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UC Davis
Institutional Animal Care and Use Committee (IACUC)

Title: Acceptable Maximum and Absolute Maximum Volumes for Research Compound Administration

I. Purpose:

The purpose of this policy is to provide researchers with the acceptable maximum (routine) and absolute maximum (one-time) volumes of fluids or compounds that may be administered to animals at a single anatomical site for research purposes. These guidelines apply to healthy, adult animals and are not intended for clinical application. When immature or clinically debilitated animals are used, veterinary staff must be consulted, as the volume guidelines provided in the table below may not be applicable.

II. Background:

The administration of excessive dose volumes may produce pain, excitement, and altered physiological parameters (e.g., serum electrolyte imbalance, increased blood pressure, and increased respiratory rate) and alter compound absorption.

III. Policy:

The following table provides acceptable maximum and absolute maximum daily cumulative volumes in **mL/kg body weight** for route of administration, unless otherwise specified.

Acceptable maximum volumes of administration are **bolded**. These are **routine, acceptable volumes of administration** that typically can be given multiple times to the same animal.

For select species and routes of administration, *absolute maximum volumes*[a] are *italicized* and provided in parenthesis. These *absolute maximum volumes* denote less frequent administration such as one-time dosing.

These volume guidelines must be followed unless otherwise specified in your approved IACUC protocol.

Species	Oral, Gavage	Subcutaneous [a]	Intraperitoneal Intracoelomic	Intramuscular[a]	Intravenous Bolus (slow injection)
Mice	**10**[6] (*50*)[2]	**10**[2] (*40*)[2]	**20**[14] (*80*)[2]	**0.05**[a,b,6] (0.1)[a,b,6] Note: this route is not recommended in this species	**5**[2] (*25*)[6]
Rats	**10**[6] (*40*)[2]	**5**[6] (*10*)[2,14]	**10**[6] (*20*)[6]	**0.1**[a,b,6] (*0.2*)[a,b,6] Note: this route is not recommended in this species	**5**[6] (*20*)[6]
Rabbits	**10**[6] (*15*)[2]	**1**[14] (*2*)[14]	**5**[6] (*10*)[6]	**0.25**[2,6] (*0.5*)[d,2,6]	**2**[6] (*10*)[6]
Guinea Pigs	**10**[6] (*20*)[6]	**5**[6] (*10*)[6]	**10**[6] (*20*)[6]	**0.1**[a,b,6] (*0.2*)[a,b,6]	**1**[6] (*5*)[6]
Hamsters	**10**[6] (*20*)[6]	**5**[6] (*10*)[6]	**10**[6] (*20*)[6]	**0.1**[a,b,6] (*0.2*)[a,b,6] Note: this route is not recommended in this species	**5**[6] (*20*)[6]
Gerbils	**10**[6] (*20*)[8]	**5**[6] (*10*)[6]	**10**[6] (*20*)[6]	**0.1**[a,b,6] (*0.2*)[a,b,6] Note: this route is not recommended in this species.	**5**[6] (*20*)[6]
Dogs	**5**[6,14] (*15*)[6]	**1**[6] (*2*)[6]	Note: this route is not recommended in this species	**0.25**[6] (*0.5*)[d,6]	**5**[6] (*10*)[6]
Cats	**10**[6,14] (*15*)[6]	**2**[6] (*5*)[6,14]	Note: this route is not recommended in this species	**0.25**[6] (*0.5*)[d,6]	**5**[6] (*10*)[6]
Ferrets	**10**[6] (*15*)[6]	**2**[6] (*5*)[6]	**5**[6] (*20*)[6]	**0.25**[6] (*0.5*)[d,6]	**5**[6] (*10*)[6]

