LD_{50} or MLD/kg

Toxins	Man	Monkey	Mouse	Guinea Pig	Rabbit
Botulinum toxin A	10 ng		1.2 ng	(0.6 ng)	(0.5 ng)
Botulinum toxin B			1.2 ng	0.6 ng	
Botulinum toxin C		1/3 mouse		1 x mouse	1/8 mouse
Botulinum toxin D		(40 ng)	<0.4 ng	(0.1 ng)	(0.08 ng)
Botulinum toxin E (proteolytically activated)		(1.1 ng)	1.1 ng	(0.6 ng)	1.1 ng
Botulinum toxin F					
Tetanus toxin	<2.5 ng		7 ng	~ 2 ng	(.05–5 ng)
Shigella dysenteriae neurotoxin		(4.5 ng)	1.3 µg	>9 µg	0.9 ng
Diphtheria toxin	≤100 ng		200 µg	160 ng	
Abrin			600 ng		
Cl. perfringens Epsilon toxin (trypsin activated)			250 ng 1 µg		
Staphylococcal Alpha toxin			40–60 µg		1.3 µg
Ricin			3 µg		
Pseudomonas aeruginosa exotoxin A			3 µg		
Streptolysin O — O-labile hemolysins			10–25 µg	as rabbit	3 µg
Cl. perfringens Theta toxin — O-labile hemolysins			13–16 µg		5–8 µg
Pneumolysin — O-labile hemolysins					4.4 µg
Cereolysin — O-labile hemolysins			40–80 µg		
Listereolysin — O-labile hemolysins			3–12 µg		
and presumably likewise for similar hemolysins produced by other Clostridium and Bacillus species					
P. pestis murine toxins A or B			35 µg, ~50 µg		
B. pertussis toxin			< or <<60 µg, 5 µg		
B. anthracis. Lethal factor (with PA)			(Rat) <114 µg		
S. aureus Beta toxin			500 µg–5 mg	40–400 µg	3–30 µg ?
Cholera Toxin (i.v.)			250 µg (less enterally)		
LT (i.v.)			presumed 250 µg		
Cl. perfringens enterotoxin			300 µg		

Type of data to be used by ORDA and the Ad Hoc Working Group on Toxins in evaluating containment levels for experiments utilizing genes coding for the biosynthesis of molecules toxic for vertebrates

Appendix G specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of toxins for vertebrates. Cloning of genes coding for toxins for vertebrates that have an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxins, diphtheria toxin, Shigella dysenteriae neurotoxin) is covered under Section III-A-1 of the Guidelines and requires RAC review and NIH and IBC approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight. A list of toxins classified by LD_{50} is available from ORDA. The following procedures should be used in evaluating the toxicity of toxins not on the ORDA list. The results of the tests shall be forwarded to ORDA, which will consult with the ad hoc Working Group on Toxins prior to including a toxin on the list (See Section IV-E-1-b-(3)-(i)).

a) Data on human toxicity are paramount in fixing containment levels.

b) If human toxicity is not known, toxicity may be inferred pro tem from assays of toxicity to other primates (intravenous injection of at least four animals).

c) If neither human nor other primate toxicity is known, containment levels shall be determined from the LD_{50} of the most sensitive of

three small animal species, namely mice, guinea pigs, and rabbits using intravenous injection into at least four animals of each species (12 animals total).

The toxin used for the tests must be of good quality without substantial denaturation or chemical alteration from the most effective form. The purity must be known sufficiently to determine the content of specific agent. If the purity is in doubt the most conservative assumption should be made.

When two or more proteins act in synergy to form a toxic principle and the components are to be cloned separately under conditions that rigorously preclude the comingling of the separate clones, the LD_{50} of each component may be used to set containment conditions. Additional precautions may be desirable if synergy or potentiation ($\geq$100 fold) occur between toxins normally produced by the host-vector system and the toxin to be cloned as, for example, occurs in the case of S. aureus exotoxins enhancing the toxicity of E. coli endotoxin. If information on potentiation becomes available, the PI should contact ORDA for further guidance.