Department of Pesticide Regulation

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Director

California Notice 2022-15

TO: Pesticide Registrants and Other Stakeholders

SUBJECT: GUIDANCE ON THE UP-AND-DOWN PROCEDURE DATA EVALUATION

The Department of Pesticide Regulation (DPR) evaluates the results from acute oral toxicity testing data when registering pesticides for use in California. This testing data is used to analyze the magnitude of acute toxicity (derived from the LD50 value[1]) and to assign the acute oral Toxicity Category[2] to pesticidal active ingredients and formulated end-use products. One methodology for generating acute oral toxicity data is the Up-and-Down Procedure (UDP). This notice provides guidance to applicants for submitting acute oral toxicity data using the UDP methodology in cases of partial survival when the reported LD50 point estimate falls between 450 – 550 mg/kg.

Currently, data generated by the UDP is the predominant acute oral toxicity data submitted to DPR for registration and label amendments of pesticidal active ingredients and formulated end-use products. However, the accuracy of the data generated by the UDP methodology is reduced when all available information is not considered and there is simple adherence to the guidance and use of default AOT425StatPgm software inputs (e.g., initial and subsequent dosing levels, initial LD50 input, and the default dose-progression factor). DPR scientists have concluded that if the AOT425StatPgm is run according to current OECD (2008) and US EPA guidance (2001a, 2001b) for formulations and active ingredients with true LD50s from 350 to 1000 mg/kg, the LD50s converge on a value of 550 mg/kg and are biased toward Toxicity Category III. A formulated product or pesticidal active ingredient may be more toxic than what the default UDP results convey. The evaluation and application of these data as submitted can potentially lead to

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[1] LD50 is the dose of a toxicant (or concentration for LC50) that results in the death of 50% of a group of test animals. The LD50 value is one way to quantify the toxic potential and severity of a specified chemical, compound, or mixture.

[2] US EPA Label Review Manual Chapter 7: Precautionary Statements. US Environmental Protection Agency, Office of Pesticide Programs, Registration Division. Revised March 2018. <epa.gov/sites/production/files/2018-04/documents/chap-07-mar-2018.pdf>

Table 1. Acute Toxicity Categories				
Study	Category I	Category II	Category III	Category IV
Acute Oral	$LD_{50} \leq 50$ mg/kg	$LD_{50} > 50 - 500$ mg/kg	$LD_{50} > 500 - 5,000$ mg/kg	$LD_{50} > 5,000$ mg/kg
Acute Dermal	$LD_{50} \leq 200$ mg/kg	$LD_{50} > 200 - 2,000$ mg/kg	$LD_{50} > 2,000 - 5,000$ mg/kg	$LD_{50} > 5,000$ mg/kg
Acute Inhalation	$LC_{50} \leq 0.05$ mg/l	$LC_{50} > 0.05 - 0.5$ mg/l	$LC_{50} > 0.5 - 2$ mg/l	$LC_{50} > 2$ mg/l

less health protective registration and regulatory decisions. As such, DPR will be taking a conservative approach in cases of partial survival when the reported LD50 point estimates falls between 450 – 550 mg/kg.

To provide guidance to stakeholders on data submission, DPR has developed the following information on how it will evaluate acute oral toxicity data generated by the UDP methodology as well as requirements for data submission and UDP test recommendations.

DPR requirements for UDP Data Submission in the case of partial survival

To better support registration decisions for both active ingredients and formulated products, DPR requires that all submitted acute oral toxicity data derived using the UDP methodology in the cases of partial survival with the maximum-likelihood point estimate at the boundary of Toxicity Category II and III, should include the following information:

1. **Justification for establishing the starting dose at the default value of 175 mg/kg.** In general, the UDP guidance suggests a starting dose of 175 mg/kg. However, the starting dose should be modified to account for any prior information of the lethality of a pesticidal active ingredient or formulated product (e.g., LD50 on the technical grade). If any information is available to make a more accurate selection of starting dose, it should be applied to the UDP test (US EPA, 2001a). Pesticide registrants or the conducting laboratory should provide a reasonable justification in the registration materials submitted to DPR for using the default value, including their efforts in finding (or not finding) prior toxicity data on which to base the starting dose.

2. **Justification for establishing the dose progression factor at the default value of 3.2.** All available information, including that on structurally related substances and results of any other toxicity tests that provide information on the slope of the dose-response curve should be used to define an appropriate σ (sigma; slope is the reciprocal of sigma). For test substances known to have steep slopes, dose progression factors smaller than the default should be chosen (OECD, 2008). Pesticide registrants or the conducting laboratory should provide a reasonable justification for using the default dose progression factor in the DPR registration materials.

3. **Justification for selecting the initial LD50 input.** As with the starting dose, all available information, including information on structurally related substances and results of any other toxicity tests on the test material, should be used to approximate the initial LD50 input for AOT425StatPgm to achieve the most accurate UDP result (US EPA, 2001a). Pesticide registrants or the conducting laboratory should provide a reasonable justification for estimating the initial LD50 for the test substance in the registration materials submitted to DPR.

Suggestions for Registrants

DPR suggests alternate approaches to conducting UDP studies to maximize the efficiency and accuracy of the study and test results. DPR's suggestions are within the specifications detailed in the US EPA guidelines for conducting UDP tests but can reduce or eliminate the data uncertainties involved with partial survival at the boundary of Toxicity Category II and III. DPR retains responsibility for reviewing UDP data submitted in support of pesticide registration and will assign the appropriate Toxicity Category based on the data.

