University of Louisville
Institutional Animal Care and Use Committee
Policies and Procedures

IACUC Standard Procedures for Rodents

Policy: This policy describes the IACUC's approved standard procedures for rodents (mice, rats, hamsters, or guinea pigs) that investigators may easily incorporate into their IACUC *Proposals*. Principal Investigators (PI) performing procedures as described in this policy may utilize the checkboxes in the procedures section of the IACUC's *Proposal* form rather than describing the procedures in full. If the performance of the procedure will deviate from the descriptions in this policy, including administration or blood collection volumes, then the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. All procedures, including the IACUC's standard procedures, must be included in the IACUC *Proposal* form and approved by the IACUC prior to performance. It is the responsibility of the PI to ensure that all laboratory personnel responsible for procedures are appropriately trained and qualified. The Comparative Medicine Research Unit (CMRU) veterinary staff is available to provide training at no charge; training can be scheduled through the IACUC's website.

Rationale: This policy has been drafted to ensure consistency in the performance of common non-surgical procedures, as well as reduce the administrative burden to investigators and the IACUC in drafting and reviewing IACUC *Proposals*.

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Original Approval: 10 December 2020
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f. Fur

Procedures, Guidelines, and Exceptions:

I. **Agent Administration:** Common agent administration techniques for rodents are described below. Table 1 lists the recommended needle size and ***maximum*** administration volume for each route. **Note:** If administration volumes will exceed the maximum volumes stated in Table 1 below, the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Administration volumes in excess of the maximum volumes listed here must be included in the procedural description and scientifically justified in the IACUC *Proposal*.

a. **Intraperitoneal (IP) Injection** [*Mice, Rats, Hamsters, Guinea Pigs*]
Restrain the animal and tilt backwards so that the head is lower than the hind end and its abdomen is exposed. Insert the needle into *the animal's* lower right quadrant of the abdomen (to avoid the cecum and urinary bladder) at about a 30-degree angle with the needle bevel up. Pull back on the plunger to ensure negative pressure and that no abdominal organs have been punctured prior to injecting. If any fluid is aspirated, the solution is contaminated and must be discarded and the procedure repeated with a new syringe and needle. If no fluid is aspirated, depress the plunger to administer the solution into the peritoneal cavity.

b. **Subcutaneous (SC) Injection** [*Mice, Rats, Hamsters, Guinea Pigs*]
Mice, hamsters: Insert the needle with the bevel up into the skin fold between the thumb and finger created by the restraining hand over the scapular region. Pull back the syringe plunger to aspirate the syringe. If air is aspirated, the needle has gone through the skin and out the other side and must be redirected. If negative pressure is aspirated, depress the plunger to administer the substance in a steady motion between the skin and the body wall.
Rats, guinea pigs: Restrain the animal and tent loose skin over the dorsum. Insert the needle with the bevel up into the skin tent and pull back the syringe plunger to aspirate the syringe. If negative pressure is aspirated, depress the plunger to administer the substance in a steady motion between the skin and the body wall.

c. **Intradermal (ID) Injection** [*Mice, Rats, Hamsters, Guinea Pigs*]
Anesthetize the animal and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex. Clip a patch of hair on the animal's back and clean the injection site with alcohol. Insert the needle ~1 mm into skin with the bevel up, holding the needle parallel to the skin. Do not aspirate but administer the substance slowly. Proper injection results in a small, persistent skin welt. The substance should go between the layers of the skin and not underneath the skin.

d. **Tail Vein Intravenous (IV) Injection** [*Mice & Rats*]
Pre-warm the animal for 5-10 minutes to dilate the tail vessels using a heating device placed under the cage or a commercially available warming box, then place the animal into a restraint device. Alternatively, the tail can be submerged in warm (37-40°C) water for 30 seconds. *Animals must be constantly monitored for signs of heat distress or injury.* Clean the injection site with alcohol. Hold tail and, if desired, apply digital pressure to the vessels at the proximal tail to act as a temporary tourniquet. Insert the needle into the lateral tail vein, holding the syringe parallel to the tail. It is easiest to inject at approximately 1/3 of the tail length from the tail tip and move proximally if additional attempts are needed. If

visualization is difficult, a light may be used to illuminate the vessels. *It is vital to ensure there are no bubbles in the syringe or injection solution.* Do not aspirate the syringe, which will collapse the vessel. Administer the substance in a slow, fluid motion, releasing any digital pressure before administration. Too rapid administration can cause vascular overload or rupture of the vein from excessive pressure. Remove the needle, gently apply pressure to the injection site with gauze, and ensure hemostasis before returning the animal to its cage.

