OVERVIEW

Up-and-Down Procedure Methodology

New Replacement Test Guideline for Acute Oral Toxicity

Introduction:

The Environmental Protection Agency (EPA) is seeking comments of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel on the regulatory applicability of the Up-and-Down Procedure (UDP) for acute oral toxicity. The US has developed this UDP as an improved alternative method suitable to meet regulatory needs for acute oral toxicity. Accordingly, this method will replace the traditional acute oral toxicity test in OPPTS Harmonized Test Guideline 870.1100. Acute toxicity constitutes the adverse health effects that occur within a short time of administration of a single dose of a chemical. Such studies provide information on the health or environmental hazards likely to arise from short term exposure and are usually an initial step in the evaluation of the toxic characteristics of a substance. Acute toxicity is used to identify doses associated with target organ toxicity and lethality that may be referable to humans; serve as the basis for hazard classification and labeling of chemicals; provide information about the mode of toxic action of a substance; and guide diagnosis and treatment for acute toxicity.

An evaluation of the performance and relevance of the revised UDP was conducted by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The revised method was found to be acceptable as a substitute for the conventional LD50 test for acute oral toxicity. In addition, the Organization for Economic Cooperation and Development (OECD) has accepted the revised UDP guideline for use as an alternative method for testing acute toxicity. The replacement OPPTS 870.1100 is fully harmonized with the new OECD test guideline.

Use of Acute Oral Toxicity under FIFRA:

The Office of Pesticide Programs (OPP) at the EPA regulates pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Food, Drug and Cosmetic Act (FDCA). All pesticides must satisfy basic data requirements set forth in 40 CFR Part 158 for purposes of assessing risk for registration, reregistration and other regulatory functions. In addition, the EPA is charged with assessing the risks of pesticides under the Endangered Species Act. The acute toxicity test is usually performed as the initial test for a variety of hazard and risk assessment functions for human health and the environment. (This topic is discussed in a separate document titled “Use of the Up-and-Down Procedure for Acute Oral Toxicity in OPPTS.”

In OPP, results of the UDP for acute oral toxicity testing will be applied to hazard classification, determination of precautionary label language, assessing the need for worker training and personal protective clothing and equipment, requirements for child resistant packaging, and assignment of pesticide products to general use categories or restricting use to certified applicators. Results of acute oral toxicity performed according to the UDP will also be used for environmental hazard and risk assessment for nontarget terrestrial mammals and endangered species and may lead to classification for environmental hazard and/or risk mitigation requirements.

Use of Acute Oral Toxicity under TSCA:

The Office of Pollution Prevention and Toxics (OPPT) at EPA regulates industrial chemicals under the Toxic Substances Control Act (TSCA). TSCA distinguishes between "existing" chemicals (those that have been in production and appear on the TSCA Inventory) and "new" chemicals (chemicals not appearing on the Inventory which are the subject of Premanufacture Notifications (PMNs) submitted to EPA for review under section 5 of TSCA). TSCA does not impose data development requirements per se on new or existing chemicals, although EPA can obtain needed testing via authorities under TSCA (e.g., TSCA Sections 4 and 5). Thus new chemical notifications from industry are not required to include toxicity test data, although any available data must be provided in the notification. Data are submitted in approximately 10% of new chemical notices. In such submissions, acute toxicity studies are commonly provided. OPPT uses acute oral toxicity data to provide a basic understanding of acute effects and to serve as a starting point for human hazard and risk assessments focused on occupational and general population exposures.

The Up-and-Down Procedure Test Guideline:

The replacement OPPTS 870.1100 test guideline features the new UDP guideline. This guideline includes a main test and a limit test. Endpoints of the revised 870.1100 guideline are a point estimate (LD50) and confidence interval and onset, severity and reversibility of toxic signs observed in the test animals.

This replacement OPPTS 870.1100 calls for testing to be performed in a single sex to reduce variability in the test population and uses sequential dosing techniques for additional statistical power, while achieving significant reductions in animal use compared to the traditional test. The UDP is not applicable to chemicals with delayed toxicity. A flexible stopping rule limits the number of animals in the main test, while allowing the method to be applied to chemicals with a wide range of slopes of the dose-response curve. Setting initial doses at sublethal levels ensures that LD50 values are not underestimated, while reducing distress in the animals. The test performs best when all available information about the chemical is used to help determine initial dosing and the dose progression or spacing. Use of dedicated computer software facilitates the execution and calculation phases of the test. This guideline also calls for use of OECD guidance on humane endpoints to reduce pain and suffering of the test animals (OECD, 2000). As in the traditional acute test, this replacement OPPTS 870.1100 provides clinical observations of 14 days and limit testing at 5000 mg/kg.

