NOTE: The European Centre for the Validation of Alternative Methods (ECVAM) has prepared a comprehensive background review document (BRD) for the Cytosensor (CM) test method and has agreed to make it publicly available for review and comment. However, they have not been able to obtain agreement for release of this document from companies that produced unpublished data that are included in the BRD. Therefore, the following draft proposed recommendations are based on data obtained from three peer reviewed publications (Balls et al. 1995, Gettings et al. 1996, Brantom et al. 1997). Subsequent to these peer review activities, ICCVAM will work with ECVAM (which is conducting a peer review of CM by the ECVAM Scientific Advisory Committee), the Japanese Center for the Validation of Alternative Methods, and Health Canada to develop final test recommendations for CM.

Draft Proposed ICCVAM Recommendations for the Cytosensor Test Method: Uses and Limitations

Use of the Cytosensor Test Method (INVITTOX Protocol Number 102) to Identify Ocular Corrosives and Severe Irritants

The database of 53 water-soluble surfactants tested using INVITTOX Protocol 102 includes 21 surfactant chemicals and 32 surfactant-containing formulations tested across seven different laboratories. Most of the 32 formulations, which are limited to cosmetic and personal care products, contain one or more surfactants at a final concentration of greater than five percent. There were no pesticide formulations included in the validation database. Using INVITTOX 102 to identify ocular corrosives and severe irritants among these surfactant-containing substances, the false positive rate ranged from 3-10% (1/29 to 3/29) when compared to *in vivo* results. The three false positives when using the EPA classification system are classified as Category II (n=2) or III (n=1) based on *in vivo* data. The one false positive when using the GHS and EU classification systems are Not Labeled based on *in vivo* data. The false negative rate ranged from 9-22% (2/23 to 5/23) when compared to *in vivo* results. In each case, these substances were classified as mild or moderate irritants *in vitro* based on the EPA, EU, and GHS classification systems (i.e., Category II/III, R36, or Category 2A/2B, respectively).

The nonsurfactant substances database for INVITTOX 102 consisted of 29 water-soluble nonsurfactant chemicals (n=27), which included a range of chemical classes (e.g., acids, alcohols, alkalis, and ketones), and nonsurfactant formulations (n=2) tested in seven laboratories. Using INVITTOX 102 to identify ocular corrosives and severe irritants among these nonsurfactant substances, the false positive rate ranged from 0-6% (0/18 to 1/18) when compared to *in vivo* results. The one false positive when using the EPA or EU classification systems was Category III and R36, respectively based on *in vivo* data. There were no false positives when using the GHS classification system. The false negative rate ranged from 43-55% (3/7 to 6/11) when compared to *in vivo* results. Three substances were false negatives when using the EPA classification system and were classified *in vitro* as either Category II/III (n=2) or IV (n=1). Five substances were false negatives when using the GHS classification system and were classified *in vitro* as either

Category 2A/2B (n=4) or Not Labeled (n=1). Six substances were false negatives when using the EU classification system and were classified *in vitro* as either R36 (n=5) or Not Labeled (n=1).

Based on these data and test method performance, ICCVAM proposes that the Cytosensor test method can be used as a screening test to identify water-soluble substances as ocular corrosives and severe irritants (i.e., EPA Category I, GHS Category 1, EU R41) in a tiered-testing strategy, as part of a weight-of-evidence approach. A substance that tests negative with Cytosensor would need to be tested in another test method that is capable of identifying possible *in vitro* false negative severe irritants and ocular corrosives and to distinguish between moderate and mild ocular irritants. Currently, the *in vivo* rabbit eye test is the only test method capable of making such a distinction.

Use of the Cytosensor Test Method (INVITTOX Protocol Number 102) to Identify Substances Not Labeled as Irritants

Using INVITTOX 102 to identify substances not labeled as irritants among the database of 53 water soluble surfactants and surfactant-containing formulations, the false negative rate ranged from 0-2% (0/27 to 1/46) when compared to *in vivo* results. The one false negative, which occurred only for the EPA classification system, was classified as Category III based on *in vivo* data. For this substance, six rabbits were included in the *in vivo* test. One rabbit had no observable effects, three rabbits had conjunctival redness (score = 1) that cleared after one (n=1) or two days (n=2), and two rabbits had corneal opacity (score = 1) that cleared after one day. The false positive rate ranged from 50-69% (3/6 to 18/26) when compared to *in vivo* results. Three substances were false positives when using the EPA classification system and were classified *in vitro* as Category II/III. Seventeen substances were false positives when using the GHS classification system and were classified *in vitro* as Category 2A/2B (n=16) or Category 1 (n=1). Eighteen substances were false positives when using the EU classification system and classified *in vitro* as R36 (n=17) or R41 (n=1).

Using INVITTOX 102 to identify substances not labeled as irritants among the database of 29 nonsurfactant substances, the false negative rate ranged from 24-38% (5/21 to 8/21)

when compared to *in vivo* results. The false positive rate ranged from 25-40% (1/4 to 2/5) when compared to *in vivo* results.

Based on these data, ICCVAM proposes that the Cytosensor test method can be used as a screening test to identify water-soluble surfactant chemicals and certain types of surfactant-containing formulations (e.g., cosmetics and personal care product formulations, but not pesticide formulations) as substances not labeled as irritants (i.e., EPA Category IV, GHS Category NL, EU Category NL) in a tiered-testing strategy, as part of a weight-of-evidence approach. However, based on the false positive rate, a substance that tests positive with Cytosensor would need to be tested in another test method that is capable of correctly identifying possible *in vitro* false positives. Positives would also need to be additionally tested with methods that can correctly identify severe, moderate, and mild ocular irritants.

Because of the high false negative rate for Cytosensor when testing water-soluble nonsurfactant substances and formulations, Cytosensor is not recommended as a screening test to identify substances not labeled as irritants among these types of substances.

Use of the Cytosensor Test Method (INVITTOX Protocol Number 102) to Identify Either Ocular Corrosives/Severe Irritants or Substances Not Labeled as Irritants

Given that the Cytosensor test method (INVITTOX Protocol Number 102) is proposed for use as a screening test to identify both ocular corrosive/severe irritants and nonirritants, specifically for water-soluble surfactant chemicals and specific types of surfactant-containing formulations (e.g., cosmetics and personal care product formulations, but not pesticide formulations), users may want to consider using Cytosensor prior to another *in vitro* ocular test method for testing these types of substances. However, water-soluble surfactant chemicals and surfactant formulations that are not identified as ocular corrosive/severe irritants or as nonirritants with Cytosensor would need to be tested in another test method(s) capable of correctly classifying substances into each of the four hazard classification categories for EPA or GHS. Currently, the only test method accepted for these purposes is the *in vivo* Draize test. Because of the high false positive rate (> 50%) for the non-irritant decision criteria, users

may not want to use Cytosensor if the intended use is to start with identifying nonirritants.

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