1. **Set an alternate initial starting dose**

   DPR suggests that registrants select a starting dose that appropriately reflects the known or assumed toxicity of the formulated product or active ingredient as per US EPA guidance (see previous page). DPR's analysis showed that the convergence of LD50 to 550 mg/kg occurred only when the starting dose was 175 mg/kg. This anomaly can be corrected if the initial dose is set at 156 mg/kg rather than 175 mg/kg. By maintaining the dose progression factor of 3.2, the second dose would be 500 mg/kg, followed by 1600 mg/kg. If the one animal survives at 500 mg/kg and the second dies at 1600 mg/kg, then a limit test at 500 mg/kg could be performed in which four additional animals are treated with 500 mg/kg. Should 3 of 5 animals survive at 500 mg/kg then the test article would be assigned Toxicity Category III for oral hazards.

2. **Set an alternate σ (inverse of the slope of the dose-response curve)**

   A slight change in σ (sigma) can result in UDP findings that fall distinctly within either Toxicity Category II or III, thereby reducing the uncertainties with findings captured near the category boundary. For example, DPR found that setting σ to 0.34 (slope of 3) rather than the default of 0.5 (slope of 2) will result in tested doses of 175, 380, and 840 mg/kg. This change assures that if there is only partial survival at one dose, it will not be as close to the toxicity category boundary. Setting σ to a value that better reflects the steepness of the dose-response curve should be done if there is prior knowledge of a pesticide's toxicity.

3. **Conduct a Limit Test at 500 mg/kg**

   DPR will evaluate results from a limit test conducted at a 500 mg/kg dose level submitted to the department in support of product registration per US EPA guidelines (US EPA 2002). A limit test is a sequential test that uses a maximum of five animals. If 3 of 5 animals survive, the LD50 is greater than 500 mg/kg, and the test article would be assigned Toxicity Category III for oral hazards. If three animals die, the LD50 is less than 500 mg/kg, and the test article would be assigned Toxicity Category II for oral hazards.

DPR endorses the efforts to reduce animal use in toxicity testing and is especially supportive of the innovative methodologies when the data are validated and predictive of *in vivo* mammalian toxicity. As such, DPR encourages and accepts data generated by the UDP methodology for data evaluation and pesticide registration purposes. DPR recommends pesticide registrants provide data from tests conducted according to the US EPA's test guidelines which meet the pesticide registration data requirements under FIFRA (40 CFR Part 158).

If you have questions regarding the registration process, please contact the Pesticide Registration Branch Ombudsman, Mr. Aron Lindgren, at <Registration.Ombudsman@cdpr.ca.gov> or by telephone at (916) 324-3563. If you have questions regarding the evaluation of pesticides using the UDP, please contact Dr. Shelley DuTeaux, Human Health Assessment Branch Chief, at <Shelley.DuTeaux@cdpr.ca.gov> or by telephone at (916) 445-4268.

Sincerely,

Original Signed by Tulio Macedo on June 20, 2022

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    Mr. Aron Lindgren, Senior Environmental Scientist (Specialist)

Draft Guidance for Evaluating Acute Oral Toxicity Data:

Resolution of Equivocal Study Results Derived from the
Up-and-Down Procedure

June 2022

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OBJECTIVE

The Department of Pesticide Regulation (DPR) evaluates the results from acute oral toxicity testing when registering pesticides for use in California. These testing data are used to analyze the magnitude of acute toxicity (derived from the LD50 value[1]) and to assign the Toxicity Category[2] to pesticidal active ingredients and formulated end-use products. One methodology for generating acute oral toxicity data is the Up-and-Down Procedure (UDP). This guidance details how DPR evaluates UDP oral toxicity data and parameters within which DPR assigns Toxicity Categories for registration purposes.

INTRODUCTION

Acute toxicity data are required under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 7 U.S.C. 136 et seq.) for registering and regulating pesticides. Acute toxicity data provide information on the health effects, adverse reactions, and symptomology following single or short-term exposures. DPR uses these data to assess the potential hazards to humans arising from pesticide exposure. These data also provide DPR the basis for categorizing the toxicity of an active ingredient or formulated product, as well as approving the label language, such as signal words, product specific precautionary label statements, first aid statements, personal protective equipment (PPE), and restricted entry intervals (REIs). The data requirements for acute toxicity are found in Title 40 of the Code of Federal Regulations (40 CFR Part 158.500-158.510) and in Title 3 of the California Code of Regulations (3 CCR) section 6172.

Acute Oral Toxicity

Historically, the determination of the median lethal dose (LD50), or the dose that is expected to kill 50% of a test animal population, was one of the first tests conducted on a chemical to quantify its potential toxicity. The LD50 is also used as a benchmark to compare the magnitude of acute toxicity of one substance to another. The classical method for estimating the LD50 value was to orally dose individual animals in groups of five to ten per sex with varying concentrations of the test substance and observe whether the animals lived or died over a defined period following administration. The LD50 value was then derived from the dose response curve for lethality (NICEATM 2001a).

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[1] LD50 is the dose of a toxicant (or concentration for LC50) that results in the death of 50% of a group of test animals. The LD50 value is one way to quantify the toxic potential and severity of a specified chemical, compound, or mixture.

