Relationship Between Adverse Ocular Effects and Their Reversibility

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Introduction

Accidental eye injury is the leading cause of visual impairment in the United States. (BLS 2004). In 2003, eye injuries from chemicals and their products (6,080) accounted for 16% of all eye injuries (36,940) reported as the cause of Days Away From Work for employees. The rabbit eye test (Draize et al. 1944) was developed to test the ocular hazard potential of new chemicals or chemical products thereby warning humans from potential accidental exposure. Sensitivity to animal use and concerns about the reliability of this test method have led to a search for alternative *in vitro* test methods for ocular hazard assessment (e.g., cell-based models, organotypic models, hemodynamic models). However, until validated alternatives are accepted as complete replacements, the Draize test will continue to be required by U.S. Federal regulatory and European agencies for ocular hazard evaluation. One of the main concerns with this test method is the pain and/or discomfort that may be produced in the test rabbits. In spite of efforts designed to screen substances for potential corrosive or severe ocular irritant properties prior to animal testing (e.g., eliminating pH extremes and dermal corrosives from testing), the potential for discomfort resulting from materials with unknown properties remains. According to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 405 (OECD 2002), any adverse effects noted during the rabbit eye test should be monitored for reversibility for up to 21 days, which allows for the identification of both irreversible (e.g., corrosion) and reversible ocular effects. As shown in **Table 1**, the United Nations Globally Harmonized System of Classification and Labeling of Chemicals (GHS) utilizes a single hazard category (i.e., Category 1) to identify substances that cause serious/irreversible effects on the eye, based on results in the Draize rabbit eye test (UN 2003). The GHS mandates that the observation period during the Draize test be sufficient to evaluate reversibility or irreversibility of any observed effects, but not to exceed 21 days post-exposure. Early termination (prior to day 21) is considered acceptable (a) for humane reasons (e.g., severe distress), (b) when a corneal opacity (CO) score of 4 is recorded at any time, (c) when at least 2 out of 3 rabbits tested have a mean score (days 1 to 3) for CO ≥ 3 or iritis ≥ 1.5, or (d) when reversal of observed effects is established (i.e., zero score for all endpoints). One mechanism to alleviate or avoid post-application ocular pain and distress could be the identification of endpoints that could be used to justify early study termination (i.e., < 21 days). To this end, NICEATM conducted an analysis of available Draize eye test data to evaluate the relationship between an adverse ocular effect and reversibility within 21 days.

Table 1. GHS Ocular Hazard Classification System

Category	Criteria for Classification
Category 1: Irreversible or serious eye damage	At least 1 animal with a CO score of 4 at any time At least 1 animal with effects not expected to reverse or that do not fully reverse within 21 days At least 2 animals with a mean CO score ≥ 3 and/or an iritis score ≥1.5
Category 2A: Irritating to eyes	At least 2 of 3 animals with mean scores for one of more of the following: CO ≥1 – Iritis ≥ 1 – Redness ≥ 2 – Chemosis ≥ 2 and the effects fully reverse within 21 days
Category 2B: Mildly irritating to eyes	When the effects listed for Category 2A fully reverse within 7 days
Nonirritating	When the substance does not meet the criteria for Category 1, 2A, or 2B

CO = corneal opacity; GHS = United Nations Globally Harmonized System for Classification and Labeling of Chemicals (UN 2003)

In Vivo Rabbit Eye Test Database

Individual rabbit data from Draize eye tests using from 1 to 6 rabbits were obtained for 1005 studies (n=3924 rabbits tested) from several different sources (e.g., publications, U.S. Federal regulatory agencies, individual scientists, private and public organizations/companies). Studies were conducted in accordance with OECD Test Guideline 405 (OECD 2002), which was based on the method originally described by Draize et al. (1944). The methodology involves instillation of 0.1 mL of the test substance (e.g., liquids, solutions, and ointments) into the conjunctival sac of an albino rabbit eye; one eye is treated while the other eye serves as the untreated control. The eye is examined at selected time intervals after exposure and any injuries to the cornea, conjunctiva, and the iris are scored. Scoring is subjective and based on a discrete, arbitrary scale (**Table 2**) for grading the severity of ocular lesions. The scores for observed ocular injuries range from 1 to 2 for iris effects, from 1 to 3 for conjunctival redness and discharge, and from 1 to 4 for corneal effects and conjunctival chemosis. A score of zero is assigned when the eye is normal and no adverse effects are observed. The eyes are typically examined at 24-hour intervals for at least 72 hours after application of the test substance for adverse effects to the cornea, conjunctiva, and iris, and the length of the post-treatment observation period should be sufficient to evaluate reversibility of any of the observed effects, but generally does not exceed 21 days.

