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Animal models for Testing Antidotes Against an Oral Cyanide Challenge

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Animal models for Testing Antidotes Against an Oral Cyanide Challenge

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Abstract

Cyanide (CN) is ubiquitous in many living organisms, commonly used in manufacturing processes (dyes, pigments, chelating agents, various nitriles, monomers, resins, fibers, case hardening, electroplating, extraction of precious metals, and fumigation), observed in accidental poisonings from natural or manmade products (smoke inhalation, cyanogens in apple seeds, peach pits, cherry pits, etc.) and as a possible terror threat agent. A higher priority has been placed on CN treatment and antidote evaluations in recent years, possibly as a result of CN being associated with fires, suicide, homicide, judicial execution, assassinations, chemical warfare operations, and as a terrorist threat. The purpose of this review is to identify the similarities and differences between human and animal species exposed orally to cyanide and provide documentation and justification for species model selection under the FDA Animal Rule. Review of CN absorption, metabolism, toxicokinetics, anatomic and physiologic assessment in various small and large animal species was conducted with focus placed on the advantages of each in CN research being provided in this review.

Introduction

Cyanide ion (CN^-) is ubiquitous in many organisms, as cyanogens in pits of fruit such as cherries and peaches as well as in almonds and lima beans. CN can be released during ingestion and metabolism of these materials. The most commonly available forms of CN are the solid salts of sodium, potassium, and calcium cyanide (NaCN , KCN , and CaCN , respectively). They are widely used in manufacturing processes, such as dyes, pigments, chelating agents, various nitriles, monomers, resins, fibers, case hardening, electroplating, extraction of precious metals, and fumigation (Ballantyne & Marrs, 1987; Salem & Katz, 2005). Sources of toxicity and pathology from CN include accidental poisonings from natural or manmade products, fires, suicide, homicide, judicial execution, assassinations, chemical warfare operations, and terrorist threats (Romano et al., 2008; Klaussen et al., 1986; Casadei et al., 1984; Medical Aspects of Chemical Warfare (2008)). The rate of toxicity and lethality are dependent upon

amount ingested or concentration and length of exposure. For survivors, longer term neurological conditions may be present, as reported in many countries (Jackson, 1994; Okafor et al., 2002). The objective of this review is to identify similarities and differences between human and animal species exposed orally to cyanide and provide information for species model selection under the FDA Animal Rule, when used in efficacy and safety studies to test therapeutic compounds and treatment regimens against oral CN poisoning.

Research on medical interventions for exposure to chemical warfare agents has a long history within Department of Defense (DoD) agencies and organizations in the United States (U.S.) as well as those in other countries. However, the increased risk of a terrorist attack in the U.S. involving chemical agents has created new challenges for the civilian community as well. This, combined with the frequency and severity of chemical industrial and occupational accidents, drives a vibrant chemical injury research community outside of DoD. As such, the National Institutes of Health (NIH) within the U.S. Department of Health and Human Services (HHS) is taking leadership in pursuing the development of new and improved medical countermeasures designed to prevent, diagnose, and/or treat the conditions caused by potential and existing toxic chemical threat agents. The ultimate goal of this effort is to enhance the Strategic National Stockpile and better prepare health care professionals for an emergency event involving the release of these threats.

Within the NIH, the threat agents' scientific and technology base is represented by the Countermeasures Against Chemical Threat (CounterACT) program (www.ninds.nih.gov/counteract) which integrates cutting-edge research with the latest technological advances in science and medicine to support basic, translational, and clinical research aimed at the discovery and/or identification of better therapeutic medical countermeasures. The CounterACT program is a trans-NIH effort, involving partnerships with several NIH Institutes and Centers that bring specific expertise related to the varied effects of the toxic chemicals studied.