[2] US EPA Label Review Manual Chapter 7: Precautionary Statements. US Environmental Protection Agency, Office of Pesticide Programs, Registration Division. Revised March 2018. https://www.epa.gov/sites/production/files/2018-04/documents/chap-07-mar-2018.pdf

Table 1. Acute Toxicity Categories				
Study	Category I	Category II	Category III	Category IV
Acute Oral	$LD_{50} \leq 50$ mg/kg	$LD_{50} > 50-500$ mg/kg	$LD_{50} > 500-5,000$ mg/kg	$LD_{50} > 5,000$ mg/kg
Acute Dermal	$LD_{50} \leq 200$ mg/kg	$LD_{50} > 200-2,000$ mg/kg	$LD_{50} > 2,000-5,000$ mg/kg	$LD_{50} > 5,000$ mg/kg
Acute Inhalation	$LC_{50} \leq 0.05$ mg/l	$LC_{50} > 0.05-0.5$ mg/l	$LC_{50} > 0.5-2$ mg/l	$LC_{50} > 2$ mg/l

A standard practice in toxicology is to use rats and other laboratory species as surrogates to quantify and qualify potential toxicity in humans. While human data are the best indicator of potential health effects in humans, animal studies provide a reasonable and predictive alternative. An analysis of the historical database demonstrated similarities in LD50 values between laboratory species and humans for a small set of chemicals: Substances that are not toxic in the rat are often not toxic in humans, and substances that are highly toxic in the rat are often highly toxic in humans (NICEATM 2001a).

The US Environmental Protection Agency (US EPA) published test guidelines for acute toxicity using the standard LD50 protocol in October 1982 as part of Subdivision F of the Pesticide Assessment Guidelines (US EPA 1982) and in September 1985 as part of 40 CFR part 797. Since 1987, the methods have been further refined to reflect the goal of reducing animal use in toxicity testing.

History of the Up-and-Down Method

The Up-and-Down procedure (UDP) for determining acute oral toxicity was first described in detail and validated by Bruce (1985, 1987). This is a sequential test method where one animal (typically female) is dosed at the estimated LD50 (or at 200 or 500 mg/kg if no prior toxicity data is known). If the animal dies or exhibits severe morbidity, a second animal is dosed at a lower level. If the first animal survives, a second animal is dosed at an appropriately higher level. Dosing continues until four animals are dosed after the first reversal (usually a minimum of 6 animals) (Bruce 1985, 1987).

The Revised Up-and-Down-Method

Efforts to revise the UDP occurred from 1991 – 2001, with the peer review and publication of guidance documents from US EPA, the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the Organisation for Economic Co-operation and Development (OECD) (US EPA 2001a, NICEATM 2001a, 2001b, OECD 2001).

The UDP main test protocol now consists of a single ordered dose progression in which animals are dosed one at a time at 48-hour intervals. The first animal receives a dose a step below the level of the estimated LD50. If the default dose progression factor of 3.2 (slope of 2.0) is utilized and the animal survives, the dose level for the next animal is increased by 3.2 times the original dose. If it dies, the dose for the next animal is decreased by the same dose progression factor. A combination of stopping criteria is used to keep the number of animals tested low while adjusting the dosing pattern to reduce the effect of an inaccurate starting dose. Testing ends when the stopping criteria are satisfied; the LD50 and the confidence intervals are estimated based on the status of all animals at the termination of the test (NICEATM 2001a). A Limit Test is a sequential test that generally uses a maximum of five animals and a maximum dose of 2000 mg/kg (Figure 1). The selection of a sequential test plan increases the statistical power of the test and intentionally biases the procedure toward rejecting the results if the LD50 is close in magnitude to the limit dose (NICEATM 2001a). The probability of accurately assigning the LD50 and the associated Toxicity Category will increase as the actual LD50 approaches the magnitude of the limit dose (NICEATM 2001a).
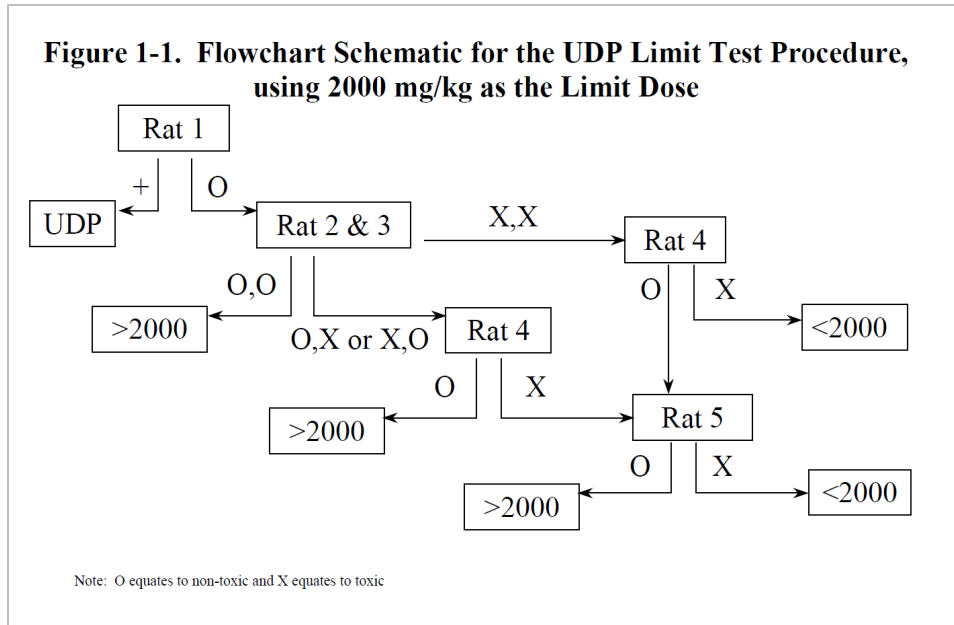
Figure 1. Flowchart schematic for the UDP Limit Test Procedure. From The Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals, Volume 2 of 2 (NICEATM 2001a). Animals that survived are designated as "O" and those that died are designated as "X" above.

