

USE OF PHARMACEUTICAL AND NON-PHARMACEUTICAL GRADE SUBSTANCES IN ANIMAL RESEARCH

1. Policy statements

The intent of this policy is to ensure that substances used in animals do not compromise the health and welfare of animals and/or interfere with the interpretation of research results.

2. DEFINITIONS

Animals	All live fish, amphibians, reptiles, birds, non-human mammals, and cephalopods.
Clinical Use	The use of pharmaceuticals for medical purposes e.g. use of antibiotics to treat bacterial infection or analgesic to manage pain and distress.
Food and Drug Administration (FDA)	The Food and Drug Administration (FDA) is an agency within the U.S. Department of Health and Human Services. With respect to this policy, FDA is responsible for protecting the public health by assuring that human and veterinary drugs, and vaccines and other biological products and medical devices intended for human use are safe and effective.
Health Science Authority (HSA)	The Health Sciences Authority is the Singapore national authority regulating health products; managing the national blood bank, transfusion medicine and forensic medicine expertise; and providing critical forensic and analytical laboratory services. HSA regulate therapeutic products in Singapore under the Health Products Act (HPA) and its regulations, including the Health Products (Therapeutic Products) Regulations 2016. All therapeutic products/pharmaceuticals for human use will require registration with HSA before they can be supplied in Singapore.
Research use	<ol style="list-style-type: none"> Using the scientific method to test substances to determine how they function in an experimental situation (e.g. testing novel in-house synthesized substances or testing approved pharmaceuticals for new uses). Use of pharmaceuticals for research purposes e.g., use of doxycycline to induce gene expression in transgenic mice.

3. Scope

This policy applies to all animal work reviewed and approved by the NUS IACUC.

4. Guidelines

4.1 General principles on the use of pharmaceutical grade substances

- 4.1.1 A pharmaceutical-grade substance is defined as any active or inactive drug, biologic, or reagent, for which a chemical purity standard has been established by a recognized national or regional Pharmacopeia (e.g., the U.S. Pharmacopeia (USP), British Pharmacopeia (BP), National Formulary (NF),

European Pharmacopoeia (EP), Japanese Pharmacopeia (JP), etc.). These standards are used by manufacturers to help ensure the products are of the appropriate chemical purity and quality, in the appropriate solution or substance, to ensure stability, safety, and efficacy.

- 4.1.2 **Clinical Use:** Whenever possible, pharmaceutical-grade substances must be used for the clinical treatment of animals and to prevent or reduce/eliminate animal pain or distress, even in acute procedures. Non-pharmaceutical grade substances should only be used in clinical treatment after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product.
- 4.1.3 **Research Use:** For research purposes, i.e., when the substances are used to accomplish the scientific aims of the study, pharmaceutical-grade substances must be used if available, and suitable unless scientifically justified. Use of pharmaceutical grade substances for research with clinical relevance is also preferred since the test substance would be manufactured according to standards appropriate for human use. If the use of non-pharmaceutical-grade substances is essential for the conduct of science (e.g. the drug itself is under investigation), the goal of the IACUC is to consider the health and well-being of the animals while aiding the researcher in minimizing potentially confounding experimental variables and maximizing reproducibility of the research.
- 4.1.4 Cost savings alone is not an acceptable justification for using non-pharmaceutical-grade substances.
- 4.1.5 Reagent formulations are listed below in the order of preferred use, with the highest grade/ purity being listed first.
1. Pharmaceutical grade or equivalent (any of these formulations is acceptable):
 - i. USP/NF, BP, or other pharmacopeia recognized pharmaceutical grade substances used in a needed dosage form
 - ii. Pharmaceutical substances from registered agencies e.g. HSA and FDA
 2. Food grade (oral use only) ^a
 3. USP Reference Standards ^a
 4. Analytical grade bulk chemical (highest grade or purity) used to formulate a needed dosage form ^a
 5. Other grades and sources of substances ^{a, b}

Note:

^a Scientific justification required.

