



Recommended Dose Volumes for Common Laboratory Animals

IQ 3Rs Leadership Group - Contract Research Organization Working Group

NOTE: This document includes dose volume guidelines that have been researched and published as well as standards that have gained acceptance through empirical use across multiple members of the IQ 3Rs leadership group (LG) and partner CROs.

Goal: The goal of this project was to develop and implement a harmonized guideline for ideal and maximum dose volumes for various routes for the most common laboratory animals in biomedical research based primarily on scientific literature.

Anticipated Benefit: Distribution and implementation of shared guidelines should 1) reduce experimental variables between studies that are/may be conducted, and in so doing, enhance the consistency and quality of the resulting experimental data, 2) enhance animal welfare and foster the 3Rs and 3) enhance biopharma and CRO collaborations/partnerships in designing and executing animal studies efficiently and in the spirit of employing best current practices.

Introduction: The 3Rs IQ Leadership group proposes that each institution accept the ideal or optimum dose volume as industry standard. These dose volumes are considered physiologically acceptable and are based on scientific evaluation of safety for dosing with sterile solutions such as physiologic saline. When other excipients are used, the dose volumes may need to be lowered. In addition the animal's health may warrant further consideration of safety. The maximum dose volumes listed in the table are simply the maximum reported in the literature, but are not scientifically determined to be safe or physiologically appropriate.

IACUC/Ethical Oversight Body: It is the Institutional Animal Care and Use Committee (IACUC) or equivalent ethical review committee that typically assumes responsibility for the development and implementation of such guidelines to govern animal care and use in research. We recommend that each institution require their IACUCs/equivalent ethical committee to review and carefully consider the scientific justification for dose volumes exceeding the ideal dose volumes listed in the table, prior to approval.

The authors of this guideline hope that this document will be of value as a reference for institutions to develop and/or refine internal guidelines and will facilitate inter-institutional harmonization of dose volume guidelines.



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Species	Dose Volume	Oral ^{2, 3, 4, 6} (ml/kg)	*IV bolus ^{2, 5, 6} /slow inj ¹ (ml/kg)	+IP ^{1, 2, 3, 6} (ml/kg)	+SC ^{2, 3, 6} (ml/kg)	+IM ^{1, 2, 3} (ml/kg)
Mouse	Ideal	10	5 bolus	5-10	5	0.05 total ml/site (2 sites/day)
	Maximum	20	25 slow injection	20	20 (divided in 2-3 sites)	0.1 total ml/site (2 sites per day)
Rat Hamster Gerbil	Ideal	10	5 bolus	5-10	5	0.1 total ml/site (2 sites/day)
	Maximum	20	20 slow injection	20	10 (divided in 2-3 sites)	0.2 total ml/site (2 sites/day)
Guinea Pig	Ideal	10	1 bolus	10	5	0.1 total ml/site (2 sites/day)
	Maximum	20	5 slow injection	20	10 (divided in 2-3 sites)	0.2 total ml/site (2 sites/day)
Rabbit	Ideal	10	1-5 bolus	3-5	2.5	0.25
	Maximum	20 (empty stomach)	10 slow injection	10 (rare)	10 (divided in 2-3 sites)	0.5 (Max 1 ml limit)
Cat Ferret	Ideal	10	5 bolus	5	2	0.25
	Maximum	15	10 slow injection	20	5	0.5 (Max 1 ml limit)
Marmoset	Ideal	10	2.5-5 bolus	5	2	0.25
	Maximum	15	10 slow injection	20	5	0.1 total ml/site (2 sites/day)
Dog	Ideal	5-8	1-5 bolus	1	1	0.25
	Maximum	15	10 slow injection	20	2 (divided in 2-3 sites)	0.5 (Max 3 ml limit)
Macaque	Ideal	5-8	1-5 bolus	3	1	0.25
	Maximum	15	10 slow injection	10	2 (divided in 2-3 sites)	0.5 (Max 2 ml limit)
Minipig	Ideal	10	1-5 bolus	1	1	0.25
	Maximum	15	10 slow injection	20	3 (divided in 2-3 sites)	0.5 (Max 5 ml limit per site)

* Bolus injections are typically dosed in less than 1 minute. Slow intravenous injections are typically dosed over 3-10 minutes. Solution properties such as tonicity, pH, etc. must be taken into account when approaching the volume limits or determining the volume to be infused IV. The recommended working range for pH is 4.5 to 8.0. The order of degree of tolerance of pH for different dosing routes is oral > intravenous > intramuscular > subcutaneous > intraperitoneal. Animal health must also be taken into consideration, such as kidney function and cardiovascular function. These systems must be normal to handle increased fluid volumes.

⁵ When larger volumes are to be administered, consider the rate of metabolism of osmolytes with respect to route of administration since apparently innocuous solutions can be rapidly metabolized leaving an equivalent of water to dilute body fluids, which can result in hyponatremia depending on the rate of administration and/or total volume administered.

+ When administering a solution IP, SC or IM, the viscosity, concentration, tonicity, and pH of the solution need to be taken into account.



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Annotated Dose Volume References (**bold=most often cited**):

1. Diehl, K.H., Hull, R., Morton, D. Pfister, R., Rahemampianina, Y., Smith, D., Vidal, J-M., and Vorstenbosch, C. (2001). A Good Practice Guide to the Administration of Substances and Removal of Blood Including Routes and Volumes. *Journal of Applied Toxicology*. 21, 15-23.

