3.0 SUBSTANCES USED FOR VALIDATION OF THE IRE 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 any limit to the range of responses that can be evaluated by the *in vitro* test method.

Of the seventeen IRE reports considered in developing this BRD, four contained or the authors provided sufficient *in vitro* and *in vivo* data for an accuracy analysis[1]. These four reports are the CEC Collaborative Study (1991), Balls et al. (1995), Gettings et al. (1996), and Guerriero et al. (2004).

A total of 149 substances and formulations were evaluated in the four studies, of which 25 were commercial products or formulations. In the Guerriero et al. (2004) SOT study, substances that were unspecified substituted chemicals, such as pyridines, were initially placed under the single test substance name "substituted pyridines," but were assigned code numbers for differentiation with respect to data analysis. However, following the individual animal and *in vitro* IRE data submission from that study, GlaxoSmithKline granted permission to use the actual chemical names of the tested substances and provided that information along with MSDS sheets for the tested substances. **Sections 3.1.1** through **3.1.4** address the rationale for the chemicals or products tested in each of these studies.

3.1.1 CEC Collaborative Study (1991)

The Commission of the European Communities sponsored a collaborative study on possible alternative methods to the *in vivo* eye irritation test. This study was commissioned by the Division Control of Chemicals, Industrial Risks and Biotechnologies of Directorate General Environment, Nuclear Safety, Civil Protection and the Health and Safety Directorate of Directorate General Employment Industrial Relations and Social Affairs. The aim of this pilot study was to obtain reliable information about the relationship between the *in vivo* eye irritation test of Annex V and several alternative test methods (five *in vitro* assays, including an *ex vivo* IRE test method and a HET-CAM model). Twenty-one test substances were chosen to cover a full range of irritation potential. These test substances were supplied to each of three participating laboratories by the Fund for Replacement of Animals in Medical Experiments (FRAME) via a single supplier (Aldrich Chemical Company Limited, UK). Each test substance was derived from a single chemical batch. Ten of the 21 chemicals were selected from a list of 30 supplied by FRAME and the other 11 chemicals were selected because they were tested in a previous skin irritation study by the EC.

[1] The ability of the IRE test method to accurately identify test substances classified as corrosive or severe irritants 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 severe irritant is provided in **Section 4.0**.

3.1.2 Balls et al. (1995)

In the European Commission (EC)/British Home Office (HO) validation study, 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 the OECD Test Guideline (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 were found in the ECETOC data bank that met the established criteria. However, this selection was determined to be inadequate due to the relatively low number of solid substances, the insufficient number of moderate to severe irritants, and the lack of pesticides. To 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 (primarily from unpublished studies) that met the established criteria, 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 removal of the solid material from the eye one hour after application (rather than being allowed to remain continuously). Thus, the study protocols 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 Gettings et al. (1996)

This report described results from Phase III of the CTFA Evaluation of Alternatives Program, a three-phase program that evaluated promising *in vitro* alternative test methods in relation to the *in vivo* rabbit eye test. Each phase of the program evaluated a specific product type; Phases I and II evaluated hydro-alcoholic and oil/water formulations, respectively, while Phase III evaluated surfactant-based personal care cleansing formulations. The rationale for using these surfactant-based formulations was that most commercial personal care products consist of several ingredients, and that there was a need in industry to predict correctly the irritation potential of complex mixtures. The 25 products tested in Phase III were representative surfactant-containing cleansing formulations, such as hair shampoos, liquid soap, eye make-up remover, and bubble bath. The selected formulations were chosen to provide a range of ocular irritancy responses in the *in vivo* rabbit eye test (from non-irritating

to moderately irritating, which is the highest level of irritancy generally achieved by this class of products. However, ten of the formulations with irritancy in the upper end of the desired MAS range (0 to 45) in a single animal test were diluted to 25% (v/v in distilled water) of 10 of the products to provide a wider distribution of irritant responses.

3.1.4 Guerriero et al. (2004)

Guerriero and his colleagues at GlaxoSmithKline (GSK) and SafePharm Laboratories (Derbyshire, United Kingdom) presented a study at the 2002 Society of Toxicology (SOT) Meeting that evaluated 30 pharmaceutical process materials (e.g., lactam, quinidine, acetophenone, sulfonamide, benzylamine, guanidine, piperazine) using the IRE test method as described in this BRD and compared the results to data obtained *in vivo* using the Draize rabbit eye test conducted concurrently. At the 2004 SOT meeting, Guerriero et al. (2004) presented a study using the IRE test method in which they tested 14 additional substances from the ECETOC database (ECETOC 1998). The rationale for the use of these pharmaceutical process chemicals was based on the potential exposure of pharmaceutical process workers to these substances and concern for worker safety in this environment. The ECETOC substances were used to expand the database. Although the test substances reported in the Guerriero et al. (2002, 2004) studies were originally coded and generic chemical names were used (e.g., substituted pyridine) as described in **Section 3.1,** the data obtained in that study and the actual names of the chemicals were eventually provided to NICEATM with permission from GlaxoSmithKline. Chemical and product classes were assigned to the test substances, and this information was used in the performance analyses.