^b Tissue or cell culture grade reagents are not regulated

4.2 Use of pharmaceutical-grade substances

- 4.2.1 Pharmaceutical-grade substances must be reconstituted according to the product insert.
- 4.2.2 Any added excipient, vehicle, diluent, or other substances must be of pharmaceutical-grade. Commonly used solvent/vehicle such as normal saline are often available in pharmaceutical-grade. Pharmaceutical-grade substances adulterated by non-pharmaceutical-grade additives will render the preparation non-pharmaceutical-grade.
- 4.2.3 Reconstitution or formulation must be done under aseptic conditions. Sterilized pipette or pipettor tips must be used in the transfer of diluent or vehicles.

4.3 Use of non- pharmaceutical-grade substances

Pharmaceutical-grade products should be used, when available, for all animal-related procedures (Guide). The use of non-pharmaceutical-grade compounds in experimental animals will only be

acceptable by IACUC in the following situations: Scientific justification with supporting documentation (e.g., product inserts, screenshots of product with catalogue number from commercial sites etc) must be provided for the use of non-pharmaceutical-grade substances.

Use of non-pharmaceutical grade substances must be compliant with applicable national regulatory guidelines and requirements, and the requirements of relevant funding agencies. Best practices for administration of substances should be followed when injecting non-pharmaceutical grade compounds into animals.

4.3.1 Justifications the IACUC will consider for approval include but are not limited to:

- 4.3.1.1 The pharmaceutical-grade product is not available in the appropriate concentration or formulation for the route of administration.
- 4.3.1.2 The substance is experimental in nature (i.e., the substance itself is under investigation).
- 4.3.1.3 The substance is required to generate data that is part of an ongoing study or that is comparable to previous work. Experiments are encouraged to determine if currently available pharmaceutical-grade versions of the substance can be used, as use of pharmaceutical-grade substances will likely be more readily translatable to subsequent human use.

4.3.2 When non-pharmaceutical grade substances are used, the following must be fulfilled/addressed:

- 4.3.2.1 The method of preparation and storage of formulations is appropriately considered with the aim of maintaining their stability and quality (i.e., to prevent inadvertent co-administration of infectious agents or contaminants).
- 4.3.2.2 Appropriate labelling (i.e., drug name, concentration, and preparation and use-by/expiration date) is provided.

4.4 Factors to consider when non-pharmaceutical-grade substances are being considered for use

Scientific justification must be provided with use of the following: (i) non-pharmaceutical-grade substances, (ii) pharmaceutical-grade substances adulterated with non-pharmaceutical-grade additives, excipients, vehicles, or diluent, and (iii) pharmaceutical-grade substances not appropriate for the species and route(s).

When non-pharmaceutical-grade substances, or pharmaceutical-grade substances adulterated with non-pharmaceutical-grade excipients are used, consideration should be given to the grade, purity, sterility, pH, osmolality, stability, site and route of administration, formulation, compatibility, toxicity, pyrogenicity, side effects and pharmacokinetics of the preparations to be administered.

The following factors affecting the health and well-being of animals must be considered when using non-pharmaceutical-grade substances. Considerations for these factors are to be documented in the animal use protocol.

4.4.1 Vehicle, diluent, and solvent

Pharmaceutical-grade vehicle and diluent should be used when available. When non-pharmaceutical-grade vehicle or diluent is used, it should be sterile, physiologic (see section 4.4.2-8 below for details) and non-toxic or used at a level safe for animals as appropriate for the route of administration. For example, pharmaceutical grade saline should be used as it is more physiologically compatible than Phosphate Buffered Saline.

4.4.2 Grade and purity.

The highest-grade equivalent chemical reagent should be used and formulated aseptically.

4.4.3 Sterility

All formulations must be sterilized prior to use, such as by autoclaving. Filtration through a 0.22 µm filter just prior to use is preferred for sterilizing heat sensitive formulations. Preparation under strict aseptic conditions without subsequent sterilization requires scientific justification.