Table 2. Scale of Scores for Grading the Severity of Ocular Lesions[1]

Lesion	Score[2]
Cornea	
A. Opacity – Degree of density (area which is most dense is taken for reading	
Scattered or diffuse area – details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
B. Area of cornea involved	
One quarter (or less) but not zero	1
Greater than one quarter but less than one-half	2
Greater than one-half but less than three quarters	3
Greater than three quarters up to whole area	4
Iris	
A. Values	
Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage; gross destruction (any one or all of these)	2
Conjunctiva	
A. Redness (refers to palpebral conjunctiva only)	
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
B. Chemosis	
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of the lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4
C. Discharge	
Any amount different from normal (does not include small amount observed in inner canthus of normal rabbits	1
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Discharge with moistening of the lids and considerable area around the eye	3

[1]From Draize et al. (1944)

[2]Scores of 0 are assigned for each parameter if the cornea, iris, or conjunctiva are normal.

Analysis Of Reversibility Of Ocular Lesions

Individual rabbit data were searched for rabbits with CO score ≥ 3 or iritis scores of 2 on days 1, 2, 3, 7, or 14 (these days were sampled as they are the recommended observation time points according to OECD TG 405 [OECD 2002]). A total of 743 (19% [743/3924]) rabbits were identified with a CO score ≥ 3 and 245 (6% [245/3924]) rabbits were identified with an iritis score of 2. **Tables 3** and **4** detail the proportion of the CO and iritis data that were used for the analysis. Two different types of analyses were conducted:

- A "snapshot" evaluation, in which scores at discrete time points of 1, 2, 3, 7, or 14 days post-treatment (i.e., CO = 3 or 4, or iritis = 2) were considered
- A "temporal" evaluation, in which mean scores on days 1-3 post-treatment (i.e. CO ≥ 3 or iritis ≥ 1.5) were evaluated, along with the trend of scores over the 3 day period for each rabbit (i.e., unchanged, increasing, decreasing, or discordant).

The percentage of scores that reversed by day 21 from each time point was then calculated, with reversibility defined as a score of zero on or before day 21 (e.g., rabbits with a CO score of zero on day 21 were added to those with no score on day 21, but for which early reversal had been established, and divided by the total number of rabbits at that time point). Binomial confidence intervals were also calculated to provide an estimate of variability.

Table 3. Relative Proportion of Rabbit Eye Test Data Available for Analysis – Corneal Opacity

CO Score	Day	Total Number of Rabbits (% of database[1])	Total Number of Rabbits Used for Analysis[2] (% of total[3])	Total Number of Rabbits Not Used for Analysis[4] (% of total[3])
3	1	170 (4.3)	65 (38.2)	105 (61.8)
	2	172 (4.4)	74 (43.0)	98 (57.0)
	3	221 (5.6)	83 (37.6)	138 (62.4)
	7	153 (3.9)	56 (36.6)	97 (63.4)
	14	152 (3.9)	67 (44.1)	85 (55.9)
4	1	132 (3.4)	25(18.9)	107 (81.1)
	2	157 (4.0)	29 (18.5)	128 (81.5)
	3	177 (4.5)	33 (18.6)	144 (81.4)
	7	221 (5.6)	51 (23.1)	170 (76.9)
	14	208 (5.3)	115 (55.3)	93 (44.7)

CO = corneal opacity

[1]n = 3924 rabbits

[2]Total number of rabbits where CO scores of 0-4 on day 21 were recorded, or reversibility (i.e., CO = 0) was established.

[3]% of the total number of rabbits with the relevant CO score at this time point

[4]Total number of rabbits where testing was terminated prior to day 21, and reversibility had not been established.

Table 4. Relative Proportion of Rabbit Eye Test Data Available for Analysis – Iritis Score = 2

Day	Total Number of Rabbits (% of database[1])	Total Number of Rabbits Used for Analysis[2] (% of total[3])	Total Number of Rabbits Not Used for Analysis[4] (% of total[3])
1	147 (3.7)	75 (51.0)	72 (49.0)
2	95 (2.4)	68 (71.6)	27 (28.4)
3	130 (3.3)	51 (39.2)	79 (60.8)
7	122 (3.1)	45 (36.9)	77 (63.1)
14	58 (1.5)	30 (51.7)	27 (48.3)

IR = iritis

[1]n = 3924 rabbits

[2]Total number of rabbits where iritis scores of 0-2 on day 21 were recorded, or reversibility (i.e., iritis = 0) was established.

[3]% of the total number of rabbits with iritis = 2 at this time point

[4]Total number of rabbits where testing was terminated prior to day 21, and reversibility had not been established.

