9.0 OTHER SCIENTIFIC REPORTS AND REVIEWS

9.1 Reports in the Peer Reviewed Literature

A search of MEDLINE, TOXLINE, and Web of Science showed 14 additional scientific publications with BCOP test method results (Gautheron et al. 1992; Vanparys et al. 1993; Rachui et al. 1994; Rougier et al. 1994; Sina et al. 1995; Cassidy and Stanton 1997; Chamberlain et al. 1997; Bruner et al. 1998; Ubels et al. 1998, 2000, 2002, 2004; Cooper et al. 2001; Jones et al. 2001), as well as nine review articles (e.g., reports from a BCOP workshop) that discussed the assay and seven background articles (e.g., basis of the test method).

These studies were not included in previous sections of the BRD because they lacked sufficient information (e.g., substance names, *in vivo* data) for an evaluation of accuracy or reliability to be conducted. The first publication on the BCOP assay (Gautheron et al. 1992) was excluded because, for most of the substances tested in this study, only opacity results were reported. Eight studies lacked other necessary information with which to conduct an accuracy or reliability analysis. Vanparys et al. (1993), Rachui et al. (1994), Rougier et al. (1994), Cassidy and Stanton (1997), Bruner et al. (1998), Cooper et al. (2001), and Jones et al. (2001) lacked sufficient *in vivo* data. Sina et al. (1995) did not include the names of the substances tested. Additionally, the purpose of four studies (Ubels et al. 1998, 2000, 2002, 2004) was to investigate potential improvements of the BCOP assay, and test results were not compared to *in vivo* reference data.

In addition to these 14 studies, a retrospective evaluation of BCOP data was conducted by the Interagency Regulatory Alternatives Group (IRAG) (Chamberlain et al. 1997), in which eight data sets were submitted by eight laboratories on a total of 242 discrete (not all unique) substances. Due to the observation that at least two of the IRAG data sets had been published in reports reviewed in previous sections of the BRD, the data and other information in the IRAG report were not included to avoid duplication of BCOP studies and data. Additionally, detailed *in vivo* data, which are necessary for the analyses performed in **Section 6.0** of this document, were not received in response to NICEATM's requests for such data.

The correlative analyses conducted by Gautheron et al. (1994) and Balls et al. (1995) as a measure of test method performance are summarized below. These analyses were not included in **Section 6.0** since they are not relevant to an accuracy analysis of the BCOP data in relation to the EPA (1996), EU (2001), or GHS (UN 2003) classification systems for *in vivo* rabbit eye test data.

These 15 studies, presented in alphabetical order, are reviewed in the following subsections. In addition, summaries are provided for a 1997 workshop on the BCOP assay, along with two review articles on the test method. A list of recent poster presentations on the assay also is provided.

9.1.1 Balls et al. (1995)

Under the auspices of the British Home Office and Directorate General XI of the European Commission, a validation study on proposed alternatives to the *in vivo* rabbit ocular toxicity test method was conducted. The goal of the evaluation was to identify at least one non-whole animal test method that could be proposed to regulatory authorities as a replacement for the currently accepted *in vivo* eye irritation test method. A total of 52 substances were evaluated in 60 tests in two to five laboratories. Four of the test substances were evaluated at two different concentrations and two substances were evaluated at three different concentrations. The ocular irritancy potential of the test substances were ranked in terms of MMAS (which ranged from 0 to 108). *In vivo* data for 46 of the test substances, which were generated in compliance with OECD TG 405, were obtained from published sources. *In vivo* data for 14 of the test substances were obtained from concurrently conducted studies, which were in compliance with OECD TG 405. *In vivo* data in the report were presented as MMAS.

This study conducted correlative analyses of the BCOP scores and the *in vivo* MMAS scores. The Spearman's rank correlation test and Pearson's correlation analysis were used to compare *in vivo* MMAS with BCOP scores and mean adjusted BCOP scores (i.e., individual scores > 200 were adjusted to 200). Spearman's rank correlation coefficients and Pearson's correlation coefficients were calculated for each participating laboratory for all test substances and separately for water-soluble substances, surfactants, solids, solutions, and liquids. **Table 9-1** presents the correlation coefficients obtained for the different analyses. Mean opacity scores and mean permeability scores also were compared to *in vivo* MMAS scores; however, the results of these correlations are not included here.

9.1.2 Bruner et al. (1998)

Three variations of the original BCOP test method protocol were used in an attempt to optimize the assay for cosmetic formulations:

1. The exposure time was increased to 24 hours.
2. Test substances were applied and rinsed four times during the 24 hours of exposure.
3. Corneas were examined histologically.

Various cosmetic formulations were tested with different concentrations of organic acid in both water-in-oil and oil-in-water emulsions. The pH of the emulsion water phase was varied to test effects of pH on corneal injury. Effects of a metal oxide also were tested. The composition of the formulations was not revealed. *In vivo* rabbit eye test data were not reported; rather, human eye tolerance tests were performed for some formulations. Endpoints of the human studies were lacrimation, stinging, conjunctival redness, and conjunctiva and cornea fluorescein staining. The authors reported individual opacity and permeability results, and light microscopy data for the BCOP studies. Due to the lack of *in vivo* rabbit eye data and the fact that only mild formulations were evaluated, these data were not considered during the analysis of the performance of the BCOP (**Sections 6.0** and **7.0**).

Table 9-1 *In Vitro*/*In Vivo* Range of Correlations Reported in Balls et al. (1995)

Score index[1]	Range of Pearson's Correlation Coefficients[2]	Range of Spearman's Correlation Coefficients[2]
Full set of test substances (59)		
BCOP-Nonadjusted Scores	0.411 - 0.490	0.520 - 0.571
BCOP-Adjusted Scores	0.508 - 0.553	0.522 - 0.573
Substances soluble in water (30)		
BCOP-Nonadjusted Scores	0.477 - 0.625	0.326 - 0.448
BCOP-Adjusted Scores	0.446 - 0.554	0.326 - 0.448
Substances insoluble in water (18)		
BCOP-Nonadjusted Scores	0.160 - 0.336	0.581 - 0.690
BCOP-Adjusted Scores	0.359 - 0.446	0.582 - 0.690
Surfactants (12)		
BCOP-Nonadjusted Scores	0.772 - 0.895	0.685 - 0.825
BCOP-Adjusted Scores	0.772 – 0.895	0.685 - 0.825
Solids (20)		
BCOP-Nonadjusted Scores	-0.061 - 0.142	0.020 - 0.328
BCOP-Adjusted Scores	0.025 - 0.297	0.022 - 0.328
Solutions (14)		
BCOP-Nonadjusted Scores	0.586 - 0.771	0.546 - 0.689
BCOP-Adjusted Scores	0.558 - 0.775	0.543 - 0.693
Liquids (26)		
BCOP-Nonadjusted Scores	0.521 - 0.690	0.664 - 0.770
BCOP-Adjusted Scores	0.521 - 0.690	0.664 - 0.770

[1]Adjusted scores refers to the analysis in which individual BCOP scores > 200 were adjusted to 200. Balls et al. (1995) reports individual correlation coefficients for each laboratory.
[2]A correlation coefficient was calculated for each of the five participating laboratories; only the range of correlation coefficients obtained for the five laboratories is presented here.