4.4.4 pH

The pH range of the internal environment of animals is very narrow (e.g., pH 7.4 ± 0.4 in human). Formulations with high or low pH should be adjusted or diluted with a buffer if possible. Formulations of high or low pH given by the intra-muscular, subcutaneous, intraperitoneal, ocular, inhalation and intratracheal routes may cause irritation, pain, or tissue necrosis. Formulations outside of this the physiologic pH range should not be used, but if required, must be scientifically justified, and given in a small volume. The effect of pH on the solubility of the substance should also be considered as precipitation may occur when mixed with blood, resulting in vascular occlusion, emboli, and thrombosis of local and distant capillary beds.

4.4.5 Osmolality

Normal plasma or serum has an osmolality in the range of 285-295 mOsm/kg (osmolarity range of 300-310 mOsm/L). The formulation should be isotonic or adjusted if it is not. Non-isotonic formulations may either cause red blood cell crenation or haemolysis when administered via the intravenous route or may cause localized tissue damage and associated pain upon injection. If approved by the IACUC, non-isotonic formulations should be administered slowly and in small volume.

4.4.6 Stability

The stability of the formulation should be considered to ensure that the dose is effective and no harmful degradation by-products may compromise the experimental results or the welfare of animals. Methods of storage to best deter degradation, such as in dark bottles for light-sensitive substances, refrigeration or freezing for substances unstable at room temperature, etc. should be determined. Formulations should be used as soon as possible, or within a week if the shelf-life of the preparation is unknown and depending on the usage of the substance i.e., possible contamination after multiple use from the same vial. Use of fresh preparation should be considered if any unstable substance is included in the formulation. The preparation must be labelled with the name, concentrations, date of preparation and expiry or use by date of the formulation and should be discarded if changes in color and/or precipitation occur.

4.4.7 Pharmacokinetics

Information on the pharmacokinetics of the substance should be noted where available. Such information will assist in determination of the dose, route and frequency and efficacy of the substance. The information should be properly documented and made available to IACUC upon request.

4.4.8 Toxicity, pyrogenicity, compatibility and side effects

The toxicity, pyrogenicity, compatibility and side effects available in the literature should be reviewed. These factors may cause pain and distress to animals post-administration, and measures to be taken to minimize pain and distress should be documented. Distinction should be made between the intended effects and potential side effects. Toxic substances should be used at the lowest possible effective doses for the intended effect and the earliest possible humane endpoints should be used. Pyrogens, such as endotoxins, may cause fever when injected into an animal. Purity and sterility of a

non-pharmaceutical grade substance do not guarantee that pyrogens are not present. Pyrogenicity is a potential experimental variable that PI should be aware of when using nonpharmaceutical grade drugs. Pyrogens can be avoided by using purified and characterized substances and diluents in preparation of the final formulation.

Substance that is not compatible may cause tissue damage when administered to animals. The above and other side effects in animals should be monitored daily.

4.4.9 Pilot Study

An efficient way to reduce the negative impacts on laboratory animals is the use of a pilot study. If a novel substance is being tested or a substance is being administered in which the safety in animals is unknown, in vitro safety testing using cells or tissues culture should be performed first before using in animals. Following this a pilot study using a minimum number of animals should be conducted to determine the dose and induced adverse effects. This would in turn help determine humane end point criteria specific to the project.

5. References

1. [Guide for the Care and Use of Laboratory Animals. National Research Council, 2011.](#)
2. [Guidelines on the Care and Use of Animals for Scientific Purposes. National Advisory Committee for Laboratory Animal Research. 2022](#)
3. https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b14_pharmaceutical_compounds.pdf
4. <https://www.aaalac.org/accreditation-program/faqs/#B9>