The guideline uses the maximum likelihood estimation method for calculation of the LD50 and profile likelihood methods for estimation of most confidence intervals. A point estimate of the LD50 is only a rough descriptor of the toxicity of a chemical to a population. The confidence interval is an integral part of a statistical evaluation of toxicity data and its use will be increasingly more important since the number of animals used in testing is being decreased for animal welfare reasons. The number of animals used in a test is one of several features reflected in the width of the confidence interval. Generally, when fewer animals are used, confidence intervals are wider. The width of the confidence interval would determine appropriate use of the data for classification purposes, in risk assessment, or for comparison of toxic potential of two substances, etc. Calculation of this type of confidence interval is computationally intensive. Because of the small sample sizes and the adaptive stopping rule used in the test, the confidence interval is approximate. However, simulations based on an assumed probit form for the dose-response relationship indicate that if the slope of the dose response curve for lethality of the test population is 2 - 4 or greater, a nominal 95% confidence interval will generally have coverage of

at least 90%. At a slope of 2, many but not all scenarios had coverage of 90% or better. At a slope of 4, coverage was generally 95% or better. In some cases, the test method may not provide a point estimate, but will indicate a range of lethality. In those cases, the range estimate corresponds to a confidence interval. The guideline does not provide information about the slope of the dose-response curve.

Laboratories performing acute toxicity testing using the replacement OPPTS 870.1100 may use special software available through the EPA web site which assists the user in setting test doses, determining when the stopping rules have been satisfied, and in calculating the LD50 and confidence interval. The software will run on a personal computer and is easy to use. A manual for using the software package is also available on line and a Toxicology Summary of Test Performance is available to inform study directors and regulatory reviewers of the strengths and limitations of the revised test method in terms of the essential characteristics of the performance of the maximum likelihood LD50 estimate and the confidence intervals (ICCVAM, 2001a). In addition, a workshop is planned to orient users to the new test method.

ICCVAM Peer Review:

ICCVAM is a standing committee of the federal government, established through the National Institute of Environmental Health Sciences and with representation from 15 research and regulatory agencies, including EPA. The Committee facilitates the development, validation, and regulatory acceptance of new test methods that aid the ability to assess hazard and risk and, where possible, to reduce, refine and replace the use of animals in toxicological testing. ICCVAM has developed general criteria and a process for validation and regulatory acceptance of test methods which includes some considerations for their regulatory use (ICCVAM, 1997). In addition, ICCVAM has developed and updated guidance for the submission of new test methods for consideration in the ICCVAM validation review process (ICCVAM, 1999).

An ICCVAM Acute Toxicity Working Group, composed of federal employees from various member agencies, interacted with a design team and prepared the final submission package for peer review. The design team which includes scientists and statisticians from OPPTS/EPA, Consumer Product Safety Commission, and Procter & Gamble refined a UDP guideline for acute oral toxicity previously available through ASTM and OECD by performing thousands of computer simulations to assess the performance of the revised test method. An independent peer review panel assembled through ICCVAM deliberated in a public meeting on July 25, 2000. The peer panel evaluated the extent to which established validation and acceptance criteria had been addressed, and developed conclusions regarding the usefulness and limitations of the revised UDP. The panel also responded to the following questions:

> Has the revised UDP been evaluated sufficiently, and is its performance satisfactory to support its adoption as a substitute for the currently accepted UDP and as a substitute for the conventional LD50 test for acute oral toxicity (OPPTS 870.1100, 1998)?
>
> With respect to animal welfare, does the revised UDP adequately consider and incorporate where scientifically feasible, procedures to refine, reduce, and/or replace animal use?