The Biomedical Advanced Research and Development Authority (BARDA) was established within HHS by the Pandemic and All-Hazards Preparedness Act of 2006 to pursue advanced development

of lead compounds discovered and developed in the CounterACT program. The CounterACT and BARDA programs collaborate to bring new, safe and more effective treatments for use during chemical emergencies and disasters. Some of the chemicals of concern include chemical warfare agents and pesticides (e.g., sarin, parathion) that cause seizures and neuropathology, metabolic poisons and agents (e.g., cyanide, hydrogen sulfide), agents that target the respiratory tract (e.g. chlorine, phosgene), and vesicating agents that cause blisters (e.g., sulfur mustard, Lewisite). Cyanide research projects supported by the CounterACT program are ongoing at Research Centers of Excellence and at several academic sites across the nation. This research, in has resulted in several promising lead candidate therapeutics poised for advanced development.

FDA Animal Rule

FDA regulations (21 CFR 314.600, FDA, 2012a; 21 CFR 601.90, FDA, 2012b, FDA, 2015) govern the approval of new drugs or biological products when human efficacy studies are neither ethical nor feasible. In other words, the FDA can approve or license a drug or biological product based on well controlled and conducted animal efficacy studies when it is not feasible or ethical to conduct studies in humans, as long as this approval is supported by studies demonstrating the product is safe for use in humans. With the advent of the FDA “Animal Rule”, new cyanide antidotes will be approved via this regulatory pathway (FDA, 2015). The animal model selected under the “Animal Rule” must be well characterized and must simulate the disease condition observed in humans as closely as possible. Species selection for CN efficacy and safety studies are not dictated under the “Animal Rule”, but remain the responsibility of the investigators who must select and justify the species used.

Cyanide

The CN^- is a singularly, negatively charged ion consisting of a carbon and a nitrogen atom joined by triple bond. The most toxic form of cyanide is free cyanide, which includes CN^- and hydrogen cyanide (HCN) in either a gaseous or aqueous state. HCN in solution at physiological conditions is largely

undissociated and readily passes physiologic barriers (WHO, 2004; Newhouse, 2010). CN readily reacts and forms simple salts with alkali earth cations and ionic complexes of varying strengths with numerous metal cations with stability dependent on the cation and pH. The salts of sodium, KCN and CaCN, are quite toxic and highly soluble in water, and they hydrolyze readily in moist conditions yielding CN^- and forming HCN (rapidly absorbed in stomach because unionized HCN and acid pH facilitates absorption with pK_a of 9.2) in the acidic environment of the stomach and the neutral environment of the small intestine (Newhouse, 2010). The cyanide ion also combines with sulfur to form thiocyanate (SCN). SCN is approximately seven times less toxic than CN but is very irritating to the lungs, as SCN chemically and biologically oxidizes into carbonate, sulfate, and ammonia (ICMI, 2012).

CN via various routes of exposure (inhalation, intravenous, ingestion, or dermal) causes cytotoxic- (intracellular), histotoxic- (pathological term) hypoxia (Marrs et al., 2007; Gupta, 2009; Ballantyne and Salem, 2005). While normal blood oxygen levels may exist initially, tissues are not able to utilize oxygen due to inhibition of mitochondrial cytochrome oxidase, which is critical for oxidative metabolism and energy production. In all species the highly reactive CN^- has its primary toxicological effect by binding to metallic co-factors in metalloenzymes inhibiting enzyme and cell function (Ballantyne & Marrs, 1987). CN first binds to the protein portion of cytochrome oxidase, then forms a relatively stable but reversible complex with the ferric form of iron in the enzyme (Ballantyne & Marrs, 1987; Romano et al., 2008). Through formation of this complex, CN inhibits electron transfer to molecular oxygen and hence blocks adenosine triphosphate (ATP) formation (Ballantyne & Marrs, 1987; Romano et al., 2008).