Results

Corneal Opacity

As shown in **Table 5**, individual rabbit CO scores of 4 on days 1, 2, 3, 7, or 14 resolved (i.e., reversed to zero within 21 days) 24% (6/25), 28% (8/29), 15% (5/33), 4% (2/51), and 3% (3/115) of the time, respectively, while CO scores of 3 on days 1, 2, 3, 7, or 14 resolved 40% (26/65), 39% (29/74), 35% (29/83), 11% (6/56), and 9% (6/67) of the time, respectively. **Table 6** indicates that mean CO scores ≥ 3 for days 1, 2, and 3 resolved 18% (12/65) of the time, with the lowest incidence of reversibility (11% [2/18]) occurring when the mean CO score = 4.

Iritis

As shown in **Table 7**, an iris score of 2 on days 1, 2, 3, 7, or 14 resolved 71% (53/75), 66% (45/68), 57% (29/51), 36% (16/45), and 7% (2/30) of the time, respectively. **Table 8** indicates that mean iritis scores ≥ 1.5 on days 1, 2, and 3 resolved 60% (36/60) of the time, with the lowest incidence of reversibility (44% [16/36]) occurring when the mean iritis score = 2.

Table 5. Snapshot Analysis of Reversibility of Ocular Lesions – Corneal Opacity

CO Score	Day	Day 21 CO Score 0	1	2	3	4	No score (early reversal[1])	No score (no reversal[2])	Number of Usable Animals	% Reversed within 21 days[3] (95% CI)
3	1	**6[4]**	7	10	5	17	**20**	105	65	**40.0 (28.0 – 52.9)**
	2	**7**	6	9	10	20	**22**	98	74	**39.2 (28.0 – 51.2)**
	3	**8**	14	7	11	22	**21**	138	83	**34.9 (24.8 – 46.2)**
	7	**3**	11	12	14	13	**3**	97	56	**10.7 (4.0 – 21.9)**
	14	**0**	6	15	27	13	**6**	85	67	**9.0 (3.4 – 18.5)**
4	1	**0**	1	3	0	15	**6**	107	25	**24.0 (9.4 – 45.1)**
	2	**0**	2	2	2	15	**8**	128	29	**27.6 (12.7 – 47.2)**
	3	**1**	1	3	4	20	**4**	144	33	**15.2 (5.1 – 31.9)**
	7	**1**	3	3	7	36	**1**	170	51	**3.9 (0.5 – 13.5)**
	14	**0**	1	5	13	93	**3**	93	115	**2.6 (0.5 – 7.4)**

CI = binomial confidence interval; CO = corneal opacity

[1]Early reversal: The test was terminated prior to day 21, with a score of zero recorded on the last day.

[2]No reversal: The test was terminated prior to day 21 with a nonzero score recorded on the last day, and therefore these data could not be used in the calculations.

[3]Calculated by summing the bolded numbers for each time point (number of rabbits with a CO score of zero on day 21 and those with no score on day 21, but early reversal), and then dividing by the number of usable animals.

[4]Number of animals in this group

Table 6. Temporal Analysis of Reversibility of Ocular Lesions – Corneal Opacity

CO score (days 1-3)	Day 21 CO Score 0	1	2	3	4	No score (early reversal[1])	No score (no reversal[2])	Number of Usable Animals	% Reversed within 21 days[3] (95% CI)
3-3-3[4]	**2[5]**	6	4	3	12	**5**	58	32	**21.9 (9.3 – 40.0)**
4-4-4[6]	**0**	1	2	0	13	**2**	85	18	**11.1 (1.4 – 34.7)**
Mean ≥ 3, increasing[7]	**1**	1	2	4	5	**1**	39	14	**14.3 (1.8 – 42.8)**
Mean ≥ 3, decreasing[8]	**0**	0	0	0	0	**1**	10	1	**100 (5.0 - 100)**
Mean ≥ 3, discordant[9]	**0**	0	0	0	0	**0**	2	0	**-**
Combined	**3**	8	8	7	30	**9**	194	65	**18.5 (9.9 – 30.0)**

CI = binomial confidence interval; CO = corneal opacity

[1]Early reversal: The test was terminated prior to day 21, with a score of zero recorded on the last day.

[2]No reversal: The test was terminated prior to day 21 with a nonzero score recorded on the last day, and therefore these data could not be used in the calculations.

[3]Calculated by summing the bolded numbers for each time point (number of rabbits with a CO score of zero on day 21 and those with no score on day 21, but early reversal), and then dividing by the number of usable animals.