9.1.3 Cassidy and Stanton (1997)

Six organosilicon compounds (siloxane polymers) were evaluated undiluted. The essential protocol components (e.g., preparation and treatment of corneas, opacity and permeability measurements) were the same as those used for Gautheron et al. (1994), except that the corneas were examined histologically. Five corneas were used per test substance, three corneas were used for an untreated control group, and two corneas were treated with a positive control (ethanol). The classification system is the same as that used for the Gautheron et al. (1994) study.

The test substances were hexamethyldisiloxane, polydimethylsiloxane, aminofunctional silicone A, aminofunctional silicone B, phenylsilsesquioxane fluid, and silicone ether. These substances are widely used in personal care formulations. The source and CASRNs of these compounds were not provided. Test substances were not coded.

The *in vivo* data were obtained according to OECD rabbit eye irritation testing guidelines, or their EU/EPA equivalent. However, only the *in vivo* irritancy grades are reported in the publication. Two nonirritants, two minimal irritants, one moderate irritant, one moderate to severe irritant, and one extremely severe irritant were evaluated, but it is not clear what *in vivo* ocular irritancy classification system was used for these classifications. NICEATM

contacted the study authors for the detailed *in vivo* data (i.e., raw scores for corneal opacity, iritis, conjunctival redness and chemosis for each animal) for this study; however, corporate clearance to release the data was not received. This study was not included in **Sections 3.0 – 7.0** of the BRD, because the raw *in vivo* scores for the rabbit studies, which are necessary to assign EPA (1996), EU (2001) and GHS (UN 2003) ocular irritancy classifications, could not be obtained.

Mean opacity and mean permeability values were reported, in addition to total BCOP scores (mean opacity value +15 x mean OD_{490} value). The BCOP scores were classified into the following three irritancy grades: nonirritant to mild (0-25), moderate (25.1-55), and severe (> 55).

For these six substances, the sensitivity and the specificity of the BCOP was 100%, using two classes of irritancy (nonirritant and irritant).

9.1.4 Chamberlain et al. (1997)

The eight laboratories that submitted BCOP data for the IRAG evaluation provided data on 242 substances encompassing a wide variety of chemical and product classes. These substances are summarized by laboratory in **Table 9-2** and represent the classifications reported in the IRAG evaluation study report. The specific substances tested were not provided in the IRAG evaluation. Neither were any physicochemical characteristics.

Table 9-2 Substances Tested in IRAG-Reviewed Studies

Laboratory Number	Number of Substances Tested	Substance Type or Class
Lab 3[1]	43	Full range of chemical classes; industrial raw materials and intermediates
Lab 4	12	Personal care products
Lab 5	21	Fragrance gels
Lab 6[2]	25	Surfactant-containing materials
Lab 7[3]	52	22 liquids; 22 solids; 8 surfactants
Lab 8	39	20 surfactants; 12 surfactant-based lotions; 7 shampoos
Lab 9	20	Industrial chemical intermediates
Lab 10	30	Miscellaneous organic chemicals from ECETOC (1992) database on eye irritants
Total:	242 discrete (not all unique) substances	

[1]Gautheron et al. (1992).
[2]From CTFA Phase III study (Getting et al. 1996).
[3]EC Interlaboratory study (Gautheron et al. 1994).

The protocols used by the different IRAG laboratories followed that used in Gautheron et al. (1994) with the following exceptions:

- The volume of test substance (both liquids and solids) applied to the cornea was reported as 0.5 mL or 0.75 mL.
- The exposure time of liquids varied (10, 30, or 60 minutes) depending on the laboratory.

- Some laboratories used positive controls (acetone in three laboratories for liquid test substances and imidazole in one laboratory for solids).
- Different laboratories used different numbers (3 to 6) of corneas per test substance.

Although these variations in BCOP protocols were described in Chamberlain et al. (1997), it was not noted which specific protocol was used by each of the eight laboratories.

Most submissions calculated a BCOP score that combined the opacity and permeability values using the same formula as the EC (Gautheron et al. 1994) and EC/HO (Balls et al. 1995) studies. However, one submission considered the opacity and permeability scores separately and assigned an *in vitro* irritation score (mild, moderate, severe) based on the greater of the two values. While the scoring procedure of the different laboratories was discussed, actual BCOP scores were not provided in the IRAG evaluation (Chamberlain et al. 1997).

The IRAG-reviewed studies used Pearson's correlation to compare the MAS of each laboratory's test substance set to the BCOP scores. The relationship between the BCOP scores and MAS for each laboratory test set was graphed on a scatterplot diagram and Pearson's correlation coefficients were determined. Pearson's correlation coefficients were calculated for some individual *in vivo* endpoints (e.g., cornea opacity, conjunctivae redness, conjunctivae discharge, swelling, days to recover, and iris); however, different *in vivo* endpoints were used for the correlation analysis of different laboratories.

The *in vivo* reference data used for the IRAG evaluation were submitted by each participating laboratory for the substances it had tested. Although the IRAG reviewers requested a description of the *in vivo* test method used by each laboratory, specific protocols or guidelines used to produce the *in vivo* eye irritation data were not discussed in the IRAG report (Chamberlain et al. 1997). *In vivo* MAS were used to produce scatterplots and perform the statistical analyses that compared the *in vivo* and *in vitro* data of each laboratory; however, only a range of MAS was reported for each laboratory.

BCOP data and results were presented in a way that maintained the confidentiality of the specific substances tested and the identity of the participating laboratories. Thus, neither original data nor individual BCOP scores were provided in the IRAG report. Instead, scores are graphically presented in scatterplots that compare the BCOP scores with the *in vivo* MAS of test materials for a specific laboratory. Pearson's correlation analysis was used to compare the MAS of each laboratory's test substance set to the BCOP scores. The data were analyzed according to guidelines developed by a separate IRAG working group (Scala and Springer 1997) for acceptance and evaluation of data submitted for comparing *in vitro* with *in vivo* data.

Pearson's correlation coefficients were calculated for each participating laboratory for the test substances evaluated by that laboratory. **Table 9-3** presents the correlation coefficients obtained for the different laboratories. Sensitivity, specificity, positive and negative predictivity, and false positive and negative rates were not determined or discussed. The

IRAG evaluation did not consider test method reliability in its assessment of the BCOP assay. It is not known whether the BCOP studies were conducted in compliance with GLP guidelines.