Revision	Author	IACUC approval date
Last updated	Dr Foo Tet Wei, John	14 August 2013
P215.01. Revisions to update policy format and content based on the NPG subcommittee recommendation	NPG subcommittee 2020	18 January 2021
P215.02. Reviewed to:	Dr Chew Li Li	3 January 2023
1. ensure policies are in compliance with the NAELAR Guidelines 2022 and the updated Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004; and	Dr Chew Li Li	
2. up-to-date with current practices	IACUC	
Changes made to:		
1. section 4.1.3 to update to "For research purposes, i.e., when the substances are used to accomplish the scientific aims of the study, pharmaceutical-grade substances must be used if available, and suitable unless scientifically justified."; and		
2. section 4.2.2 to update to "Any added excipient, vehicle, diluent, or other substances must be of pharmaceutical-grade."; and		
3. section 4.3 to add "Best practices for administration of substances should be followed when injecting non-pharmaceutical grade compounds into animals"; and		
4. section 4.4 to add "When non-pharmaceutical-grade substances, or pharmaceutical-grade substances adulterated.....toxicity, pyrogenicity, side effects and pharmacokinetics of the preparations to be administered.; and		
5. under section 5, updated to "Guidelines on the Care and Use of Animals for Scientific Purposes. National Advisory Committee for Laboratory Animal Research. 2022"		
P215.03.	Dr Chew Li Li	19 Aug 2024
1. Annex B, FAQ question 5 updated to include a table of acceptable and unacceptable justification for the use of NPG.		
2. Broken hyperlinks updated		

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ANNEX A: A Guide to the availability and procurement of pharmaceutical grade substances

1. Search the various databases below for chemicals/substances to determine if they are available in pharmaceutical grade

Pharmaceutical grade substances can be purchased from vendors in Singapore, as well as from the US, Australia, and other countries.

- a. [HSA \(Health Sciences Authority\) website](#) for registered human therapeutic products to determine if a pharmaceutical grade substance is available in Singapore.
 - i. The search can be based on either “Product Name Containing” or “Active Ingredient(s) Containing”.
 - ii. Using the generic name or the product name (for example, use paracetamol instead of Panadol) will yield different results.
 - iii. The search results will provide information on the availability of pharmaceutical grade versions of the substance, its registration number under HSA and the Registrant.
 - iv. Clicking on the “Registration No” of individual entry in the search results page will provide the list of active ingredients and additional information such as product inserts (if available) for each product. This information can aid researchers in considering if any of the product ingredients may interfere with the study.
**Note: The information on the constituents of each product may not be exhaustive. Researchers may want to contact the registrant for more details if needed.*
- b. In addition to HSA databases, the following overseas databases can be used to check for the availability of pharmaceutical grade substances too.

For veterinary products sold in the United States:

- i. <https://www.fda.gov/industry/structured-product-labeling-resources/electronic-animal-drug-product-listing-directory>.
- ii. Click on “Electronic Animal Drug Product Listing Directory” (first line of text)
- iii. Open the zip file
- iv. Open the Excel spreadsheet

For human products sold in the United States: <https://www.accessdata.fda.gov/scripts/cder/daf/>

2. For ease of procurement, researchers may purchase pharmaceutical grade products from NUH pharmacy. A NUS Purchase Order¹ is needed when purchasing pharmaceutical grade product from NUH pharmacy for research or training purposes. Please indicate “purpose of purchase” on the form and get the PI/lecturer to sign on the copy.

Researchers may write to the following personnel from NUH pharmacy for more details and quotations if needed.

Email contacts:

ken.goh.p.h@alpshealthcare.com.sg

ng.jia.min@alpshealthcare.com.sg

lim.sook.wei@alpshealthcare.com.sg

* Please go to the [NUH Pharmacy website](#) for the latest updates if needed.

3. Researchers may approach CM vets for assistance if required.

¹ Researchers may refer to please refer to the link below for NUS Procurement Mini Guide:

<https://share.nus.edu.sg/cpr/Document%20Library/NUS%20Procurement%20Mini%20Guide.pdf>

For more information on procurement procedures, researchers may contact CPO at askCPO@nus.edu.sg

ANNEX B: FAQ on the use of pharmaceutical grade substances in animal work

1 Why must pharmaceutical grade substances be used for animal work?

Pharmaceutical-grade substances, when available, must be used to avoid toxicity or side effects that may compromise the health and welfare of vertebrate animals or may interfere with the interpretation of research results.