Decreased cellular utilization of oxygen leads to an increase in venous oxygen levels. The brain, heart, and other oxygen-sensitive tissues are very susceptible to this inhibition of oxygen utilization which results in cellular or histotoxic hypoxia (Ballantyne & Marrs, 1987; Romano et al., 2008). In addition, because the mitochondria cannot utilize oxygen to generate ATP in the presence of CN, a decreased utilization of pyruvate by the mitochondria leads to anaerobic metabolism with resulting lactic acid production causing metabolic acidosis. Consequences of severe metabolic acidosis caused by lactic

acidosis leads to central nervous system (CNS) disturbances in perception and consciousness (Ballantyne & Marrs, 1987). The homeostatic mechanism to buffer lactic acidosis results in a progressive decrease in plasma bicarbonate (Ballantyne & Marrs, 1987).

CN is sequestered in the erythrocytes and a smaller portion transported in plasma to target organs. The plasma level is considered predictive of tissue level and the ratio of cyanide in erythrocytes to cyanide in plasma is 199:1 (Romano et al., 2008). Whole blood and serum cyanide levels are similar for different species for a given route of exposure (ICMI, 2012; Klaussen et al., 1986).

In a ^{14}C -labeled CN pharmacokinetic study in which 24 mg /kg of KCN and 2 mmoles of HCl were administered via stomach tube to dogs (Christel et al. , 1977; ATSDR (2006), respiratory arrest occurred within 3 minutes with a plasma cyanide concentration of 40 $\mu\text{mol/mL}$. At a plasma concentration of 70 $\mu\text{mol/mL}$, cardiac arrest occurred 5 min after respiratory arrest (Christel et al., 1977; ATSDR, 2006). In severe CN poisoning, the effects are even more complex, with autonomic shock resulting from the release of biogenic amines and possibly CN inhibition of nitric oxide synthetase, also a heme-based molecule (Ballantyne & Marrs, 1987; Romano et al., 2008).

CN also affects calcium channels of the heart, resulting in increased intracellular calcium, and vasoconstriction of the pulmonary arteriolar and coronary vessels, in turn resulting in electrocardiographic anomalies, cardiac failure and a decrease in cardiac output. CN can also inhibit carbonic anhydrase, thus affecting neuronal transmission (Ballantyne & Marrs, 1987; Romano et al., 2008). Other direct or secondary effects associated with CN are reactions with the ferric and carbonyl group of enzymes (e.g., catalase, peroxidase, phosphatase, tyrosinase, ascorbic acid oxidase, xanthine oxidase, hemoglobin), sulfhydryl compounds (e.g., cystine, mercaptopyruvate, glutathione), and proteins (e.g., iron containing proteins, carbonic anhydrase, succinic dehydrogenase, hemoglobin). Once bound, CN inhibits the reactions that these enzymes or proteins catalyze blocking product formation (Ballantyne & Marrs, 1987).

CN is reported to increase intracellular calcium, generate reactive oxygen species, enhance N-methyl-D-aspartate (NMDA) receptor function, interact with cystine to produce 2-ICA (2-

iminothiazolidine-4-carboxylic acid) and 2-ACA (2-aminothiazolidine-4-carboxylic acid). It is associated with memory loss, convulsions, elicit dopaminergic toxicity, lactic acidosis, mitochondrial ADP ribosylation, and hyperammonemia (Ballantyne & Marrs, 1987). CN inhibition may be due to affinity to Schiff base intermediates (e.g., ribulose diphosphate carboxylase) and 2-keto-4-hydroxy glutarate aldolase involving formation of a cyanohydrins intermediate (Salem & Katz, 2005). CN alters carbohydrate metabolism resulting in increased glycogenolysis, shunting of glucose to the pentose phosphate pathway by decreasing the rate of glycolysis, inhibition of the tricarboxylic acid cycle, and marked metabolic acidosis (WHO, 2004).