Table 9-3 Summary Evaluation of BCOP Data Submitted to IRAG

Laboratory (No. of Materials Tested)	Substance Type or Class	Range of *In Vivo* MAS	Pearson's Correlation (*r* value)
Lab 3 (43)	Full range of chemical classes; industrial raw materials and intermediates	0 - 81.5	0.72
Lab 4 (12)	Personal care products	1 - 28	0.78
Lab 5 (21)	Fragrance gels	17.9 - 40.0	0.35/0.31[1]
Lab 6 (25)	Surfactant-containing materials	0 - 40	0.79/0.79[2]
Lab 7 (52)	22 Liquids; 22 Solids; 8 Surfactants	0 - 84	0.66
Lab 8 (39)	20 Surfactants; 12 Surfactant-based lotions; 7 Shampoos	0 - 64	0.56
Lab 9 (20)	Industrial chemical intermediates	0 - 110	0.74
Lab 10 (30)	Miscellaneous organic substances from ECETOC (1992) database on eye irritants	1.67 - 108	0.55

[1]Pearson's correlation coefficients for BCOP scores at 10 minutes/30 minutes.
[2]Pearson's correlation coefficients for log BCOP/BCOP permeability only.

As described in the introduction to Section 9.1, the IRAG study was not included in previous sections of this document due to the observation that at least two of the IRAG data sets had been published in reports reviewed in previous sections of the BRD; the data and other information in the IRAG report were not included to avoid duplication of BCOP studies and data. Additionally, BCOP and detailed *in vivo* data, which are necessary for the analyses performed in **Section 6.0** of this document, were not received in response to NICEATM's requests for such data.

9.1.5 Cooper et al. (2001)

The BCOP assay was performed essentially as described by Gautheron et al. (1992), except that corneal swelling and histological evaluation were added as endpoints, and various exposure times and dilutions were used. Seven shampoo formulations of mild to extreme *in vivo* irritancy were evaluated. BCOP scores alone tended to underpredict the irritancy of the substances investigated; however, the authors noted that histological evaluation provided useful information.

In vivo data were not available for all substances. For some substances, modified Draize rabbit eye test data (MAS) were available. Mean opacity, mean permeability, and mean BCOP scores were reported. Additionally, corneal swelling percentages and results of the histological evaluation were reported. Since the *in vivo* test results were expressed as MAS, the data provided in this report could not be used to evaluate the accuracy of the BCOP for detecting ocular corrosives and severe irritants according to the GHS (UN 2003), EPA (1996), or EU (2001) classification systems. NICEATM contacted a representative from the corresponding author's organization for detailed *in vivo* data and was informed that these data were not readily available.

9.1.6 Gautheron et al. (1992)

This is the first publication on the BCOP assay. Many protocol components are the same as those for Gautheron et al. (1994); however, the protocol lacks some refinements used in the latter study, such as combining the opacity and permeability values into a total *in vitro* score, and assigning irritancy grades to test materials based on ranges of scores. For these reasons, the study was not included in the accuracy analyses (**Section 6.0**) of this document.

Forty-one liquids (e.g., alcohols, solvents, volatile organics, and other chemical classes with varying physicochemical characteristics) and six solids (acids, anionic surfactant, cationic surfactants) were tested for which chemical names are provided. Fifteen process intermediates also were tested but their structures/names were not provided.

The *in vivo* reference data used were from the published literature or from in-house studies. Data were standardized to four irritancy grades (mild, mild/moderate, moderate, and severe). Only opacity values were reported for the 47 reference substances; values were classified into four groups (mild: 0-20 opacity units; mild/moderate: 21-40; moderate: 41-70; severe: ≥ 71). Opacity and permeability values were reported for the 15 process intermediates. *In vitro* opacity grades were compared with *in vivo* irritancy grades for the 47 reference substances. There were six false negatives (SDS and some medium chain length alcohols). For opacity alone (44 substances), the Spearman's rank correlation coefficient was 0.73.

9.1.7 Gautheron et al. (1994)

The test method performance analyses conducted for this study are summarized here. An *in vitro/in vivo* comparison using BCOP and MAS scores was conducted as follows. Mean *in vitro* scores of the 52 test substances were compared first with *in vivo* MAS scores and then with day 1 scores using the Spearman rank correlation test. The correlation between BCOP and *in vivo* MAS scores was $r = 0.64$, while the correlation between BCOP and *in vivo* day 1 scores was $r = 0.73$. The authors decided to use *in vivo* day 1 scores for all further correlation calculations:

- *in vitro* opacity scores versus *in vivo* day 1 scores: $r = 0.67$
- *in vitro* permeability values versus *in vivo* day 1 scores: $r = 0.60$
- total BCOP scores for liquids plus surfactants versus *in vivo* day 1 scores: $r = 0.78$
- total BCOP scores for solids versus *in vivo* day 1 scores: $r = 0.62$

In vitro/in vivo comparison using irritancy categories: Substances tested *in vitro* were classified into two categories based on their *in vitro* score: irritant (score ≥ 25.1) and nonirritant (score ≤ 25.0). Substances categorized as nonirritant *in vivo* were those classified as practically nonirritant, minimally irritant, or mildly irritant with the Kay and Calandra system (Kay and Calandra 1962). Substances categorized as irritant *in vivo* were those classified as moderately irritant, severely irritant, and extremely irritant with the Kay and Calandra system. A two-by-two contingency table was constructed to determine concordance, sensitivity, and specificity values. The values for concordance, sensitivity, and specificity were the same, 85%, since the BCOP assay overpredicted and underpredicted four substances. The false positive rate was 15% (4/26 substances) and the false negative rate was 15% (4/26 substances). According to the study authors, the BCOP test method performed reasonably well at distinguishing irritating from nonirritating substances. **Table 9-4** provides a comparison of *in vivo* and *in vitro* data for irritants classified as severe or stronger in Gautheron et al. (1994) using either the Kay-Calandra (1962) or EEC (1984) classification systems.

Table 9-4 Comparison of *In Vivo* and *In Vitro* Data for Irritants Classified as Severe or Stronger in Gautheron et al. (1994) Using Either the Kay-Calandra (1962) or EEC (1984) Classification Systems

Substance Name (Physical Form)	*In Vivo*					*In Vitro*	
	MAS	Day 1 Score	Days to Reverse	K-C class[1]	EEC[2]	BCOP Score	BCOP Class[3]
Dibenzoyl-L-tartaric acid (S)	33.7	33.7	21	Mod	R41	120.5	Sev (I)
Sodium oxalate (S)	47.0	47.0	IRR	Sev	R36	4.8	Mild (NI)
Imidazole (S)	54.3	48.0	IRR	Sev	R36	87.9	Sev (I)
Quinacrine (S)	52.3	52.3	IRR	Extr	R36	31.1	Mod (I)
Hexadecyltrimethyl-ammonium bromide (SF)	69.0	49.7	IRR	Extr	R36	66.4	Sev (I)
Benzethonium chloride (SF)	76.3	67.0	IRR	Extr	R41	133.9	Sev (I)
Promethazine hydrochloride (S)	103.0	82.3	IRR	Max	R41	120.5	Sev (I)

Abbreviations: I = Irritant; IRR = Irreversible; MAS = Maximum average score; NI = Nonirritant; S = Solid; SF = Surfactant.

[1]Kay and Calandra (1962): Mod = Moderately irritant; Sev = Severely irritant; Extr = Extremely irritant; Max = Maximally irritant.

[2]EEC (1984) risk categories for ocular irritancy: R36 = Irritant; R41 = Severely irritant.

