3.0 SUBSTANCES USED FOR VALIDATION OF THE BCOP 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 23 BCOP reports considered in developing this BRD, only eight contained or provided sufficient *in vitro* and *in vivo* data for an accuracy analysis[1]. These eight reports are: Gautheron et al. (1994), Balls et al. (1995), Swanson et al. (1995), Gettings et al. (1996), Casterton et al. (1996), Southee (1998), Swanson and Harbell (2000), and Bailey et al. (2004).

A total of 161 substances and formulations were evaluated in the eight studies, of which 69 were commercial products or formulations. **Sections 3.1.1** through **3.1.8** address the rationale for the chemicals or products tested in each of these studies.

3.1.1 Gautheron et al. (1994)

In the EC interlaboratory assessment of the BCOP assay, 52 substances were studied, including 22 liquids, 22 solids, and eight surfactants (both solids and liquids). The substances were selected to:

- represent a broad range of chemical classes and structures (e.g., alcohol, polycyclic aromatic hydrocarbon, acid, base, ether, phenol, halogenated hydrocarbon)
- include a wide range of solubilities
- cover the range of ocular irritancy categories *in vivo*, from nonirritant to severe eye irritant (i.e., MAS scores ranging from 1.3 to 103)

One of the test substances, thiourea, was found to be extremely toxic via ocular exposure by Balls et al. (1995), killing the three rabbits on which it was tested. Thiourea was excluded from the accuracy and reliability analyses for the Balls et al. (1995) study. For consistency, thiourea also was excluded from the accuracy and reliability analyses for the Gautheron et al. (1994) study. Therefore, the final list of test substances included a total of 51 substances available for the accuracy and reliability analyses in **Sections 6.0** and **7.0**. However, for the EPA (EPA 1996), EU (EU 2001), and GHS (UN 2003) classification systems, three, three, and two of the *in vivo* studies, respectively, did not provide sufficient data to assign an ocular irritancy classification.

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[1] The ability of the BCOP 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 EC/HO validation study, the test substances were initially selected from the 1992 ECETOC Eye Irritation Reference Chemicals Data Bank (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 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 used for the accuracy and reliability analyses in **Sections 6.0** and **7.0**. For the EPA (EPA 1996), EU (EU 2001), and GHS (UN 2003) classification systems, six, nine, and five of the *in vivo* studies, respectively, did not provide sufficient data to assign an ocular irritancy classification.

3.1.3 Swanson et al. (1995)

Twenty full-strength industrial and household cleaning formulations were evaluated undiluted to determine the utility of the BCOP assay to predict the ocular irritation potential of these types of products. The substances were surfactant-based aqueous product formulations with pH values ranging from 1 to 14. Product types include toilet bowl cleaner, floor cleaner, meat room degreaser, all-purpose cleaner, bathroom cleaner, pot and pan cleaner, floor stripper, glass cleaner, and metal cleaner. However, only a subset of nine of these substances could be included in the accuracy evaluations described in **Section 6.0**, since *in vivo* ocular irritation classifications (i.e., EPA 1996, EU 2001, UN 2003) could not be assigned to 11 substances (see **Section 4.0**) that had been evaluated using a modified

rabbit eye test protocol which used a 30 μL test substance volume instead of the 100 μL volume on which the EPA, EU, and GHS ocular irritancy classification systems are based.

3.1.4 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 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 nonirritating to moderately irritating), which is the highest level of irritancy generally achieved by this class of products. Because it was found that a majority of the formulations produced irritant responses either in the middle (MAS ~ 45) or the nonirritating (MAS ~ 0) end of the Draize ocular irritation range, a decision was made to test dilutions (25% v/v in distilled water) of 10 of the products at the middle of the range to have a more uniform distribution of irritant responses. While there were 25 substances available for the accuracy and reliability analyses in **Sections 6.0** and **7.0**, for the EPA (EPA 1996) and EU (EU 2001) classification systems, two of the *in vivo* studies did not provide sufficient data to assign an ocular irritancy classification.

3.1.5 Casterton et al. (1996)

Ninety-seven test substances were selected primarily based on the availability of historical *in vivo* rabbit eye data. Fifteen of the test substances evaluated in the BCOP test method were selected from the formulations tested in the CTFA Evaluation of Alternatives Program – Phase III, and 48 were selected from the substances included in the ECETOC Eye Irritation Reference Chemicals Data Bank (ECETOC 1992). Twenty-one test substances were Amway products with *in vivo* data, while the remaining substances were surfactant raw materials with *in vivo* data available from the suppliers. A secondary rationale was to evaluate a wide range of chemicals and products, both industrial and consumer. However, detailed *in vivo* reference data were available for only a subset of 56, 54, or 55 of these substances for the EPA (EPA 1996), EU (EU 2001), and GHS (UN 2003) classification systems, respectively, as described in **Section 4.0**.