In 2001, Westat (Rockville, MD) developed the Acute Oral Toxicity (Guideline 425) Statistical Program (AOT425StatPgm) to perform the statistical calculations associated with the OECD Guideline for conducting the Acute Oral Toxicity: Up-and-Down Procedure (OECD 2001) and for use with the US EPA guidelines for acute oral toxicity using UDP (US EPA 2001b). The AOT425StatPgm recommends a dose level for each animal, determines when dosing should stop, and calculates the LD50 point estimate and confidence interval. An executable version of the software as well as a user guide and recommendations for data quality assurance are available at https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/acute-oral-toxicity-and-down-procedure.

DPR USE OF UDP ACUTE ORAL TOXICITY DATA

US EPA recommends that pesticide registrants provide data from tests conducted according to the agency's Test Guidelines which meet the pesticide registration data requirements under FIFRA (40 CFR Part 158). US EPA endorsed the UDP as the preferred method for testing acute oral toxicity in 2002 (US EPA 2002). However, data from these tests was not commonly submitted for pesticide registration until several years later. Over the last 15 years, UDP has been employed with an increasing frequency as the testing method of choice, especially with the recent federal directive to reduce animal testing (US EPA 2019).

In California, data generated using UDP is the predominant acute oral toxicity data submitted for registration and label amendments of pesticidal active ingredients and formulated end-use products. DPR has been analyzing UDP acute oral toxicity data using for approximately 16 years. The first registration dataset using the AOT425StatPgm was received in 2006. In that

time, there have been rare instances when DPR's interpretation of the UDP data and assignment of Toxicity Category was more conservative than that submitted by the pesticide registrant. Out of 500 submitted UDP studies using the main test protocol, 23 pesticides were assigned an LD50 value of 550 mg/kg by the AOT425StatPgm. For 15 of these pesticides, the LD50 was reported as 550 mg/kg and toxicity was classified as Category III for oral hazards despite having greater than 50% mortality of the test animals.

To further investigate the assignment of Toxicity Category in instances of partial survival, DPR conducted an audit and analysis of UDP data submitted for registration purposes (Appendix A). Two important observations from DPR's analysis are that 1) the AOT425StatPgm software is based on the OECD toxicity categories rather than those of US EPA, and that 2) conducting laboratories overwhelmingly use the default starting dose of 175 mg/kg and dose progression factor of 3.2 rather than modifying those values based on all available toxicity information. DPR scientists have concluded that if the AOT425StatPgm is run according to current OECD (2008) and US EPA guidance (2001a, 2001b) for formulations and active ingredients with true LD50s from 350 to 1000 mg/kg, the LD50s converge on a value of 550 mg/kg and are biased toward Toxicity Category III. This imprecision is especially evident in cases of partial survival with a maximum-likelihood point estimate at the boundary of Toxicity Category II and III (LD50 > 50-500 mg/kg and LD50 > 500-5000 mg/kg respectively) and when the confidence intervals span the same toxicity categories.

The accuracy of the data generated by the UDP methodology is reduced when all available information is not considered and there is simple adherence to the guidance and use of default AOT425StatPgm software inputs, such as the initial and subsequent dosing levels, the initial input of LD50, and the default dose-progression factor. Comments were submitted to NICEATM that highlighted these concerns during the peer review of proposed revisions to the UDP methodology (NICEATM 2001a). Specifically, in test cases where an LD50 of 550 mg/kg was assigned to results with either a 25% or 75% animal mortality, commenters noted that these datasets should have been expected to give different estimates of the minimum lethal dose (MLD) (NICEATM 2001a; Pate 1989). The commenters encouraged revising the guidance to instead calculate the MLD by limiting the slope to the maximum practical value or by taking the mid-point of the profile likelihood confidence interval (NICEATM 2001a; Pate 1989).

DPR supports the efforts to reduce animal use in toxicity testing and is especially supportive of the innovative methodologies when they are validated and predictive of *in vivo* mammalian toxicity. As such, the department continues to encourage and accept data generated by the UDP methodology for pesticide registration purposes. However, there are important implications for protecting human health if UDP data are generated in a rote or inaccurate manner:

- Because of the tendency of AOT425StatPgm to generate LD50s at 550 mg/kg, even with greater than 50% mortality at that dose, a formulated product or pesticidal active ingredient may be more toxic than what the default UDP results convey.

- Bridging to already registered products with LD50s of 550 mg/kg will perpetuate the error.[3]

- Using inaccurate UDP results for acute dermal toxicity waivers (using oral data to waive acute dermal toxicity) may also perpetuate the error.

- Pesticides may be inappropriately prioritized for comprehensive human health risk assessment if the prioritization utilizes ranked LD50 values as a measure of toxicity.

Each of these situations can potentially lead to less health protective registration and regulatory decisions. As such, DPR will be taking a conservative approach in cases of partial survival when the reported LD50 point estimates falls between 450 – 550 mg/kg. The guidance for how DPR will evaluate UDP data in these cases and recommendations to reduce the data uncertainty follow.

DPR EVALUATION OF UDP DATA IN THE CASE OF PARTIAL SURVIVAL

DPR will be taking a conservative approach when utilizing US EPA's UDP guidance (US EPA 2001a, 2001b) when evaluating acute oral toxicity datasets submitted in support of pesticide registration. Cases involving partial survival at the boundary of Toxicity Category II and III will be evaluated on a case-by-case basis, and will generally conform to the following specifications:

- DPR considers 550 mg/kg a boundary dose level that separates Toxicity Category II and Toxicity Category III because of the inherent uncertainty in the AOT425StatPgm– generated LD50 point estimate.

- If there is partial survival (< 50% survive and ≥ 50% mortality) at a reported LD50 between 450 – 550 mg/kg, DPR will assign Toxicity Category II for oral hazards (see Decision Matrix below).

- Because of the inherent uncertainty in the LD50 as a point estimate, DPR will include an analysis of confidence intervals to confirm assignment of Toxicity Category per US EPA guidance (US EPA 2001a, 2001b; see Confidence Intervals below).