[3]BCOP data were grouped into three classes (Mild irritant = 0-25; Moderate irritant = 25.1-55; and ≥ 55.1) and two classes (Nonirritant [NI] = < 25.0 and Irritant [I] = ≥ 25.1).

Regarding interlaboratory reproducibility, this study found that 82.7% of the test substances were classified the same by all laboratories when using a three-category system. In this system, substances were classified into one of the following categories: mild irritant (BCOP score 0-25), moderate irritant (25.1-55) and severe irritant (≥ 55.1).

9.1.8 Jones et al. (2001)

The BCOP assay was performed as described by Gautheron et al. (1992), except that corneal swelling and histological evaluation were added as endpoints, and various exposure times and dilutions were used. Ten shampoos containing anionic or amphoteric surfactants and seven conditioner formulations containing cationic surfactants were evaluated. *In vivo* irritant categories (mild, moderate, substantial) were based on Draize scores and any other information that was available, such as market history. NICEATM contacted the corresponding author for detailed *in vivo* data and was informed that these data were not readily available. Mean opacity, mean permeability, and mean BCOP scores were reported. Additionally, corneal swelling percentages and some histological results were reported. BCOP classifications correlated poorly with the *in vivo* irritancy categories used for this set of substances. The assay overpredicted the irritancy of the conditioners, but could discriminate between shampoos with different *in vivo* irritancies.

9.1.9 Rachui et al. (1994)

The BCOP protocol used for this study was essentially the same as Gautheron et al. (1994). Thirty-eight cosmetic and personal care test materials obtained from Maybelline, Inc. were tested. Examples include creams, refresher sprays, oil sprays, lotions, shower gels, bath oils, eyeliners, mascara, and eye creams. Mean BCOP scores (opacity + 15 x O.D.) were reported and classified into 3 grades: mild (0-25); mild/moderate (25.1-55); and severe (≥ 55.1).

This study was not included in the accuracy analyses (**Section 6.0**) because the *in vivo* data were obtained from a modified Draize eye irritation protocol (i.e., 0.03 mL of test substance). Scores for 24, 48, and 72 hours were reported and irritant grades assigned; however, the *in vivo* ocular irritation classification scheme was not described. *In vivo* data were not available for all substances tested *in vitro*. For 32 substances, 24-, 48-, 72- hour scores are reported. Seventeen substances were classified as nonirritants, and two were classified as mild. The method of comparing *in vivo* and *in vitro* results is not clear or well-described. However, the study reports that BCOP grades correlated to available *in vivo* grades for 25 of 28 (89%) substances, without clearly explaining how these results were obtained or providing sufficient *in vivo* data to verify the accuracy calculation.

9.1.10 Rougier et al. (1994)

The essential protocol components (e.g., preparation and treatment of corneas, opacity and permeability measurements) were the same as those used by Gautheron et al. (1994). However, the authors did not note the number of corneas used per test substance, whether any controls were used, or whether the substances were tested undiluted or diluted. Spearman's correlation coefficients were calculated for BCOP scores and *in vivo* MAS for the 20 surfactants and the 21 cosmetic formulations. No other measures of accuracy were noted. An *in vitro* classification system was not provided

Twenty surfactants and 21 cosmetic formulations were evaluated. The surfactants were identified, and included nonionic, anionic, amphoteric, and cationic types. The types of cosmetic formulations included eye make-up remover, make-up remover, shampoos, and one shower gel. The components of the formulations were not provided. Seven of the

surfactants were purchased from Sigma; however, the sources of the other materials were not provided. CASRNs were not provided. Test substances were not coded.

Historical *in vivo* data from in-house Draize rabbit eye tests were used as reference data. MAS and the average score at seven days are reported for each substance in the publication. Irritancy grades were not provided. Detailed *in vivo* data were not obtained for this study, which prevented its inclusion in earlier sections of this document. NICEATM could not readily find current contact information for the study authors.

BCOP scores (opacity value +15 x O.D. value) were reported for all substances tested, but irritancy grades were not assigned. Spearman's correlation coefficients were calculated for BCOP scores and *in vivo* MAS for the set of 20 surfactants and the set of 21 cosmetic formulations. The Spearmann's correlation coefficient for BCOP scores and MAS for the 20 surfactants was 0.75. The Spearmann's correlation coefficient for BCOP scores and MAS for the 21 surfactant-based cosmetic formulations was 0.79.

The performance characteristics of the BCOP assay for all 41 substances using two classes of irritancy (nonirritant and irritant) was reported as: concordance = 93% (38/41 substances); sensitivity = 91% (20/22 substances); false negative rate = 9% (2/22 substances); specificity = 95% (18/19 substances); false positive rate = 5% (1/19 substances).

9.1.11 Sina et al. (1995)

The protocol was identical to Gautheron et al. (1994) except that 0.5 mL of test material was applied to corneas and exposure was for 30 minutes. Thirty-seven test substances representing a broad range of pH, solubility, and *in vivo* irritation potential were tested. Most substances were synthetic intermediates isolated during manufacture of pharmaceuticals. Chemical names, structures, and classes are not provided in paper.

Few details were provided on the conduct of the *in vivo* reference studies. However, MAS and Kay/Calandra classifications were reported for each substance. Mean BCOP scores (opacity + 15 x O.D.) were reported and classified into four grades: nonirritating/ mild (0-15); mild (> 15-25); moderate (> 25-55); severe (≥ 55.1). The correlation between BCOP and *in vivo* classes for 36 substances was 89%. Specificity (36) was reported as 90%. Sensitivity (36) was reported as 88%. The Spearman correlation coefficient for *in vitro* and *in vivo* scores (32 substances) was 0.74. The Pearson correlation coefficient for *in vitro* and *in vivo* scores (32 substances) was 0.62. NICEATM contacted Dr. Sina for additional data and information on the various studies he published; it was found that many of the *in vivo* studies were stored on microfiche in company archives, so additional data were not readily available.

9.1.12 Ubels et al. (1998)

This study investigated the effect of hydration on corneal opacity using the modified BCOP assay reported by Casterton et al. (1996). The authors note that corneal opacity can result from an increase in corneal hydration (i.e., corneal swelling or edema) or from damage to the cornea (e.g., precipitation of corneal proteins), and that it might be useful to distinguish between these two causes of opacity, since the former is sometimes reversible while the latter

is often irreversible. The study evaluated 10 substances previously studied by Casterton et al. (1996) that are known to cause opacity in the BCOP assay. Hydration measurements (i.e., comparison of wet and dry cornea weights), corneal light absorbance at 570 nm, and light and electron microscopy data were reported. The authors concluded that corneal hydration measurements would be a useful addition to the BCOP assay.

9.1.13 Ubels et al. (2000)

This study investigated the effect of reduced treatment times (30 seconds and 1 minute) on corneal opacity, permeability, and hydration using a modified BCOP assay (Casterton et al. 1996). Effects of irritants on the corneal endothelium were also examined. This study examined 13 substances previously studied by Casterton et al. (1996). Hydration measurements, corneal light absorbance at 570 nm, and electron microscopy data were reported. For most of the substances, the reduced treatment times resulted in lower corneal opacity and hydration values. The authors suggested that the shorter exposure times might provide results in the BCOP assay more predictive of human response to eye irritants. Based on the electron microscopy data, the authors also found that certain irritants damage the corneal endothelium. Some endothelial damage also was found for untreated corneas that had simply been mounted in the BCOP corneal holder, suggesting the need for optimization of the corneal holder.