These preparations are made under Good Manufacturing Practices (GMP) to meet the international standards for administration to humans and animals. These are a set of stringent practices outlining the methods, facilities, and controls used in manufacturing, processing and packing of a drug product. They are used to assure that a product is safe for use, and that it has the ingredients and dose it claims to have.

2. My area of research is in basic sciences. Why do I need to use pharmaceutical grade substances in my animal work?

The IACUC developed this policy in order to ensure that the health and welfare of vertebrate animals are not compromised. The policy states that pharmaceutical grade substance must be used when available and suitable for research purposes. Scientific justification must be provided for IACUC review and approval if non-pharmaceutical-grade substances are used in animal work. Please refer to section 4.3 and 4.4 of the IACUC policy [P215 on Use of Pharmaceutical and Non-Pharmaceutical Grade Substances in Animal Research](#) and question 3 below for more details on the use of non-pharmaceutical grade substances.

NUS Animal Care and Use Program is AAALAC accredited. Under the [AAALAC accreditation program](#), pharmaceutical grade substances should be used for animal research when available and suitable.

3. What is the difference between substances for clinical use and substances for research use?

Substances for clinical use (such as anesthetics, analgesics, euthanasia agents, antibiotics, etc.) always have pharmaceutical grade preparations available commercially. These substances are used for medical treatment and reduction or alleviation of pain and distress. They are accepted by medical community as safe and effective and available to the general public through product labelling.

Sometime, substances for research use (test substances) are not commercially available in pharmaceutical grade preparations. The substances are test products of unproven benefit in the applications they are being tested (eg. in-house synthesized test substances).

4. The pharmaceutical grade version of my test substance costs more. Can I use the research grade version instead?

Cost savings alone is not an adequate justification for the use of non-pharmaceutical-grade substances in animals. However, unavailability or shortages of pharmaceutical-grade substances may lead to cost increase and the IACUC may consider these as plausible justifications for the use of the non-pharmaceutical-grade substitution.

5. Under what circumstances may the IACUC approve the use of non-pharmaceutical-grade compounds?

Details on why the available pharmaceutical grade substances are unsuitable for your study must be provided for IACUC review and approval. Examples of circumstances that the IACUC might approve include when

- a. the pharmaceutical grade substances are unavailable in appropriate concentration or formulation for your research. For example, pharmaceutical grade substances that are only available in tablet form may contain other substances not suitable for injection.
- b. an equivalent/appropriate vehicle/additives used in the pharmaceutical grade formulation is unavailable as a standalone for use as a vehicle control.

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- c. If pharmaceutical grade substances contain additives that may impact animal wellbeing.
- d. non-pharmaceutical grade substances required to generate data that are part of an ongoing study or that are comparable to previous work*.
- * Note: experiments are encouraged to determine if currently available pharmaceutical-grade versions of the substance can be used.

Some examples of acceptable and unacceptable justifications as follow:

Acceptable justification for use of NPGs	Unacceptable justification for use of NPGs
PGs are not available	NPGs are cheaper.
1. Continuation/direct comparison with previous data/results from the lab needed. 2. Comparison with recently published data from other laboratories needed, to be reviewed on a case-by-case basis. The publication(s) should be published within the past 10 years.	Comparison with old, published data from other laboratories needed
Pharmaceutical grade compounds not available in appropriate concentration or formulation for the specific route of administration e.g. 1. PGs in oral tablet form inappropriate for injections; or 2. PGs concentration too low. Volume for administration will be too large for the required concentration via the route of administration used.	The route of administration does not require PGs e.g. immersion for fishes or oral administration
Pharmaceutical grade compounds formulated with special excipients that are not available as standalone for use as vehicle controls.	NPG excipients or vehicles are used. Hence, even if PGs substances used, the NPG excipients or vehicle will render the preparation non-pharmaceutical grade.
Pharmaceutical grade compounds may contain additives that may interfere with the study model or cause animal welfare concerns. Some examples are: i. clinically, mannitol is given with cisplatin as a prevention for nephrotoxicity. However, mannitol is also a diuretic and could potentially cause dehydration in animals. ii. some common excipients such as magnesium stearate and poly(ethylene glycol) 400, were shown to affect intestinal drug absorption of atenolol in Wistar rats Many additives are potential toxicants at high doses in animals, though safe in humans at therapeutic doses, including commonly used excipients such as cyclodextrins, dextrans, and polyethylene glycol ² .	NPGs are purer than PGs

