Biomedical Protocols for Free-ranging Brown Bears, Gray Wolves, Wolverines and Lynx

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PREFACE

Compilation of this document was initiated by the Norwegian Directorate for Nature Management in order to establish recommended protocols for capture, chemical immobilization, anaesthesia and radiotagging of free-ranging brown bears (*Ursus arctos*), wolves (*Canis lupus*), wolverines (*Gulo gulo*) and lynx (*Lynx lynx*). In addition, procedures to ensure proper sampling of biological materials for management, research and banking purposes have been included.

The current protocols are based on more than 2,000 captures of free-ranging brown bears, wolves, wolverines and lynx carried out during the last two decades in Scandinavia. Some of the results have been published as peer reviewed papers, conference presentations, theses and reports. However, a large amount of data are still on file and will be published in the future. In addition, comprehensive reviews of the world literature on brown bears, wolves, wolverines and lynx have been carried out in order to include pertinent information from other sources.

Specific and mandatory requirements for sampling in Norway and Sweden, respectively, are outlined in the appendices.

The protocols have been approved by all ongoing research projects on brown bears, wolves, wolverines and lynx in Scandinavia.

We thank the contributors for their cooperative efforts. We also thank the Norwegian Directorate for Nature Management for their support.

This document will be updated on a regular basis and will be available in pdf format at: <u>http://www.rovviltportalen.no/content.ap?thisId=500026811</u>

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INTRODUCTION

Chemical immobilization of wild animals is a form of veterinary anaesthesia conducted under the most difficult circumstances. Anaesthetic drugs are never completely devoid of toxicity and induction of anaesthesia invariably carries a risk to the life of even healthy patients. The risk of severe side effects, injuries and death can never be completely eliminated. In addition, several immobilizing drugs are toxic and potentially lethal to humans.

Chemical immobilization of free-ranging wildlife should only be considered if it is necessary to accomplish research or management goals, and should be carried out by a team of professionals with proper training, experience and expertise in wildlife capture, veterinary anaesthesia, animal handling and basic first aid and CPR techniques. If captures are carried out by darting from a helicopter, the skill of the pilot and the crew members is of paramount importance for a successful outcome.

All captures need to be properly planned. If possible, chemical immobilization of brown bears, wolves, wolverines and lynx should be carried out in winter or spring, on snow-covered ground. High ambient temperatures, open water and bare ground make captures more difficult and will increase the risk of accidents and mortality. Although a number of different capture techniques are available, darting from a helicopter is the most efficient, safest and probably least stressful method in most species and situations. Net-gun capture of wild animals is not recommended due to the extreme risk of helicopter accidents and due to animal welfare considerations.

DRUGS AND DOSES FOR CHEMICAL IMMOBILIZATION AND ANAESTHESIA

Brown bears

Brown bears are usually captured in early spring, shortly after they emerge from their dens. Although brown bears are sometimes chemically immobilized during summer or shortly before denning, such captures are more difficult due the lack of snow cover, open water, high ambient temperatures and increased dose requirements.

Brown bears are darted from a helicopter using a remote drug delivery system (Dan-Inject®). Currently, the following standard doses of medetomidine (M) (Domitor®, Zalopine®) and tiletamine-zolazepam (TZ) (Zoletil®) are used for immobilization of free-ranging bears in April-May: Yearlings (15-45 kg) 1.25 mg M + 62.5 mg TZ; small bears (2-3 years, 45-70 kg) 2.5 mg M + 125 mg TZ; adult females and small males (70-120 kg) 5 mg M + 250 mg TZ; medium-sized adult males (120-200 kg) 10 mg M + 500 mg TZ; large males (> 200 kg) 15 mg M + 750 mg TZ. A fixed M:TZ ratio is used so that doses can be split or combined. The doses and darts are made up as follows:

- 1.25 mg M + 62.5 mg TZ (yearlings): 1 ml of Zalopine® and 1.8 ml of sterile water are used to dissolve 500 mg of Zoletil®; split into 8 doses; use 2 ml darts with 1.5 x 25 barbed needles (Dan-Inject®)
- 2.5 mg M + 125 mg ZT (small bears): 5 ml of Domitor® and 0.8 ml of sterile water are used to dissolve 500 mg of Zoletil®; split into 4 doses; use 2 ml darts with 2.0 x 30 mm barbed needles (Dan-Inject®)
- 5 mg M + 250 mg ZT (adult females and small males): 5 ml of Domitor® and 0.5 ml of Zalopine® are used to dissolve 500 mg of Zoletil®; split into 2 doses; use 3 ml darts and 2.0 x 40 mm barbed needles (Dan-Inject®)

- 10 mg M + 500 mg TZ (medium-sized adult males): 1 ml of Zalopine® and 1.5 ml of sterile water are used to dissolve 500 mg of Zoletil®; one dose; use 3 ml dart and 2.0 x 40 mm barbed needle (Dan-Inject®)
- 15 mg M + 750 mg TZ (large adult males): 1 ml of Zalopine® and 0.5 ml of sterile water are used to dissolve 500 mg of TZ; make up three vials that are split into two doses; use 3 ml darts and 2.0 x 40 mm barbed needles (Dan-Inject®)
- For capture of bears late in the fall: Consider increasing the dose by 25-50 % and using longer needles.

Tiletamine-zolazepam used to be the drug combination of choice for immobilization of several bear species. Tiletamine-zolazepam has a wide margin of safety and has no major cardiopulmonary or thermoregulatory side effects in bears. The main disadvantage of this combination is extended recoveries. There is no reversal agent for tiletamine, and the use of a benzodiazepine antagonist like flumazenil (Anexate®), for reversal of zolazepam, in animals immobilized with high doses of tiletamine-zolazepam is not recommended. However, in combination with medetomidine, the effective dose of tiletamine-zolazepam can be reduced by as much as 75%, and atipamezole can then be used to shorten the recoveries. The physiologic effects of medetomidine-tiletamine-zolazepam have been studied in several bear species, and this drug combination is well tolerated by healthy individuals. Based on trials in captive brown bears scheduled for euthanasia, we consider that toxic effects from standard doses of medetomidine-tiletamine-zolazepam in healthy bears are very unlikely. A life-threatening situation should not be expected, even if a massive overdose (i.e. five times more than recommended) is administered by accident.

Wolves

Wolves are usually immobilized from a helicopter in winter on snow-covered ground. All animals ≥ 6 months of age, regardless of sex and body mass, are darted with 500 mg tiletamine-zolazepam (Zoletil®) per animal using a remote drug delivery system (Dan-Inject®). A 3 ml dart syringe with a 1.5 x 25 mm barbed needle (Dan-Inject®) is used. Recent trials indicate that the dose of tiletamine-zolazepam can be reduced to 250 mg per animal, although administration of medetomidine may be required to induce complete immobilization (see below). In trapped wolves, the lower dose of tiletamine-zolazepam is recommended. Mean (range) body weights in wolves > 18 months old captured in Scandinavia were 48 (38-52) kg for males and 39 (35-44) kg for females. Juveniles 7-10 months old weighed 34 (24-42) kg.

Wolverines

Adult wolverines and juveniles (> 8 months) are usually immobilized from a helicopter or in the den. Animals are darted with an initial dose of 4 mg of medetomidine (Zalopine®) + 100 mg of ketamine (Narketan 10®) per animal using a remote drug delivery system (Dan-Inject®). A 1.5 ml dart syringe with a 1.5 x 25 mm barbed needle (Dan-Inject®) is used. If the sex of an adult animal is known, an initial dose of 3 mg of medetomidine (Zalopine®) + 75 mg of ketamine (Narketan 10®) is sufficient for females (9-11 kg). The initial dose for adult males (14-16 kg) should be kept to 4 mg medetomidine (Zalopine®) + 100 mg ketamine (Narketan 10®). Cubs (up to 5-6 kg) are manually restrained, weighed and immobilized with 0.1 mg/kg of medetomidine (Domitor®) + 5 mg/mg of ketamine (Ketalar®) i.m. (induces 30-40 min of safe immobilization) A blood gas study showed that arterial oxygenation was impaired in adult and juvenile wolverines anaesthetised with the above doses. The anaesthesia was conducted at high altitudes (500-1,300 m above sea level) and altitude was responsible

for 30% of the reduction in arterial oxygenation. Further studies are needed to evaluate whether a lower medetomidine dose would improve arterial oxygenation. Supplemental oxygen is recommended.