9.1.14 Ubels et al. (2002)

This study represents a continuation of the work reported in Ubels et al. (2000). It describes the design and use of a redesigned corneal holder. The authors note potential limitations of the conventional corneal holder: 1) it has a circular opening 17 mm in diameter, yet the bovine cornea is oval shaped and has dimensions of about 24 mm vertically and 30 mm horizontally; 2) it has flat inner surfaces, whereas the bovine cornea is convex or curved. These elements of the corneal holder reportedly force the bovine cornea into an unnatural shape when mounted in the holder, causing the cornea to wrinkle. The authors also noted damage to all three corneal cell layers (epithelium, stroma, and endothelium) where the cornea comes in contact with the circular edge of the holder opening.

Recognizing some of the potential limitations of the conventional corneal holder, the authors designed a new corneal holder with dimensions that better fit the bovine cornea and maintain its natural shape during the BCOP assay. The new holder was designed to contact the 2 to 3 mm rim of sclera left around the bovine cornea during dissection, rather than the corneal tissue. The authors report that this refined corneal holder does not cause wrinkling of the mounted bovine cornea, nor does it damage the cell layers around the edge of the cornea.

The following test substances were studied in this evaluation: acetone, 1% benzalkonium chloride, isopropanol, and 30% trichloroacetic acid. Hydration measurements, corneal light absorbance at 570 nm, and electron microscopy data were reported.

9.1.15 Ubels et al. (2004)

This study is a continuation of the authors' evaluation of the utility of a redesigned corneal holder for use in the BCOP assay. Previous studies have suggested that the new holder is an improvement over the conventional holder based on comparisons of corneal opacity,

hydration, and endothelial morphology (Ubels et al., 2000, 2002). This study provides a comparison between the conventional holder and the redesigned holder with respect to corneal permeability. The effects of acetone, isopropyl alcohol, 1% sodium hydroxide, 30% trichloroacetic acid, and 30% sodium dodecylsulfate on corneal permeability were compared between the two corneal holders. The authors contend that the lack of damage seen with the redesigned holder (as opposed to the damage to the cornea reportedly induced by the conventional holder) reduces the level of permeability, as well as reducing measurement variability.

9.1.16 Vanparys et al. (1993)

The essential protocol components (e.g., preparation and treatment of corneas, opacity and permeability measurements) were the same as those used for Gautheron et al. (1994). Six corneas were used per test substance and three corneas were used for an untreated control group. The classification system differed slightly from Gautheron et al. (1994) in that: nonirritant = BCOP score of 0 to 3; mild irritant = 3.1 to 25; moderate irritant = 25.1 to 55; and severe irritant > 55. Concordance, specificity, and sensitivity were calculated for two scenarios: 1) the *in vivo* and *in vitro* irritancy grades were divided into two groups; 2) the irritancy grades were divided into four groups.

Fifty pharmaceutical and commercially available substances were evaluated representing both liquids (miscible and immiscible) and solids (soluble and insoluble). Examples include piperidines, epoxides, furans, thiazoles, nitrophenyls, imidazole, Tween 20 & 80, shampoos, and alcohols. Nine of the substances were in-house compounds (i.e., candidate drugs) and 15 were pharmaceutical process intermediates. Chemical names and physical state are provided in the publication. Test substances were not coded. CASRNs and the source of materials were not provided.

For the in-house substances and the pharmaceutical intermediates, historical *in vivo* data from the Draize test were available at Janssen Pharmaceutica. For the commercially available substances, *in vivo* data were obtained from the literature or from Draize tests (OECD 1987) performed at J. Simon Laboratories. The *in vivo* ocular irritancy grades of the test substances were nonirritant (13 materials), mild (6), mild/moderate (2), moderate (10), and severe (19) based on an internal classification scheme (not an accepted regulatory classification system). The only *in vivo* data in the publication were these irritancy grades.

Mean opacity and mean permeability values were reported, in addition to total BCOP scores (mean opacity value +15 x mean O.D. value). The BCOP scores were classified into the following four irritancy grades: nonirritant (0 to 3), mild (3.1 to 25), moderate 25.1 to 55), and severe (> 55). Concordance, specificity and sensitivity were calculated for two scenarios: 1) *in vivo* and *in vitro* irritancy grades were divided into two groups; and, 2) irritancy grades were divided in four groups

When *in vivo* and *in vitro* irritancy grades were grouped into two categories (negative = nonirritant and mild irritants; and positive = moderate and severe irritants), the concordance was 96% (48/50 substances), specificity was 95% (18/19 substances), and sensitivity was

97% (30/31 substances). The false positive rate was 5% (1/19 substances) and the false negative rate was 3% (1/31 substances).

When four *in vivo* and *in vitro* eye irritancy grades (nonirritant, mild, moderate, and severe) were used, 36 of 50 (72%) *in vivo* grades were correctly predicted with the BCOP assay. Twelve (24%) substances (alcohols and other highly permeable substances) were overpredicted in the BCOP assay, while two (4%) solids were underpredicted.

Of the 19 substances classified as severe irritants *in vivo* by the investigators, the BCOP assay correctly predicted all 19 as severe irritants.

9.1.17 1997 Bovine Corneal Opacity and Permeability Technical Workshop

In November 1997, the Institute for *In Vitro* Sciences (IIVS) convened a workshop that addressed the state-of-the-art of the BCOP assay with a focus on how it met certain regulatory acceptance criteria. The proceedings of this workshop were published in 1998 (In Vitro & Molecular Toxicology 11(4):315 to 351) in an article entitled "Report from the Bovine Corneal Opacity and Permeability Technical Workshop – November 3-4, 1997." This report summarizes the talks and discussions of the workshop, which included:

- An Historical Perspective (summarized by J.F. Sina and P. Gautheron)
- The Bovine Corneal Opacity and Permeability Assay: An Alternative Protocol (summarized by P. Casterton)
- Considerations for Histological Examination of Bovine Corneal Tissue (summarized by M.G. Evans)
- The Bovine Corneal Opacity and Permeability Assay: Observations on Assay Performance (summarized by J.W. Harbell and R.D. Curren)
- Experience with Other Isolated Eye Models: Isolated Rabbit Eye (IRE) (summarized by L. Earl)
- The Use of Prediction Models with Non-Animal Eye Irritation Tests (summarized by L. Bruner)
- Workshop Summary (summarized by R.D. Curren and J.W. Harbell)

In the Workshop Summary, Drs. Curren and Harbell addressed several of the criteria used by ICCVAM to assess the validation status of an alternative test method. The BCOP assay was discussed in terms of its scientific and regulatory rationale, the relationship of the test method endpoints to the biologic effect of interest, available protocols, extent of intra- and inter-laboratory variability, test method performance using reference chemicals (prediction models), and assay constraints.

