(Bagley et al., 1994); as well as an independent study of surfactants and surfactant-containing formulations (Triglia et al., 1989). Many of these studies were subsequently reviewed by the Interagency Regulatory Alternatives Group, as part of a workshop review to evaluate the results of voluntary data submissions of *in vitro* methods to predict Draize scores (Harbell et al., 1997).

Gettings et al. (1996) evaluated the results of 34 different *in vitro* assays in testing 25 surfactant-based formulations for the prediction of Draize scores. The *in vitro* tests were ranked by discordance and separation index (i.e., the ability of the test to rank the toxicity of the 25 chemicals with the same relative rank as the Draize test). The NHK NRU test was not among the *in vitro* tests with the lowest discordance and highest separation index. Triglia et al. (1989), testing 12 surfactant-based formulations, suggested that sensitivity and specificity of the NHK NRU were sufficient for the test to be used as a screening tool as part of a battery of *in vitro* tests. Likewise, Harbell et al. (1997), in evaluating six data sets containing 9-45 surfactant or surfactant-containing materials, concluded that the NHK NRU had sufficient performance in predicting Draize scores that the assay could be used as a screen or adjunct over the range of toxicities found in personal care and household products.

4.4 Reliability of the NHK NRU Test

The reliability of the NHK NRU assay has been less well documented than that of the 3T3 NRU assay; however several reports have described the intralaboratory and interlaboratory variability of the test. Triglia et al. (1989) reported that 10 cytotoxicity trials in a single laboratory using the surfactant sodium lauryl sulfate (SLS) at five different concentrations produced coefficients of variation (CVs) <18% for all but the lowest concentration. (The average NRU_{50} [i.e., concentration reducing NRU to 50 % of control value] from one laboratory in these trials was 4.4 µg/ml; twelve years later the same laboratory has an average NRU_{50} for SLS of 4.4 +/- 0.97 µg/ml). Triglia et al. (1989) also reported interlaboratory variability for 12 compounds replicated in four laboratories. The interlaboratory CVs for the NRU_{50} means ranged from 19% - 60%. More recently, as part of the Interagency Regulatory Alternatives Group evaluation, Harbell et al. (1997) analyzed data from two laboratories that tested 22 materials in a blind fashion. NRU_{50} values for these materials showed an excellent interlaboratory correlation of 0.99. Dickson et al. (1993) also reported on variability for the NHK NRU assay and found that the NRU_{50} values for SLS tested in four different keratinocyte isolates were nearly identical at 66.7, 67.5, 70.9 and 73.4 µg/ml. Dickson et al. (1993) used a 24 h exposure rather than the 48 h exposure used for the other tests described in Sections 4.3 and 4.4.

5.0 CONCLUSION

This document provides guidance for using *in vitro* basal cytotoxicity assays to reduce the number of animals required for the conduct of *in vivo* lethality assays. The recommended approach takes advantage of the relationship between *in vitro* IC_{50}s and *in vivo* LD_{50}s derived from the RC for 347 chemicals (Halle and Spielmann, 1992; Halle, 1998). Detailed protocols for two recommended NRU assays, one using a rodent cell line, BALB/c 3T3 cells, and one using primary human cells, NHK, are included. Guidance is also provided for qualifying these tests, or any other *in vitro* cytotoxicity assay, for use with the RC regression to predict the starting dose for lethality assays.

ACKNOWLEDGEMENT

The authors wish to thank Dr. Elke Genschow with the Centre for the Documentation and Validation of Alternative Methods, Federal Institute for Health Protection of Consumers and Veterinary Medicine (Germany) for performing the correlation analysis for Figure 1 and the regression analyses for Figures 3 and 4.