Lynx

Adult lynx and juveniles (> 5 months) are either immobilized from a helicopter or captured using box traps or snares set around fresh roe deer kills. Hunting dogs are sometimes used to chase the lynx into a tree. Adults (males 18-26 kg, females 14-19 kg) are darted with an initial dose of 4 mg of medetomidine (Zalopine®) + 100 mg of ketamine (Narketan 10®) per animal using a remote drug delivery system (Dan-Inject®). In adults captured in box traps (calm animals) and in juveniles (6-12 months 9-16 kg, yearlings 12-21 kg), the doses can be reduced by 25 and 50 %, respectively. A 1.5 ml dart syringe with a 1.5 x 25 mm barbed needle (Dan-Inject®) is used. Kittens (4-5 weeks of age; mean body mass 1.5 kg) are captured by hand in the lair, weighed and immobilized with 0.1 mg medetomidine/kg (0.1 ml Domitor®) + 5 mg ketamine/kg (0.1 ml Ketalar®) i.m.

Supplemental dose

Supplemental dosing depends on the situation, species and whether anaesthesia is required or not. Animals that are not down 15 minutes after the initial dose, are redarted with a full dose (all species). If the animal is down but incompletely immobilized, administration of additional drugs is usually necessary.

Brown bears: In large bears (adult females and adult and subadult males), darting with either a full dose or half the initial dose is recommended for safety reasons. In yearling bears and small bears 1 mg of medetomidine (Domitor®) can be given i.m. by hand syringe injection.

Wolves: Wolves are usually easy to handle, even if they are not completely immobilized (which is often the case after darting with 250 mg of tiletamine-zolazepam). To reduce stress and to facilitate sampling, 1 mg of medetomidine (Domitor®) i.m. is recommended to induce complete immobilization.

Wolverines and lynx: If the animal is down but incompletely immobilized, 25-50 % of the initial dose can be given i.m. by hand syringe injection.

In case of a prolonged procedure or signs of spontaneous recovery, 0.5-1.0 mg of medetomidine (Domitor®) i.m., can be used to keep juvenile and adult wolves, wolverines and lynx and yearling bears immobilized for another 15-30 minutes. For safety reasons, 2 mg of medetomidine (Domitor®) should be combined with 1-2 mg/kg of ketamine (Narketan 10®) in adult bears. If extra time is needed to finish surgery or other painful procedures, medetomidine-ketamine should always be administered. Due to the long elimination time, additional tiletamine-zolazepam should not be used, unless for safety reasons in large bears.

CHASING, TRAPPING AND STRESS

Animals that have not been captured from (or chased by) a helicopter, are usually naïve when approached and darting can be performed within a few minutes of observation if the snow condition and the area are optimal (ice-covered lakes, clear-cuts, open terrain etc). Animals that have been captured before (especially wolves) will usually run for cover when they hear the helicopter and are much more difficult to approach. To avoid stress and physiological side effects (hyperthermia) during immobilization, intensive chasing should be kept to a minimum and the total time of pursuit should never exceed 30 minutes. In lynx, which are sometimes captured in box traps or foot snares or after being chased into a tree by hunting dogs, special care should be given to avoid accidents or physiological side effects due to lenghty procedures.

