United States Department of the Interior

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

In Reply Refer To:
Mail Stop 300
#20030295

AUG 2 7 2003

Dr. Kenneth Olden
Director, National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, North Carolina 12233

Dear Dr. Olden:

We are responding to your letter of March 21, 2003, to Secretary Norton on testing recommendations developed by the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM), pursuant to Section 3(e)(4) of the ICCVAM Act of 2000 (Public Law 106-545). As you know, the Department of the Interior (DOI) has very limited regulatory authority related to chemical registration. We conduct some registration studies on therapeutics for aquaculture and have a substantial effort that assesses the hazards of various environmental contaminants (e.g., pesticides, industrial chemicals, metals) to natural resources (invertebrates, fish, wildlife and their supporting habitat). The documents describing *In Vitro Methods for Assessing Acute Systemic Toxicity* and the *Revised Up-and-Down Procedure for Determining Acute Oral Toxicity of Chemicals* were reviewed by scientific staff of the Bureau of Land Management, U.S. Fish and Wildlife Service, National Park Service, and the U.S. Geological Survey (USGS). Below please find comments on application and utility of these methods by the Department.

In Vitro Methods for Assessing Acute Systemic Toxicity -
The development of *in vitro* methods for the prediction of acute oral toxicity of chemicals is a worthy initiative that is gaining international momentum. At present, this effort is focused on cytotoxicity studies in mammalian cell culture lines, and the extrapolation of these data to domesticated laboratory rodents, and humans. Much of our work is focused on invertebrates, fish and wildlife whose responsiveness to toxic agents is often different from that of laboratory mammals, both from a sensitivity and mechanistic standpoint. Unfortunately, efforts to develop *in vitro* methods to predict acute oral toxicity in these nontraditional test species have been hampered for several reasons. Invertebrate and fish cell lines generally exhibit poor responsiveness to xenobiotics, and research with cell lines from other groups of species is too limited to draw conclusions about their potential utility. Scientists within the DOI recognize these problems and recommend additional work with nontraditional cell lines and test species. The development and validation of methods for extrapolation of laboratory mammal acute toxicity data to invertebrates, fish and wildlife is also warranted. Such data would potentially Reduce the number of animals used in *in vivo* acute toxicity studies and might also assist in *ecotoxicological risk assessments.*

Revised Up-and-Down Procedure for Determining Acute Oral Toxicity of Chemicals -
There are practical concerns limiting the number of animal subjects used in acute oral toxicity tests. However, the weak quality of inference obtained when using the Revised Up-and-Down Procedure may have serious natural resource consequences. Estimating the median lethal dose from six or seven animals (National Institutes of Health Publication Number 02-4501: Executive Summary and Appendix F) is dubious for species whose sensitivity and response characteristics are poorly known. If inadequate numbers of individuals are used to generate acute toxicity data, the precision of the trial (confidence interval about the median lethal dose estimate) is so poor that one could argue that the test subjects were wasted. The Revised Up-and-Down Procedure does not generate a slope of the dose-response relationship and thus has very limited utility in describing this relationship and ecological effects at concentrations found in the environment. However, the Revised Up-and-Down Procedure could serve as a corroborative method in studies comparing toxicity among species whose sensitivity and response characteristics are well known.

In view of the limited authority of DOI in the area of chemical registration, we do not have relevant test methods for which the new ICCVAM test regulations can be substituted. Nonetheless, we can report that the Up-and-Down Procedure has been used by some scientists in the USGS involved in registration studies on therapeutics for potential use in aquaculture. Use of the Revised Up-and-Down Procedure will be considered by scientists within DOI for range finding and pilot studies, although thorough ecological risk assessments require more rigorous test methods. Thank you for providing us the opportunity to comment on these new toxicological testing recommendations.

Sincerely,

Charles G. Groat
Director