e. **Oral Gavage (PO)** [*Mice, Rats, Hamsters, Guinea Pigs*]
Manually restrain the animal. Measure the gavage needle against animal's body to ensure proper needle length. The gavage needle should measure from the oral cavity to the xiphoid process/last rib. Mark the needle at that length and do not insert it further, which may perforate the stomach. Gavage needles can be stainless steel or plastic and straight or curved but must have a non-traumatic tip and be appropriate size for the animal (see Table 1). Place tip of gavage needle in animal's mouth and advance it to the back of the oral cavity while extending the animal's head and neck vertically to create a straight line with the body. Pass the gavage needle slowly to the measured point, letting it fall without resistance down the esophagus. *Never force the gavage needle, which can cause traumatic injury such as esophageal perforation and require euthanasia.* If any resistance is felt, pull the needle out and place it again. Once the gavage needle is properly placed, administer the substance slowly and carefully remove the needle. Return the animal to the cage and monitor for labored breathing or other signs of distress. Recheck the animal in 12-24 hours after dosing.

f. **Intramuscular (IM) Injection** [*Mice, Rats, Hamsters, Guinea Pigs*]
Restrain the animal to allow access to a hind limb. A restraint device or second technician may be needed to ensure proper placement of the injection. Clean the injection site with alcohol. Palpate the quadriceps muscle and insert the needle with the bevel up into the muscle belly, directed away from the femur and sciatic nerve. Injection can be placed in either the cranial thigh musculature or caudal thigh musculature. Pull back the syringe plunger to aspirate the syringe. If no blood is aspirated, depress the plunger to slowly administer the substance. *Due to the limited muscle mass of rodents, only a very small volume can be comfortably and practically administered IM. Consequently, this technique is typically not recommended, particularly for mice or hamsters, and requires additional justification in the IACUC Proposal.*

Table 1: Recommended needle size and ***maximum*** administration volume for common injection techniques in rodent species. **Note:** If administration volumes will exceed the maximum volumes stated in the table below, the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Administration volumes in excess of the maximum volumes listed here must be included in the procedural description and scientifically justified in the IACUC *Proposal*.

	IP		SC		ID		IV		PO		IM	
	Needle size	Volume (ml/kg)	Needle size	Volume (ml/kg)	Needle size	Volume (ml/ site)	Needle size	Volume (ml/kg)	Needle size, length	Volume (ml/kg)	Needle size	Volume (ml/kg /site)
Mice	25-27g	<10	25-27g	<5*	27-30g	<0.05-0.1	27-30g	<5**	18-22g 1-1.5”	<5-10	27g	<0.05
Rats	23-25g	<10	23-25g	<5	27-30g	<0.05-0.1	25-27g	<5**	16-20g, 1.5-3”	<5-10	25g	<0.05
Hamsters (dwarf)	25-27g	<10	25-27g	<5	27-30g	<0.05-0.1	27-30g	<5**	18-22g 1-1.5”	<5-10	25g	<0.05
Hamsters (Syrian)	23-25g	<10	23-25g	<5	27-30g	<0.05-0.1	25-27g	<5**	18-20g 1.5-2”	<5-10	25g	<0.05
Guinea Pigs	23-25g	<10	23-25g	<5	27-30g	<0.05-0.1	25-27g	<5**	14-18g, 1.5-3”	<5-10	25g	<0.05

*Up to 40 ml/kg can be administered in mice
**Up to 2-4 ml/kg/hr can be given IV for continuous rate infusions

II. **Blood Collection:** Common phlebotomy techniques for rodents are described below. Non-terminal blood collection is limited to 1% of body weight per collection or 1.5% of body weight total over a 14-day period with multiple draws. If an approved *Proposal* requires blood collection greater than this amount for scientific reasons, fluid volume replacement must be considered. It is recommended to withdraw only the minimum amount of blood required to meet experimental needs. Table 2 provides maximum collection volumes for rodent species. For estimation purposes, each drop of blood is ~50 µL; however, it is recommended to measure out the approved collection volume prior to phlebotomy if not designated on the collection tube. **Note:** If collection volumes will exceed the maximum volumes stated in Table 2 below, then the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Collection volumes in excess of the maximums outlined in this policy may require fluid replacement; investigators are strongly encouraged to consult with a CMRU veterinarian in regard to fluid replacement volumes and types prior to *Proposal* submission.