HANDLING AND MONITORING OF IMMOBILIZED ANIMALS

Immobilized animals should be monitored and clinically examined by professionals with experience in wildlife medicine. Possible side effects include respiratory depression (drug overdose in individuals with poor body condition, aspiration of vomitus/saliva, pneumothorax due to misplaced dart), vomiting (in wolves), and thermoregulatory dysfunction. If several animals are being captured at the same time (e.g.: members of a pack, family group), they should be brought together for monitoring and processing.

To prevent aspiration of saliva or vomitus, immobilized animals should be kept in lateral recumbency with the mouth and head low relative to the body. An eye gel (Viscotears®) should be applied to the cornea to prevent drying. Animals should be protected from direct sunlight into the eyes. Preferably, a blind-fold (and ear plugs) should be used.

Thermoregulation should be monitored by frequent measurements of the rectal temperature (RT). "Normal" RT in brown bears, wolves, wolverines and lynx is thought to be 38.0-39.0°C. Hyperthermic animals (RT > 40.0 °C) should be cooled by applying snow (or water in summertime) to the axilla, groin, and/or tongue. In case of persistent hyperthermia or RT > 41.0 °C i.v. fluid therapy should be initiated (10-15 ml/kg/hr of Ringer®-acetat). Oxygen supplementation is recommended to hyperthermic animals since the oxygen demand increases 10% for each degree °C increase in body temperature. Hypothermic (RT < 36.0 °C) animals should be protected from wind and cold surfaces to avoid further cooling using a Wolverine Bag®. In case of prolonged immobilization and recovery, hypothermic animals should be administered.

Cardiorespiratory function should be monitored using a pulse oximeter (Nellcor®) with the sensor (VetSat®) applied to the tongue. A relative arterial oxygen saturation $(SpO_2) > 90$ % is considered to be clinically acceptable in a field situation. A decreasing trend or $SpO_2 < 90\%$ indicate mild to severe hypoxaemia and treatment with 5-10 mg/kg of doxapram (Dopram®). Supplemental oxygen (using a nasal tube) is recommended in all immobilized animals. A laryngoscope, endotracheal tubes and a ventilation bag should be available. The color of the mucous membranes in the mouth can be used to assess blood oxygenation. A pink or red color is normal; bluish membranes indicate hypoxaemia. The capillary refill time (CRT) can be used to assess peripheral circulation. Normal CRT is 2 seconds or less.

A small surgical kit for treating wounds and an electrical clipper should be part of the standard equipement.

TAGGING, SAMPLING AND DOCUMENTATION

Most animals are captured for tagging or sampling purposes and should be processed according to the aim of the project. Capture data should be recorded according to an established animal capture form and photos should be taken (specific instruction for wolves).

Radiocollars (VHF, GPS or sattelite) should be fitted according to the size, age and sex of the animal. The weight of the radiocollar should not exceed 2% of the animal's body mass. Brown bears: The collar should be fitted so that it can be pulled on over the head. Drop-off collars or a break-away zone (double webbing in males, single in females) should be used on all growing bears and on bears of unknown age. For adult males, which may have a greater circumference of the neck than the head, one should consider clipping hair on the neck to avoid loosing the collar. Be sure that it is possible to pass a flat hand between the collar and the neck. Wolves: Minimum collar circumference should be 44.5 cm for females and 48.0 cm for males. Be sure that there is enough space for two fingers between the collar and the neck. Wolverines: The circumference of the animal's head and neck should be measured before fitting the collar. The circumference of the collar should then be adjusted so it is slightly less than the circumference of the head, but larger than the circumference of the neck. Be sure the collar is not too tight (make room for one finger between the neck and the collar) or that it can be pulled over the head of the animal. In some cases the difference in circumference of the head and neck is very small (especially in males) and fitting the collar can be difficult. *Lynx*: The minimum collar circumference should be 26 cm for females and 30 cm for males. Be sure that at least one finger can be passed between the collar and the neck. Collars for juvenile males should have a break-away zone or an implant should be used if 30 cm is too great to be retained by the animal. All species: The transmitter should be activated by removing the magnet and should be tested with the receiver before the animal is released. Be sure that the GPS unit is working properly before any capture is initiated.