² Haschek and Rousseaux's Handbook of Toxicologic Pathology (Third Edition) Volume II, 2013, Pages 725-750. Chapter 24 - Pathology in Non-Clinical Drug Safety Assessment pg 738 (<https://doi.org/10.1016/B978-0-12-415759-0.00024-8>)

6. **Is there a pharmaceutical grade preparation of MS-222?**

Yes. Pharmaceutical grade MS-222 is available and can be purchased directly from CM pharmacy.

7. **Is USP reference standard the same as pharmaceutical-grade?**

No. USP reference standard (sometimes labelled as Pharmaceutical Secondary Standard) is not the same as pharmaceutical grade.

U.S.P. stands for United States Pharmacopeia. The Pharmacopeia is a compendium that contains monographs for medicines, dosage forms, drug substances, excipients, biologics, compounded preparations, medical devices, dietary supplements, and other therapeutics. A monograph includes the name of the ingredient or preparation; the definition; packaging, storage, and labelling requirements; and the specification. The specification consists of a series of tests, procedures for the tests, and acceptance criteria. These tests and procedures require the use of official [USP Reference Standards](#).

USP Reference Standards are integral components of monographs and other documentary standards established by USP to help ensure the identity, strength, quality, and purity of medicines and foods, and are provided primarily for quality control use in conducting the assays and tests in these documentary standards. These standards come in small vials, and enable pharmaceutical manufacturers to test their product against USP's standards to ensure it meets published specifications. They are not the same as pharmaceutical grade.

USP Reference Standards are **not** for use in humans or animals as drugs or medical devices. They are intended for test and assay use only as per USP compendia. You may be able to use USP Reference Standards for analytical or laboratory applications other than those specified in USP compendium.

Similar principles apply to the [European Pharmacopoeia \(EP\) Reference Standards](#) and other Reference Standards.

8. **How do I know if a substance is pharmaceutical grade?**

Pharmaceutical grade substances are generally identified by their compliance with international quality standards and requirements, such as the ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines, national pharmacopoeia/formularies (e.g. BP, USP/NF, EP) and the Handbook of Pharmaceutical Ingredients. The declaration of compliance is normally indicated on the certificate of analyses and/or specification documents accompanying the supply of these substances. There are no specific labelling/packaging requirements for these substances.

If a drug or substance is used in people or animals there is usually a pharmaceutical grade preparation available. These preparations are manufactured under GMP (Good Manufacturing Practices) by a pharmaceutical company (e.g., Eli Lilly, Pfizer, Hospira), acquired from human and veterinary medical supply companies and often require a prescription.

The Health Sciences Authority ([HSA](#)) maintains [databases of registered human therapeutics products and medical devices in Singapore](#). The United States Food and Drug Administration maintains a list of approved substances for [veterinary](#) and [human](#) use that may be purchased from the US. [The European Union](#), [Australia](#), [Japan](#), and other countries may also have databases.

Powders and other substances purchased from chemical storehouses (e.g. Sigma) are **not** pharmaceutical grade. The bottles will often be labelled with a disclaimer such as, "For laboratory use only. Not for drug, household or other uses." Reference standards are often labelled United States Pharmacopeia (USP) Reference Standard, or Pharmaceutical Secondary Standard.

9. **I have a bottle of powdered substance. How do I tell if it is pharmaceutical grade?**

Solid pharmaceutical grade substances can be supplied in lyophilized bottle. One such example is the antibiotic Ceftriaxone (trade name: Tricefin) as shown in the picture on the left below. Instructions on how to properly reconstitute the drug for injection will be found on the label or product inserts. As long as the manufacturer's instructions are followed (e.g. correct sterile diluent is used), the final preparation will be considered pharmaceutical grade.



