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Review

Nonexperimental Xenobiotics: Unintended Consequences of Intentionally Administered Substances in Terrestrial Animal Models

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Abstract

Summary: Review of the use of nonexperimental xenobiotics in terrestrial animal models and the potential unintended consequences of these compounds, including drug-related side effects and adverse reactions.

Key words: Xenobiotics; drug-related side effects; adverse reactions; animal models; review

Xenobiotics are chemicals or compounds that are foreign to a biologic system. Exposure to xenobiotics may occur through application or inoculation of pharmacologic agents or other chemicals intentionally introduced as part of the routine conditioning or experimental procedure. The effect or toxicity of a xenobiotic is based on the dose, route, and disposition. Absorption, distribution, biotransformation, and excretion all affect xenobiotic disposition [1]. In addition, host barriers (that is, the skin, lungs, and alimentary tract, and the physical and chemical properties of the xenobiotic also affect its toxicity. The xenobiotic and its metabolites may cause physiologic alterations in the animal and thus affect the outcome of the experiment by altering immune function, cardiovascular parameters, or organ systems or by acting as a mutagen and/or a teratogen [1, 2]. Examples of nonexperimental xenobiotics can include antiinfectives; commonly administered anesthetics, analgesics, sedatives, and tranquilizers; specific topical treatments; and surgical scrub agents that can be absorbed trans-dermally.

Pharmaceutical agents are administered to laboratory animals for a variety of reasons. For example, pharmaceuticals are administered to induce and maintain anesthesia, provide analgesia, prevent or treat disease, or activate an inducible promoter that turns on or off specific genes. While their administration may be necessary, they are ancillary to the primary experimental goal. Pharmaceuticals can result in physiological changes distinct from those expected from their principal mechanism of action, or they can alter the metabolism of other chemicals and therefore alter experimental results. Importantly, effects induced by pharmaceutical agents can frequently be dose and species dependent. Antiinfectives; anesthetics, tranquilizers, sedatives, and analgesics; topical agents; other medications given intra-orally, intra-tracheally, or topically; and surgical scrub agents will be covered in this chapter. Commonly used experimental compounds such as doxycycline for conditional mutants, Bromodeoyuridine (BrDU) for cell mapping, luciferase for in vivo bioluminescent imaging, and solvents (eg, Dimethyl sulphoxide (DMSO) and Polyethylene glycol (PEG)) will not be discussed herein as they are beyond the scope of this review [3].

Antiinfectives

The potential for antimicrobial compounds to influence physiologic responses is well established. Because these compounds are commonly administered to terrestrial animal models, it is essential to recognize the nature and scope of their potential side effects. Antibiotic administration can be toxic to a variety of animals. Aminoglycoside antibiotics, including gentamicin, amikacin, and dihydrostreptomycin/streptomycin, are

nephrotoxic and ototoxic, can induce neuromuscular blockade, and can produce negative inotropic effects in both cardiac and arterial muscle [4–7]. Vancomycin is also known to cause nephrotoxicity. While the exact mechanism is undefined, studies in laboratory animals incriminate oxidative effects on the proximal renal tubule [8]. Hepatoxicity is associated with some penicillins, sulfamethoxazole/trimethoprim, erythromycin, and several other macrolides [9]. Procaine, used in some penicillin formulations, has been shown to be toxic to guinea pigs, mice, and rabbits [10]. Some fluoroquinolones are known to damage cartilage [11] and can cause hepatotoxicity [12]. High doses of lincomycin can disrupt myocardial conductance and is arrhythmogenic in dogs [13]. Some antibiotics can affect the endocrine system. For example, sulfamethoxazole (SMZ) induces thyroid hyperplasia and hypertrophy in mice, rats, and dogs, and causes a decrease in free thyroxine in dogs and mice [14, 15]. In mice, SMZ caused a marked elevation in thyroid-stimulating hormone and a decrease in circulating free thyroxine after feeding a commercially available rodent diet impregnated with trimethoprim-SMZ after 2 weeks [14]. Subacute exposure of imipenem/cilastatin in Wistar rats may cause nephrotoxicity and increase the risk for developing uroliths; further, this can induce an oxidative stress status and histopathological changes in the testis and alter spermatogenesis in a dose-dependent manner [16, 17].