This reference provided consensus figures based on peer-reviewed, published literature whenever possible and internal guidelines when this was not possible. The species captured included mouse, rat, rabbit, dog, macaque, marmoset and minipig, all common laboratory species used in toxicity studies. The routes captured included oral, subcutaneous, intraperitoneal, intramuscular, intravenous bolus (over 1 minute) and intravenous slow injection (over 5-10 minutes). Ideal volumes were considered good practice in terms of animal welfare and practicality, but possible maximum dose volumes were also listed. It was noted that some of the maximum dose volumes listed appeared high when compared with good practice. Thus, the maximum volumes should have consideration by an ethical committee, especially for repeat dose studies. If maximum volumes are exceeded, animal welfare or scientific implications may result and reference to a responsible veterinarian should be made. Co-authors include Hull and Morton. The paper was a result of an initiative between EFPIA and ECVAM. Recommendations refer to the normal animal and special consideration is needed during lactation and pregnancy.

Note: Intravenous infusion of a single occasion would be less than 10% of the circulating blood volume over 2 hours using warmed (body temperature) fluids. Intradermal volume for all species was of 0.05-0.1 ml with adjuvant, dependent on the thickness of the skin. Subcutaneous and intramuscular dosing is limited to two sites per day.

2. Gad, SC , Spainhour CB, Shoemake C, et. al. 2016. Tolerable Levels of Nonclinical Vehicles and Formulations Used in Studies by Multiple Routes in Multiple Species With Notes on Methods to Improve Utility, *International Journal of Toxicology*, Jan 2016: 1-84.
This article provides a very comprehensive list of vehicles and formulations that are tolerated in a variety of laboratory animal species. Table 2 includes Volume Guidelines for Administration of Compounds by Routes of Administration to Laboratory Animals and is referenced here.
3. Hull, R.M. (1995). Guideline Limit Volumes for Dosing Animals In The Preclinical Stage Of Safety Evaluation. *Human & Experimental Toxicology*. 14: 305-307. This reference provided upper dose volume limits of normal practice for conventional repeat dose toxicity studies by oral and parenteral routes. The species listed included mouse, rat, hamster, guinea pig, rabbit, dog, primate (large= e.g., baboon, Cynomolgus monkey) and primate (small- e.g., marmoset). The routes included oral, subcutaneous, intraperitoneal,



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intramuscular, intravenous bolus and intradermal. These volumes were agreed upon by the Toxicology Subcommittee of the Association of the British Pharmaceutical Industry (APBI). The referenced limits listed were common practice and did not capture the absolute maximum dose volumes. The guidelines are for routine multiple dose preclinical toxicity studies in the species commonly used for this purpose. Note: Intravenous infusion of a single occasion would be less than 10% of the circulating blood volume over 2 hours using warmed (body temperature) fluids. Intradermal volume was 0.5 ml/site for all species.

4. Li, P., and Zhao, L. (2007). Developing Early Formulations: Practice and Perspective. *International Journal of Pharmaceutics*. 341, 1-19. This paper is focused on developing early formulations for discovery, pharmacokinetic and general toxicology studies. The authors represent two US pharmaceutical companies, Novartis and Sanofi-Aventis. The paper goes into depth on the approaches to dosage form, solubility enhancements, and recommends excipient use for oral and intravenous dosing and is a more relevant reference for a pharmaceutics scientist. There is a table on p. 3 that covers the normal dose volumes for oral and intravenous dosing in mouse, rat, rabbit, rhesus monkey and dog. The dose volumes listed were within the ideal dose volumes from the above table for all species.
5. Mann, W.A. and L.B. Kinter. Characterization of maximal intravenous dose volumes in the dog (*Canis familiaris*). *General Pharmacology* 24:357-366, 1993.
6. **Morton D.B., Jenning, M., Buckwell, A., Ewbank, R., Godfrey, C., Holgate, B., Inglis, I., James, R., Page, C., Sharman, I., Verschoyle, R., Westall, L., and Wilson, A.B. (2001). Refining Procedures for the Administration of Substances. Report of the BVAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals*. 35, 1-41.** This report was drawn from the scientific community from industry, academia, and animal welfare organizations. The aim was to refine administration procedures and improve animal welfare and science. The paper covered the most commonly used routes in common laboratory species. The focus on this paper was not dose volumes, but rather emphasized general principles of “good practice,” refinement for individual routes and procedures, and special considerations for wild animals. The dose volume guideline (p. 12) provided the maximum volumes for common routes of administration in common laboratory species. Though the table indicates it is the maximum volume, the intent is that the dose volume is the maximum volume for ideal or good practice dose volumes. The committee felt that a single guideline volume was applicable to most species for any given route. The routes included oral, intraperitoneal, intravenous bolus (over 1 minute), subcutaneous, intradermal.

Note: IV infusions were to avoid volumes greater than 4 ml/kg/h. Intradermal volume was 0.05-0.1 mL/site. Importantly, the authors list the maximum intramuscular dose of 0.05 ml/kg/site for rodents, rabbits and small nonhuman primates. Diehl and Hull list the dose volume per site without factoring in the animal body weight. Intraperitoneal dosing in non-rodents (excluding fish) is not recommended,



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nor does it recommend intraperitoneal dosing in pregnant rodents. Intramuscular dosing in rats, mice or hamsters is not recommended unless there are exceptional circumstances. (p.18).