3.1.6 Southee (1998)

The selection of the 16 test substances in this BCOP study was based on including substances that represented a range of physical forms and irritancy and also had high quality *in vivo* eye irritation data. The test substances were selected from substances included in the ECETOC Eye Irritation Reference Chemicals Data Bank (ECETOC 1992). Fourteen of the substances had sufficient *in vivo* data to assign EPA (EPA 1996) and EU (EU 2001) classifications, while 15 of the substances had sufficient *in vivo* data to assign GHS (UN 2003) classifications.

3.1.7 Swanson and Harbell (2000)

Thirteen test substances were selected to evaluate the effect of increasing concentrations of ethanol and other solvents on the ocular irritancy of insect repellent formulations, while maintaining a constant concentration of the active ingredient. However, detailed *in vivo* reference data were available for only a subset of nine these substances, as described in **Section 4.0**.

3.1.8 Bailey et al. (2004)

The 16 test substances in this study were selected to evaluate whether the BCOP assay was useful for predicting the ocular irritation potential of unique petroleum products (e.g., lubricant additive packages, base stocks, cutting fluids, solvents, monomers). Test substances included solids, nontransparent, transparent, and semiviscous or viscous liquids. Thirteen of the substances had sufficient *in vivo* data to assign EPA (EPA 1996) and EU (EU 2001) classifications, while 14 of the substances had sufficient *in vivo* data to assign GHS (UN 2003) classifications.

3.2 Rationale for the Number of Substances Tested

The rationale for the number of substances tested in the studies is not known.

3.3 Chemicals or Products Evaluated

Descriptive information for each of the substances tested in the BCOP assay was obtained, to the extent possible, from the information provided in the study reports. When provided, the specific information extracted for each substance included its name, source/supplier, purity, CASRN, product class, concentration tested, and the study citation. No attempt was made to identify the source/supplier or the purity of a substance if the information was not included in the study report. 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. **Appendix B** provides the available information on the name, CASRN, and chemical/product class of each substance evaluated in the BCOP test method. Components of the formulations tested in the BCOP assay are also provided in **Appendix B**, to the extent this information was available. **Tables 3-1** and **3-2** provide the chemical and product classes, respectively, of the test substances evaluated with the BCOP assay. Because the purity, source/supplier, and concentration of substances tested in multiple laboratories varied depending on the testing laboratory, this study specific information is provided **Appendix B** with the BCOP test method data.

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

Chemical Class	# of Substances
Acyl halide	3
Alcohol	22
Aldehyde	1
Alkali	3
Aluminum compound	1
Amide	2
Amidine	6
Amine	10
Amino acid	4
Boron compound	1
Carboxylic acid	17
Ester	12
Ether/Polyether	9
Formulation	69
Heterocyclic compound	12
Hydrocarbon	18
Imide	2
Inorganic salt	6

Chemical Class	# of Substances
Ketone	12
Lactone	3
Nitrile compound	1
Nitro compound	2
Oil	1
Onium compound	12
Organic salt	3
Organic sulfur compound	5
Organophosphate	1
Organosilicon compound	1
Phenol	1
Polycyclic compound	3
Terpene	1
Wax	1

As shown in **Table 3-1**, the chemical classes with the greatest amount of *in vitro* BCOP data are alcohols, carboxylic acids, esters, formulations, heterocyclic compounds, hydrocarbons, ketones, and onium compounds. Other chemical classes tested include amines, ethers/polyethers, inorganic and organic salts, and organic sulfur compounds. The formulations tested include hair shampoos, personal care cleansers, detergents, bleaches, insect repellents, petroleum products, and fabric softener.

As shown in **Table 3-2**, the most common product classes tested in the BCOP assay are chemical/synthetic intermediates, cleaners, drugs/pharmaceuticals/therapeutic agents, petroleum products, solvents, shampoos, and surfactants. Other product classes tested include detergents, insect repellents, lubricants, personal care cleansers, pesticides, and plasticizers.

3.3.1 Gautheron et al. (1994)

Regarding descriptive information about the test substances, the EC interlaboratory study report includes specific chemical names of the 52 test substances, but not chemical and product classes. The physical form, and the CASRN of the test substances also are provided. Liquids were tested undiluted, while surfactants were tested at 10% in assay medium. Solids were tested either as a solution or suspension at 20% in assay medium. However, chemical characteristics, purity, and stability of the test substance in the test medium were not described.