A microchip (e.g. Indexel®) should be implanted s.c. in the hump of brown bears or at the base of the right ear in all the other species. The microchip should be tested with the scanner (Indexel®) after implantation. Tattooing and application of ear tags depend of the species, age of the animal and aim of the project. *Brown bears*: One plastic ear tag and a lip tattoo in all animals. *Wolves*: Numbered plastic tags in both ears only in animals that are not radiocollared. *Wolverines*: One aluminium ear tag (Sweden) and a lip tattoo in all animals. *Lynx:* One plastic ear tag (Norway) and lip tattoo in all adult animals. An ear tattoo in 5-week-old kittens. *All species*: Body measurements should be recorded according to the animal capture form.

Blood can be sampled from the jugular (all species), cephalic (wolves, lynx), or the femoral (all species) vein using evacuated plastic tubes and multisample needles (e.g. VenoSafe®, Venoject® II). The number of samples are specified in Appendix 1-4. Blood for genetic studies (5 ml EDTA) should be stored at -20 °C until shipment to the laboratory. Tubes without anticoagulant for serology should be kept at room temperature for 1-2 hours to ensure complete coagulation. Serum should then be separated by centrifugation (1500 g for at least 15 minutes) and transferred to 2 ml cryogenic vials (Nalgene®). Serum for banking (serology and back-up) is stored at -20 °C until shipment to the laboratory.

In brown bears and wolverines, the rudimentary first premolar is extracted for age determination. The tooth is preserved in 96% alcohol in a 2 ml cryogenic vial (Nalgene®).

Hair should be collected with pliers and transferred to 15 ml sterile plastic tubes (Sarstedt®) (brown bears and wolves) or 5 ml sterile cryogenic vials (Nalgene®) (wolverines and lynx). Hair samples can be preserved by drying (in paper envolopes) or by freezing at -20

°C. Skin biopsies are taken from the inside of the ear using a sterile dermal biopsy punch (Miltex®) (6 mm in brown bears and 4 mm in all other species) and transferred to 2 ml cryogenic vials (Nalgene®) and preserved by adding 96% ethanol.

In brown bears and wolves, feces is collected by inserting the index finger into the rectum using latex gloves. The feces is transferred to 50 ml sterile plastic tubes (Sarstedt®). In wolverines and lynx, feces is sampled by inserting a sterile cotton swab (Transwab®) into the rectum.

Depending on the situation and the study protocol, other biological materials should be sampled according to current standards in veterinary medicine or specific instructions from the laboratory.

ANALGESIA AND ANAESTHESIA FOR SURGERY

In brown bears, wolverines and lynx, surgical anaesthesia is induced by the recommended immobilizing drugs and doses. In wolves darted with 250 or 500 mg of tiletamine-zolazepam (Zoletil®), 0.025-0.05 mg/kg of medetomidine (Domitor®) i.m. is required to achieve surgical anaesthesia. Wolf pups (4-6 weeks old) are anaesthetized with 0.04 mg/kg of medetomidine (Domitor®) combined with 5 mg/kg of ketamine (Narketan 10®) i.m. The drugs can be given in the same syringe. [A separate protocol for gas anaesthesia of pups is available.] For post operative analgesia, 4 mg/kg of carprofen (Rimadyl®) is administered s.c. as soon as possible after immobilization is induced and before surgery is initiated (all species)