Signs and symptoms of cyanide toxicity

CN exposure can be fatal in seconds to minutes and is dependent on the compound, dose, time, and exposure route in all species. For example: HCN solution demonstrates a decreasing order of lethal toxicity with intravenous (IV) infusion equal to (=) intramuscular (IM) greater than (>) ocular > intraperitoneal (IP) > oral (PO) > percutaneous (PC). With KCN, an IV infusion > IM > IP > PO > ocular > PC, while a NaCN IV infusion = IM > IP > PO > ocular > PC (Ballantyne & Marrs, 1987). Systemic effects of CN intoxication are present in organ systems most sensitive to low oxygen levels. The organs primarily affected are the CNS (brain), the cardiovascular system (heart and blood vessels), and the pulmonary system (lungs). The CNS is the most sensitive target organ to cyanide poisoning, with cardiovascular effects requiring higher cyanide doses than those necessary for CNS effects (NIOSH, 2012). In humans, early symptoms of cyanide poisoning, reported after HCN inhalation or ingestion of CN salts, include lightheadedness, giddiness, rapid breathing, nausea, vomiting, feeling of neck and chest constriction and suffocation, confusion, restlessness, dyspnea, cyanosis, hypotension, bradycardia, and sinus or AV-nodal arrhythmias anxiety (NIOSH, 2012; Medical Aspects, 2008; Meredith et al., 1993). If severe, progression to stupor, coma, muscle spasms, opisthotonus, convulsions, seizures, fixed and dilated pupils, and death. While cardiac irregularities are often noted, the heart invariably outlasts respiratory

function (Salkowski & Penney, 1994). Death is typically due to respiratory arrest of central origin (NIOSH, 2012).

Clinical features of oral CN poisoning in humans present with a delay in the onset of symptoms from several minutes to 2 hours. After swallowing CN, very early symptoms include irritation of the tongue and mucus membranes. The secondary stage of poisoning presents impaired consciousness, coma, and convulsions. The skin becomes cold, clammy, and moist, and the pulse becomes weaker and more rapid. Opisthotonus and trismus may also be observed. Late signs of CN toxicity include hypotension, complex arrhythmias, cardiovascular collapse, pulmonary edema, and death (Ballantyne & Marrs, 1987). KCN can cause irritation to the eyes, skin, and upper respiratory system, lassitude, headache, confusion, nausea, vomiting, and changes in respiration (Marrs et al., 2007; ATSDR, 2006; ECETOC, 2005).

Differences between Species

Anatomical, physiological, and metabolic differences between the species can play a role in CN intoxication. The solubility and dissolution rate of drugs are higher when they are in the ionized form (Ballantyne & Marrs, 1987). Passive membrane absorption for lipophilic drugs is favored in the unionized state. Therefore, the pH of the fluids throughout the gastrointestinal (GI) tract is critical in the dissolution, solubilization, and absorption processes of ionizable drugs (Ballantyne & Marrs, 1987). The source of hydrogen ions in the GI tract is secretion by the parietal cells of the stomach. Secretion is controlled by both neural (vagus) and hormonal (gastric) feedback mechanisms (Guyton, 1991). When the acidic content of the stomach reaches the duodenum, it stimulates the secretion of an alkaline fluid from the pancreas rich in bicarbonate anions (pH 8). This fluid, along with the bile and alkaline fluids, secreted by the mucosal lining of the small intestine, neutralizes the acidic contents reaching the duodenum. As for CN, in the acidic state of the gastric and gastro-intestinal region, HCN (pKa 9.2, a weak acid) is formed in the unionized state and is rapidly absorbed. Likewise, ingested simple salts of CN are rapidly converted to HCN in this acidic environment, which facilitates rapid absorption.

Representative pH values for humans, rhesus monkeys, swine, beagle dogs, and rabbits are presented in Table 2. In dogs, the gastric acid secretion rate at the basal state is low. Therefore, the stomach pH of the dog can be as high as its duodenal contents in the unstimulated state. Following stimulation (i.e., food, histamine), gastric acid secretion rates in dogs exceed those of the human and pig. In humans, the stomach pH after food is initially higher due to the strong buffering action of food. However, the pH returns to a low value after about one hour. Hence, the delay in CN symptoms reported in humans after ingestion.