The panel's conclusions were as follows:

UDP Primary Test

> "The performance of the revised UDP Primary Test is satisfactory and exceeds the performance of OECD TG 401 in providing, with fewer animals, both an improved estimate of the LD50 for the purpose of hazard classification and more accurate information on acute toxicity. In particular, the use of 0.5 log units for dose spacing is reasonable and appropriate based on experience and the results of computer simulations. Three disadvantages of the revised UDP Primary Test recognized by the Panel are: a) the increased length of time needed to conduct a study; b) the increased costs per test material evaluated; and c) the increased complexity of the protocol."

UDP Limit Test

> "The revised UDP Limit Test at 2000 or 5000 mg/kg is expected to perform as well as or better than the Limit Test in OECD TG 401, with a reduction in the number of animals needed to conduct a test."

Animal Welfare Considerations

> "The revised UDP Primary Test and the revised UDP Limit Test will reduce the number of animals used, but will not replace the use of animals. The Panel could not reach a consensus on the overall issue of refinement. However, the OECD Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluations (OECD, 2000), referenced in the revised UDP Guideline, provides an element of refinement."

During a public teleconference on August 21, 2001, the peer review panel was asked to:

> "evaluate the usefulness and limitations of the test guideline and determine the sufficiency of quality assurance procedures used to test the performance of the software developed to be used with the guideline as well as its adequacy to perform the calculations required to complete the procedure and provide LD50 and confidence interval values."

Detailed conclusions, and strengths and limitations of the UDP, as determined by the panel, are presented in the final ICCVAM report (ICCVAM, 2001b) and are as follows:

> The Panel endorsed the proposed procedure for calculating the confidence interval (CI) for the estimated LD50. However, the Panel recommended the inclusion of language in the UDP guideline and software to fully describe the limitations and uncertainties of the proposed method, and to provide appropriate cautions for interpretation of test results. The Panel noted that statistical techniques are evolving and recommended the future development of alternative approaches, such as nonparametric methods, be encouraged.
>
> The Panel concluded the software program was appropriate and suitable for establishing test doses, determining when to stop the test, estimating the LD50, and providing a CI for the LD50.

On October 10, 2001, the main body of ICCVAM reviewed and endorsed the peer review panel's findings and provided specific recommendations concerning its strengths and limitations

for regulatory use in the US. The final ICCVAM report (ICCVAM, 2001b), conveying the results of the peer review and the recommendations of the main ICCVAM body was issued November 2001. The ICCVAM agreed with the UDP peer panel that the revised UDP test guideline is acceptable as a substitute for the conventional LD50 test for acute oral toxicity for the purpose of hazard classification and for obtaining certain information on acute toxicity. The ICCVAM also agreed with the peer panel that the revised UDP will refine and reduce animal use and further concluded:

> "the revised UDP is an appropriate method for generating a point estimate for the LD50 for use in hazard classification and in estimating a confidence interval for the LD50 under specified circumstances. The revised UDP does not provide information about the slope of the dose response curve for lethality. If other human health or ecological risk assessment information is desired, including hazard dose-response and slope information, a different test should be conducted."

The new OPPTS 870.1100 test guideline for the UDP is that reviewed by the ICCVAM and reflects their conclusions and recommended modifications.

References.

ICCVAM. 1997. Validation and Regulatory Acceptance of Toxicological Test Methods. A Report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods. NIH publication No. 97-3981. National Institute of Environmental Health Sciences. Research Triangle Park, NC. Available on web site at http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/iccvam.html.

ICCVAM. 1999. Evaluation of the Validation Status of Toxicological Methods: General Guidelines for Submission to ICCVAM.. NIH publication No. 99-4496. National Toxicology Program Center for the Evaluation of Alternative Toxicological Methods. Research Triangle Park, NC. Available on web site at http://iccvam.niehs.nih.gov/docs/guidelines/subguide.htm.

ICCVAM. 2001a. The User Manual and the Toxicology Summary of Test Performance are currently available on the ICCVAM web site at http://iccvam.niehs.nih.gov/methods/udpdocs/udprpt/udp_ciprop.htm

ICCVAM. 2001b. The Revised Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals. NIH publication 02-4501. National Institute of Environmental Health Sciences. Research Triangle Park, NC.

OECD. 2000. Revised Draft Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. Organization for Economic Cooperation and Development, OECD Environmental Health and Safety Publications, Series on Testing and Assessment, No. 19. OECD, Paris. Available on web site at http://www.oecd.org/ehs/test/monos.htm.