SURGICAL PROCEDURES FOR IMPLANTATION OF INTRAPERITONEAL RADIOTRANSMITTERS

The animal is kept in dorsal recumbency. An appropriate area caudal to the umbilicus is clipped and swabbed with chlorhexidine in 60% ethyl alcohol (Klorhexidin®). To avoid excessive heat loss at low ambient temperatures (below -10 C), clipping should not be done. Instead an antiseptic cream (Brulidine®) is rubbed into the fur along the midline and the hair is parted to expose the skin. For access to the peritoneal cavity, a ventral midline incision is made using standard surgical procedures. The weight of the implant (Telonics®) should not exceed 2% of the body mass of the animal. The radiotransmitter should be tested with the receiver before implantation. Implants should be gas sterilized (ethylene oxide) or disinfected by soaking in 10 mg/ml benzalkonium chloride (non proprietary) for at least 24 hours. They should be prewarmed and, in the case of chemically disinfected implants, thoroughly rinsed with sterile saline before being placed aseptically into the peritoneal cavity. The incision is closed in two layers with absorbable sutures (Vicryl®), using a simple interrupted pattern for the Linea alba (US 1 in all all bears except yearlings, US 0 in juvenile and adult wolves, wolverines, lynx and yearling bears and US 2-0 in wolf pups, wolverine cubs and lynx kittens; use sutures with round needle) and a interrupted horizontal mattress pattern for the skin (US 0 in all bears except yearlings and US 2-0 in all other animals; use sutures with a cutting needle). The skin wound is covered with a spraydressing (OpSite®). Before surgery the animal is injected with a "long-acting" combination of procaine penicillin and benzathine penicillin at 100.000 IU/kg (100 mg/kg) i.m. (PENI-kél L.A. 15+15®) in order to reduce the risk of postoperative wound infections.

REVERSAL OF IMMOBILIZATION

For reversal of immobilization in animals that have received medetomidine-combinations, 5 mg of atipamezole (Antisedan®) i.m. or s.c. per mg of the total dose of medetomidine administered. Due to the long elimation time of tiletamine-zolazepam, atipamezole should not be given until 40-50 min after darting. In an emergency, atipamezole can be given at any time but recovery may then be rough with possible incoordination, excitation and convulsions. Such an animal can be calmed by administration of midazolam (Midazolam®) i.m. (suggested dose 0.1-0.2 mg/kg).

Immobilized animals can usually be left to recover at the site of capture. Possible side effects and dangers during and immediately after recovery include vomiting (wolves), hypothermia (especially in animals with small body mass relative to body surface or in case of extended procedures), hyperthermia (due to extensive chasing prior to capture, sun and/or high ambient temperatures), intraspecific strife (attack by pack members, males attacking other males, males trying to mount immobilized females in estrus, males attacking dependent young), open water, lack of fear, traffic, and poaching. All wolves should be observed by trained personel until full recovery is evident. This may take 4-6 hours or more in wolves immobilized with 500 mg of tiletamine-zolazepam. It is highly recommended that all radio-instrumented animals are checked the day after capture.

OTHER TREATMENT

Captured animals with health-threatening diseases should be treated according to accepted standards in veterinary medicine. In animals with severe or terminal illness, euthanasia should be considered. Vaccination of free-ranging carnivores in Scandinavia is currently not recommended.

NECROPSY PROCEDURES

In case of a capture-related mortality, the carcass should be sent to a diagnostic laboratory for complete necropsy (Sweden: Statens Veterinärmedicinska Anstalt, Uppsala; Phone: + 46 18674000. Norway: Veterinærinstituttet, Trondheim; Phone: + 47 73580727). To ensure rapid cooling, skinning and evisceration should be considered. If transportation to the laboratory is not possible within 24-48 hours, the carcass should be frozen. As an alternative, a field necropsy can be carried out by a veterinarian after consultations with the laboratory.

LEGAL ASPECTS

All captures have to be approved by the appropriate animal etichal committee (Norway: Utvalg for forsøk med dyr; Sweden: Forsöksdjuretiska nämnden) and the wildlife management authority (Norway: Direktoratet for naturforvaltningen; Sweden: Naturvårdsverket). The use of motor vehicles may require special permits from local, regional and/or national authorities. Prior to starting capture activities, the police, animal welfare and wildlife authorities should be informed according to the permit. The use of radio-tememetry equipment reguires a permit (Norway: Post- og teletilsynet; Sweden: Post- och telestyrelsen). Immobilizing agents are prescription drugs and must be used by or on the the order of a licensed veterinarian (Norway: Statens legemiddelverk; Sweden: Läkemedelverket). Some of these drugs are also controlled substances, i.e. drugs that are capable of being abused, for which specific regulations apply. In Norway, non-veterinarians can legally use immobilizing agents if a valid veterinarian/client/patient relationship is established; i.e. the veterinarian should ensure that the animal in question is under his/her care. In Sweden a special permit is required for non-veterinarians (Jorbruksverket).