In the stomachs of the nonhuman primate, swine, and dog, the cranial (cardiac) region has a higher pH value than the pyloric region, because the parietal cells tend to be localized in the lower part of the stomach (Kararli, 1995; Guyton, 1991). Generally, in the small intestine, although the pH values are variable from animal to animal, the measured pH becomes progressively more alkaline in the distal portions within the same animal. In the monkey, the pH value of the distal ileum was found to be the lowest among the animals studied (Kararli, 1995). The pH values in the large intestine tend to be more acidic than the pH values recorded from the small intestine, due to fermentation (Kararli, 1995). While smaller animals (mice, rats, rabbits) are most suitable for determining the mechanism of drug absorption and bioavailability values from powder or solution formulations, larger animals (dogs, swine, and monkeys) are often used to assess absorption from formulations and the safety and efficacy of potential therapeutics and treatment regimens. The understanding of physiological, anatomical, and biochemical differences between the GI tracts of different animal species can help lead to the selection of the correct animal model to mimic the bioavailability of compounds in the human (Kararli, 1995). In addition to metabolic differences, the anatomical, physiological, and biochemical differences in the GI tract of the human and common laboratory animals reported in Tables 1 and 2 can cause significant variation in CN or drug absorption (Patterson, 2012). Among the physiological factors, pH, bile, pancreatic juice, mucus, fluid volume, and content can modify dissolution rates, solubility, transit times, and membrane transport of drug molecules. The microbial content of the GI tract can significantly affect the reductive metabolism and enterohepatic circulation of drugs and colonic delivery of formulations. The

transit time of dosage forms can be significantly different between species due to different dimensions and propulsive activities of the GI tract. The lipid/protein composition of the enterocyte membrane along the GI tract can alter binding as well as passive, active, and carrier-mediated transport of drugs. The location and number of Peyer's patches can also be important in the absorption of large molecules and particulate matter (Kararli, 1995).

In the human, NHP, swine, and dog, the stomach is of the glandular type, is lined with cardiac, gastric, and pyloric mucosa, and contains parietal and chief cells (Kararli, 1995). The small intestine is the major site for the absorption of nutrients and drugs (Kararli, 1995). This is accomplished through the enormous surface area within this organ. The luminal surface of the small intestine is covered by villi. Columnar absorptive cells or enterocytes cover over 90% of the cell population on the villi surface (Kararli, 1995). The luminal surface of the enterocytes contains fine extensions called microvilli. The surface area of a mucosal cylinder the size of the small intestine would be approximately 33 m². This value increases to 120 m² with the villi and microvilli extensions. The villi have characteristic shapes and patterns in different species (Kararli, 1995). For example, villi are finger shaped in the pig and human while, in stump-tailed monkeys (*Macaca* species), the villi are tongue shaped in the duodenum and finger shaped in the jejunum and ileum. The number of microvilli per unit area of villi surface shows some variation among different animals. In the dog the number is 34 (Kararli, 1995). However, after taking into account the sizes of the villi, the effective surface area per unit villus surface becomes a constant value of around 25 μm². In the dog jejunum, compared with the ileum, the villi and microvilli are longer and wider and the enterocytes are more numerous per unit weight of tissue (Kararli, 1995).

The colon plays a major role in the absorption of water, Na⁺, and other minerals. The colon contains the largest population of microorganisms in the GI tract and is the major site of production and absorption of volatile fatty acids in the human, pig, and dog (Kararli, 1995). The luminal surface of the colon does not contain villi but is divided into geographical areas by transverse furrows. The colonic enterocyte differs slightly from that of the small intestinal variety. Colonic microvilli are less closely packed. There are some significant species differences in the anatomy of the colon. In humans, the colon

length varies from 90 to 150 cm and consists of the ascending, transverse, descending, and sigmoid sections. All sections of the colon in humans, monkeys, and pigs are sacculated (Kararli, 1995).