Importantly, several classes of antibiotics can affect immune function, including tetracyclines [18], aminoglycosides [19], fluoroquinolones [20], trimethoprim-SMZ, and chloramphenicol [21]. Other antibiotics indirectly induce toxicity by altering the normal (commensal) flora of the gastrointestinal tract [22], thus causing changes in the microbiome [23]. In addition, antibiotic use can eliminate commensal bacteria, allowing for colonization and overgrowth of harmful bacteria. That being stated, it is critical to ensure that animals receive therapeutic doses and not be underdosed, particularly when drugs are delivered in water sources [24]. For example, guinea pigs, hamsters, gerbils, and rabbits may develop fatal enterotoxemias when treated orally with antibiotics, such as penicillin, permitting colonization and proliferation of either toxin-producing *Clostridium difficile* or *C. spiroforme*, dependent on the animal species, the route of administration, and the dose [25, 26]. Whether by direct or indirect action, antibiotics can also influence the pharmacokinetics and metabolism of other agents. For example, the fluoroquinolones compete with GABA receptors and therefore can interfere with studies involving the central nervous system [27]. Bacitracin, gentamicin, and nystatin alter cecocolonic motility and increase fecal excretion of dry matter and water in rats, and amoxicillin/clavulanate alters intestinal motility in humans [28]. Macrolide antibiotics can inhibit hepatic metabolism of some compounds by forming complexes with them, can directly inactivate cytochrome P450, or can alter the enteric flora, impacting compound bioavailability [29]. Pazufloxacin and meloxicam may induce oxidative damage in rabbits, including a decrease in reduced glutathione levels and significant lipid peroxidation compared with levels in untreated controls. Pazufloxacin also induces glutathione peroxidase activity [30]. Therefore, the concurrent administration of antibiotics with test compounds should be carefully considered. Lastly, natural antimicrobial peptides are being used with greater frequency due to the increased antimicrobial resistance to traditional antibiotics. These small cationic peptides have broad antimicrobial activity. Examples include magainin from frogs and indolicidin from cows; notably, toxicity problems have been shown with these compounds [31]. Antimicrobial peptides will likely become more commonly used in comparative medicine clinically; thus, it will be important to understand the physicochemical properties and potential side effects of these compounds.

Parasiticides can interfere with normal metabolic processes and may impact research outcomes. Ivermectin, commonly used as both an anthelmintic and an acaricide, is toxic to certain dog breeds and mouse strains due to a lack of p-glycoprotein, a member of the adenosine triphosphate-binding cassette transporter protein superfamily that is normally present in the blood–brain barrier [32]. Fenbendazole, a benzimidazole anthelminthic that disrupts parasite tubulin–microtubule equilibrium, is commonly used to treat oxyuriasis in rodents. Fenbendazole interferes with motor function in mice as measured by rotarod, induces select hepatic cytochrome P450 isoforms known to activate procarcinogens, prolongs microglial activation, induces release of striatal dopamine, attenuates the loss of astrocytes, and induces weight loss in F344 rats injected intrastriatally with Lipopolysaccharide (LPS) [33–35]. In addition, several anthelmintics can induce immunomodulatory effects. Ivermectin, levamisole, and thiabendazole are immune potentiators. In 1 report, ivermectin activated a tamoxifen-regulated Cre recombinase fusion protein in murine T cells [36]. Levamisole stimulates cell-mediated immune responses, enhances the rate of T-lymphocyte differentiation, and increases the activity of effector lymphocytes [37, 38]. In contrast, fenvalerate, oxfendazole, and aminocarb are immune suppressors. Fenbendazole and dieldrin can have both immunostimulatory and immunosuppressive actions depending on experimental conditions and the specific immune function evaluated [39]. Fenbendazole has been shown to depress splenic B-cell proliferation, especially in aged mice, by decreasing mRNA and protein expression of a B-lymphocyte transcription factor. Fenbendazole also increases anti-DNA antibody in the (NZB X NZW)F1 model of autoimmunity without altering disease progression and inhibits subcutaneous growth of a human Burkitt lymphoma cell line in SCID mice when coadministered with a vitamin supplement [35, 40–42]. Fipronil alters cytochrome P450 enzyme activities in rats, including the induction of hepatic phase I cytochrome P450 enzymes in a dose-dependent manner. This may cause an increased potential to interact with compounds that are substrates of these enzymes [43].