Sheep and Goats	**20**[14]	**5**[14]	Note: this route is not recommended in this species	**5**[b,e,14]	**5**[8,14]
Bovine	**20**[14]	**5**[14]	Note: this route is not recommended in this species	**10**[b,e,14]	**5**[8,14]
Horses			Note: this route is not recommended in this species	**10**[b,e,14]	
Macaques	**5**[6] (*15*)[6]	**1**[6] (*2*)[6]	**3**[6] (*10*)[6] Note: this route is not recommended in this species	**0.25**[6] (*0.5*)[d,6]	**5**[6] (*10*)[6]
Marmosets	**10**[6] (*15*)[6]	**2**[6] (*5*)[6]	**5**[6] *(20)*[6]	**0.25**[6] (*0.5*)[a,b,6]	**2.5**[6] (*10*)[6]
Minipigs	**10**[6] (*15*)[6]	**1**[6] (*2*)[2]	**1**[6] (*20*)[6]	**0.25**[b,e,6] (*0.5*)[b,e,6]	**5**[6] (*10*)[6]
Pigs	**20**[14]	**5**[14]	**10**[14]	**5**[b,e,14]	**5**[14]
Birds (<u>domestic</u> fowl)	**5**[8]	**5**[26]	**10-15** mL total per injection (midline, halfway between cloaca and sternum)[9] Note: this route is not recommended in this species	**1-2** mL total per injection (pectoral muscles; divided into multiple injection sites)[9]	**2-3** mL total per injection (brachial vein)[9]
Fish	**2** g/kg[c,8]	**1**[8]	*(10)*[8]	*(0.05)*[b,8]	**5**[8] (2)[8]

Frogs	Note: this route is not recommended in this species	**1**[8]	*(10)*[8]	(*0.05*)[b,8]	**5**[8] (2)[8]

[a]For non-aqueous compounds, consideration must be given to the time of absorption before re-dosing. **Generally, no more than two intramuscular sites should be used per day, but there may be reasonable exceptions such as for larger species and only when specified within an IACUC-approved protocol**. **Subcutaneous sites should be limited to two to three sites per day**. Please note, the reference for SQ administration does not include injections of Freund's adjuvant and other irritants that must be assessed based on specific study designs.
[b]Volumes in mL per site.
[c]Administered via gel capsules
[d]Intramuscular injections should not exceed the following <u>total volumes of injection</u>, by species (unless otherwise approved per protocol): rabbits, cats, ferrets ≤ 1mL[6]; macaques ≤ 2mL[6]; dogs ≤3mL[6].
[e]Intramuscular injections should not exceed the following <u>total volumes per site</u>, by species (unless otherwise approved per protocol): minipigs ≤ 5mL[6]; sheep, goats, pigs ≤ 5mL[14]; horses, cattle ≤ 10mL[14].

IV. <u>Additional routes, techniques, and volumes of administration:</u>

A. Epidural, Intrathecal, and Intracranial[8]
   1. Epidural and intrathecal administration should only be performed on heavily sedated animals and by staff specifically trained in these techniques.
      i. Epidural, Mammals: 0.15–0.2 mL/kg (6 mL total volume in animals up to 35 kg)
      ii. Intrathecal, Mammals: 0.075-0.1 mL/kg (intrathecal volumes are typically 50% of epidural volumes)
   2. Intracranial injections require additional equipment, training, and project-specific considerations; please consult Campus Veterinary Services for guidance.

B. Intranasal and Intratracheal
   1. Additional best practices apply when using intranasal and intratracheal routes. To minimize respiratory complications, compounds should be administered slowly with close monitoring of respiratory rate and effort. Campus Veterinary Services may be consulted for questions on suitability of specific substances administered via these routes to minimize irritation or damage to respiratory tissues.
   2. Intranasal injection should be split into two or more aliquots and administered in alternating nostrils with at least one minute between administration to each nostril to allow drug absorption and airway clearing prior to administration in the contralateral nostril.

3. Maximum recommended intranasal volumes per day:
    i. Adult rodents: 0.020-0.025 mL per nostril (total volume per animal 0.05 mL[7,13,14,15])
    ii. Adult dogs, cats, rabbits, ferrets, swine, nonhuman primates: 0.1 mL-0.25 mL per nostril (total volume per animal 0.2-0.5 mL[8,14])
4. Maximum recommended intratracheal volumes:
    i. Adult mice, rats, hamsters, guinea pigs: 0.5 mL per animal[12,13]
        - Adult guinea pigs can also be administered an absolute maximum of 0.2 mL as a one-time intratracheal dose[13]
    ii. Adult rabbits: 0.2 mL per animal[13]
        - Adult rabbits can also be administered an absolute maximum of 0.5 mL as a one-time intratracheal dose[13]

C. Intradermal[11,13,14].
    1. The maximum intradermal dose for all species is 0.05-0.10 mL/site and dependent on skin thickness.
    2. Please see footpad injections below for additional information regarding this administration site.