Regarding the discussion of available protocols, the authors noted that the original test method protocol was designed to assess the potential eye irritation of pharmaceutical intermediates. As use of the assay spread to different laboratories and testing of different types of materials, the protocol changed to accommodate the different physical and chemical characteristics of different test substances. Certain aspects of the protocol, such as exposure and post-exposure times, can vary depending on the test material or objective of the study. The authors concluded that it is very likely that no single exposure protocol and prediction

model could provide accurate prediction of ocular irritation across all chemical classes and physical forms of test substances.

The authors also noted that histopathological evaluation of the corneas appears to be very useful; however, further development and refinement of this procedure was recommended at the time of the publication. Histology allows for an evaluation of the depth and type of injury, which could be used to evaluate the potential for recovery.

Regarding variability in the BCOP assay, the authors noted that reproducibility within and among laboratories appeared to be acceptable based on a number of in-house evaluations and multinational studies. Proposed sources of variability include variations in technical approach and potential differences in the corneas related to their source.

Constraints of the assay also were discussed. The authors noted that some laboratories have reported a decline in quality of the bovine corneas obtained during the summer months. The thicker epithelial layer of the bovine cornea, in comparison with human and rabbit corneas, was noted as a possible constraint that could potentially lead to an underestimation of irritancy for some substances. Also, the authors noted limitations of the currently used opacitometer, which provides a center-weighted reading of corneal opacity; they recommended development of a more accurate device for measurement of corneal opacity that could account for opacity over the whole surface of the cornea.

9.1.18 Review Articles on the BCOP Assay

Sina (1994) reviewed the steps taken by Merck Research Laboratories (West Point, Pennsylvania) to validate the BCOP assay for the purpose of screening chemicals (e.g., pharmaceutical intermediates and raw materials) to which workers would be exposed in a pharmaceutical manufacturing setting. The author discussed the initial development of the BCOP assay for this purpose, the results of an interlaboratory evaluation, and how results from the BCOP assay compare to other alternative eye irritation test methods.

Sina and Gautheron (1994) reviewed their experiences with developing a test battery to evaluate ocular irritation of substances. The BCOP assay, three cytotoxicity assays, and a few inflammation assays (e.g., chemotaxis, arachidonic acid cascade) were evaluated. In a study of 43 in-house materials representing a variety of chemical classes (aromatic and organic acids and bases, alcohols, esters, peptides, inorganic salts), the authors found that the accuracy of the BCOP *In Vitro* Irritancy Score in predicting Kay-Calandra class was greater than 80%. However, two of the false negatives in the BCOP assay resulted from substances that produced no irritation in the rabbit eye test until after 48 hours. The cytotoxicity assays did not perform very well across a range of chemical classes. The authors noted that the inflammation assays were still under development.

9.1.19 Poster Presentations

Over the past five years, numerous poster presentations have been given on the BCOP assay, which depict the ways in which the assay has evolved in recent years. Although it is not possible to summarize all of these presentations here, they are listed below by year of presentation to show that the assay has been applied to many different types of substances

(e.g., alkaline dry detergent products, hypochlorite-containing solutions, fragranced formulations, oxidizing/reactive cleaning products, petrochemical products, and fragrance mixtures). In studying different types of substances with the BCOP assay, optimal exposure and post-exposure times have been defined for certain types of substances. For example, a protocol using a 25% (v/v) aqueous dilution and 30-minute exposure was recommended for the surfactant-based products tested by Cater et al. (2001). Some of these posters (e.g. Curren et al. 2000a, 2000b) also demonstrate the usefulness of adding histopathological assessment to the BCOP assay. Another significant use of the BCOP assay has been to compare results of a product series with a selected "benchmark" that had been previously tested *in vivo* and had a well-established market history (e.g., Cater et al. 2001). A majority of the poster presentations can be obtained from the Institute for *In Vitro* Sciences (Gaithersburg, Maryland; website: http://www.iivs.org/).

2000

Curren R, Evans M, Raabe H, Ruppalt R, Harbell J. 2000a. Correlation of histopathology, opacity, and permeability of bovine corneas exposed *in vitro* to known ocular irritants. Veterinary Pathology 37(5):557.

Curren RD, Evans MG, Raabe HA, Ruppalt RR, Harbell JW. 2000b. An histopathological analysis of damage to bovine corneas *in vitro* by selected ocular toxicants. Presented at the 2000 Society of Toxicology meeting.

Swanson JE, Harbell JW. 2000. Evaluating the eye irritancy potential of ethanolic test materials with the bovine corneal opacity and permeability assay. The Toxicologist 54(1):188-189.
(*Note:* S.C. Johnson & Son, Inc. submitted *in vitro* and *in vivo* data to NICEATM for this poster. This study was included in **Sections 3.0 – 7.0** of this document.)

2001

Cater KC, Raabe HA, Mun G, Harbell JW. 2001. Corporate validation program for predicting eye irritation of surfactant formulations *in vitro*. The Toxicologist 60:99.

Rees WM, Swanson JE, Burdick JD, Hilgers DS, Harbell JW. 2001. Evaluating toxic synergism in hypochlorite-containing solutions using the bovine corneal opacity and permeability (BCOP) assay. The Toxicologist 60:99.

2002

Burdick JD, Merrill JC, Spangler TC, Moyer GO, Harbell JW. 2002. Use of histological examination in bovine corneal opacity and permeability (BCOP) assay for assessing the ocular irritation potential of fragranced formulations. The Toxicologist 66:244.

Cater K, Nusair T, Merrill JC, Harbell JW. 2002. Exploratory *in vitro* eye irritation study of marketed alkaline laundry detergents by BCOP assay and pH/reserve alkalinity (RA) parameters. The Toxicologist 66:244.

Cuellar N, Merrill JC, Clear ML, Mun G, Harbell JW. 2002. The application of benchmarks for the evaluation of the potential ocular irritancy of aerosol fragrances. The Toxicologist 66(1-S):243-244.
(*Note:* S.C. Johnson & Son, Inc. submitted *in vitro* data and other information to NICEATM for this poster. See **Section 9.2** for a summary of this poster and **Appendix G** for the submitted information.)

2003

Cater K, Mun G, Moyer G, Merrill J, Harbell JW. 2003. Exploratory *in vitro* eye irritation study of marketed alkaline dry laundry detergents by BCOP assay and pH/reserve alkalinity (RA) parameters. The Toxicologist 72:220.

Cuellar N, Lloyd PH, Swanson JE, Merrill JC, Clear ML, Mun G, Harbell JW, Bonnette KL. 2003. Evaluating the eye irritancy of solvents in a simple fragrance mixture with the bovine corneal opacity and permeability assay. The Toxicologist 72:312.

Gran BP, Swanson JE, Merrill JC, Harbell JW. 2003. Evaluating the irritancy potential of sodium percarbonate: a case study using the bovine corneal opacity and permeability (BCOP) assay. The Toxicologist 72:220.
(*Note:* S.C. Johnson & Son, Inc. submitted *in vitro* data and histology figures to NICEATM for this poster. See **Section 9.2** for a summary of this poster and **Appendix G** for the submitted information.)

