3.0 SUBSTANCES USED FOR VALIDATION OF THE ICE TEST METHOD

3.1 Rationale for the Substances or Products Selected for Use

In vitro ocular test method validation studies should, ideally, evaluate an adequate sample of test substances and products from chemical and product classes that would be evaluated using the *in vivo* rabbit eye test method. Test substances with a wide range of *in vivo* ocular responses (e.g., corrosive/severe irritant to nonirritant) also should be assessed to determine limits to the range of responses that can be evaluated by the *in vitro* test method.

Five reports contained sufficient *in vitro* and *in vivo* data for accuracy analyses[1]. These five reports are Prinsen and Koëter (1993), Balls et al. (1995), Prinsen (1996), Prinsen (2000) and Prinsen (2005).

As noted in **Section 2.2.5**, the ICE test method has been used for a wide range of test substances with different physicochemical characteristics. However, highly hydrophobic compounds and certain solids may require alternative testing strategies to ensure that contact with the corneal surface is maximized (Balls et al. 1995). There is no mention in any of the following studies of modification to the ICE protocol employed to account for this issue.

3.1.1 Prinsen and Koëter (1993)

The chemicals tested in this study were used in a previous study sponsored by the Commission of the European Communities (CEC 1991) to evaluate several *in vitro* ocular toxicity methods, including IRE and HET-CAM. These same chemicals were used by Prinsen and Koëter (1993) to provide comparative data and to determine the suitability of the chicken as an alternative to the rabbit as an eye donor for the isolated eye test.

3.1.2 Balls et al. (1995)

In the EC/HO validation study (Balls et al. 1995), the test substances were initially selected from the 1992 European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Reference Data Bank for ocular irritation (ECETOC 1992) based on the following criteria:

- Substances should be single chemicals (no mixtures).
- Substances should be available at high purity and stable when stored.
- The *in vivo* rabbit eye test data should have been generated since 1981 according to OECD TG 405 and in compliance with GLP guidelines.

Other criteria specific to the conduct of the studies are noted in the study report (Balls et al. 1995).

Originally, 60 substances that met the established criteria were found in the ECETOC data bank. However, this selection was determined to be inadequate due to the low number of solids, the insufficient number of moderate to severe irritants, and the lack of pesticides. To

[1] The ability of the ICE test method to accurately identify test substances classified as corrosive or a severe irritant is provided in **Section 6.0**. A description of the criteria and guidelines used by regulatory agencies to classify a substance as a corrosive or a severe irritant is provided in **Section 4.0**.

avoid additional animal testing, the validation study management team attempted to locate high quality rabbit eye study data within the commercial sector. Subsequently, based on the availability of additional data that met the established criteria (obtained primarily from unpublished studies), the original list was modified to include more solids, some pesticides, and substances representing moderate to severe degrees of irritation. During the validation study, it was discovered that 14 of the reference substances had been tested by a protocol that involved rinsing or removing the solid material from the eye one hour after application, rather than allowing it to remain continuously. Thus, the study protocol for these substances had not adhered to OECD TG 405. These 14 substances were retested *in vivo* and it was found that one, thiourea, was extremely toxic, killing the three rabbits on which it was tested. Based on this response, thiourea was excluded from the list of reference substances.

The final list of test substances included a total of 51 substances, four of which were tested at two different concentrations and two of which were tested at three concentrations, for a total of 59 different tests.

3.1.3 Prinsen (1996)

This report described the use of the ICE test method as a prescreen for severe eye irritants at TNO. All substances tested at TNO, from the time that the ICE was implemented as a prescreen up to the report date (1992-1994), are discussed in this report. Therefore, it appears that substances were tested as they were submitted to TNO by industrial, cosmetic, and food manufacturing companies for testing and subsequent regulatory classification. Thus, there was no specific rationale in the use of these substances.

3.1.4 Prinsen (2000)

The four substances tested for this report were siloxane polymers and surfactants, selected as part of phase II of a reference standard validation project conducted at TNO. No specific rationale was provided for the selection of any particular substance.

3.1.5 Prinsen (2005)

This report contained ICE test method data for 50 substances submitted to TNO, subsequent to those tested in Prinsen 1996. Again, no specific rationale for the use of any of these substances was provided.

3.2 Rationale for the Number of Substances Tested

No rationale was provided for the number of substances tested in any of the studies.