Decision Matrix

DPR evaluated the extent of the bias toward 550 mg/kg when using the default dose progression factor and starting value with an exploratory analysis using the AOT425StatPgm software. This analysis yielded the observation that the AOT425StatPgm software automatically assigns the LD50 to a dose if it is the only dose with partial survival. DPR then performed probability calculations to assess the likelihood that pesticides with true LD50s in Toxicity Category II will result in an estimated LD50 of 550 mg/kg. While using the default values, pesticides with a true LD60 of 550 mg/kg will have an LD50 estimated at 550 mg/kg 84.5% of the time (Appendix A). Pesticides with true LD75s of 550 mg/kg will have an LD50 estimated at

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[3] Bridging refers to the use an existing data set to characterize the hazard for another chemical for which there is little or no existing data. Generally, bridging can be supported when there is existing data on a product to address an endpoint for a proposed product so that data do not need to be generated in each case (US EPA 2012).

550 mg/kg 68% of the time if no animals die at 175 mg/kg. Even pesticides with a true LD90 at 550 mg/kg will have their LD50 estimated at 550 mg/kg 34.4% of the time if no animals die at 175 mg/kg. DPR developed the following matrix (Table 1) to use as a guide to assign Toxicity Categories for acute oral toxicity based on UDP data when there is partial survival at the boundary of Toxicity Category II and III.

Table 1. Decision Matrix for Assigning Toxicity Category with Partial Survival

Number of mortalities per total animals tested at LD50	Reported LD50 (AOT425StatPgm)		
	450 mg/kg	500 mg/kg	550 mg/kg
DPR Proposed Toxicity Category			
1/5	Category II/III	Category III	Category III
1/4	Category II	Category III	Category III
1/3	Category II	Category II	Category III
2/5	Category II	Category II	Category II
2/4	Category II	Category II	Category II
2/3	Category II	Category II	Category II
3/5	Category II	Category II	Category II
3/4	Category II	Category II	Category II
4/5	Category II	Category II	Category II

Confidence Intervals

DPR's analysis has shown that the default starting values in the UDP guidance documents and the AOT425StatPgm User's Guide (US EPA 2001a, 2001b, 2003) are biased towards generating LD50s in the range of Toxicity Category III. This is most apparent for chemicals with true LD50s between 350 – 1000 mg/kg and for which the conducting laboratories used the default starting dose and dose progression factor. Therefore, there are uncertainties inherent in relying on the AOT425StatPgm-generated LD50 as a point estimate of acute oral toxicity. To alleviate some uncertainty, DPR will consider the confidence intervals generated by the AOT425StatPgm in the weight of evidence and as a plausible bound on the true value of the LD50 for a specific active ingredient or formulated product.

The width of the confidence interval is a result of the underlying variability in the dose-response data and the total number of animals tested. Wider intervals imply less precision in the estimate of the LD50 and the likelihood that repeat testing under identical conditions would result in a different LD50 value. US EPA specifically outlined the use of confidence intervals in evaluating acute oral toxicity:

> "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, in a situation where an LD50 estimate falls so close to a classification boundary that the confidence limits (or

bracketing range) include values well below the boundary value, Agency reviewers must take a conservative approach, and classify the test material in the more toxic category. Under these circumstances, the 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, they would also have to take into consideration the presence or absence of symptoms of toxicity in the test animals, particularly in situations when severe and/or life-threatening reactions occur at lower dose levels with subsequent recovery and no mortality" (US EPA 2001a, p. 7).

Therefore, DPR will consider the confidence interval along with other measures of the validity of the test results, such as availability of dose response of the test population's tolerance to the pesticide in evaluating UDP acute oral toxicity data as per US EPA guidance (US EPA 2001a).

DPR REQUIREMENTS FOR UDP DATA SUBMISSION IN THE CASE OF PARTIAL SURVIVAL

To better support registration decisions for both active ingredients and formulated products, DPR requires that all submitted acute oral toxicity data derived using the UDP methodology in the cases of partial survival with the maximum-likelihood point estimate at the boundary of Toxicity Category II and III, should include the following information:

1. **Justification for establishing the starting dose at the default value of 175 mg/kg.** In general, the UDP guidance suggests a starting dose of 175 mg/kg. However, the starting dose should be modified to account for any prior information of the lethality of a pesticidal active ingredient or formulated product (e.g., LD50 on the technical grade). If any information is available to make a more accurate selection of starting dose, it should be applied to the UDP test (US EPA, 2001a). Pesticide registrants or the conducting laboratory should provide a reasonable justification in the registration materials submitted to DPR for using the default value, including their efforts in finding (or not finding) prior toxicity data on which to base the starting dose.

2. **Justification for establishing the dose progression factor at the default value of 3.2.** All available information, including that on structurally related substances and results of any other toxicity tests that provide information on the slope of the dose-response curve should be used to define an appropriate σ (sigma; slope is the reciprocal of sigma). For test substances known to have steep slopes, dose progression factors smaller than the default should be chosen (OECD, 2008). Pesticide registrants or the conducting laboratory should provide a reasonable justification for using the default dose progression factor in the DPR registration materials.

3. **Justification for selecting the initial LD50 input.** As with the starting dose, all available information, including information on structurally related substances and results of any other toxicity tests on the test material, should be used to approximate the initial LD50 input for AOT425StatPgm to achieve the most accurate UDP result (US EPA, 2001a). Pesticide registrants or the conducting laboratory should provide a reasonable

justification for estimating the initial LD50 for the test substance in the registration materials submitted to DPR.