Humans have a poorly defined cecal region, which is continuous with the colon. The transit time of food or other objects to pass through the gastrointestinal tract in the human varies but generally takes about 30 to 50 hours. It has been reported to take about 1 hour for the gastric emptying of 50% of a meal and about 2 hours for complete stomach emptying (Kim, 1968). The small intestine transit time has been reported as 1 to 2 hours for 50% of the contents to empty (Kim, 1968). The colon has reported taken 12 to 50 hours for emptying with wide variation in individuals (Ghoshal et al., 2012).

Given the oral route of exposure, detoxification of CN is influenced by how much and how quickly the CN is delivered to sites of detoxification, the species variation in detoxification metabolic pathways, and the enzymes such as mercaptopyruvate sulfurtransferase (Table 2) and thiosulfate sulfurtransferase (rhodanese), which are associated with detoxification (Ballantyne & Marrs, 1987; Patterson, 2012; Aminlari et al, 2007). Table 2 provides a comparison of 3-MPST activities in different species. As noted from the table, there can be significant activity differences between the animal models relative to humans. Toxicity observed is a balance of CN absorption, metabolism, detoxification, and elimination, with CN⁻ circulating in the bloodstream in excess of elimination, binding cytochrome c oxidase a³ and other enzyme systems.

Laboratory animal species

The following sections describe and compare the advantages of various common laboratory animal species used to study CN toxicity, focusing on the structure and function of the gastrointestinal tract because of its relevance to oral CN testing.

Nonhuman primate

In the monkey, the stomach is of the glandular type and is lined with cardiac, gastric, and pyloric mucosa (Guyton, 1991; NRC, 1988; Wolfe-Coote, 2005). Both gastric and pyloric mucosa contains parietal and chief cells. The diameter of the small intestine in the rhesus monkey is 1.2 to 2 cm (Kararli,

1995). Most primates are mixed feeders that consume large amounts of fruit or other reproductive parts of plants, supplemented by leaves, seeds, insects, and other animal matter for protein. Based on the NHP's diet, feeding specializations include special adaptations of masticatory (chewing) apparatus and cranial anatomy for processing of hard seeds and hard paricarps (outer shells), increased number of rugae (the hard palate palatal cross ridges; humans have no more than four), taste sensitivities, and cheek pouches (Kararli, 1995; Suckow, 2002; Wolfe-Coote, 2005).

The liver may consist of a few to multiple lobes depending on the species or subspecies. The stomach and small intestine are similar to those of humans and are divided the same, with the same functions (Suckow, 2002; Fox et al., 2002; Wolfe-Coote, 2005). The large intestine is similar to that of humans except, depending on the diet, the cecum may be more prominent or the large intestine sections varied. Likewise, the GI tract will vary from humans based on the dietary intake and the need for fermenting or bacterial decomposition.. For example, the Colobines are foregut fermenters with enlarged sacculated stomachs, Strepsirrhini are tree foliage consumers, and Lepilemurs, Avahi, and Indri, are cecal or colon fermenters with enlarged hindguts (Fox et al., 2002; Wolfe-Coote, 2005). Many species fall in between. Species such as capuchins, which depend on animal matter and other rich energy feedstuffs, have a GI system that is similar to that of humans (Fox et al., 2002; Wolfe-Coote, 2005).

Swine

The pig digestive system is nearly identical to that of a human, both evolutionarily and dietarily (Kararli, 1995; Suckow, 2002; McAnulty et al., 2012; Swindle, 2007). Similar to humans, swine masticate their food before it enters the esophagus. As an omnivore, the pig has a classic vertebrate digestive system with an extremely convoluted intestinal tract that extracts the maximum amount of nutrients from the vegetation in the diet. (Suckow, 2002; McAnulty et al., 2012). The pig stomach is of glandular type and is lined with cardiac, gastric, and pyloric mucosa (Suckow, 2002; Swindle, 2007; McAnulty et al., 2012). Unlike the human, the pig stomach is two to three times larger, and the cardiac mucosa occupies a greater portion of the stomach (Suckow, 2002; McAnulty et al., 2012). Both gastric