a. **Submandibular Blood Collection** [*Adult Mice*]
Restrain the animal by grasping the skin along its back and ensure the skin is taut over the mandible. The intersection of a line drawn straight down from the lateral canthus of the eye and straight back from the commissure of the lips (note: usually a hairless dot over the mandible is present here) is used as a landmark for the puncture site. Insert an 18-20 gauge needle or 5 mm lancet to the shallow depth of 1-2 mm just caudal to the dimple and then pull out to start the flow of blood from the facial vein. Collect the sample and then release manual restraint to stop the flow of blood. Ensure bleeding has stopped before returning the animal to the home cage.

b. **Tail Vein Blood Collection** [*Mice & Rats*]
Place the animal in an appropriate restraining device and clean the blood collection site with alcohol. If the lateral tail vein is difficult to visualize, the animal may need to be pre-warmed using a heating device placed under the cage, a commercially available warming box, or by submerging the tail in warm (37-40°C) water for 30 seconds. *Animals must be constantly monitored for signs of heat distress or injury.* Using a sterile needle or lancet, nick the lateral tail vein approximately 1/3 of the tail length from the tail tip. It is recommended to use a 25-gauge needle or 4 mm lancet for mice and a 22-gauge needle or 6 mm lancet for rats. Collect the approved blood volume into an appropriate container. Gently apply pressure to the injection site with gauze to facilitate hemostasis before returning the animal to its cage. A clotting agent such as styptic powder may be applied if needed. If multiple collections are needed, alternate sides of the tail and move proximal towards the base of the tail.

c. **Lateral Saphenous Vein Blood Collection** [*Mice, Rats, Hamster, Guinea Pigs*]
Restrain the animal manually or using a restraint device. Clip the hair on the lateral aspect of a hind limb. Clean collection site with alcohol. Extend the leg and apply gentle pressure at the caudal aspect of the knee joint to occlude the vessel. Prick the lateral saphenous vein with a needle, which runs dorsally and laterally over the tarsal joint. It is recommended to use a 25-27 gauge needle for mice and hamsters and a 23-25 gauge needle for larger rodents. Collect the approved blood volume into an appropriate container. Release occluding pressure and hold gentle pressure with gauze over the puncture site to stop bleeding. Ensure hemostasis before returning the animal to the home cage.

d. **Jugular Blood Collection** [*Rats, Hamsters, Guinea Pigs*]
Anesthetize the animal and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex. Position the animal in dorsal recumbency, clip the hair on the ventral neck to aid in identification of landmarks, and wipe the skin with alcohol. Using the non-dominant hand, restrain the animals so the forearms are pulled back, the skin is pulled taught across the neck, and head is hyperextended upwards. Insert a 25-gauge needle with the bevel up, medial to the point of the shoulder and advance towards the jugular furrow. Once the skin has been pierced, apply negative pressure as the needle is advanced until blood is aspirated. Collect the sample and then gently apply pressure with gauze over the puncture site to facilitate hemostasis before recovering the animal and returning to the home cage.

e. **Cranial Vena Cava Blood Collection** [*Hamsters, Guinea Pigs*]
Anesthetize the animal and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex. Position the animal in dorsal recumbency, clip the hair on the ventral neck to aid in identification of landmarks, and wipe the skin with alcohol. Insert a 25-gauge needle at a 30-degree angle between the manubrium and point of the shoulder, cranial to the first rib. Direct the needle towards the head of the femur on the contralateral side. Once the skin has been pierced, apply negative pressure as the needle is advanced until blood is aspirated. Collect the sample and then gently apply pressure with gauze over the

puncture site to facilitate hemostasis before recovering the animal and returning to the home cage.

f. **Cardiocentesis** [*Mice, Rats, Hamsters, Guinea Pigs*]
Anesthetize the animal and wait until the animal reaches a deep plane of anesthesia as observed by lack of pedal reflex. Insert a 22-25 gauge needle with the bevel up through the skin and below the xiphoid process at midline at an approximately 10-30 degree angle. Direct the needle into the chest cavity and towards the heart. A 1" needle is typically sufficient for a mouse, but larger rodents may require a 1.5-2" needle to puncture the heart. Apply negative pressure by pulling back on the syringe plunger until blood is aspirated. Collect enough blood to exsanguinate the animal (4-5% of body weight) and observe cessation of heart and respiratory rate or another secondary method of euthanasia must be performed. *This technique should only be performed on a deeply anesthetized animal as a terminal collection.*
Note: Exsanguination via cardiocentesis can also be performed immediately following euthanasia (e.g., carbon dioxide asphyxiation) for both terminal blood collection and as a secondary method of euthanasia.