3.3 Chemicals or Products Evaluated

A total of 175 test substances were evaluated in the five studies, of which 90 were individual chemicals and 85 were commercial products, formulations or other mixtures. Chemical classes tested included alcohols, acids, hydrocarbons, inorganic chemicals, acyl halides, alkalis, esters, heterocyclics, ketones, and organophosphates; commercial products or formulations tested included detergents/surfactants, pesticides, solvents, silicone powder, ink, paint, toilet cleaners, and thermal paper coatings.

Physicochemical properties for each of the substances tested was obtained from information provided in the published reports and submitted data. No attempt was made to review original records to determine additional information about the test substances. Information, including substance name, CASRN, chemical and/or product class, concentration(s) tested, purity, supplier or source, and literature reference using the test substance are provided in **Appendix B**. However, if a product class was not assigned in the study report, this information was sought from other sources, including the National Library of Medicine's ChemID Plus database. Chemical classes were assigned to each test substance using a standard classification scheme, based on the National Library of Medicine Medical Subject Headings (MeSH) classification system (available at http//www.nlm.nih.gov/mesh) that ensures consistency in classifying substances among all *in vitro* ocular test methods under consideration.. A substance could be in more than one chemical or product class. **Tables 3-1** and **3-2** show the chemical classes and some of the product classes of the test substances evaluated with the ICE test method. All of the product classes are included in **Appendix B**.

Table 3-1 Chemical Classes Tested in the ICE Test Method

Chemical Class	# of Substances	Chemical Class	# of Substances
Acetate	**1**	**Inorganic Chloride Compound**	**1**
Acid	**5**	**Inorganic Salt**	**3**
Acyl halide	**1**	**Inorganic Silver/ Nitrogen Compound**	**1**
Alcohol	**15**	**Ketone**	**4**
Aldehyde	**2**	**Lactone**	**1**
Alkali	**3**	**Lipid**	**1**
Amide/Amidine	**7**	**Nitrile**	**1**
Amino Acid	**1**	**Nitro Compound**	**1**
Boron Compound	**1**	**Not Classified**	**85**
Carbohydrate	**2**	**Onium Compound**	**8**
Carboxylic Acid	**12**	**Organic Silicon Compound**	**2**
Ester	**10**	**Organic Sulfur Compound**	**3**
Ether	**1**	**Organometallic**	**2**
Heterocyclic	**9**	**Organophosphrous Compound**	**1**
Hydrocarbon	**5**	**Polycyclic**	**4**
Imide	**2**	**Polyether**	**3**
Inorganic Chemical	**1**	**Urea Compound**	**1**

Table 3-2 Product Classes Tested in the ICE Test Method

Product Class	# of Substances	Product Class	# of Substances
Adhesive	**2**	**Fertilizer**	**1**
Antifungal	**2**	**Food Additive**	**1**
Antihistamine	**1**	**Fungicide/Germicide**	**1**
Anti-infective	**3**	**Industrial Chemical, Intermediate or Formulation**	**20**
Antiseptic	**2**	**Not Classified**	**23**
Caustic Agent	**4**	**Optical Resolution Agent**	**1**
Chlorination by-product	**1**	**Paint**	**4**
Cleaner	**8**	**Pesticide/Herbicide**	**15**
Copolymer	**3**	**Preservative**	**6**
Cosmetic Ingredient	**1**	**Pharmaceutical Compound**	**5**
Detergent	**8**	**Raw Material**	**9**
Developer	**1**	**Reagent**	**4**
Disinfectant	**5**	**Resin**	**2**
Dyes & Stains	**10**	**Silicone Resin**	**1**
Elastomer	**2**	**Soap**	**9**
Enzyme Inhibitor	**1**	**Surfactant**	**25**
Enzyme Solution	**3**	**Solvent**	**37**

As shown in **Table 3-1**, the chemical classes with the greatest amount of ICE data are alcohols, carboxylic acids, esters and heterocyclics. Of the 175 substances included in **Appendix B**, 85 substances, including formulations and mixtures of unidentified composition, could not be assigned a specific chemical class.

As shown in **Table 3-2**, the most common product classes tested in the ICE assay are industrial chemicals, solvents, soaps/surfactants and pesticides/herbicides . Other product classes tested include dyes and stains, and raw materials. Of the 175 substances included in **Appendix B,** 23 substances could not be assigned a product class.