Picture on the left: Pharmaceutical grade Ceftriaxone powder for injection (i.m. injection/i.v. infusion). Picture on the right: USP purity grade Heparin sodium salt, considered non-pharmaceutical grade.

Powdered substances to be used for injection supplied in a screw, flip-top or other non-aseptic container (picture on the right) are usually not pharmaceutical grade. Powders and other substances purchase from chemical storehouse such as Sigma are not pharmaceutical grade even if they are “USP” or “Pharma grade”.

10. **I have a bottle of chemical labelled “USP” which was bought from Sigma. Is this pharmaceutical grade?**

Generally, powders and other substances purchased from chemical storehouses are not pharmaceutical grade even if they are “USP” or “Pharma grade”. These chemicals are often USP reference standard which is not equivalent to pharmaceutical grade (see question 5 for more information on USP reference standards). The bottles will usually be labelled with a disclaimer such as, “For research use only”.

11. **My test substance is a pharmaceutical grade veterinary or human drug. I need to dilute or change its formulation for my animal work. How will this affect the pharmaceutical grade formulation of the substance?**

Pharmaceutical grade drug refers to the active ingredients that creates the pharmacological effect.

Pharmaceutical grade formulation refers to the final preparation that is administered to the animals. This will include any diluents, excipients, vehicles etc.

Veterinary and human drugs that are reconstituted according to the product insert remain pharmaceutical-grade formulation.

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The IACUC's policy is that pharmaceutical grade drug should be used, even if non-pharmaceutical grade vehicle, diluent, or excipient is used. Please refer to the policy [P215 Use of Pharmaceutical and Non-Pharmaceutical Grade Substances in Animal Research](#), section 4.4.1 Vehicle, diluent, and solvent for details.

12. **I have some pharmaceutical grade drug in tablet form. Can they be used for injection for my animal work?**
Pharmaceutical grade drugs or substances in tablet form is designed for oral administrations and may contain fillers and other excipients that help to stabilise the active ingredients. They may not be appropriate for injection into animals.
13. **I have some expired pharmaceutical grade drug. Can I use them for my animal work?**
The use of expired pharmaceuticals and non-pharmaceutical grade products is not consistent with acceptable veterinary practice and does not constitute adequate veterinary care.

Drugs administered for euthanasia, anesthesia, analgesia, and emergency purposes **must** not be used beyond their expiration date, even if a procedure is terminal.

Other drugs may be used for non-survival procedures, provided that:

- expired drugs are marked as "Expired — Use ONLY in TERMINAL Procedures",
- they are stored in a different location (cabinet, drawer, etc.) than materials used for survival procedures until disposal,
- the use of expired medical devices and devices/ materials does not adversely affect the animal's well-being or compromise the validity of the scientific study, and

Details are described in the IACUC Policy titled [P214 Expired Devices, Materials, Drugs and Fluid](#).

14. **What is an excipient?**
Pharmaceutical excipients are substances that are included in a pharmaceutical dosage form not for their direct therapeutic action, but to aid the manufacturing process, to protect, support or enhance stability, or for bioavailability or patient acceptability.
15. **What is the difference between PBS and saline?**
"0.9% Sodium Chloride (NaCl) for Injection", also referred to as normal saline or physiological saline, is a sterile solution containing water for injection and sodium chloride (NaCl) to render it isotonic. It is suitable for IV injection.

The use of pharmaceutical grade saline is preferred in animal work since it is isotonic and is specifically sold for injection into animals. It is manufactured using Good Manufacturing Practice (GMP) and comes in a sterile container ready for *in vivo* use. Another example of pharmaceutical grade diluent or buffered media that is appropriate for injection is *PLASMA-LYTE 148 Injection*. These products are preferred as a diluent or vehicle, since they are specifically designed for injection.

If PBS must be used as a diluent or vehicle, careful attention must be made to the pH, tonicity and osmolality of the solution. Methods for addressing sterility, removal of particulates, and pyrogens should be described in the protocol.