Anesthetics, Tranquilizers, Sedatives, and Analgesics

The induction and maintenance of general anesthesia leads to significant physiological alterations, principally of the cardiovascular, pulmonary, neuroendocrine, immune, and nervous systems. Tranquilizers, anesthetics, and analgesic combinations are usually selected, which minimize physiologic disturbance of the system under study. This choice is most critical when experimentation is conducted while the animal is under anesthesia, as most significant cardiopulmonary alterations return to normal following recovery. However, some anesthetics can induce physiological and behavioral changes distinct from their cardiopulmonary effects, which may persist long after the animal has awakened from anesthesia. For example, rats exposed to 1 minimum alveolar concentration sevoflurane had altered brain protein-level expression for up to 28 days postanesthetic exposure [44].

Anesthetics can be directly toxic. Anesthetics such as tribromoethanol, xylazine, the combination of ketamine and xylazine, and a variety of inhalant anesthetics have been shown to induce tissue injury. When administered at clinically

relevant doses, and depending on the species and route of administration, examples of tissue injury include pulmonary parenchymal damage, muscle necrosis, peritonitis, corneal calcium deposition and ulceration, lymphocyte and Kupffer cell damage, ileus, and keratoconjunctivitis sicca [45–54]. Lesions associated with the use of tribromoethanol in mice are speculated to have resulted from improper anesthetic preparation or the concentration administered rather than from any inherent toxicity of the anesthetic [55, 56]. Methoxyflurane is nephrotoxic in F344 rats, causing a dose-related diabetes insipidus syndrome [57]. Hepatic toxicity is caused during the metabolism of desflurane, enflurane, halothane, and isoflurane due to tissue acetylation that create neo-antigens against which an antibody-mediated immune response may occur [58]. Sevoflurane anesthesia increases plasma inorganic fluoride concentrations [58]. Neurotoxicity in neonatal mice and rats has been shown with isoflurane, nitrous oxide, propofol, sevoflurane, and midazolam [59–63].

Anesthetics may enhance or inhibit the toxicity of other agents. Barbiturates and xylazine induce hepatic cytochrome P450-metaboloizing enzymes that may influence the metabolism of other chemicals [64]. Tribromoethanol, ketamine and xylazine in combination, isoflurane, meloxicam, and buprenorphine reduce fetal growth [4]. Enflurane, halothane, and methoxyflurane have been shown to inhibit cytochrome P450-dependent type I substrates [65]. The authors speculated that methoxyflurane, because of its high lipid solubility, could have long-lasting effects.

The effects of anesthetics on the immune system are well recognized. Halothane, methoxyflurane, isoflurane, sevoflurane, desflurane, tribromoethanol, ketamine-xylazine in combination, chloral hydrate, pentobarbital, and urethane have been shown to reduce the responsiveness of lymphocytes to mitogens and decrease their chemotactic, phagocytic, and transforming capabilities as well as their ability to synthesize RNA and protein; inhibit cell-mediated cytotoxicity, neutrophil and monocyte chemotaxis, and neutrophil phagocytosis; and influence T-cell adhesion properties as well as their inflammatory response [66–73]. A variety of anesthetic agents and opioid analgesics, including tribromoethanol, ether, fentanyl, halothane, isoflurane, ketamine-xylazine, morphine, sufentanil, and sevoflurane, may reduce the cytotoxic activity of natural killer (NK) cells in the postoperative period [74–76]. Hypo-responsiveness of NK cells lasts for at least 11 days after anesthesia [77]. Following anesthesia, NK cells fail to respond normally to interferon (INF) or poly I: C, an inducer of endogenous INF synthesis [75, 76, 78]. Anesthetic-induced NK-cell activity depression strongly accelerated progression of spontaneous lung metastasis produced by the 3LL Lewis lung carcinoma and B16 melanoma [79, 80]. Conversely, meloxicam, but not buprenorphine, has been shown to reduce B16 melanoma lung metastases in C57BL/6 mice [81]. Ketamine and thiopental have been shown to inhibit the production of endogenous proinflammatory cytokines, including tumor necrosis factor-α, interleukin (IL)-1, IL-6, and IL-8, and increase the production of the antiinflammatory cytokine IL-10 [82].