3.3.1 Prinsen and Koëter (1993)

In this study, 21 substances were tested. Substances were provided by the Fund for the Replacement of Animals in Medical Experiments (FRAME) through Aldrich Chemicals. All substances were tested undiluted, except for acetic acid, silver(I)nitrate, sodium fluorescein, and sodium hydroxide, which were tested at a concentration of 10%, 3%, 20%, and 1% (w/v) in demineralized water, respectively. No explanation was provided for the dilutions tested. No chemical class or physicochemical characteristic (e.g., pH) information was provided, but this information was gathered based on the listed supplier for each test substance.

3.3.2 Balls et al. (1995)

In this study, the substances tested were classified as acids (4), an acyl halide (1), alcohols (9), an aldehyde (1), an alkali (1), esters (6), heterocyclics (3), hydrocarbons (2), inorganics (4), ketones (3), an organophosphate (1), pesticides (5), surfactants (6), and miscellaneous (6). The authors provided CASRNs, chemical class, sources, catalog numbers, purity, form tested, and concentration tested in the report

3.3.3 Prinsen (1996)

In this study, ICE test results for 44 substances were correlated to *in vivo* rabbit ocular irritation test results. The substances tested included formulations (3), pesticides (4), detergents (3), silicone powders (2), a lubricant (1), ink (4), paint (1), a liquid nylon product (1), solvents (10), thermal paper coatings (2), toilet cleaners (2), and individual chemicals (11). The composition of the products was not provided. There were 33 liquids, 9 solids, 1 paste, and 1 gel. No other information on physicochemical characteristics (e.g., pH) was provided.

3.3.4 Prinsen (2000)

This report contained ICE test method data for four substances: cetylpyridinium bromide (6%), cyclohexylamino-functional polymethylsiloxane (PMS), dimethylcyclopentasiloxane and Triton X-500 (5%). The EU classification for each substance was provided but the corresponding rabbit eye test data were not provided. Therefore, the EPA and GHS classifications for these substances could not be determined.

3.3.5 Prinsen 2005

In this study, ICE test results for 50 substances were correlated to *in vivo* rabbit eye test results. None of these substances was classified to a particular chemical class. The substances tested included cleaners (1), copolymers (8), disinfectants (2), dyes (2), elastomers (2), enzyme solutions (3), paints (3), pesticides (1), raw materials (8), resins (2), silicone resins (1) and soaps: surfactants (6). Eleven of the substances were not classified as to product class. Of the substances tested, 28 were liquids, 13 solids, 7 emulsions and the form tested was not provided for 2 substances.

3.4 Coding Procedures Used in the Validation Studies

The coding procedures used in the reviewed literature references were evaluated only by the information provided in the published reports. No attempt was made to obtain original study records to assess these procedures.

3.4.1 Prinsen and Koëter (1993)

No specific coding mechanisms for the substances tested are detailed, and none appear to have been used. Because only one laboratory performed the ICE test method in this study (the author's laboratory), an interlaboratory evaluation was not feasible

3.4.2 Balls et al. (1995)

Test substances and participating laboratories were each assigned a numeric code in order for subsequent data analysis to be performed without knowledge of the identities of the test

substance or the laboratory. The total number of aliquots of each test substance required for the full study was determined. Computer software was then used to generate random codes for the total number of samples, so that a unique number could be assigned to each sample.

3.4.3 Prinsen (1996)

The substances used in this study were mostly proprietary compounds. While the identity of these proprietary compounds was not provided in the publication, physicochemical properties were provided for each substance, which included chemical or product class. No specific coding methods for the substances are detailed, and do not appear to have been used. Because only one laboratory performed the ICE in this study (the author's laboratory), an interlaboratory evaluation was not feasible.

3.4.4. Prinsen (2000)

The substances used in this study were surfactants and siloxane polymers. It appears that test substances were each assigned a numeric code, although the coding mechanism was not described. Because only one laboratory performed the ICE in this study (the author's laboratory), an interlaboratory evaluation was not feasible.

3.4.5 Prinsen (2005)

The substances used in this study were mostly proprietary compounds. While the identity of these proprietary compounds was not provided in the publication, physicochemical properties were provided for each substance, which included the product class. It appears that test substances were each assigned a numeric code, although the coding mechanism was not described. Because only one laboratory performed the ICE in this study (the author's laboratory), an interlaboratory evaluation was not feasible.