SUGGESTIONS FOR REGISTRANTS

DPR suggests that there can be alternate approaches to conducting UDP studies to maximize the efficiency and accuracy of the study and test results. All suggestions are within the specifications detailed in the US EPA guidelines for conducting UDP tests but can reduce or eliminate the data uncertainties involved with partial survival at the boundary of Toxicity Category II and III. These are general recommendations. DPR maintains the responsibility of reviewing UDP data submitted in support of pesticide registration and will assign the appropriate Toxicity Category based on the data.

1. **Set an alternate initial starting dose**
   DPR suggests that registrants select a starting dose that appropriately reflects the known or assumed toxicity of the formulated product or active ingredient as per US EPA guidance (see previous page). DPR's analysis showed that the convergence of LD50 to 550 mg/kg occurred only when the starting dose was 175 mg/kg. This anomaly can be corrected if the initial dose is set at 156 mg/kg rather than 175 mg/kg. By maintaining the dose progression factor of 3.2, the second dose would be 500 mg/kg, followed by 1600 mg/kg. If the one animal survives at 500 mg/kg and the second dies at 1600 mg/kg, then a limit test at 500 mg/kg could be performed in which 4 additional animals are treated with 500 mg/kg. Should 3 of 5 animals survive at 500 mg/kg then the test article would be assigned Toxicity Category III for oral hazards.

2. **Set an alternate σ (inverse of the slope of the dose-response curve)**
   A slight change in σ (sigma) can result in UDP findings that fall distinctly within either Toxicity Category II or III, thereby reducing the uncertainties with findings captured near the category boundary. For example, DPR found that setting σ to 0.34 (slope of 3) rather than the default of 0.5 (slope of 2) will result in tested doses of 175, 380, and 840 mg/kg. This change assures that if there is only partial survival at one dose, it will not be as close to the toxicity category boundary. Setting σ to a value that better reflects the steepness of the dose-response curve should be done if there is prior knowledge of a pesticide's toxicity.

3. **Conduct a Limit Test at 500 mg/kg**
   DPR will evaluate results from a limit test conducted at a 500 mg/kg dose level submitted to the department in support of product registration per US EPA guidelines (US EPA 2002). A limit test is a sequential test that uses a maximum of five animals. If 3 of 5 animals survive, the LD50 is greater than 500 mg/kg, and the test article would be assigned Toxicity Category III for oral hazards. If three animals die, the LD50 is less than 500 mg/kg, and the test article would be assigned Toxicity Category II for oral hazards.

CONCLUSION

The Up-and-Down Procedure (UDP) for generating acute oral toxicity data is performed in the interest of limiting the number of animals used and for maximizing the dose range tested. However, at the present time, when 50% or less of the animals survive at 550 mg/kg, DPR will conservatively assign the hazard designation in the interest of being health protective.

REFERENCES

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APPENDIX A.

AUDIT AND ANALYSIS OF ACUTE ORAL TOXICITY DATA GENERATED BY THE
UP-AND-DOWN PROCEDURE (UDP) IN CASES OF PARTIAL SURVIVAL

BACKGROUND

A recent audit of the rat acute oral toxicity studies generated using the UDP methodology and the AOT425StatPgm software program revealed an anomaly inherent in the default dosing progression and the resulting LD50s. This anomaly resulted in study data whose interpretation introduced excessive uncertainty in the establishment of an appropriate Toxicity Category for hazard identification. In these instances, an LD50 value of 550 mg/kg was assigned to the test substance although more than 50% of the animals died and the 95% confidence interval ranged across two toxicity category designations. Uncertainty arose in the effort to distinguish between a Toxicity Category II and III designation. Toxicity Category III hazard designation stipulates that 500 mg/kg <LD50< 5000 mg/kg. To further evaluate the uncertainties, a more extensive two-phase analysis was conducted on the UDP data submitted to DPR.

METHOD

Phase 1

An audit of more than 500 UDP datasets submitted to DPR in support of pesticide registration was conducted. The audit involved reviewing and cataloging all UDP data from these datasets including the following:
- Test article, indicating active ingredient, percent active ingredient, and/or formulated product name
- EPA Registration number
- Study Number
- Conducting laboratory
- Dosing levels in mg/kg, including starting dose
- Total number of animals tested at each dose level
- Total number of animals who died at a dose level
- The AOT425StatPgm UDP-reported LD50 level in mg/kg
- The AOT425StatPgm UDP-generated confidence intervals in mg/kg

Phase 2

DPR conducted an exploratory analysis of the UDP to determine the reason so many studies resulted in an LD50 of exactly 550 mg/kg. To begin this analysis, DPR performed simulations in the AOT425StatPgm to test what different combinations of data would result in an estimated LD50 of 550 mg/kg. These simulations were meant to be exploratory and not a comprehensive simulation of every possible combination of data. This is because the purpose of the analysis was to understand how the UDP studies would generally end up with an LD50 estimate of 550 mg/kg, not to quantify any specific estimate or probability relating to these results.

Second, DPR examined the registrant submitted UDP studies to better understand how many had LD50 estimates of exactly 550 mg/kg, and to see what the data of these studies typically looked like. Finally, DPR used these data to better describe what probability of Toxicity Category misclassification with these UDP studies. Specifically, DPR calculated how likely it was for pesticides with true LD50s below 500 mg/kg to have an LD50 estimate of exactly 550 mg/kg when using the UDP default values and AOT425StatPgm software. To do this, DPR

created a table (A.3) of the probability of partial survival at 550 mg/kg when 550 mg/kg is a higher than a 50% lethal dose for a hypothetical pesticide.