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

Product Class	# of Substances
Adhesive	1
Agricultural chemical	2
Antifreeze agent	1
Bactericide/Fungicide/ Disinfectant/Germicide	11
Beverage	1
Bleach	3
Chelating agent	2
Chemical/synthetic intermediate	28
Cleaner	15
Cleanser (personal care)	13
Coupling agent	1
Cutting fluid	2
Degreaser	1
Dessicant	1
Detergent	11
Drug/Pharmaceutical/ Therapeutic agent and/or Metabolite	17
Dry cleaning preparation	1
Dye, in manufacture of	3
Emulsifier	1
Etching and/or electroplating	2
Explosive	1
Fabric softener	1
Fertilizer	1

Product Class	# of Substances
Flame retardant	1
Flavor ingredient	3
Food additive	1
Herbicide	3
Insect repellant	8
Lubricant/lubricant additive	6
Paint, lacquer, varnish (component)	1
Pesticide	8
Petroleum product	16
Photographic chemical/ developing agent	2
Plant growth regulator	2
Plasticizer	4
Preservative	2
Reagent	5
Shampoo (hair)	14
Soap	3
Solvent	34
Surfactant	39
Anionic surfactant	3
Cationic surfactant	6
Nonionic surfactant	5
Thermometer fluid	1

3.3.2 Balls et al. (1995)

The 51 substances tested in the EC/HO validation study, included a wide range of chemical and product classes. For each test substance, the authors provided a CASRN, chemical class, source/supplier, catalog number, purity, form tested, and concentration tested in the study report.

3.3.3 Swanson et al. (1995)

Twenty full-strength industrial and household cleaning formulations were evaluated undiluted in this study. The materials were surfactant-based aqueous product formulations with pH values ranging from 1 to 14. Product types include toilet bowl cleaner, floor cleaner, meat room degreaser, all-purpose cleaner, bathroom cleaner, pot and pan cleaner, floor stripper, glass cleaner, and metal cleaner. The authors of this study provided the components (percent composition) and pH of each formulation. The ingredients that contribute to irritancy were provided in the study publication. The formulas are from S.C. Johnson & Son, Inc. and JohnsonDiversey, Inc.

3.3.4 Gettings et al. (1996)

In this study, 25 surfactant-based cleaning formulations were evaluated in the BCOP assay, with each product tested as a 10% (w/v) solution of the formulation that had been tested *in vivo* at either 100% or a dilution of 25%. Generic names of the formulations were provided in the study report, such as Baby Shampoo No. 1, Mild Shampoo, Liquid Soap No. 1, Gel Cleaner, Skin Cleaner, Bubble Bath, and Eye Make-Up Remover. The components of each formulation were provided in the study report, including percent concentration (w/w). However, the sources/suppliers of the formulations were not provided.

3.3.5 Casterton et al. (1996)

The only descriptive information about the test substances provided in the study report is the name of each chemical or formulation tested in the BCOP assay. However, some descriptive information, such as CASRNs and chemical/product classes, could be readily obtained from other sources for a majority of the test substances.

3.3.6 Southee (1998)

In the study report for the European Community Prevalidation Study of the BCOP assay, the authors provided the chemical name, CASRN, source/supplier, catalog number, purity, form tested, and concentration tested for each test substance.

3.3.7 Swanson and Harbell (2000)

Ethanol and 12 ethanol-containing insect repellent formulations were evaluated in this study. The concentration of the active ingredient was the same in all the formulations, but the concentration of ethanol and other organic solvents varied among the formulations. The authors of this study provided the components (percent composition) of each formulation. The test substances were obtained from S.C. Johnson & Son, Inc.

3.3.8 Bailey et al. (2004)

The study report provided the name and a physical description of each test substance, and the pH for liquid materials. Information about product classes also was provided.

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 Gautheron et al. (1994)

Coding of test substances was used during the EC study. Chemicals were sampled, coded, and shipped by an independent company (MCS-Pharma, Erstein, France). The study participants were aware of the identities of the substances to be tested, and, for safety reasons, received substance codes and safety sheets to be used in case of an emergency.

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 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 Swanson et al. (1995)

The formulations were coded when tested in the BCOP assay. Test substances were assigned a numeric code by S.C. Johnson & Son, Inc., and testing was performed by laboratory personnel without knowledge of the identities of the formulations.

3.4.4 Gettings et al. (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 specific for each distribution of the sample. 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 test substances provided in each shipment.

3.4.5 Casterton et al. (1996)

Coding procedures were not discussed in this study report.

3.4.6 Southee (1998)

Test substances were each assigned a numeric code, specific for each of the three testing laboratories. BIBRA performed the coding and distribution of the test substances, which were tested blind by each of the testing laboratories. Each laboratory tested 10 coded substances on two separate occasions. The results from each laboratory were sent to BIBRA for data analysis (i.e., comparison with the proposed prediction model). The codes were broken in October 1997 for subsequent statistical analysis of the data.

3.4.7 Swanson and Harbell (2000)

The formulations were coded when tested in the BCOP assay. Test substances were assigned a numeric code by S.C. Johnson & Son, Inc., and testing was performed by laboratory personnel with knowledge that the formulations were solvent-based (primarily ethanol) insect repellents.

3.4.8 Bailey et al. (2004)

The substances were coded when tested in the BCOP assay. Testing was performed by laboratory personnel without knowledge of the identities of the formulations.