Table 2: Maximum blood collection volumes for common rodent species.
Note: If collection volumes will exceed the maximum volumes stated here, then the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used.

	Example Body Weight (BW)	1% BW per single collection	1.5% BW total over 14 days	4-5% BW terminal collection
Mice	20 g	0.2 ml	0.3 ml	0.8-1.0 ml
Rats	300 g	3.0 ml	4.5 ml	12.0-15.0 ml
Hamsters (dwarf)	30 g	0.3 ml	0.45 ml	1.2-1.5 ml
Hamsters (Syrian)	120 g	1.2 ml	1.8 ml	4.8-6.0 ml
Guinea Pigs	900 g	9.0 ml	13.5 ml	36-45 ml

III. **Animal Identification:** Common animal identification techniques for rodents are described below. More information including the advantages and disadvantages of these methods is available in the IACUC's *Rodent Identification* policy. Table 3 summarizes the recommended ages for the identification methods and whether anesthesia or analgesia is recommended.

a. **Subcutaneous Transponder Placement** [*Mice, Rats, Hamsters, Guinea Pigs*]
Anesthetize the animal and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex. Position the animal in ventral recumbency and wet the injection site over the shoulder blades with 70% alcohol to part the fur. The microchip and implantation cannula must be appropriately sized for the species (ideally 16g or smaller for rodents), encapsulated in biocompatible material, and sterilized prior to implantation. Most microchips can be purchased sterilized and pre-loaded into disposable delivery systems. Tent the skin at the injection site and insert the implantation needle subcutaneously into the inter-scapular space. Deliver the microchip and then slowly withdraw the needle while manually pinching the skin to ensure the microchip stays under the skin. If needed, the injection site

may be closed with medical grade tissue glue (e.g., Vetbond™). Recover the animal and return it to the home cage. This procedure is not recommended for neonatal mice due to the size of the implant but is acceptable for *weanlings and adults*.

b. **Toe Tattooing** [*Mice, Rats, Hamsters, Guinea Pigs*]
Use an appropriate restraint method for the age and species. Wipe the desired paw pad with an alcohol wipe. Transfer animal tattoo paste (e.g., Ketchum Manufacturing green tattoo paste) onto a sterile surface such as gauze squares or into a sterile secondary container (e.g., Eppendorf tube) to prevent contaminating the stock tube. Dip a 27-30 gauge hypodermic needle tip into a small amount of the tattoo paste and then superficially puncture through the toe pad corresponding to the desired number. A 30-gauge needle is recommended for mice, while up to a 27-gauge needle may be used for larger rodents. A new needle should be used for each animal and replaced if it becomes dull or barbed. Return the animal to its cage and do not clean excess paste off the toe pad. An example identification chart is included in Appendix I, but identification systems can vary based on needs and preferences. This procedure can be performed on all ages of rodents and is the preferred method for identifying neonatal mice.

c. **Tail Tattooing** [*Mice & Rats*]
Use an appropriate restraint method or anesthetize the animal. Wipe the tail tattoo site with an alcohol wipe. To manually apply a tattoo, use the tip of a 27-30 gauge sterile hypodermic needle to abrade the dermis of the dorsal tail in the shape of the desired identifier, careful to avoid the tail vasculature. Transfer animal tattoo paste (e.g., Ketchum Manufacturing green tattoo paste) onto a sterile surface such as gauze squares or into a sterile secondary container (e.g., Eppendorf tube) to prevent contaminating the stock tube. Dip the needle into a small amount of tattoo paste and apply it to the abraded area. Gently blot the excess paste from the tail with gauze. A new needle should be used for each animal and replaced if it becomes dull or barbed. Alternatively, commercially available animal tattooing methods such as the AIMS™ or Labstamp® rodent tattoo systems may be used according to the manufacturer's specifications. A dose of analgesia such as meloxicam or buprenorphine is recommended prior to starting the procedure. This procedure can be performed on all ages of mice and rats.