Rabbits are commonly used for surgical models in biomedical research and are exquisitely susceptible to postoperative anorexia and weight loss. When evaluating gastrointestinal side effects associated with the pain management of Dutch Belted rabbits, buprenorphine, meloxicam, and incisional infiltration with bupivacaine were assessed during a 7-day postoperative period following routinevariohysterectomy [83]. All rabbits showed decreased pellet consumption, fecal production, and weight on day 1 after surgery. This effect was severe in some rabbits that received bupivicaine; therefore, treatment of this entire group with metoclopramide, fluids, and hay was instituted to reverse gut stasis. No significant difference in feed consumption and fecal production was observed between the buprenorphine- and meloxicam-treated groups. On the basis of these results, meloxicam appears to be a suitable alternative or adjunct to buprenorphine for alleviating postoperative pain with minimal risk of anorexia and gastrointestinal ileus in rabbits [83].

The effects of anesthetics on cardiovascular function are well known. At concentrations used clinically, most volatile anesthetics and many injectable anesthetics depress myocardial contractile force of the heart. The mechanisms underlying the negative inotropic effects of these agents partially involve the effect of calcium on the myofibrillar apparatus [84]. Volatile anesthetics, including halothane, isoflurane, and sevoflurane, have an inhibitory effect on both vascular and tracheal smooth muscle, leading to both vascular and airway dilatation [85–87]. These agents attenuate and prevent airway smooth muscle constriction when exposed to allergen and leukotriene-D4 [88]. These effects are mediated by influencing calcium sensitivity [85, 86].

It is worth highlighting some unintentional consequences associated with isoflurane use, as it is one of the most commonly used inhalant anesthetics in the laboratory animal setting due to its high safety profile for both the user and patient for procedures of all levels of invasiveness. It has been shown in the literature that exposure of isoflurane in mice may cause a decrease in cognitive performance [89]. Conversely, isoflurane and sevoflurane (1 minimum alveolar concentration for 2 hours) demonstrated enhanced performance in mice after 24 hours of exposure but not after 7 days [90, 91]. It has been shown that even brief (<1 minute) exposure of isoflurane anesthesia to mice 21–45 days old results in significant locomotor and behavior deficits for up to 5 hours postinhalation [92]. In addition, measurable benefits (eg, diminished anxiety-like responses to procedures like tail biopsy) are not evident compared with nonanesthetized control mice. A growing body of literature has identified additional undesirable outcomes of isoflurane on neonatal and young adult mice. This inhalant has been shown to have neurodegenerative effects and can impact cognitive function, spatial learning, and memory [93–98]. Interestingly, isoflurane exposure may have positive effects contributing to antiinflammatory responses in organs, subcutaneous tissues, and skin [99]. For example, pre- and posttreatment with isoflurane and other volatile anesthetics has been shown to reduce brain edema and improve neurobehavioral function in mice with traumatic brain injury or induced subarachnoid hemorrhage; [100–102] decrease renal and myocardial ischemic-reperfusion injury in mice [103, 104]; and attenuate sepsis-induced lung inflammation [105]. The mechanism by which this antiinflammatory effect is exerted is still unknown, but isoflurane has been shown to decrease several proinflammatory cytokines involved with neutrophil recruitment. [106–108]

The widely used α2-adrenergic agonist, xylazine, lowers basal plasma insulin concentrations and abolishes the rise in insulin following glucose administration, resulting in elevations in fasting glucose and glucose intolerance in multiple species [109–112].