ADDENDUM

Use of the Up-and-Down Procedure
For Acute Oral Toxicity in OPPTS

Use of the Up-and-Down Procedure for Acute Oral Toxicity under Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)

Assignment of Pesticides to Toxicity Categories:

40 CFR 156.10 provides for hazard labeling of pesticides; Part 152.160 provides for classification of pesticides; and Parts 152.170, 152.171, and 152.175 provide for restricted use of pesticides. Historically, Agency reviewers have tended to consider only the LD50 value in assigning a pesticide formulation to a toxicity category in terms of its oral or dermal toxicity. The Agency reviews have also assumed that these traditional acute toxicity studies could be relied upon to provide relatively manageable confidence intervals. Confidence limits associated with the LD50 values have generally been reported by the performing laboratories. They are usually included in Agency review summaries.

Pesticide active ingredients and formulations are categorized for acute oral toxicity using four categories with toxicity thresholds between 50 mg/kg and 5000 mg/kg. In addition, in the future, EPA will implement the new Globally Harmonized System (GHS) which uses five categories with different cut points. The Up-and-Down Procedure will allow for a smooth transition to this system since it provides point estimates of LD50. The Agency then uses these toxicity classifications to make determinations regarding restricted or general use, hazard signal word requirements, requirements for personal protective equipment, precautionary statements, and statements of practical treatment. Worker training and protection is also initiated based on hazard classification.

The basic endpoint used for such classification is the LD50 value. With the use of acute toxicity testing protocols that minimize the numbers of animals tested, it becomes more important for Agency toxicologists to consider not only the findings of a study, but also its inherent statistical limitations, in any interpretation and regulatory decision. As a result, situations where an LD50 estimate falls so close to a classification boundary that the confidence limits include values well below the boundary value, would be evaluated on a case by case basis. Under these circumstances, toxicology reviewers would normally feel comfortable with the use of 90% confidence limits, as there would then be only a 5% probability that the LD50 value would be below the lowest value of the confidence interval range. However, the reviewer would also have to take into consideration the presence or absence of signs of toxicity in the test animals, particularly in situations when severe or marked reactions occur at lower dose levels with subsequent recovery and no mortality. When the UDP provides a range instead of a point estimate for the LD50, this implies that any value within the range is equally plausible for the actual LD50. For such results, agency reviewers take a conservative approach and use the low end of the range for classification.

Requirements for Child Resistant Packaging:

FIFRA Section 25(c)(3) authorizes the Agency to establish Child-Resistant Packaging standards, consistent with those under the authority of the Poison Prevention Packaging Act (Public Law 91-601), to protect children from serious injury or illness resulting from accidental ingestion or

contact with pesticides. Child Resistant Packaging is required for residential use products with an oral LD50 value of 1500 mg/kg and less, or meeting any of the other toxicity criteria in 40 CFR 157.22(a). If there is a 5% probability that the oral LD50 value is at or lower than 1500 mg/kg, then a toxicology reviewer would recommend the use of Child Resistant Packaging. Taking into consideration the emphasis on protecting children from serious injury or illness, the Agency toxicologist would also evaluate the occurrence and severity of toxicological signs in an acute oral LD50 study at doses below which mortality occurs.

Data and Information for Use in Acute Poisoning Incidents:

The toxic signs of pesticides are characterized in the course of acute toxicity testing. Acute toxicity test results are the primary source of information about basic pesticide toxicity provided to poison control centers across the United States for use in assessing pesticide poisoning incidents and prescribing appropriate treatment. Thus, it is important to the Agency that the study reports describing a UDP toxicity test also report all the toxic signs that are observed in the test animals during the conduct of each test.

Biological Pesticides:

The Office of Pesticide Programs (OPP) has issued policies which provide incentives for registrants to develop and formulate safer pesticides. Biological pesticides provide inherently safer alternatives to many chemical pesticides currently in use. Use of reliable limit tests at 5000 mg/kg enable biological pesticides to qualify for categorization as safer pesticides with a minimum of toxicity testing.