D. Retro-orbital[15]
    1. Retro-orbital administration maximum values: adult mice 0.15 mL, neonatal mice 0.01 mL.

E. Rectal[16]
    1. Rectal administration maximum values: mouse <0.5 mL.

F. Intraosseous[11]
    1. Intraosseous administration maximums: mouse 0.20 mL, rat and guinea pig 0.50 mL, gerbil 0.10 mL, hamster 0.30 mL, rabbit 1-5 mL.

G. Footpad Injections[17,18,19,20]
    1. When scientifically justified, footpad injections are most often completed in mice and rats. It is contraindicated to complete footpad injections in rabbits due to the lack of the anatomic structure in their feet (no footpads).
    2. Acceptable maximum volume of injection is 0.05 mL intradermally into the footpad for mice and up to 0.1 mL intradermally into the footpad for rats.
    3. Application of isopropyl alcohol or other appropriate disinfectant over the injection site is recommended.
    4. Because of prehension of food and manipulation of other environmental implements with the front feet, footpad injections should only be performed in the hind feet.
    5. Additionally, footpad injections in the hind feet should be limited to only the R or L foot to allow for compensatory use of the unaffected contralateral limb.
    6. Multiple injections must be scientifically justified and previously approved in the protocol with an interval of at least 2 weeks or more recommended between each injection.

7. It is recommended that injected animals are housed on soft bedding (ex. ALPHA-Dri® bedding).
8. It must be ensured that animals are able to reach food and water and that animals are monitored daily for pain or distress and/or complications at the injection site.
9. For additional considerations regarding footpad injections, please contact Campus Veterinary Services for guidance.

H. Hydrodynamic Intravenous (Fast, Large Bolus) Injections[21,22,23]
1. Hydrodynamic injections are most commonly performed in mice.
2. Hydrodynamic injections - while similar to IV injections in terms of intravenous access and use of the same anatomical structures (ex. lateral tail veins in mice) - have unique considerations as volumes are often larger and the administration speed is faster than a routine, slow bolus IV injection (see above chart).
3. Maximum volumes typically represent 7-10% of body weight and the speed of administration is typically within 5-10 seconds.

I. Other Non-Specified Routes of Administration
1. For additional species and routes of administration not included in the information above, please contact Campus Veterinary Services for guidance.

V. <u>Procedures:</u>

1. For values noted as mL/kg, multiply the appropriate number above times the animal's body weight in kg to obtain the acceptable or absolute maximum volume of administration.
2. Absolute maximum volumes of administration denote uncommon volumes of administration (ex. one-time dose) as repeated doses at this higher range can result in adverse effects that may confound studies.
3. In addition to the volume of administration, one must also consider the character of the solution. Known irritants such as Freund's adjuvant must be delivered according to specific IACUC approved guidelines and protocols.
4. Solutions above pH 8.0 and below pH 4.5 should be diluted or buffered when given for routes other than intravenous - even if this means exceeding guidelines - provided that veterinary and IACUC approvals have been obtained.
5. Ideally, injectable products should be formulated as isotonic solutions (osmolality of about 300 mOsm/kg). The degree of hypertonicity has been related to the sensation of pain and an upper limit of 600 mOsm/kg is proposed to minimize hypertonicity-induced pain[5].
6. One must consider any possible effects attributable to the vehicle alone (e.g., dimethyl sulfoxide (DMSO) and polyethylene glycol).
7. Larger intravenous volumes of drugs administered in isotonic crystalloid vehicles may be permissible with IACUC review and approval.
8. The highest-gauge (smallest) needle size appropriate for volume and material delivery should always be selected. Typically, each animal should receive injections using a new, sterile needle with ideally no reuse on multiple animals. Factors supporting the use of one needle per animal include the relatively small gauge of needles required for most research animals (ex. rodents) and the higher likelihood of dulling in smaller bore needles with serial reuse. Dull needle use can contribute to pain and discomfort at the injection site, potentially

confounding study data. Additionally, it is not recommended to reuse needles when administering compounds intravenously or intraperitoneally/intracoelomically, nor is it allowed to reuse needles for animals on USDA-covered protocols[24]. Reasonable exceptions exist such as reusing the same, intact, non-damaged needles for oral gavage in rodents of the same cage and reusing intact, non-damaged larger bore needles for up to 10 cattle when administering vaccines[25]. Overall, however, it is ultimately best practice to use one new sterile needle per animal. Additionally, whenever possible, the needle used for puncturing drug container membranes and drawing drugs into a syringe should not be used for injection as this process dulls the needle and increases injection site pain.