Select anesthetics have been shown to exert effects on the neuroendocrine system. Reported anesthetic-induced alterations include both increased and decreased cortisol and catecholamine secretion; increased concentrations of serum growth hormone, thyroxine, antidiuretic hormone, ACTH,

and renin; and decreased secretion of luteinizing hormone, aldosterone, and testosterone [113–118]. The combination of ketamine and etomidate has been shown to downregulate the hypothalamic-pituitary-adrenal axis in mice [119]. The metabolism of etomidate in dogs can cause corticosteroid suppression due to the accumulation of metabolites in the brain or periphery [120, 121]. Etomidate also significantly inhibits androgen synthesis and LH-stimulated androgen production in the Leydig cells of rats [122]. The combination of propofol and ketamine in rabbits results in increased levels of cortisol and corticosterone [123], while propofol alone has been shown to increase corticosterone levels and induce long-term increase in the frequency of hippocampal miniature postsynaptic currents in neonatal rats [124].

Anesthetics and analgesic agents may also influence behavior. High doses of buprenorphine are associated with pica in rats [125]. Cognitive performance in behavioral assays may be impaired or enhanced by volatile anesthetics. For example, rats had impaired performance in a previously trained spatial memory task for up to 2 weeks after isoflurane–nitrous oxide anesthesia, whereas impairment lasted only 2 days during the acquisition of a new task [126–129]. Opioids have been shown to indirectly hinder tumor growth by controlling pain while promoting tumor growth and metastasis via their direct effects. In addition, nonsteroidal antiinflammatory drugs have been shown to have a significant impact on tumor growth and metastasis, primarily by reducing angiogenesis and cell proliferation while promoting apoptosis. The review article "Influence of Pain and Analgesia on Cancer Research Studies" provides an in-depth review of these immunomodulatory affects [130].

Topical Anesthesia/Analgesia

Certain topical treatments also fall under the category of anesthetics or analgesics given that they are designed to be a less invasive means of alleviating pain after surgical or experimental procedures.

Unintended effects have been described following the use of select topical agents, which can be of critical importance to experimental outcome even though they are often clinically inapparent.

The administration of local anesthetics inhibits lymphocyte capping; depresses adhesion, phagocytosis, and the production of superoxide anions and hydrogen peroxide in neutrophils; reduces both the number and function of CD4+ and CD 19+ cells; alters lymphocyte secretion of INF, tumor necrosis factor, IL-1, and soluble IL-2 receptor following stimulation by a variety of mitogens; and increases plasma endothelin-like immunoreactivity [79, 131–134]. Local anesthetics including procaine, lidocaine, butacaine, tetracaine, and dibucaine have been shown to enhance the toxicity of the bleomycin derivative, peplomycin [135]. The application of the topical anesthetic benzocaine is associated with methemoglobinemia in a variety of species [136]. A 2-second burst of anesthetic spray or direct application of 56 mg of benzocaine increased methemoglobin concentrations sufficiently to substantially alter cardiovascular and pulmonary function [136, 137]. Recent work has shown that lidocaine, when mixed with an injection of sodium pentobarbital euthanasia solution, reduced abdominal writhing in rats; similar results were found when bupivacaine was mixed with euthanasia solution [138].

Other topical treatments can include vapocoolants, liquid analgesics, and alcohol-based agents, several of which have been attempted for preemptive relief of discomfort prior to tail and digit biopsy [139–144]. Cetacaine is a fast-acting, long-lasting topical anesthetic that combines benzocaine (fast-onset, short-acting) with butamben (intermediate-onset, intermediate-acting) and tetracaine (slow-onset and long-acting) to provide a potentially broader therapeutic range of topical effect. Cetacaine is specifically labeled for use on mucous membranes; therefore, it was not unexpected to find that this agent appeared to have minimal to no anesthetic effect when applied to intact skin and tissue. Jones et al found that pretreatment of mouse tails by submersion for 2 minutes into a solution of lidocaine, bupivacaine, or a combination of the two did not seem to permit sufficient penetration into tail tissue to ensure adequate localized analgesia [142]. Similarly, Freeman found no significant differences in hot-plate withdrawal times between untreated mice and those treated with lidocaine, bupivicaine, or combinations of the two when delivered by tail submersion in DMSO [141]. Reports of methemoglobinemia due to oropharyngeal use of topical analgesics have been published in both humans and various species of laboratory animals, including mice [136, 145–147]. A very common behavioral observation after application of topical agents is for mice to lick the tip of the tail, leading to the likely ingestion of Cetacaine. Due to the extensively studied concerns of methemoglobinemia when ingested or applied topically to the mucosa of the oropharynx, the use of Cetacaine may pose a further safety hazard to laboratory mice after application.