[4]CO = 3 on days 1, 2, and 3

[5]Number of animals in this group

[6]CO = 4 on days 1, 2, and 3

[7]Mean CO ≥ 3, with scores increasing over days 1-3 (e.g., CO = 2, 3, 4 on days 1, 2, 3, respectively)

[8]Mean CO ≥ 3, with scores decreasing over days 1-3 (e.g., CO = 4, 3, 2 on days 1, 2, 3, respectively)

[9]Mean CO ≥ 3, with scores varying over days 1-3 (e.g., CO = 2, 4, 3 on days 1, 2, 3, respectively)

Table 7. Snapshot Analysis of Reversibility of Ocular Lesions – Iritis Score = 2

Day	Day 21 Iritis Score 0	1	2	No score (early reversal[1])	No score (no reversal[2])	Number of Usable Animals	% Reversed within 21 days[3] (95% CI)
1	**15[4]**	5	17	**38**	72	75	**70.7 (59.0 – 80.6)**
2	**16**	7	16	**29**	27	68	**66.2 (53.7 – 77.2)**
3	**12**	7	15	**17**	79	51	**56.9 (42.4 – 70.6)**
7	**11**	12	17	**5**	77	45	**35.6 (21.9 – 51.2)**
14	**2**	8	20	**0**	28	30	**6.7 (0.8 – 22.1)**

CI = binomial confidence interval; IR = iritis

[1]Early reversal: The test was terminated prior to day 21, with a score of zero recorded on the last day.

[2]No reversal: The test was terminated prior to day 21 with a nonzero score recorded on the last day, and therefore these data could not be used in the calculations.

[3]Calculated by summing the bolded numbers for each time point (number of rabbits with an iritis score of zero on day 21 and those with no score on day 21, but early reversal), and then dividing by the number of usable animals.

[4]Number of animals in this group

Table 8. Temporal Analysis of Reversibility of Ocular Lesions – Corneal Opacity

IR score (days 1-3)	Day 21 Iritis Score 0	1	2	No score (early reversal[1])	No score (no reversal[2])	Number of Usable Animals	% Reversed within 21 days[3] (95% CI)
2-2-2	**8[4]**	5	15	**8**	56	36	**44.4 (27.9 – 61.9)**
Mean ≥ 1.5, increasing[5]	**0**	1	2	**9**	10	12	**75.0 (42.8 – 94.5)**
Mean ≥ 1.5, decreasing[6]	**3**	0	1	**8**	5	12	**91.7 (61.5 – 99.8)**
Mean ≥ 1.5, discordant[7]	**0**	0	0	**0**	0	0	**-**
Combined	**11**	6	18	**25**	71	60	**60.0 (46.5 – 72.4)**

CI = binomial confidence interval; IR = iritis

[1]Early reversal: The test was terminated prior to day 21, with a score of zero recorded on the last day.

[2]No reversal: The test was terminated prior to day 21 with a nonzero score recorded on the last day, and therefore these data could not be used in the calculations.

[3]Calculated by summing the bolded numbers for each time point (number of rabbits with an iritis score of zero on day 21 and those with no score on day 21, but early reversal), and then dividing by the number of usable animals.

[4]Number of animals in this group

[5]Mean IR ≥ 1.5, with scores increasing over days 1-3 (e.g., IR = 1, 2, 2 on days 1, 2, 3, respectively)

[6]Mean IR ≥ 1.5, with scores decreasing over days 1-3 (e.g., IR = 2, 2, 1 on days 1, 2, 3, respectively)

[7]Mean IR ≥ 1.5, with scores varying over days 1-3 (e.g., IR = 2, 1, 2 on days 1, 2, 3, respectively)

Conclusions

Based on these data:

- Mean CO scores ≥ 3 and mean iritis scores ≥ 1.5 on days 1-3 reverse 18.5% (9.9% to 40% [95% confidence interval]) and 60% (46.5% to 72.4%) of the time, respectively. According to the GHS classification system, a study can be terminated and the substance labeled Category 1 (severe irritant/corrosive) if 2 of 3 rabbits have mean CO scores ≥ 3 and mean iritis scores ≥ 1.5 on days 1-3, or if a single animal has a CO score = 4 at any time.
- There is a marked reduction in the likelihood of reversibility of corneal damage when a CO score of 3 is present in a rabbit on or after day 7 (i.e., the damage reverses up to 11% of the time). Thus, terminating a study based on a rabbit with a CO score of 3 on or after day 7 may reduce pain and suffering without significantly altering the resulting hazard classification assigned to a test substance.
- An iritis score of 2 in a rabbit on day 14 only reverses 6.7% (0.8% to 22.1%) of the time. Thus, terminating a study based on a rabbit with an iritis score of 2 on day 14 may also reduce pain and suffering without significantly altering the resulting hazard classification assigned to a test substance.
- In a rabbit, a CO score = 4 at any time point reverses from 3% to 28% of the time, with the greatest reversal rate occurring on days 1 (24% [9.4% to 45.1%]), 2 (27.6% [12.7% to 47.2%]), and 3 (15.2% [5.1% to 31.9%]). However, these analyses are based on relatively small numbers of animals available, particularly for the early time points.

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NIEHS National Institute of Environmental Health Sciences

ICCVAM The Interagency Coordinating Committee on the Validation of Alternative Methods

NICEATM The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

More information on ICCVAM and NICEATM can be accessed at http://iccvam.niehs.nih.gov/