

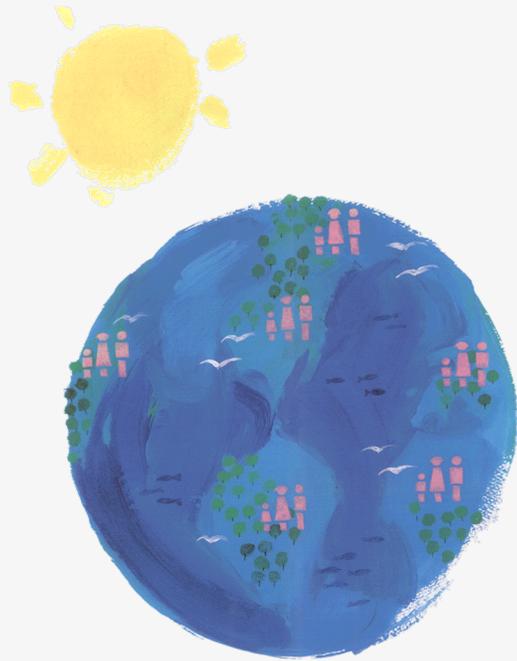
NICEATM

*National Toxicology Program
Interagency Center for the Evaluation of
Alternative Toxicological Methods*

ICCVAM

*Interagency Coordinating Committee on
the Validation of Alternative Methods*

Current Acute Oral Toxicity Regulatory Testing Requirements and Procedures



Amy Rispin, Ph.D.
U.S. EPA

ICCVAM Peer Review Panel Meeting
May 23, 2006
NIH - Natcher Center
Conf Room B
Bethesda, Maryland



Statutes and Regulations Requiring Acute Oral Toxicity Testing

Agency	Authority	Regulation	Guideline
EPA	FIFRA (1947) TSCA (1977)	40CFR	OPPTS 870.1100 UDP
CPSC	FHSA (1964)	16CFR1500.3	-
OSHA	OHSA (1970)	16CFR1500.3	-
EU	Council Directive 67/548/EEC	Commission Directive 2004/73/EC	Annex VI
OECD	-	-	Guideline 425 UDP Guideline 423 ATC Guideline 420 FDP

CFR: Code of the Federal Register; CPSC: Consumer Product Safety Commission; EPA: Environmental Protection Agency; EU: European Union; FDA:; FHSA: Federal Hazardous Substances Act; FIFRA: Federal Insecticide, Fungicide, and Rodenticide Act; OECD: Organisation for Economic Co-ordination and Development; OPPTS: EPA, Office of Prevention, Pesticides, and Toxic Substances; OSHA: Occupational Safety and Health Administration; TSCA: Toxic Substances Control Act ; UDP: Up-And Down Procedure; ATC: Acute Toxic Class Method; FDP: Fixed Dose Procedure



The *In Vivo* Acute Oral UDP Toxicity Test

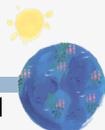
- **Staircase design in which single adult albino female rats unless otherwise indicated, are dosed in sequence at 48 hr intervals**
- **Default dosing scheme is 1.75, 5.5, 17.5, 55, 175, 550, and 5000 or 2000 mg/kg. Default starting dose is 175 mg/kg**
- **Current recommended sources for estimating the starting dose:**
 - Physical chemical properties
 - Results from other in vitro or in vivo toxicity tests on the substance (specifically, results from in vitro cytotoxicity assays are mentioned)



The *In Vivo* Acute Oral UDP Toxicity Test

■ Limit Test:

- Sequential testing
- Dose at 5000 mg/kg or 2000 mg/kg
- 3 - 6 animals



The *In Vivo* Acute Oral UDP Toxicity Test (cont)

- **Main study proceeds until first of three statistically determined stopping rules is met:**
 - 3 consecutive animals survive at limit dose (2000 or 5000 mg/kg)
 - 5 reversal occur in any six consecutive animals tested
 - 4 or more animals have followed the first reversal and the specified likelihood-ratios exceed the critical value. Three likelihood-ratios are calculated: a likelihood at an LD50 point estimate; a likelihood at a value below the point estimate (divided by 2.5); and a likelihood at a value above the point estimate (multiplied by 2.5). The ratios of the likelihood are examined to determine whether they exceed a critical value.



The *In Vivo* Acute Oral ATC Toxicity Test

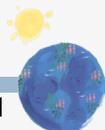
- The purpose is to classify a test substance into the appropriate GHS category for acute oral toxicity for classification and labeling; done by estimating the range of the LD₅₀ values for a test substance rather than calculating a point estimate of the LD₅₀
- Based on a stepwise administration of test substance to three adult albino rats at a time at one of a number of fixed doses: 5, 50, 300, and 2000 mg/kg / 5000 mg/kg
- **Limit Test:**
 - Sequential, 3/6 animals, 2000/5000 mg/kg.



The *In Vivo* Acute Oral ATC Toxicity Test (cont)

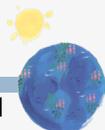
■ Main Study:

- Starting dose is selected so that at least some animals die at that dose. If no information to base starting dose is available, default starting dose of 300 mg/kg is used.
- Test at the next higher dose, or test at the next lower dose is determined by the outcome of the three animals tested at the starting dose.
- If the starting dose is 300 mg/kg and two or three animals die or are in a moribund state, the next step is to administer 50 mg/kg to three more animals.