The interest in assessing vapocoolants, as compared with systemic anesthesia, was derived from anecdotal reports, and the National Institutes of Health recommends that these "skin refrigerant" agents would be effective in providing some measure of comfort following tissue sampling. These agents are often used in human and sports medicine to temporarily assist with pain control associated with injections, minor surgical procedures, sprains, bruising, and muscle injury; thus their use in certain animal species has been extrapolated to have potential efficacy [148–153]. Vapocoolants are typically delivered by aerosol application, and the cooling effect is thought to be the direct result of immediate evaporation of the alcohol-based ingredients. The intended mechanism of action is for the rapid onset of cold to temporarily numb the skin by decreasing nerve conduction, thus interrupting the stimuli to the brain that typically would process the sensation of pain.

Although ethyl chloride has long been used as a vapocoolant for pediatric human patients and in other patients receiving injections or catheter placements, it has been shown to be less effective compared with other local anesthetics [154–156]. Additionally, inappropriate use of ethyl chloride has the potential to cause tissue damage and frostbite [157]; therefore, the labeled use advocates for protection of surrounding skin with petroleum jelly (Vaseline). In mice, despite the application of Vaseline along the majority of the tail, animals sprayed with ethyl chloride exhibited greater reaction after tail biopsy and increased licking and grooming of the tail (likely due to the detected temperature change by freezing of the tail), and ethyl chloride treatment led to the greatest amount of inflammatory infiltrate in distal tail tissues [99].

Work by Jones et al assessed outbred preweanling Non-Swiss Albino mice at 17 day old with application of ethyl chloride spray, buprenorphine (0.05 mg/kg subcutaneously), and 30-second immersion in 0.75% bupivicaine to determine if these agents provided appropriate analgesia for tail biopsy [142]. The group found that local anesthetics were ineffective in alleviating pain associated with tail biopsy and further stated that topical ethyl chloride spray resulted in an increase in tail grooming

behavior for as long as 60 minutes compared with other treatment groups. Importantly, this same group verified that submersion of tails into ice-cold ethanol, currently endorsed by various institutional guidelines [158–162], also is not effective at providing topical analgesia [142]. Paluch et al sought to evaluate effects of vapocoolant (95% 1,1,1,3,3 -pentafluoropropane and 5% 1,1,1,2-tetrafluoroethane) for toe clipping procedures in C57Bl/6 J mice. This group applied vapocoolant on digits of young pups (7–17 d.o.) and determined that the immediate change in tissue temperature appeared to be distressful and often fused digits together. They also noted that treated animals elicited vocalizations, limb withdrawal, prolonged tissue swelling, and increased blood loss following toe clipping, likely related to subsequent hypothermia-induced vasodilation. This group ultimately did not recommend the use of a vapocoolant, as the adverse effects on the animals markedly outweighed any potential benefits [144]. Overall, the use of vapocoolant, ethyl chloride spray, is ineffective for providing cryoanalgesia and may have serious drawbacks to routine use in laboratory rodents [99].

Other Medications Given Intra-orally, Intra-tracheally, or Topically

Styptic powder, or other hemostatic powders, as a means to stop bleeding after biopsy or incisions has not been widely assessed in animals. The concern in application is related to animals grooming the agent from the site and ingesting the chemicals, with little known about possible side effects. Recent studies from human literature have analyzed hemostatic powders and not found any negative impact on humans [163].