9. A large intramuscular injection into a small muscle mass may force the dose into fascial planes and subcutaneous tissues that may accelerate lymphatic drainage and may also cause pressure necrosis or nerve damage. Small gauge needle size and small volumes of injection are preferred and should be appropriate to the size of the animal. Preference should be given to larger muscle groups (e.g., quadriceps, triceps) whenever possible to reduce these risks.
10. For parenteral routes of administration (a route other than oral), it may be less irritating to administer the dose halved in two separate locations. For studies requiring repeated parenteral dosing, the same site should not be used for two consecutive administrations.

VI. Resources:

1. The Care and Feeding of an IACUC: The Organization and Management of an Institutional Animal Care and Use Committee - Whitney K. Petrie and Sonja L Wallace, CRC Press, 2015.
2. Diehl, et al. Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes. J Appl Toxicol. Jan-Feb 2001;21(1):15-23. doi: 10.1002/jat.727.
3. Hull. Guideline limit volumes for dosing animals in the preclinical stage of safety evaluation. Toxicology Subcommittee of the Association of the British Pharmaceutical Industry. Hum Exp Toxicol. Mar 1995;14(3):305-7. doi: 10.1177/096032719501400312.
4. Gad, et al. Tolerable levels of nonclinical vehicles and formulations used in studies by multiple routes in multiple species with notes on methods to improve utility. Int J Toxicol. Mar-Apr 2016;35(2):95-178. doi: 10.1177/1091581815622442.
5. Usach, et al. Subcutaneous injection of drugs: literature review of factors influencing pain sensation at the injection site. Adv Ther. Oct 2019;36(11):2986-2996. doi: 10.1007/s12325-019-01101-6.
6. IQ Consortium. Recommended Dose Volumes for Common Laboratory Animals. 2016.
7. Hanson, et al. Intranasal administration of CNS therapeutics to awake mice. J Vis Exp. Apr 2013;(74):e4440. doi:10.3791/4440
8. Turner PV, et al. Administration of substances to laboratory animals: routes of administration and factors to consider. J Am Assoc Lab Anim Sci. Sep 2011;50(5):600-613. PMID: 22330705
9. Bowling Green University, Office of Research and Compliance, Appendix 9 Injection Sites, Maximum Volumes and Needle Size
10. University of Montana, IACUC, Injection Routes, Needle Sizes, Volumes
11. Liberty University, IACUC, Guidelines Regarding the Use of Injections in Laboratory Animals

12. Boston University, IACUC, Administration of Drugs and Experimental Compounds in Mice and Rats
13. Indiana University, IACUC, Policy for Dose Volumes in Laboratory Animals
14. Washington State University, IACUC, Guideline #10: Drug and Chemical Administration
15. University of California, San Francisco, IACUC, Routes and Volumes of Administration in Mice
16. University of Cincinnati, IACUC, Guideline for Fluid Administration and Blood Collection
17. University of California, Santa Barbara, IACUC Guideline: Antibody Production
18. University of California, Berkeley, Guidelines for the Production of Antibodies in Laboratory Animals
19. The University of North Carolina, Chapel Hill, IACUC, Policy on Foot Pad Injection in Mice and Rats
20. National Institutes of Health, Guidelines for the Use of Adjuvants in Research, Special Emphasis on Freund's Adjuvant
21. Huang, et al. Technical improvement and application of hydrodynamic gene delivery in study of liver diseases. Front Pharmacol. Aug 2017;(8):591. doi: 10.3389/fphar.2017.00591
22. University of California, San Francisco, IACUC Hydrodynamic Delivery in Rodents
23. Suda, et al. Hydrodynamic gene delivery: its principles and applications. Molecular Therapy. Oct 2007;(15):2063-69. doi:10.1038/sj.mt.6300314
24. University of Wisconsin, Animal User Requirement #2: The Reuse of Needles in Research Animals
25. Reuter, et al. Needle management contributes to beef quality. Noble Research Institute. Nov 2013.
26. Morton, et al. Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Lab Anim. Jan 2001;(35):1-41. doi:10.1258/0023677011911345