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

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, Chemical Abstracts Service Registry Number (CASRN), chemical and/or product class, physicochemical properties, and literature reference for the substances tested in the IRE test method are shown in **Appendix B**. A chemical class was assigned for each test substance based on information found in the literature reference. If a chemical class was not assigned in the literature reference, the information was retrieved from the National Library of Medicine's ChemID Plus database.

As shown in **Table 3-1**, the chemical classes with the greatest amount of *in vitro* IRE data are surfactant-based formulations (25), alcohols (21), heterocyclic/aromatic compounds (18), acids (17), organic compounds (16), and carboxylic acids (16). Other chemical classes tested include inorganic chemicals, alkalis, ketones, esters, ethers, amines, amides, aldehydes, carboxylic acids, hydrocarbons, organometallics, and an organophosphate. The formulations tested include hair shampoos, personal care cleansers, detergents, bleaches, and a fabric

softener. Of the 149 substances included in **Appendix B**, all were categorized within one or more chemical class.

As shown in **Table 3-2**, the most common product classes tested in the IRE assay are chemical intermediates/raw materials (38), solvents (38), soap and surfactant-based products (28), solubilizers/emulsifiers/lubricants (14), shampoo and hair care products (12), herbicides/pesticides (12). fungicide/germicide (11), flavor additives/food ingredients (8) and detergents (8). Of the 149 substances included in **Appendix B**, all were categorized within one or more product class.

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

Chemical Class	# of Substances	Chemical Class	# of Substances
Acetate/Ester	**13**	**Halogenated compound**	**1**
Acid	**17**	**Hydrocarbon**	**2**
Alcohol	**21**	**Imide**	**2**
Acyl halide	**1**	**Inorganic**	**9**
Aldehyde	**2**	**Ketone**	**8**
Alkali	**4**	**Lactone**	**1**
Amide	**5**	**Onium**	**12**
Amine	**14**	**Organic**	**16**
Amino acid	**1**	**Organometallic**	**2**
Amidine	**2**	**Organophosphate**	**1**
Boron compound	**1**	**Nitrile**	**2**
Carboxylic acid	**16**	**Nitro compound**	**4**
Cyclic hydrocarbon	**1**	**Sulfur containing**	**9**
Glycol	**0**	**Polycyclic compound**	**1**
Ester	**13**	**Surfactant, anionic**	**2**
Ether	**10**	**Surfactant, cationic**	**8**
Heterocyclic/Aromatic	**18**	**Surfactant, nonionic**	**5**
Formulation	**25**	**Surfactant-based formulations**	**25**

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

Product Class	# of Substances	Product Class	# of Substances
Active pharmaceutical ingredient	**6**	**Fungicide/Germicide**	**11**
Antiseptic/Disinfectant	**4**	**Household cleaner**	**1**
Caustic agent	**7**	**Plasticizer**	**7**
Chemical intermediate/ Raw material	**38**	**Shampoo/Haircare**	**12**
Detergent	**8**	**Soap/Surfactant**	**28**
Herbicide/Pesticide	**12**	**Solubilizer/Emulsifier/ Lubricant**	**14**
Flavor additive/Food ingredient	**8**	**Solvent**	**38**
Fragrance/Perfume	**5**	**Tanning agent**	**1**

3.4 Coding Procedures Used in the Studies

The coding procedures used in the IRE validation studies were reported in the literature. No attempt was made to review original records to assess these procedures. Based on the available information, the only reports that identified using coded chemicals were Balls et al. (1995) and Gettings et al. (1996).

3.4.1 CEC Collaborative Study (1991)

Substances evaluated in the CEC collaborative pilot study were coded, but the identity of those substances was provided to each laboratory before the study.

3.4.2 Balls et al. (1995)

In the EC/HO study, Balls et al. allocated a numeric code for each test substance. In addition, each participating laboratory in the study was allocated a unique code number to permit analysis of the data without knowledge of which laboratory actually performed the test. The number of aliquots required for each substance by all of the participating laboratories was carefully determined and random codes were generated by computer software for the total number of substances and each sample was then assigned a unique number

3.4.3 Gettings et al. (1991; 1996)

A two-part system was developed to ensure that the identity of the test substances remained unknown during testing. The first part of the identification consisted of a sample ID that was unique for each test material to be distributed. The sample ID consisted of a two letter and one number combination. If additional samples were needed, the number was increased in sequence. The two letter code was chosen at random, but was unique to each sample and laboratory. The second part of the identification consisted of a sample number (which ranged from 1 to 12). The sample numbers corresponded to the 12 test substances provided in each shipment.

3.4.4 Guerriero et al. (2004)

Substances evaluated in the GSK studies by Guerriero and his colleagues were assigned generic nomenclature for proprietary reasons and were coded numerically for the purpose of differentiating similar chemicals with various chemical substitutions (e.g., substituted pyridine). The generic nomenclature, however, was provided to the testing laboratory before the study.

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