RESULTS AND DISCUSSION

Phase 1

Over 500 UDP acute oral toxicity datasets were audited. Of these, there were 23 datasets for which at least 50% of the animals died at a dose level of 550 mg/kg. Table A.1 shows a typical finding of the treatment results in the 23 datasets in which this partial survival anomaly is apparent. For most of these datasets, the LD50 value was 550 mg/kg, with a 95% confidence interval is 197 to 863 mg/kg.

Table A.1. Example of Greater than 50% Mortality at the Reported LD50

Dosing Progression (mg/kg)	No. animals dying out of total tested
175	0/3
550*	3/4
1750	1/1

*Reported by AOT425StatPgm as the LD50; CI 197 – 863 mg/kg

Summarized findings of the audit of LD50 results for UDP datasets that reported partial survival are in Table A.2, below. DPR's analysis found that 15 of the 23 datasets had a reported LD50 of 550 mg/kg, even though there was greater than 50% mortality of the animals at that reported LD50. These 15 test substances were also designated as Toxicity Category III for oral hazards by the conducting laboratory and/or the pesticide registrant. Two additional datasets reported deaths at the highest test dose of either 1750 or 2000 mg/kg and a resulting LD50 greater than 550 mg/kg. The latter likely did not meet the stopping criteria and should have been rejected during the UDP data quality assurance process. One study tested four dose levels and the LD50 was estimated using regression of animal mortality at the tested doses and was < 550 mg/kg.

Table A.2. Analysis of UDP Studies with Partial Survival and Greater than or Equal to (≥) 50% Mortality at the AOT425StatPgm reported LD50

DPR Study Number	Test Substance Dose in mg/kg (no. animals died/ total tested at dose level)			LD50 in mg/kg (AOT425StatPgm-reported value)	Confidence Interval	
	Low	Mid	High		Lower bound mg/kg	Upper bound mg/kg
1. 237399	174 (0/3)	550 (3/4)	1740 (1/1)	550	196.1	864
2. 243783*	175 (1/4)	550 (3/4)	2000 (2/2)	311.4	114	872
3. 247255	175 (0/3)	550 (3/4)	1750 (1/1)	550	197.1	863
4. 254658	175 (0/3)	550 (2/4)	1750 (2/2)	550	267.7	1410
5. 254987	175 (0/2)	550 (2/3)	2000 (2/2)	550	124.2	1170

Table A.2. Analysis of UDP Studies with Partial Survival and Greater than or Equal to (≥) 50% Mortality at the AOT425StatPgm reported LD50

DPR Study Number	Test Substance Dose in mg/kg (no. animals died/ total tested at dose level)			LD50 in mg/kg (AOT425StatPgm-reported value)	Confidence Interval	
	Low	Mid	High		Lower bound mg/kg	Upper bound mg/kg
6. 255954**	175 (0/2)	550 (2/3)	1750 (1/2)	795.8	0	5000
7. 261129	175 (0/3)	550 (3/4)	1750 (1/1)	550	197.1	863
8. 262432†	175 (0/2)	550 (2/3)	2000 (0/1)	853.3	0	>20,000
9. 270772	174 (0/3)	550 (3/5)	1740 (2/2)	550	237.7	1010
10. 276384	175 (0/3)	550 (2/4)	2000 (2/2)	550	265.4	1520
11. 282317	175 (0/3)	550 (3/4)	1750 (1/1)	550	197.1	863
12. 283139	175 (0/3)	550 (3/4)	1750 (1/1)	550	197.1	863
13. 289303	175 (0/2)	550 (2/3)	2000 (2/2)	550	124.2	1170
14. 292891	175 (0/3)	550 (2/4)	1750 (2/2)	550	267.7	1410
15. 298355	175 (0/3)	550 (3/4)	1750 (1/1)	550	197.1	863
16. 299187	175 (0/2)	550 (2/3)	1750 (1/1)	550	92.34	2240
17. 311041	175 (0/2)	550 (2/4)	2000 (2/2)	550	191.7	1680
18. 311957	175 (0/2)	550 (2/4)	2000 (3/3)	550	209.7	1220
19. 311968	175 (0/4)	550 (3/4)	1750 (1/1)	550	235.1	835
20. 314730	175 (0/3)	550 (3/4)	1750 (1/1)	550	197.1	863
21. 316002	175 (0/3)	550 (3/4)	1750 (1/1)	550	197.1	863
22. 321535	175 (0/2)	550 (2/3)	2000 (1/1)	550	88.94	2430
23. 331490	175 (0/3)	550 (3/4)	1750 (1/1)	550	197.1	863

Shaded cells indicated study results for pesticidal active ingredients or formulated end-use products which were assigned a Toxicity Category III for oral hazards by the registrant despite having greater than 50% mortality at the reported LD50 of 550 mg/kg.
* UDP results also included 0/2 deaths at 55 mg/kg. The LD50 was derived using a maximum likelihood approach, (e.g., a form of regression analysis) mortality data at all dose levels.
** This study had partial survival at both 550 mg/kg (2/3) and 1750 mg/kg (1/2).
† Study did not meet any stopping criteria. It included partial survival at the highest tested dose, resulting in an LD50 estimate of >550 mg/kg.

Phase 2

In the exploratory simulation of UDP studies, DPR found the primary reason that so many UDP studies had an LD50 estimate of exactly 550 mg/kg was because the AOT425StatPgm software automatically assigns the LD50 estimate to a dose if it is the only dose tested with partial survival. Therefore, UDP studies with one out of five animals dying at 550 mg/kg and studies with four out of five animals dying at 550 mg/kg (and everything in between) will result in an LD50 estimate of exactly 550 mg/kg if there is no other dose with partial survival.

