



# United States Department of the Interior

U.S. GEOLOGICAL SURVEY  
Office of the Director  
Reston, Virginia 20192

In Reply Refer To:  
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Rear Admiral William S. Stokes, Executive Director  
NTP Interagency Center for the Evaluation of  
Alternative Toxicological Methods  
National Institute of Environmental Health Sciences  
National Institutes of Health  
P.O. Box 12233, Mail Code EC-17  
Research Triangle Park, North Carolina 27709

Dear Admiral Stokes:

At the request of Dr. Samuel Wilson, Acting Director of the National Institute of Environmental Health Sciences, we have examined the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Test Method Evaluation Report "In Vitro Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests." The document was formally reviewed by Dr. Joseph E. Bunnell (U.S. Geological Survey Public Health Research Biologist) and others, and we offer the following comments.

This report is presented in the general context of ongoing international efforts to reduce the number of animals used in toxicological experimentation. The test methods, viz. *in vitro* neutral red uptake (NRU) by commercially available BALB/c 3T3 mouse fibroblast (3T3) and human epidermal keratinocyte (NHK) cell lines, were evaluated in a multi-laboratory validation study. The report consists of a two volume Background Review Document describing the results and analyses generated from the test methods evaluation study, and the ICCVAM Test Method Evaluation Report (referred to hereafter as the Report) itself. Drafts of the Background Review Document and the Report were peer reviewed by a panel of 16 experts.

The studies were designed competently and in compliance with quality assurance standards set by the U.S. Food and Drug Administration and U.S. Environmental Protection Agency. The conclusions and recommendations presented in the Report flow logically from the results of the studies, and implementation of them will likely have the intended effect of ultimately reducing and refining animal use for acute oral toxicity testing. The comprehensive Background Review Document serves as a useful reference for future studies. Overall, the Report was soundly conceived, thoroughly and critically reviewed by a large number of knowledgeable scientists, and well executed.

While it appears that the Report does reflect the recommendations offered by the Peer Review Panel Report, it is not entirely clear that all of the points raised were addressed explicitly. For instance, the Panel recommended that the "rationale for testing the positive control on separate

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plates rather than on the test plates should be provided." A more thorough rationale may have been overlooked by this reviewer, but it appears that this comment was addressed simply by the statement that "The (positive control) substance should be tested concurrently with (and independent of) the test substance." On balance, however, this is a relatively small point, and does not detract from the utility and overall high quality of the Report.

On behalf of the Department of the Interior, I thank you for the opportunity to review this document.

Sincerely,

Mark D. Myers  
Director