Swanson JE, White BT, Gran BP, Merrill JC, Harbell JW. 2003. Evaluating oxidizing/reactive cleaning products in the bovine corneal opacity and permeability (BCOP) assay. The Toxicologist 72:220-221.

2004

Bailey P, Freeman J, Phillips R, Merrill J. 2004. Evaluation of the BCOP assay as a predictor of ocular irritation of petrochemical products. Presented at the 2004 Society of Toxicology meeting.
(*Note:* ExxonMobil Biomedical Sciences, Inc. submitted *in vitro* and *in vivo* data to NICEATM for this poster. This study was included in **Sections 3.0 – 7.0** of this document.)

Cater K, Patrick E, Harbell J, Schilcher S. 2004. Comparison of *in vitro* eye irritation potential by BCOP assay to erythema scores in human eye sting test of surfactant-based formulations. Presented at the 2004 Society of Toxicology meeting.

Cuellar N, Lloyd PH, Swanson JE, Merrill JC, Mun G, Harbell JW, Bonnette KL. 2004. Phase Two: Evaluating the eye irritancy of solvents in a simple fragrance mixture with the bovine corneal opacity and permeability (BCOP) assay. The Toxicologist 78(S-1):Abstract No. 1306.
(*Note:* S.C. Johnson & Son, Inc. submitted *in vitro* data and histology figures to NICEATM for this poster. See **Section 9.2** for a summary of this poster and **Appendix G** for the submitted information.)

Swanson JE, Rees WM, Hilgers DS, Merrill JC, Harbell JW. 2004. Managing toxic synergism in hypochlorite-containing cleaners using the bovine corneal opacity and permeability (BCOP) assay. Part II. Presented at the 2004 Society of Toxicology meeting.

9.2 Other Scientific Reports Received in Response to a *Federal Register* Notice

In addition to the BCOP studies identified from the literature search, several studies were obtained in response to two *FR* Notices (Vol. 69, No. 57, pp. 13859-13861, March 24, 2004, and Vol. 70, No. 38, pp. 9661-9662; available at http://iccvam.niehs.nih.gov/methods/eyeirrit.htm), requesting original BCOP test method data and *in vivo* reference data. In response to these requests, *in vitro* test method data were submitted by Johnson & Johnson Pharmaceutical Research & Development, L'OREAL, and S.C. Johnson & Son, Inc. In these three reports, insufficient *in vivo* reference data precluded their use in an assessment of the performance characteristics of BCOP compared to the GHS (UN 2003), EPA (1996) and EU (2001) ocular irritancy classification systems. IIVS submitted replicate experiment data for the BCOP results reported in Gettings et al. (1996); these data were used for an analysis of intralaboratory reproducibility in **Section 7.0**. IIVS also submitted additional analyses of the *in vivo* and BCOP data reported in Gettings et al. (1996). Johnson & Johnson Pharmaceutical Research & Development submitted data for 20 chemicals tested in the BCOP assay, comparing corneas from adult animals (> 24 months) to those of young animals (6 to 8 months). These data were provided to evaluate the impact of age of source animals for test eyes on the BCOP assay. Details of these studies are included below.

9.2.1 S.C. Johnson & Son, Inc.

In addition to two datasets included in the accuracy and reliability analyses of this document, S.C. Johnson & Son, Inc. submitted three other datasets on the BCOP assay:

1. an evaluation of the potential ocular irritancy of aerosol fragrance formulations with the BCOP assay
2. the application of benchmarks for evaluation of the ocular irritancy of solvents in a simple fragrance mixture
3. an evaluation of reactive chemistry formulations using the BCOP assay

These three datasets are provided in **Appendix G,** and briefly summarized here.

The first dataset (**Appendix G1**) provides data and supporting information for a poster presentation given by Cuellar et al. (2004) on use of the BCOP assay to study the influence of solvents on the ocular irritation potential of fragrance mixtures. The study evaluated one fragrance, six solvents, and six solvent/fragrance mixtures. In this study, the protocol was modified in the following ways: exposure times of one and three minutes were used to evaluate the test substances; and, post-exposure times of 2-, 4- and 20-hours were used for different aspects of the study. In addition, a histopathological evaluation was performed on the treated corneas. A modified rabbit eye irritation test was conducted on the same substances tested *in vitro*. Four animals were treated per test substance. After 24 hours, ocular tissues were harvested for three animals for a histopathological assessment. The remaining animal was examined up to 28 days to evaluate recovery of ocular lesions.

The authors concluded that the choice of solvent can have a major influence on the ocular irritation potential of fragrance mixtures. Some solvents in a simple fragrance produce mild irritation, while other different solvents can produce severe irritation. The authors noted that the time course of tissue scores *in vivo* was similar to the time course of the histological changes in BCOP. It was also noted that morphological changes in the keratocytes were found in both the isolated bovine corneas and the rabbit eye treated corneas.

The second dataset (**Appendix G2**) provides data and supporting information for a poster presentation given by Cuellar et al. (2002) demonstrating how the BCOP assay can be used to evaluate new formulations in relation to an appropriate reference benchmark for which the ocular irritation potential is well-characterized. This study evaluated specific aerosol formulations in comparison to ethanol/fragrance benchmarks.

The third dataset (**Appendix G3**) provides data and supporting information for a poster presentation given by Gran et al. (2003) that described use of the BCOP assay to evaluate the potential eye irritancy of sodium percarbonate, a commonly used substance in cleaning products. Sodium percarbonate is highly reactive, producing corneal epithelial peeling and other types of irritation in the rabbit eye test. The standard BCOP protocol for solids was not used in this investigation of sodium percarbonate. Based on past experience with the BCOP assay, the eye irritancy potential of more aqueous-soluble solids such as laundry powders using the standard solids protocol is vastly overpredictive of the outcome resulting from accidental human exposure. Furthermore, experience has shown that reactive/oxidizing chemistries (such as bleach, percarbonates and peroxides) have a delayed toxicity response in the assay necessitating increased post-exposure observation time.

The question the investigators faced in this case study of sodium percarbonate was what protocol parameters were needed to model the bolus exposure for an extended period that occurs in the Draize eye irritation protocol, as well as what might be expected to be a realistic maximum exposure in humans. The following parameters were chosen: a 50% suspension of the solid with a 30-minute exposure time to model the *in vivo* exposure and 10-minute exposure time to model maximum accidental human exposure. While post-exposure time in the BCOP assay is typically two hours, times of four hours and 20-24 hours were chosen for this study.

Using the protocol considerations discussed above, the BCOP assay was able to adequately predict the irritancy potential of two different concentrations of sodium percarbonate for both a realistic human exposure scenario and an *in vivo* exposure scenario. Reduction of sodium percarbonate concentration predictably reduced the irritancy potential of the end-use formulation. Histology as a third endpoint in the BCOP assay was critical in evaluating the depth and degree of injury.