The exploratory analysis also found that the default dose-progression factor and starting dose specifically biased the results toward an LD50 estimate of 550 mg/kg. This bias is illustrated by Figure A.1 where the first study uses the default starting dose and the second study uses a

slightly lower starting dose, therefore changing the estimated LD50 from 550 mg/kg to 450 mg/kg. It should also be noted that the US EPA user documentation for the AOT425StatPgm software (US EPA, 2003) describes the fact that smaller sigma values will result in increased precision for the LD50 estimate but decreased efficiency at which an LD50 will be found.

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	175	O	O
2	2	550	O	O
3	3	1750	X	X
4	4	550	X	X
5	5	175	O	O
6	6	550	X	X
7	7	175	O	O
8	8	550	X	X

Estimated LD50 = **550** (The one dose with partial response).
95% PL Confidence interval is **197.1 to 863**.

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	23	142	O	O
2	24	450	O	O
3	25	1420	X	X
4	26	450	X	X
5	27	142	O	O
6	28	450	X	X
7	29	142	O	O
8	30	450	X	X

Estimated LD50 = **450** (The one dose with partial response).
95% PL Confidence interval is **160.1 to 707**.

Figure A.1. UDP Simulation Results Demonstrating the Result of Changing the Default Starting Dose. Animals that survived are designated as "O" and those that died are designated as "X" above.

The UDP studies submitted to DPR that resulted in an LD50 estimate of 550 mg/kg were generally homogenous (Figure A.2). All had partial survival at 550 mg/kg. Approximately 70% of the studies tested exactly four animals at 550 mg/kg (Table A.2). All of these studies resulted two or three deaths among animals tested at 550 mg/kg and very wide 95% confidence intervals, ranging in width from about 600 mg/kg to 2,300 mg/kg. The review of these studies confirms the potential for the UDP main test of failing to distinguish between Toxicity Categories II and III for the test substance.
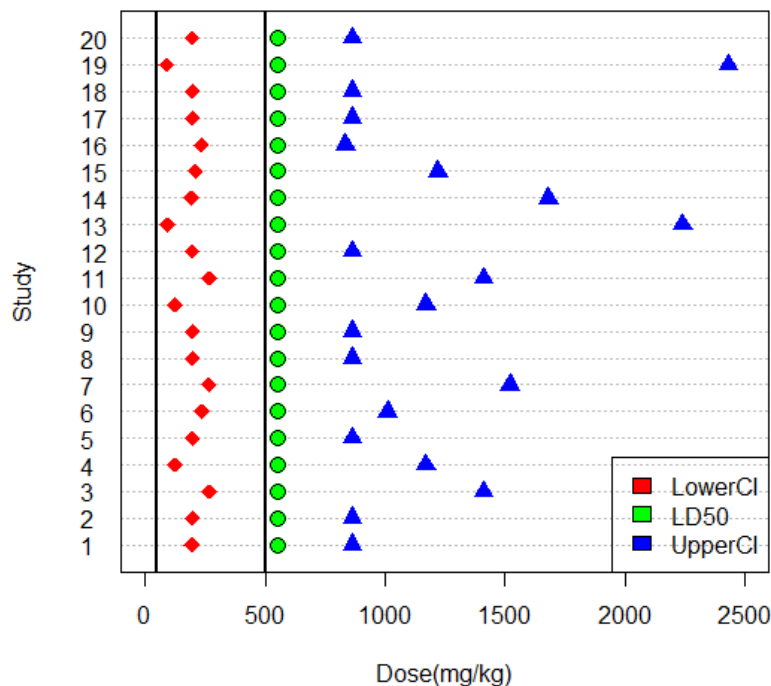

Figure A.2. UDP Studies Submitted to DPR with a Reported LD50 of 550 mg/kg

The analysis of the probability of Toxicity Category misclassification under the default values of the AOT425StatPgm software demonstrates that caution is warranted when reviewing these studies. Table A.3 displays the probability of a UDP study resulting in partial survival at 550 mg/kg when testing four animals of a hypothetical pesticide where 550 mg/kg is one of various LD percentage values. For example, this table demonstrates that a hypothetical pesticide with the true LD70 of 550 mg/kg would have partial survival at 550 mg/kg about 75 percent of the time. This highlights the high likelihood of misclassification when UDP studies result in an LD50 estimate near the Toxicity Category boundary and the need for a conservative approach when reviewing them.

Table A.3. Probability of Different Possible Number of Deaths out of Four Animals Tested at a Given Dose under Hypothetical Lethal Dose (LD) Percentages

Lethal Dose	1 out of 4 animals die	2 of 4 animals die	3 of 4 animals die	Probability of Partial Survival
LD95	0.0%	1.4%	17.1%	18.5%
LD90	0.4%	4.9%	29.2%	34.4%
LD85	1.1%	9.8%	36.8%	47.7%
LD80	2.6%	15.4%	41.0%	58.9%
LD75	4.7%	21.1%	42.2%	68.0%
LD70	7.6%	26.5%	41.2%	75.2%
LD65	11.1%	31.1%	38.4%	80.6%
LD60	15.4%	34.6%	34.6%	84.5%
LD55	20.0%	36.8%	29.9%	86.7%
LD50	25.0%	37.5%	25.0%	87.5%

CONCLUSION

DPR recently analyzed over 500 acute oral toxicity datasets generated by the UDP methodology. An audit revealed an anomaly whereby LD50 values of 550 mg/kg were assigned to multiple test substance even though more than 50% of the animals died. Further analysis showed that this anomaly and the associated data uncertainty was largely due to the use of default starting inputs in the AOT425StatPgm software program. The tests resulted in partial survival at 550 mg/kg and large confidence intervals that extended well into Toxicity Categories II and III. This analysis illustrates that if the UDP methodology is tested strictly with default values, it fails to distinguish acute oral toxicity at the boundary of Toxicity Category II and III.

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