Biochemical pest control agents are generally naturally occurring chemicals such as insect growth regulators or pheromones with nontoxic modes of action. Biochemical pesticides must be characterized by a first tier of acute, subchronic and developmental and genotoxicity data. Very often, the Agency is able to waive the subchronic or even the developmental toxicity test when it reviews the results of acute testing. In that case, a reliable LD50 value, the confidence interval, along with toxic signs, and appropriate histopathology are needed. This information can be compared to anticipated exposures in performing risk assessments for human health and the environment. Thus, even if only a limit test at 5000 mg/kg is conducted, it is important for the study report to describe the toxic signs in the test animals.

Bridging Rules for Pesticide Formulations:

Availability of reliable and suitable acute toxicity data allow the Agency to use bridging rules to waive additional tests and rely on data for substantially similar products, thus using fewer animals for testing, while maintaining levels of protection. Typically, registrants may choose to reformulate their products for purposes of eliminating classification in a highly toxic category for effects such as skin irritation by substituting less hazardous components. Much duplicative testing is eliminated when one inert is substituted for another in pesticide formulations if the new inert ingredient can be shown, using the LD50 value, to be no more acutely toxic than the original inert ingredient. In that case, the registrant may be able to test the new formulation with one new test (e.g., skin irritation), rather than submit the normal complement of acute toxicity data (acute oral, dermal, inhalation toxicity and skin and eye irritation and sensitization) for the reformulated product.

Ecological Risk Assessment for Terrestrial Mammals:

The rat is used as a surrogate for wild mammals for purposes of performing terrestrial hazard and

risk assessment. Hazard evaluation requires a good point estimate of the LD50 with confidence limits. Risk is assessed by dividing the LD50 estimate by the median expected environmental exposure. This is called the Risk Quotient method. Specific fractions of the LD50 value are used as regulatory thresholds and may lead to recommendations for risk mitigation. Use of additional characteristics of the dose-response curve including the confidence interval are also applicable in describing the weight of evidence for tier one risk assessments in assessing risks to terrestrial mammals. Confidence intervals are also necessary for estimating the overall uncertainty/variability in a distribution of risk.

The uncertainty in the LD50 estimate is an important component in estimating the overall uncertainty in a probabilistic risk assessment. To make the ecological risk assessment more robust, the Agency has developed methods for probabilistic risk assessments for pesticides. Such methods will describe ranges of risk to nontarget species and will use confidence interval and other characteristics of acute toxicity such as the slope of the dose-response curve. Policies for probabalistic risk assessment are evolving separately and will involve consideration of other testing needs under FIFRA mandates.

Endangered Species Assessments for Pesticides:

Assessment of potential risk of pesticides to endangered species requires that the likelihood of loss of any individual in the population be assessed. An Agency team systematically assesses site-specific acute risk to endangered species using the acute toxicity data for comparisons with environmental concentrations. Following initial determination of the Risk Quotient, more conservative fractions of the LD50 value are used to determine if a risk exists that needs to be addressed through protective measures.

Confidence intervals for the LD50 value are not directly used in assessing effects on endangered species because EPA assesses risks to protect individuals and not simply the typical representative (i.e., at the population mean). The slope would allow the reviewer to determine the extent of mitigations needed to achieve an endangered species no-effect level, which is what is necessary under the Endangered Species Act. No-effect levels, such as can be obtained by using the slope in conjunction with the LD50, are used for this purpose. Absent a reliable estimate of the no-effect level, a safety factor is applied to the LD50 value, and the reliability of the LD50 value, as indicated by the confidence intervals is an important feature of the test results.

Use of the Up-and-Down Procedure for Acute Oral Toxicity under Toxic Substances Control Act (TSCA)

Although many TSCA chemical testing actions do not include a requirement to conduct acute toxicity testing (such efforts generally focus on higher tier studies, e.g., subchronic toxicity, reproductive effects, etc.), acute oral toxicity testing is included in testing menus to obtain basic or "screening level" information on certain chemicals. These include two situations.

a. Higher volume/higher exposure new chemicals where TSCA section 5(e) "exposure-based" testing authorities are used to obtain a basic level of hazard and environmental fate information. Such data include acute toxicity, 28-day repeated dose toxicity, mutagenicity screen, acute ecotoxicity, biodegradation, and others.

b. High Production Volume existing chemicals (i.e., those produced and/or imported at or above 1 million lbs/yr). For these chemicals, "screening information data set" (SIDS) information is obtained, including the basic level noted above for new

chemicals as well as a 1-generation reproductive toxicity study.