

*False Negative Substances in the Isolated Chicken Eye (ICE) Test Method.**EPA Classification*

As described in Section 6.1.2.6 of the draft ICCVAM BRD (<http://iccvam.niehs.nih.gov/restrict/ocupanel/mildmod/ICE-BRD.pdf>), following the removal of substances belonging to discordant classes (i.e. alcohols, surfactants and solids, see also ICCVAM [2006]), there were six EPA ocular irritants classified as Category IV using the ICE test method (i.e. were false negatives, see **Table 2**). Among the six false negatives for the EPA system, 50% (3/6) were EPA Category III substances, 33% (2/6) were EPA Category II substances, and 17% (1/6) were EPA Category I substances.

GHS Classification

As described in Section 6.1.1.6 of the draft ICCVAM BRD (<http://iccvam.niehs.nih.gov/restrict/ocupanel/mildmod/ICE-BRD.pdf>), following the removal of substances belonging to discordant classes (i.e. alcohols, surfactants and solids, see also ICCVAM [2006]), there were three GHS ocular irritants classified as Not Classified using the ICE test method (i.e. were false negatives, see **Table 2**). Among the three false negatives for the GHS system, 33% (1/3) were GHS Category 2B substances, 33% (1/3) were GHS Category 2A substances, and 33% (1/3) were GHS Category 1 substances.

EU Classification

As described in Section 6.1.3.5 of the draft ICCVAM BRD (<http://iccvam.niehs.nih.gov/restrict/ocupanel/mildmod/ICE-BRD.pdf>), following the removal of substances belonging to discordant classes (i.e. alcohols, surfactants and solids, see also ICCVAM [2006]), there were five EU ocular irritants classified as Not Labeled using the ICE test method (i.e. were false negatives, see **Table 2**). Among the five false negatives for the GHS system, 60% (3/5) were EU Category R36 substances, and 40% (2/5) were EU Category R41 substances.

Table 2 ICE False Negative Substances¹

Substance	In Vivo Classification ²			In Vivo Scores		
	EPA	GHS	EU	N ³	Corneal Opacity: score (day cleared)	Conjunctival Redness: score (day cleared)
TNO-94 ⁴	I	1	R41	3	N=1 2(7)	N=2 3(14)
TNO-28 ⁵ (toilet bowl cleaner-1)	I	1	R41	3	None	N=1 2(7) N=1 3(28)
Methyl Cyanoacetate	II	2A	R36	3	N=1 1(2) N=1 1(7)	N=1 3(7) N=2 3(14)
TNO-9 (paint)	II	NI	NI	3	N=1 2(14)	N=1 2(2) N=1 3(3)
DMSO	III	2B	NI	3		N=1 2(3) N=1 2(4)
Methyl Cyclopentane	III	NI	NI	6		N=1 2(2)
TNO-3 (pesticide)	III	NI	NI	3		N=1 2(2) N=1 2(3)
TNO-29 (toilet cleaner-2)	III	2A	R36	3	N=1 1(2) N=1 1(3)	N=1 3(7) N=1 2(14) N=1 3(14)
TNO-52	III	2A	R36	3	N=3 1(7)	N=3 3(14)

¹ 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.

² False negatives are shaded.

³ N: Number of animals.

⁴ 1 animal with ischemic necrosis of conjunctiva; study terminated.

⁵ 1 animal with ischemic necrosis of conjunctiva.

For the purposes of this evaluation, clearing is defined in the EPA Hazard Category system as corneal opacity or iritis scores = 0 or redness or chemosis scores = 1; in the GHS and EU Hazard Category systems as corneal opacity, iritis, redness or chemosis scores = 0.