Dermatologic issues, wounds, and superficial inflammation are very often topically treated in veterinary medicine. For chronic dermatitis, particularly in laboratory rodents, a plethora of agents have been studied, many with variable efficacy. Topical treatments include triple-antibiotic ointment, pramoxine lotion, Vitamin E, povidone-iodine, and sulfadiazine and sodium hypochlorite; these have shown variable success in laboratory mice [164–169]. Disease predilection for idiopathic dermatitis has been linked to the use of topical treatments in 10- to 16-month-old female mice. In addition, mice with a C57BL/6 background deficient in the gene for inducible nitric oxide synthase have had as high as a 50% disease incidence [170]. Recently, a more popular and benign noninvasive treatment for ulcerative dermatitis has been the clipping of sharp toenails; when rodents are unable to self-injure as they scratch, lesions have time to heal [171, 172]. Lanolin is often utilized to treat dry wounds and skin with excellent outcomes in rodents with ringtail; lanolin ointment is most commonly used as a relief measure for breast-feeding women and works well in laboratory animals because it is a nontoxic and effective moisturizer thought to be benign even if ingested [173]. Radiation-induced lesions are also treated by topical agents, some of which are more efficacious than others in reducing inflammation, further injury, and pain [174].

Topical cyclosporine has been used to successfully treat atopic dermatitis in nonhuman primates [175]. Honey has been a topical treatment for wound healing for centuries and is used with efficacy in laboratory animal species [176].

Transdermal applications for analgesia (eg, fentanyl) are increasingly applied to a variety of species in veterinary medicine as a treatment method that allows for slow release of drug and minimizes animal handling and the stress of frequent/repeat dosing of pill or oral formulations [177–179]. Overdosing is typically not of concern unless animals are able to access the patches and ingest them. As an oral treatment in mice, chronic fentanyl administration induces some behavioral changes in mice but is ultimately felt to be safe under properly controlled conditions [180]. In dogs, single administration of transdermal fentanyl was felt to be safer than repeated subcutaneous injections of oxymorphone for the control of postoperative pain [181]. Side effects in dogs and sheep may include severe sedation, decreased food and water intake, hypersalivation, lateral recumbency, stereotypic behavior, and gut hypomotility with abnormal stool [182, 183]. Formulations of sustained release nonsteroidal antiinflammatory drugs (ie, meloxicam) in sheep [184] and sustained release buprenorphine in guinea pigs [185] may offer an option for prolonged pain management yet warrant further research. In growing pigs, transdermal fentanyl has been found to have variable efficacy for pain management because drug absorption from patches was unpredictable and deficient [186]. In contrast, recent studies in cynomolgus macaques have shown that therapeutic levels for fentanyl and low- and high-dose buprenorphine patches were maintained for 96, 120, and 144 hours, respectively; thus both fentanyl and buprenorphine patches achieve minimal therapeutic levels for clinically relevant periods of time and should be considered viable options for pain management [187].

Tamoxifen is commonly utilized as a chemotherapeutic agent in veterinary medicine and cancer treatments, as well as to induce gene expression in transgenic mouse models. Tamoxifen is administered for estrogen receptor positive breast cancers, but it can induce uterine endometrial cancer and nonalcoholic fatty liver disease, which may be offset by administration of antifertility drugs [188]. Side effects of tamoxifen include exacerbated neutrophil activity that may harm surrounding tissues. This is important in a range of diseases, including allergic asthma and chronic obstructive pulmonary disease in humans, as well as equine asthma (also known as recurrent airway obstruction [189]). Side effects of tamoxifen in dogs can include development of pyometra; therefore, spaying of females is recommended when undergoing treatment [190]. In mice, acute liver injury can be a fatal side effect of tamoxifen treatment; administration of medicinal herbs has shown promise to ameliorate hepatoxicity [191]. Bleomycin, another chemotherapeutic, is used to induce lung injury following intratracheal instillation. It is helpful to determine criteria for humane endpoints, like body condition scores, for monitoring and welfare when using these substances [192]. Pulmonary fibrosis can be induced by various chemicals, including bleomycin, paraquat, and polyhexamethylene guanidine-phosphate (PHMG-p), as a means of studying the toxic principles of these agents and disease treatment [193, 194].

Unanticipated ingestion of environmental materials, foreign bodies, and other agents occur with frequency in veterinary medicine and are documented in case reports that are beyond the scope of this review. However, an emerging topic that may have impact on the housing of laboratory rodents and their health is the finding that plastic materials, from which caging is often fashioned, if ingested may be harmful to reproductive parameters [195–197]. This topic is reviewed in the "Housing Systems and Husbandry/Terrestrial Models" article.

