58). The text below regarding the false negative substance supplements text on page 3.

The BCOP test method incorrectly underpredicted 9 eye hazard substances as substances that do not require eye hazard classification. All 9 substances are false negative using EPA hazard classification criteria.

Seven of the 9 false negative substances produced corneal opacity in the rabbit studies (see table below). One of the 9 false negative substances produced visible damage inside the eye (iritis) that persisted beyond 24 hours. Five of the 9 false negative substances produced conjunctival redness injuries with a severity score of at least 2 (“*diffuse, crimson red, individual vessels not easily discernable*”). One of these false negative substances (polyalkenylsuccinate ester/amine salt) produced injuries with the highest redness severity score of 3 (“*diffuse beefy red*”), with injuries that persisted beyond 3 days. Two of the 9 false negative substances produced conjunctival chemosis (edema) severity scores of at least 2 (“*obvious swelling with partial eversion of the lids*”) that persisted beyond 24 hours. One of these false negative substances (EDTA, dipotassium salt) produced a chemosis severity score of 3 (“*swelling with lids about half closed*”).

The newly added substance, DL-glutamic acid, is EPA Category III based on one rabbit with a positive score for corneal opacity.

¹ In March 2011, this document served to update the false negative substances reported for BCOP in the ICCVAM TMER (ICCVAM 2010). Since that time, additional data and information have been received, which indicate that L-aspartic acid is not a false negative substance for the EPA or GHS classification systems. Due to a CASRN assignment error, which resulted from an apparent language mistranslation, it appears that the wrong substance was tested *in vitro* in the initial validation study. It is currently thought that L-asparagine was originally tested *in vitro* and compared to the results of L-aspartic acid tested *in vivo*. Therefore, L-aspartic acid is no longer considered a false negative substance for BCOP and this document has been revised accordingly.

For the EPA classification system, the false negative rate for BCOP for all reference substances classified as EPA Category I, II, and III eye hazards is 6% (9/155). Among the 155 EPA eye hazard substances in the BCOP validation database, 63 are EPA Category I substances, 22 are EPA Category II substances, and 70 are EPA Category III² substances.

References:

ICCVAM 2010. ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products. NIH Publication No. 10-7553. Available at: <http://iccvam.niehs.nih.gov/methods/ocutox/MildMod-TMER.htm>.

² Includes 13 substances now identified as “at least EPA Category III”.

Table UPDATED¹ BCOP False Negative Substances² (in order of corneal opacity severity from most severe to least severe)

Substance (Discordant Class Y/N) ³	In Vivo Classification				In Vivo Scores							
					Corneal Opacity		Iritis		Conjunctival Redness		Conjunctival Chemosis	
	EPA	FHSA 20%	FHSA 67%	GHS	N ⁴ CO	Number of Animals- CO Score <Day Cleared ⁵	N ⁴ IR	Number of Animals- IR Score <Day Cleared ⁵	N ⁴ CR	Number of Animals- CR Score <Day Cleared ⁵ (Score to 0)	N ⁴ CC	Number of Animals- CC Score <Day Cleared ⁵ (Score to 0)
Iminodibenzyl (Y, solid)	III	Irr	Irr	NC	3/3	3-CO1 <2 days	0/3	-	0/3	-	0/3	-
Dimethylbiguanide (Y, solid)	III	Irr	Irr	NC	2/3	1-CO1 <3 days 1-CO1 <2 days	1/3	1-IR1 <2 days	2/3	2-CR2 <3 days (1 <5 days; 1 < 4 days)	0/3	[2-CC2 <1 day] (2 < 3 days)
Magnesium Carbonate (Y, solid)	III	Irr	Irr	NC	2/3	1-CO1 <3 days 1-CO1 <2 days	0/3	[2-IR1 <1 day]	0/3	[1-CR2 <1 day] (1 <2 days)	0/3	[1-CC2 <1 day] (1 < 2 days)
EDTA, dipotassium salt (Y, solid)	III	Irr	Irr	NC	1/3	1-CO1 <3 days [2-CO1 <1 day]	0/3	[2-IR1 <1 day]	3/3	3-CR2 <2 days (3 <7 days)	2/3	1-CC3 <2 days 1-CC2 <2 days [1-CC2 <1 day] (2 <3 days; 1 <2 days)
Polyalkenylsuccinate ester/amine salt (N)	III	Irr	Irr	U ⁶	2/6	2-CO1 <2 days [3-CO1 <1 day]	0/6	-	2/6	1-CR3 <2 days; CR2 <6 days [1-CR3 <1 day; CR2 <6 days] 1-CR3 <2 days [3-CR3 <1 day; CR2 <2 days] (4 >6 days; 2 <6 days)	1/6	1-CC2 <2 days [1-CC2 <1 day] (1 < 3 days; 1 < 2 days)
DL-Glutamic acid (Y, solid)	III	Irr	FTR	NC	1/3	1-CO1 <2 days [2-CO1 <1 day]	0/3	[1-IR1 <1 day]	0/3	-	0/3	[3-CC2 <1 day] (2 <3 days; 1 <2

												<i>days</i>)
Compound I (Disinfectant/ Cleaner; Unknown)	III	NL	NL	NC	1/6	1-CO1 <2 days	0/6	-	0/6	-	0/6	-
Tween 20 (N)	III	Irr	FTR	NC	0/4	-	0/4	-	2/4	2-CR2 <2 days (2 <7 days)	0/4	-
Methylcyclopentane (Y, ketone)	III	NL	NL	NC	0/6	-	0/6	-	1/6	1-CR2 <3 days (1 <7 days)	0/6	-

Abbreviations: BCOP = bovine corneal opacity and permeability; CO = corneal opacity; CC = conjunctival chemosis; CR = conjunctival redness; EPA = U.S. Environmental Protection Agency; FHSA = Federal Hazardous Substances Act; FTR = further testing required; GHS = Globally Harmonized System; IR = iritis; Irr = irritant; N = number of animals; NC = Not Classified (as irritant); NL = Not Labeled (as irritant); U = unclassifiable.

¹Updated *in vivo* classifications in bold font.

²False negative substances (shaded here) are those that test as substances not labeled as irritants *in vitro* but are mild, moderate, or severe irritant/ocular corrosives *in vivo*.

³Among the false negative substances for which chemical class and/or physical properties could be assigned, 7/9 were from discordant classes that have previously been identified for the BCOP test method (i.e., either solids [false negative] or ketones [false positive]). Chemical class information was unavailable for Compound I.

⁴Number of animals with positive responses, based on meeting or exceeding the minimum severity criteria at 24, 48, or 72 hours. The following scores are considered positive: CO or IR ≥ 1 or CC or CR ≥ 2 . Therefore, CO or IR scores of 0 and CC or CR scores of ≤ 1 are considered cleared. One-hour observations are noted as italic font in brackets, but were not used for classification.

⁵Day cleared: 1 day = cleared between 1 hour and 24 hours; 2 days = cleared between 24 hours and 48 hours; 3 days = cleared between 48 hours and 72 hours; 6 days = cleared between 72 hours and 144 hours.

⁶This substance cannot be assigned a GHS classification based on the *in vivo* data because there were not a sufficient proportion of positive animals to fulfill the GHS criteria for ocular hazard classification.