

## **Proposed Reference Substances for Validation Studies**

***[This Page Intentionally Left Blank]***

## V. Proposed Reference Substances for Validation Studies

### 1.0 Adequacy and Completeness of the Recommended List of Reference Substances

The list of proposed substances is fairly comprehensive in that the three major groups of products to which the eye is exposed (i.e., industrial chemicals, pharmaceuticals, cosmetics) are represented. Individual substances have been chosen based on: the availability of high quality *in vivo* data; commercial availability; lack of excessive hazard or prohibitive disposal costs. The substances appear to be readily available and in acceptably pure form. The range of possible ocular toxicity responses in terms of severity and types of lesions appears to be represented. Appropriately, there are presently no substances with color that will interfere with the observation of the endpoints. However, while the list covers a broad range of organic chemical classes, only two inorganic substances (sodium hydroxide and ammonium nitrate) were included. If possible, additional inorganic chemicals (including more alkali substances) that are used in consumer products should be included. Surfactants are over-represented and correspond to an area where the panel can make selective recommendations. The use of substances at different concentrations (which are included in the reference list) is important as it allows for determination of test sensitivity. However, different substance concentrations should not be included in early studies that evaluate reproducibility. The source of the *in vivo* data should be provided in the list of reference substances in each BRD. For clarity, the identity of the individuals charged with selecting the list of reference chemicals should be specified in each BRD and any potential biases among these individuals identified. Conversely, classification data for each *in vitro* test should not be included in a list of test substances that are proposed for validating *in vitro* tests, and therefore this information should be removed from the list.

Where applicable, within a chemical class, substances of lower, medium and higher molecular weight should be included (although as noted above, it is recognized that selection of substances may have been limited by the availability of high quality *in vivo* rabbit eye test data). Finally, the recommended substances should represent the entire spectrum of injury as defined by each *in vivo* test.

To declare this list adequate and complete is difficult. The current list has entirely too many substances and, thus, is unwieldy. Perhaps, a worthy effort would be to select from the list an appropriate number of specific substances that the Panel believes optimal for validation and optimization studies.

With that in mind, one possible approach for determining the adequate and most efficient number of substances could be to employ a two-stage study design for validation studies. In this two-stage approach, the first stage would be for a subset of substances to be tested in multiple laboratories to yield an estimate of test method reliability. The substances to be included in each stage would be selected from the list of recommended reference substances included in Section 12.4 of each test method BRD. In the first stage, a subset of substances (e.g., n = 10) could be tested in multiple laboratories to yield an estimate of test method reliability. Because negative substances provide little information with regard to test method

reliability, severe ocular irritants/corrosives should be the focus of this stage. Also, the nonsevere irritants or nonirritants that would be included (e.g.,  $n = 2$ ) should be moderate irritants (i.e., GHS Category 2A). This initial set of substances would cover a broad range of chemical classes, as well as encompassing the range of GHS Category 1 responses (i.e., GHS Category 1 subcategories as detailed in Section 12.4 of each test method BRD; one per chemical class and including at least one per Category 1 subcategory). Product class does not seem to be as important a factor in selecting test substances. In constructing this initial list of reference substances, the focus might be on substances to which individuals are most likely to come into contact (e.g., the 50 highest production volume non-polymeric substances in commerce). In most instances, volume of production (apart from pharmaceuticals) is a good surrogate for risk of exposure. However, it is recognized that inclusion of substances in this list is limited in part by the availability of high-quality *in vivo* rabbit eye test reference data. Therefore, representatives from the following classes would seem most appropriate for inclusion in this list: acids (organic and mineral); alkalis; amines, imines, and amides; alcohols (including polyols); ethers; esters; thiols; halides; quaternary ammonium compounds; N- and S- heterocyclics; and hydrocarbons. The list should also include a reasonable range of molecular weights, but no formulations, prototypes, or products should be included, and testing should be in several laboratories. Limiting this initial list to liquid substances (as they represent the majority of substances for which “real world” testing would be performed) would also minimize the complexity of the resulting analysis that would result from the inclusion of too many variables in this early stage.

If results from this initial stage indicate that the test method is suitably reliable, a second stage that includes a larger number of substances could be conducted to evaluate test method accuracy. During this stage, the list of substances to be tested would be expanded to include multiple representatives from each chemical class and GHS Category 1 subcategory. In addition, within each chemical class, testing substances of different physical properties (solubility, molecular weight, pH) would seem appropriate, where feasible. An issue during this stage would be the appropriate number of chemical classes necessary to assess accuracy, and the extent of generalization of results that would be anticipated across classes. A possible design might include a set of five substances per class (covering the range of irritancy responses).

Presently in each test method BRD, the criteria for selection include “substances which represent the range of known or anticipated mechanisms or modes of action for severe/irreversible ocular irritation or corrosion.” Section 1.2.2 of each test method BRD purports to discuss similarities and differences of modes and mechanisms of action between the *in vitro* test method and ocular irritancy in humans and/or rabbits. Despite a very illuminating discussion of the anatomy of the human, rabbit, bovine, and/or chicken eye, there is no discussion of mechanism of action of irritants, only a description of the effects. That criterion for agent selection should be deleted or appropriate justification provided.

Regarding health and safety concerns, laboratory personnel doing the testing should be well trained in general safety associated with handling of potentially toxic chemicals. Information regarding the test substances with respect to handling and inadvertent exposure should be readily available, if needed. Therefore, for all validation studies, Material Safety Data Sheets

(MSDS) for the recommended substances should be provided (i.e., as a coded MSDS) and prestudy safety briefings should be conducted.

## **2.0 Other Criteria that Should Be Addressed in the Selection of Reference Substances**

Substances known to induce severe lesions, *in vivo*, in the eyes of humans should be included, even in the absence of rabbit data.

***[This Page Intentionally Left Blank]***