The *In Vivo* Acute Oral FDP Toxicity Test

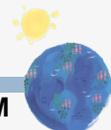
- **The principle of the test method is to use only moderately toxic doses to classify the substance according to the GHS. Lethal doses are to be avoided.**
- **Sighting Study:**
 - Starting dose expected to produce evident toxicity is selected from the fixed doses: 5, 50, 300, and 2000 mg/kg. In the absence of information, the starting dose is 300 mg/kg.
- **Limit Test:**
 - If expected to be non-toxic, a dose of 2000 or 5000 mg/kg to one animal followed by 4 additional animals.



The *In Vivo* Acute Oral FDP Toxicity Test (cont)

■ Main Study

- After sighting study identifies dose that does not cause death four additional animals are dosed. Dose levels are selected from doses of 5, 50, 300, and 2000 mg/kg (and 5000 mg/kg, if necessary).
- Next dosing is determined by the onset, duration, and severity of the toxic signs. Treatment of animals is delayed until there is confidence of survival of previously dosed animals (usually 3-4 days).
- Based on the observations one of three actions is taken: stop testing and assign the appropriated GHS hazard class; test at a higher dose; or test at a lower dose.
- For example, if at 300 mg/kg, death or toxicity is observed then Category 3. If no toxicity, then go to a dose of 2000 mg/kg and if toxicity is observed, assign to Category 4.

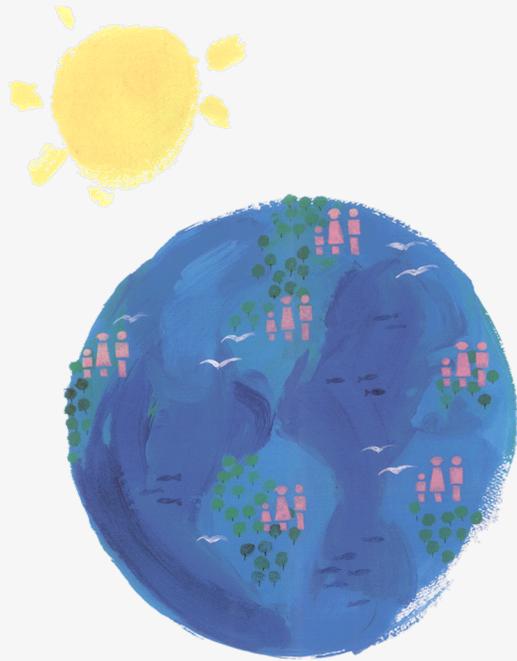


NICEATM

*National Toxicology Program
Interagency Center for the Evaluation of
Alternative Toxicological Methods*

ICCVAM

*Interagency Coordinating Committee on
the Validation of Alternative Methods*

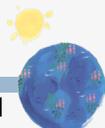


Overview of Acute Oral Toxicity Regulatory Applications



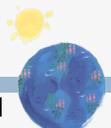
Regulatory Applications

- **Classification and Labeling**
- **Risk Assessment**
- **Risk Management**
 - Protective equipment
 - Restrictions on use



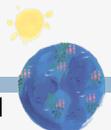
Acute Endpoints

- **Lethality ranges**
- **Point estimate of LD₅₀**
- **Confidence Interval around point estimate**
- **Toxic Signs**



Classification

- **Acute oral toxicity classification definitions and criteria vary among regulatory hazard classification systems (EPA, CPSC, DOT, EU, GHS, FHSA)**



EPA Classification System

EPA Category	<i>In Vivo</i> Acute Oral LD ₅₀ Value
I	≤50 mg/kg
II	>50 to ≤500 mg/kg
III	>500 to ≤ 5000 mg/kg
IV	>5000 mg/kg



EPA Labeling Statements

Toxicity Category	Signal Word	Statements
I	DANGER-POISON Skull & Crossbones required	Fatal if swallowed. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum or using tobacco
II	WARNING	May be fatal if swallowed. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum or using tobacco
III	CAUTION	Harmful if swallowed. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum or using tobacco
IV	CAUTION (optional)	No statements are required.



EU Classification System

R-phase (corresponding to acute toxicity classification symbol)	LD ₅₀ Range (rat)
R28 (very toxic if swallowed)	≤ 25 mg/kg
R25 (toxic if swallowed)	>25 to ≤ 200 mg/kg
R22 (harmful if swallowed)	>200 to ≤ 2000 mg/kg

CPSC Classification System

CPSC Category	<i>In Vivo</i> Acute Oral LD ₅₀ Value
1	≤50 mg/kg
2	>50 thru 5000 mg/kg



DOT Packing Group Categories

DOT Category	<i>In Vivo</i> Acute Oral LD ₅₀ Value
1	≤5 mg/kg
2	>5 to ≤ 50 mg/kg
3 (solids)	>50 to ≤ 200 mg/kg
3 (liquids)	>50 to ≤ 500 mg/kg



GHS Classification System

Category	<i>In Vivo</i> Acute Oral LD ₅₀ Value
1	≤5 mg/kg
2	>5 to ≤ 50 mg/kg
3	>50 to ≤ 300 mg/kg
4	>300 to ≤ 2000 mg/kg
5	>2000 to ≤ 5000 mg/kg
Unclassified	>5000 mg/kg



GHS Labeling Statements

Category	Symbol	Signal Word	Statements
1	Skull-Crossbones	Danger	Fatal if swallowed
2	Skull-Crossbones	Danger	Fatal if swallowed
3	Skull-Crossbones	Danger	Toxic if swallowed
4	Exclamation Point	Warning	Harmful if swallowed