9.2.2 L'OREAL

L'OREAL Advanced Research provided a dataset for an in-house porcine corneal opacity and permeability (PCOP) assay, as well as some data from the BCOP assay. The dataset includes PCOP data and *in vivo* MAS scores for 50 liquid and water-soluble compounds, and data from both the PCOP and BCOP assays for 23 substances for which there was historical

in vivo data in the form of MAS scores. The authors note that the PCOP protocol is essentially that described by Gautheron et al. (1992), with the exception of some changes related to using a different species. Detailed *in vivo* data were requested from the submitters, but they indicated that they did not have the individual irritation scores for individual animals. This data submission is provided in **Appendix G4**.

9.2.3 IIVS

Dr. John Harbell submitted supplementary analyses for the BCOP study conducted for the CTFA Phase III evaluation of surfactant-based personal care products (Gettings et al. 1996). Dr. Harbell performed a bootstrap analysis of the *in vivo* rabbit eye studies performed for this evaluation, and compared the results of the bootstrap analysis to the permeability values obtained for the 25 test substances. Six rabbits were used in the *in vivo* eye irritation studies performed for the CTFA evaluation. However, a three rabbit eye irritation protocol is now accepted for use by the OECD and the EPA. Thus, the bootstrap analysis involved determining all of the possible three-animal combinations that result from the six animal test, assessing what the classification of the study would be according to the GHS system for each three animal combination, then determining the percentage of agreement among the 20 different possible three-animal combinations. For highly irritating substances and substances that were nonirritating, the extent of agreement among the 20 combinations was high. However, for substances that produced irritation in between these two extremes, the extent of agreement was more variable.

Several graphical representations of the *in vivo* data generated for the CTFA Phase III study were provided to include the average opacity score, average iris score, average redness score, and average chemosis score obtained for each substance in the rabbit eye test. The variability of these scores for each test substance was also depicted on the graphs.

Finally, there are three graphs that show the permeability values obtained for each test substance versus the results of the bootstrap analysis discussed above.

The bootstrap analysis and graphs are provided in **Appendix G5**.

9.2.4 Johnson & Johnson Pharmaceutical Research & Development

BCOP results from tests conducted with 20 substances using corneas from adult animals (i.e., > 24 months) and young animals (i.e., 6 to 8 months) were provided, along with the reported EU and GHS classification for each substance (**Table 9-5**). The submitters state that one of the test substances (acetone) needs to be repeated, due to discordant results with this substance relative to an earlier study. For this reason, only 19 of the 20 test substances were considered in the evaluation below. Corneas (3/test substances) were treated for 10 minutes followed by a 120-minute recovery period. Medium was removed from the anterior compartment and replaced by 1 ml of a 0.4% sodium-fluorescein solution. Corneas were incubated in a horizontal position for 90 minutes at 32°C in a water-bath. After incubation, medium from the posterior chamber was removed and its optical density (OD) determined with a spectrophotometer at 490 nm, and the IVIS calculated. Experiments with corneas from young animals were performed with a specially designed cornea holder, which has a smaller diameter than the traditional holder.

Based on the data summarized in **Table 9-5** (and excluding acetone, as indicated above), and regardless of which *in vivo* classification was used (i.e., EU or GHS), the overall accuracy of the BCOP using eyes from adult animals (> 24 months) was 68%, with false positive and false negative rates of 31% and 33%, respectively. By comparison, the overall accuracy of the BCOP using eyes from young animals (6-8 months) was 74%, with false positive and false negative rates of 19% and 67%, respectively. These results provide evidence that the performance of the BCOP using eyes from younger animals may not be significantly different than using eyes from older animals. However, given their smaller size relative to those from adult animals, from which corneas are typically obtained for the BCOP, a specially designed corneal holder (i.e., smaller diameter) is required for using younger corneas.

Table 9-5 Substances Used to Evaluate the Use of Corneas from Animals of Different Ages in the BCOP Assay

Test Substance	CASRN	*In Vivo* (EU)[1]	*In Vivo* (GHS)[1]	BCOP (> 24 months)				BCOP (6-8 months)			
				Opacity	Perm.	IVS	Class	Opacity	Perm.	IVS	Class
3,3-Dimethylpentane	562-49-2	NI	NI	0.6	0.01	0.8	NON	0.0	0.02	0.3	NON
3-Methoxy-1,2-propanediol	623-39-2	NI	NI	-0.3	0.0	0.2	NON	0.6	0.02	0.9	NON
Polyethylene glycol 400	25322-68-3	NI	NI	-0.3	0.0	-0.3	NON	0.0	0.08	1.1	NON
Glycerol	56-81-5	NI	NI	-1.0	0.01	-0.9	NON	-0.7	-0.01	-0.8	NON
Methyl cyclopentane	96-37-7	NI	NI	1.0	0.43	7.5	MILD	1.3	0.26	5.2	MILD
Tween 20	9005-64-5	NI	NI	0.0	0.01	0.1	NON	0.0	-0.01	-0.1	NON
Methyl iso-butyl ketone	108-10-1	NI	NI	6.6	1.07	22.7	MILD	5.7	0.83	18.1	MILD
Toluene	108-88-3	NI	NI	6.3	3.18	54	MOD	6.0	1.46	28.0	MOD
Methyl amyl ketone	110-43-0	NI	NI	5.3	1.8	32.3	MOD	4.0	0.99	18.8	MILD
2-Methyl-1-pentanol	105-30-6	NI	2B	12.0	4.3	76.6	SEV	8.6	1.94	37.7	MOD
Ethanol	64-17-5	NI	2B	16.0	2.34	51	MOD	16.3	1.83	43.8	MOD
Sodium hydroxide (1%)	1310-73-2	R36	2B	99.7	4.16	162	SEV	135.7	3.74	191.8	SEV
Triton X-100 (5%)	9002-93-1	R36	2B	4.3	3.81	61.5	SEV	4.7	3.7	60.1	SEV
1-Octanol	111-87-5	R36	2B	10.0	5.24	88.6	SEV	10.3	1.53	33.3	MOD
2-Ethyl-1-hexanol	104-76-7	R36	2B	4.3	1.76	30.6	MOD	2.3	0.86	15.3	MILD
n-Hexanol	111-27-3	R36	2A	15.3	3.73	71.2	SEV	14.0	3.62	68.2	SEV
Acetone[2]	67-64-1	R36	2A	39	2.95	83.2	SEV	91.3	2.86	134.2	SEV
Cyclohexanol	108-93-0	R41	1	15.3	5.04	90.7	SEV	11.6	2.13	43.6	MOD
Cetylpyridinium bromide (6%)	140-72-7	R41	1	11.7	1.01	26.8	MOD	15.0	1.66	39.9	MOD
Benzalkonium chloride (10%)	8001-54-5	R41	1	92.2	4.22	155.4	SEV	105.7	4.05	166.5	SEV

[1]*In vivo* classification provided by Johnson & Johnson Pharmaceutical Research and Development

[2]Data excluded due to reported technical difficulties with this substance, which requires retesting (no data received from retest)

CASRN=Chemical Abstracts Service Registry Number; IVS=*In vitro* score; MILD=Mild irritant (IVS=3.1-25); MOD=moderate irritant (IVS=25.1-55); NI=Nonirritant; NON=Nonirritant (IVS ≤ 3); Perm.=Permeability; SEV=Severe irritant (IVS > 55.1)

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