Withdrawal times (brown bears): According to the current legislation in force in the European Union (EU), any substance to be used in food producing animals must be assessed by the European Medicines Evaluation Agency (EMEA) in order to establish Maximum Residue Limits (MRLs). After assessment, substances may be listed in one of four Annexes of Council Regulation (EEC) No 2377/90 of 26 June 1990: Annex I – substances for which a full MRL has been fixed; Annex II - substances for which an MRL is not required; Annex III - substances for which a provisional MRL has been fixed; Annex IV – substances for which no MRL can be fixed. If the animal is a "food producing animal" (i.e. an animal, domestic or wild, captive or free-living, whose flesh or products are intended for human consumption), the veterinarian or the person acting under his/her direction may *only* administer a substance listed in Annex I, II, or III. Substances in Annex IV or substances that do not have an Annex entry (I, II, or III) may not be used in food producing animals. As of June 2001, very few of the drugs currently used for wildlife immobilization are authorized for use in food producing animals in the EU. In the EU, a withdrawal period is set within the procedure of granting a marketing authorization, i.e. either by the national authority concerned (Norway: Mattilsynet; Sweden: Läkemedelsverket) or, in case of a centrally authorized product, by the EMEA. However, for substances that do not have an Annex entry, no marketing authorization can be granted for use in food producing animals.

RECOMMENDED DRUGS AND EQUIPMENT

Disclaimer: The list does not indicate approval by any authorities or manufacturer for use on wildlife. Drugs and equipment mentioned in the text can be purchased from other manufacturers than those listed.

Anexate® 0.1 g/ml, F. Hoffmann-La Roche, Basel, Switzerland Antisedan®, 5 mg/ml, Orion Pharma Animal Health, Turku, Finland Brulidine[®], Aventis Pharma, Oslo, Norway Dan-Inject[®], Børkop Denmark Domitor® 1 mg/ml and Zalopine® 10 mg/ml, Orion Pharma Animal Health, Turku, Finland Dopram®, Wyeth Lederle, Wyeth-Ayerst International Inc., Philadelphia, PA, USA Indexel®, Merial, Lyon, France Ivomec® 10 mg/ml, Merial SAS, Lyon, France Ketalar® 50 mg/ml, Warner Lambert, Morris Plains, New Jersey, USA Klorhexidin 5 mg/ml, Galderma Svenska AB, Bromma, Sweden Midazolam® 5 mg/ml, Alpharma AS, Oslo, Norway Miltex[®], Miltex GmbH, Tuttlingen, Germany Nalgene[®], Nalge Company, Rochester, NY, USA Narketan 10® 100 mg/ml, Chassot, Dublin, Ireland Nellcor® NP-20, Nellcor Inc., Pleasanton, CA, USA OpSite®, Smith & Nephew Medical Limited, Hull, England PENI-kél L.A. 15+15, Kela Laboratoria NV, Hoogstraten, Belgium Produkte für die Medizin AG, Köln, Germany Rimadyl® 50 mg/ml, Orion Pharma Animal Health, Turku, Finland Ringer®-acetat, Pharmacia & Upjohn, Oslo, Norway Sartsedt®, Sarstedt AS, Ski, Norway Telonics®, Telonics Inc., Meza, AZ, USA Transwab®, Medical Wire & Equipment Co. Ltd., Corsham, Wiltshire, UK VetSat®, Nellcor Inc., Pleasanton, CA, USA Venoject® II, Terumo Europe N.V., Leuven, Belgium Vicryl®, Ethicon, Norderstadt, Germany Viscotears®, CIBA Vision AG, Hetlingen, Switzerland Wolverine Bag®Jerven AS, Odda, Norway Zoletil® 500 mg/vial, Virbac, Carros, France Zalopine® 10 mg/ml, Orion Pharma Animal Health, Turku, Finland