Surgical Scrub Agents

Common scrub disinfectants used in veterinary medicine include iodophors and chlorhexidine. Iodophors (eg, Betadine comprised of Povidone-Iodine) inactivate a wide range of microbes, but their action is reduced in the presence of organic

matter; chlorhexidine (eg, Novalsan) is rapidly bactericidal, persistent, and active against many viruses and effective despite the presence of blood [198]. It is not uncommon for institutional guidelines to recommend triplicate applications or prolonged contact times of alternating surgical prep liquids on the skin; in essence, to repeatedly apply and then remove disinfectant with a "rinse," more commonly by using a neutral substance (typically alcohol-based or sterile water/saline) to best achieve asepsis [199–202]. Application of these types of scrub agents, particularly chlorhexidine, has been shown to be detrimental primarily in aquatic models [203]. More recent work has demonstrated that shaved skin prior to surgical incision is able to be cleared of bacterial contaminants with fewer numbers of wipes and with contemporary waterless alcohol-based agents that are left on the skin and allowed to dry [204]. Because the majority of scrub options reduced skin bacterial load without adverse impacts on healing, it is evident that effective skin preparation can be achieved using single-step or only 2 applications of scrub, rendering the triplicate skin prep method obsolete in laboratory mice [204]. The only tested skin prep agent that did not have prolonged bactericidal effects in laboratory mice was over-the-counter alcohol-based hand sanitizer, which is not recommended as a sole surgical scrub agent [204]. Combinations of other skin prep agents in mice, in conjunction with varying means for fur removal (shaving vs depilatory agent), have not shown adverse effects on tissues [205].

Although alcohol's properties of disinfection include broad-spectrum antimicrobial, antiviral, and antifungal effects [206, 207], the evaporative cooling effect is predicted to be aversive for use as a rinse, particularly in laboratory mice. Biomedical research institutions have overtly stated that alcohols (eg, 70% isopropyl alcohol [IPA] or ethanol) should be avoided for skin preparation due to concerns that hypothermia will be exacerbated in small rodents [198, 208–212]; thus, sterile saline rinse has been deemed as an alternative to alcohol for use in patient skin preparation [210].

Interestingly, after an initial cooling (hypothermic response) in laboratory animals when applied to skin, isopropyl alcohol leads to a rebound of core temperature, which may be attributed to (1) vasodilation of skin with an increase in blood flow due to a peripheral site of action, or (2) a direct action on central vasomotor control mechanisms following skin absorption [213]. In support of this theory, it has been stated that consumption of ethanol will increase heat loss through the skin by increasing peripheral blood flow [214, 215]. This response is inferred from available yet limited information from humans, where rubbing alcohol (IPA) has been used historically to attempt to reduce fevers; notably, this practice is now denounced [216, 217]. The "alcohol-bath" treatment was believed to work temporarily, as skin would cool with evaporation of the alcohol and leave a perception of relief. In reality, the physiologic response on absorption of alcohol is to signal the body to raise internal temperatures (typically by shivering), often resulting in unwanted spikes in core and skin temperatures. IPA is quickly absorbed through human skin, and if used to douse the patient to lower a fever can cause additional amounts to be further inhaled, with potential to lead to alcohol poisoning and cardiac and neurological problems [216, 218, 219]. The consideration that there may be appreciable absorption of alcohol through skin preparation in animals should serve as a precaution to the veterinary community. In particular, it may be appropriate to review practices of applying alcohol in volumes that could lead to overdosing, neurological sequalae, and potential anesthetic complications. Further studies are warranted to assess this potential complication of alcohol baths and topical applications in laboratory animal medicine. Notably, ethanol by injection can be utilized as an American Veterinary Medical Association-approved agent of euthanasia, emphasizing the importance of care in dosing and application to laboratory rodents [220, 221].

In summary, interventions employing nonexperimental therapeutic and pharmaceutical agents (xenobiotics) are an integral aspect of laboratory animal medicine. Animal species need to be evaluated carefully prior to treatments for individual factors (whether induced or spontaneous) that can affect the distribution and metabolism of therapeutic drugs [222]. It is important to be aware of the types and doses of drugs being administered, the route of delivery, and the potential interactions and side effects they may have with other provided treatments.

Conflict of Interest Statement

The authors declare they have no conflict of interests.

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