PBS, or Phosphate Buffered Saline, is a laboratory grade buffer solution containing water, sodium chloride (NaCl) and disodium phosphate (Na_2HPO_4). In some instances, potassium, or even calcium or magnesium may be added to the solution. It is a buffer solution commonly used in biological research and helps to maintain a constant pH. There are different formulations of PBS. In general, the osmolality and ion concentrations of the solutions match those of the human body. PBS has a buffer range of pH 5.8 to 8.0 at 25°C. Unless labelled as such, it is not pharmaceutical grade

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Most commercial preparations of PBS are not typically tested for pyrogens or particulates and the solution is not intended for injection. However, if use of PBS is approved by the IACUC, it is recommended that you use a sterile and endotoxin tested product such as that produced by Gibco™, which supplies PBS that is manufactured at a GMP-compliant facility registered with the FDA as a medical device manufacturer.

16. **What are the recommended pH ranges for the various routes of administration?**

The recommended pH range of the formulation for the various routes of administration can be found in the table below. The order of degree of tolerance of pH range for different dosing routes is: oral ~ intramuscular ~ intraperitoneal ~ intravenous ~ subcutaneous. More details on can be found in the table below.

Administration route	pH range	References
Oral	2–9	45
	4.5–8	54
	5–9	23
Intravenous	4–9 (bolus dosing)	29
	3–9 (continuous infusion)	29
	2–9	45
	physiologic ^a	54
Subcutaneous	physiologic ^a	45, 54
Intramuscular	2–9	45
	physiologic ^a	54
Intraperitoneal	physiologic ^a	45, 54

Administration of substances outside of the recommended pH ranges may result in tissue necrosis and vascular thrombosis.

^aApproximately pH 7.3–7.4.

Table extracted from *Administration of Substances to Laboratory Animals: Equipment Considerations, Vehicle Selection, and solute Preparation*. *Journal of the American Association for Laboratory Animal Science*. September 2011, Vol 50, No 5, 614–627

17. **What is osmolality and why is It important in my animal work**

Normal plasma has an osmolality in the range of 285–295 mOsm/kg (osmolality range of 300–310 mOsm/L) and intravenously administered compounds should generally be formulated to a range matching this osmolality as closely as possible. Agents with an osmolality greater than 600mOsm/kg cause red blood cell crenation upon intravenous injection while agents less than 150 mOsm/kg will cause hemolysis, both of which are associated with pain and physiologic disturbances. Solutions with an osmolality between 150 to 450 mOsm/kg are generally well tolerated by intravenous infusion. Agents with widely non-physiologic osmolality administered via other routes (IM, SC, IP) may result in localized tissue damage and associated pain upon injection. As the volume of injected compound increases the potential for tissue damage and pain due to non-physiologic osmolality also increases dramatically and this may impact animal welfare. Osmolarity/osmolality calculators are available on the internet to assist in calculating predicted drug osmolality/osmolality (http://www.rxkinetics.com/iv_osmolality.html).

Osmolality measurement can be done using an osmometer. As an example, osmolality can be measured using 10ul of sample using [VAPRO](#) as show below.



18. **How do I filter-sterilize substances prepared in organic solvents?**

Syringe filters are popularly used to filter-sterilize liquid preparations. The membrane used to manufacture these syringe filters will determine the type of solvent it can be effectively used during filter sterilization. Information on the chemical compatibility of the syringe filters are usually provided by the manufacturer. You may also wish to refer to the [syringe filters solvent compatibility chart](#) provided by Restek Corporation as a general guide on the chemical compatibility with different types of syringe filter membranes available.

19. **What pore size filter should I use to filter-sterilize liquid preparations?**

0.2 or 0.22µm (micron)-rated pore size membranes should be used for sterile filtration of liquid preparations.

20. **If I have more questions or need assistance, whom can I contact for help?**

If you have any questions or need assistance in obtaining a pharmaceutical grade preparation, please contact CM Pharmacy at cmpharmacy@nus.edu.sg for assistance.

- The End -