d. **Ear Punching/Notching** [*Mice, Rats, Hamsters, Guinea Pigs*]
Appropriately restrain the animal so the ears are accessible and there is limited movement of the neck/head. Use an ear punch device to remove a small piece(s) of ear tissue on the pinna. The punch/notch location should correspond with a desired identification system. If bleeding occurs, apply gentle pressure to the site with gauze and ensure hemostasis before returning the animal to its cage. The ear punch device should be maintained with a sharp cutting surface to minimize tissue injury and disinfected prior to each use. Ear punches should be no larger than 2 mm in diameter. This procedure can be performed once the ears have developed and the pinna are sufficiently pronounced (*after 14 days of age*).

e. **Ear Tagging** [*Mice, Rats, Hamsters, Guinea Pigs*]
Appropriately restrain the animal so the ears are accessible and there is limited movement of the neck/head. Wipe the pinna with alcohol and then apply a unique identifier using an ear tagging device. This is traditionally a stamped nickel tag, but may include newer commercial

options such as colored stainless-steel dots or plastic QR codes. The ear tag should be sterilized prior to application. Place the ear tag ~3mm inward from the margin of the pinna on the ventral half of the ear. Improper placement too central in the ear can cause excessive irritation and discomfort. Improper placement too distal on the pinna can cause the tag to become detached from the ear or torn out. This procedure can be performed once the ears have developed and the pinna are able to support the weight of the tag (*weaning age or older*).

f. **Fur Clipping** [*Mice, Rats, Hamsters, Guinea Pigs*]
A patch of fur on the back or side of the rodent may be shaved using an electric clipper. This method may be used to temporarily mark animals of all coat colors for 1-4 weeks at a time depending on the hair cycle.

g. **Temporary Marking** [*Mice, Rats, Hamsters, Guinea Pigs*]
Sharpies or other non-toxic markers may be used to temporarily mark rodents on the tail or fur. These marks usually only last 1-2 days and can be hard to see based on coat color. Non-toxic fur pigments are also available and may last up to 12 weeks (*e.g.*, Animal Markers).

IV. **Genotyping:** Common rodent genotyping techniques are described below. More information about these techniques is available in the IACUC's *Tissue Harvesting for Rodent Genotyping* policy.

a. **Ear Punching/Notching** [*Mice, Rats, Hamsters, Guinea Pigs*]
Ear tissue may be collected as described in the "Animal Identification" section and used for genotyping. Any variation from this method will be described separately in the proposal.

b. **Tail Biopsy** [*Mice & Rats*]
Manually restrain or anesthetize the animal and remove up to 2mm of the distal tail tip using a sterile scalpel, razor blade, or sharp scissors. Collect the sample and gently apply pressure with gauze to the biopsy site to facilitate hemostasis before returning the animal to the home cage. A clotting agent such as styptic powder or medical grade tissue glue (e.g., Vetbond™) may be applied if needed. A new sterile blade or scissors must be used for each animal or, alternatively, the instruments can be disinfected between animals with a bead sterilizer if reused. Recommendations and requirements for anesthesia and analgesia are based on the animal's age(see Table 3 below). Ice cold ethanol should be considered in pre-weaned animals as a local anesthetic. Systemic analgesia and general anesthesia is required in adult animals. Anesthetics and analgesics are commonly isoflurane and meloxicam or buprenorphine in rodents. Additional options can be found in IACUC Guidelines "Recommended Rodent Anesthetics and Analgesics".

Table 3: Anesthesia and analgesia recommendations and requirements for tail biopsy procedures based on rodent age and amount of tissue removed.

Age, Tissue Removed	Topical Anesthesia	Systemic Analgesia	General Anesthesia
<28d, up to 2mm	Consider ice cold ethanol	Recommended	
>28d, up to 2mm	Not required	Required	Required

c. **Blood** [*Mice, Rats, Hamsters, Guinea Pigs*]
Blood may be collected as described in the "Blood Collection" section and used for genotyping. Any variation from these methods will be described separately in the proposal.

d. **Saliva/Buccal Swab** [*Mice, Rats, Hamsters, Guinea Pigs*]
The animal is appropriately restrained based on age and species. A cotton swab is used to collect saliva and cheek cells from the oral cavity by rubbing the swab back and forth on the inside of the cheek. Once a sample is collected, the animal is returned to its home cage.

e. **Fecal Pellet** [*Mice, Rats, Hamsters, Guinea Pigs*]
Fecal pellets are either collected from the cage or collected directly from the animal. The animal may be manually restrained and handled for up to 1 minute to encourage passing and collection of a fresh stool sample. Alternatively, the animal may be placed into an empty, clean cage to encourage defecation. Once a sample is collected, the animal is returned to its home cage.

f. **Fur** [*Mice, Rats, Hamsters, Guinea Pigs*]
The animal is appropriately restrained based on age and species. Forceps are used to pluck a small amount of fur from the animal by pulling from the base of the hair shafts. Once a sample is collected, the animal is returned to its home cage.