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Direktoratet for naturforvaltning, Norge: http://www.dirnat.no/ EMEA: http://www.emea.eu.int/ Forsöksdjuretiska nämnden, Sverige: http://www.djurskyddsmyndigheten.se/jahia/Jahia/pid/2 Läkemedelverket, Sverige: http://www.mpa.se/index.shtml Jordbruksverket, Sverige: http://www.sjv.se/ Mattilsynet, Norge: http://www.mattilsynet.no/ Naturvårdsverket, Sverige: http://www.naturvardsverket.se/ Post- og teletilsynet, Norway: http://www.npt.no/ Statens legemiddelverk, Norge: http://www.legemiddelverket.no/ Statens Veterinärmedicinska Anstalt, Sverige: http://www.sva.se/ Utvalg for forsøk med dyr, Norge - http://www.fdu.no/fdu/om/ Veterinærinstituttet, Norge: http://www.vetinst.no/

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APPENDIX 1 – BROWN BEAR

| SAMPLE | NUMBER/VOLUME | RECIPIENT |
|--------|---------------|----------------------|
| Tissue | 2 | SVA/Björnprojektet |
| Tissue | 2 | DN/NINA |
| Hair | 1 | SVA/Björnprojektet |
| Hair | 1 | DN/NINA |
| Feces | 1 | HiHm v/Jon M. Arnemo |
| Serum | 2 ml | SVA/Björnprojektet |
| Serum | 3 x 2 ml | HiHm v/Jon M. Arnemo |
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APPENDIX 2 – GRAY WOLF

| SAMPLE | NUMBER/VOLUME | RECIPIENT |
|------------------|---------------|----------------------|
| Tissue (new) | 1 | DN/NINA |
| Tissue (new) | 1 | Skandulv/Grimsö |
| Hair (new) | 1 | DN/NINA |
| Hair (new) | 1 | Skandulv/Grimsö |
| Feces | 1 | HiHm v/Jon M. Arnemo |
| EDTA blood (new) | 1 x 5 ml | DN/NINA |
| EDTA blood (new) | 1 x 5 ml | Skandulv/Grimsö |
| Serum | 6 x 2 ml | HiHm v/Jon M. Arnemo |
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APPENDIX 3 – WOLVERINE

| SAMPLE | NUMBER/VOLUME | RECIPIENT |
|--------|----------------------------------|---------------------|
| Norway | | |
| Tissue | 2 | DN/NINA |
| Hair | 2 | DN/NINA |
| Feces | 1 | NVH v/Jon M. Arnemo |
| Serum | 2 x 2 ml adults 1 x 2 ml cubs | NVH v/Jon M. Arnemo |
| Sweden | | |
| Tissue | 2 | Järvprojektet |
| Hair | 1 | Järvprojektet |
| Feces | 1 | NVH v/Jon M. Arnemo |
| Serum | 2 x 2 ml adults 1 x 2 ml cubs | NVH v/Jon M. Arnemo |
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APPENDIX 4 – LYNX

| SAMPLE | NUMBER/VOLUME | RECIPIENT |
|--------|-----------------|---------------------|
| N | | |
| Norway | | |
| Tissue | 2 | DN/NINA |
| Hair | 2 | DN/NINA |
| Feces | 1 | NVH v/Jon M. Arnemo |
| Serum | 2 x 2 ml adults | NVH v/Jon M. Arnemo |
| | 1 x 2 ml cubs | |
| | | |
| Sweden | | |
| Tissue | 2 | Loprojektet |
| Hair | 1 | Loprojektet |
| Feces | 1 | NVH v/Jon M. Arnemo |
| Serum | 2 x 2 ml adults | NVH v/Jon M. Arnemo |
| | 1 x 2 ml cubs | |
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