References:

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4. Turner PV, et al. 2011. Administration of substances to laboratory animals: Routes of administration and factors to consider. *JAALAS* 50(5): 600-613.
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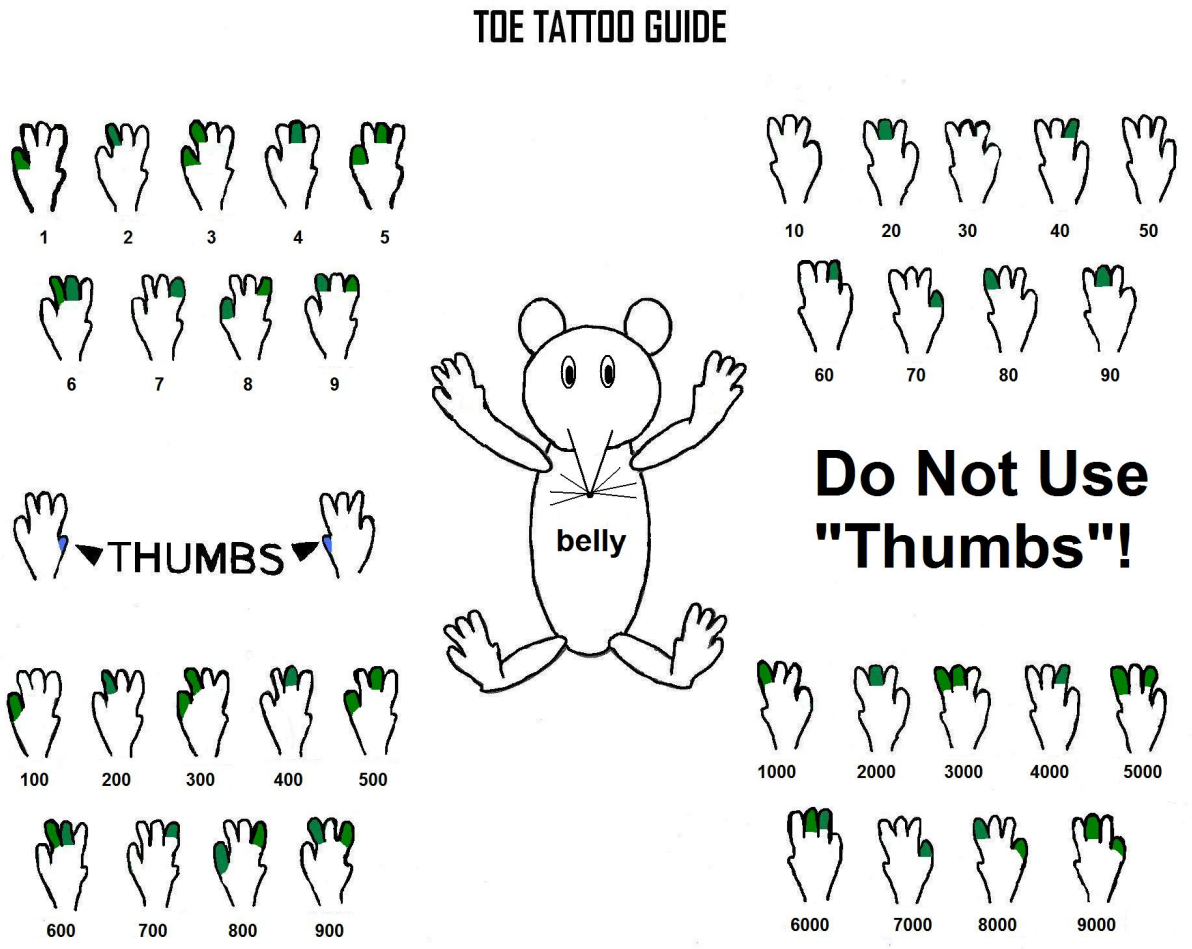
Appendix I: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