and pyloric mucosa contains parietal and chief cells. The cardiac cells secrete mainly mucus. The pig also has a simple GI tract, however, the length of the domestic pig's GI tract is longer than that of the dog (Kararli, 1995). The diameter of the small intestine in the domestic pig is 2.5 to 3.5 cm. The domestic pig's ascending colon is longer than that of the dog and the monkey (Kararli, 1995). The domestic pig's cecum is several orders of magnitude larger than that of the human (Suckow, 2002; McAnulty et al., 2012). The digestive system of the pig is physiologically similar to humans, as both are omnivores. The pig also has comparable metabolic functions, intestinal transport times, and nutrient absorption characteristics, which make them useful in basic nutritional research (Suckow, 2002; Swindle, 2007; McAnulty et al., 2012). Gastrointestinal transit time in pigs ranges from about 15 hours to 60 hours (McAnulty et al., 2012).

Gastrointestinal comparison between humans and the pig suggest that the bioavailability of compounds administered orally would be similar (Suckow, 2002; Swindle, 2007; McAnulty et al., 2012). Pigs have a pharyngeal diverticulum that opens into the dorsal wall of the pharynx near the beginning of the esophagus therefore care must be taken to avoid entering the diverticulum during intubation (Swindle, 2007). The esophageal muscle layers are mainly composed of smooth muscle until the termination of the esophagus cranial to the esophageal sphincter, where it becomes partially striated (Suckow, 2002; Swindle, 2007; McAnulty et al., 2012). The stomach has the characteristics of a monogastric with the exception of a transverse pyloric fold that serves as the "gate keeper" (Suckow, 2002; Swindle, 2007; McAnulty et al., 2012).

In general, the intestinal tract is approximately 15 times its body length (Kararli, 1995; McAnulty et al., 2012). The mesenteric vessels of the small intestine take the shape of vascular arcades in the muscularis mucosa of the intestine (not in the mesentery as with most species). The large intestine is different anatomically in the pig from other common laboratory animals and humans (Suckow, 2002; Swindle, 2007; McAnulty et al., 2012). The cecum, the ascending and transverse colon, and the proximal portion of the descending colon are arranged in a series of centrifugal and centripetal coils located in the upper left quadrant of the abdomen and termed the spiral colon (Suckow, 2002; Swindle, 2007; McAnulty

et al., 2012). The pancreas is related to the proximal duodenum as in humans and other species, with a single pancreatic duct entering the duodenal lumen distal to and separate from the common bile duct (Suckow, 2002; Swindle, 2007; McAnulty et al., 2012).

Canine

The dog has a glandular type stomach lined with cardiac, gastric, and pyloric mucosa (Ballantyne & Marrs, 1987). Both gastric and pyloric mucosa contains parietal and chief cells. The beagle dog small intestine is 225 to 290 cm long, of which the first 25 cm is the duodenum and the last 15 cm is ileum (Kararli, 1995). The digestive tract of the dog is relatively short and simple. The diameter of the small intestine in the beagle dog is approximately 2.0 to 12 cm, depending on level of fluid fill. The dog colon does not have sacculations, and the overall length is about 25 cm with a diameter of 2 cm. The dog colon is also divided into ascending (5 cm), transverse (7 cm), and descending (12 cm) portions (Kararli, 1995). In comparison, the ascending colon in humans is 20 cm in length. (Guyton, 1991; Kararli, 1995).

Rabbit

Rabbits have four pairs of salivary glands, the parotid (largest and lies laterally at the base of the ears), submaxillary, sublingual, and zygomatic (no counterpart in humans) (NRC, 1988; Fox et al., 1984; Suckow, 2002; Rollin & Kesel, 1995; Fox et al., 2002). The rabbit has three layers of striated muscle in the esophagus, which extends the length of the esophagus to include the cardia of the stomach. This is different from the esophagus of humans and most other species, which have separate portions of striated and smooth muscle down the length of the esophagus (Fox et al., 1984; Suckow, 2002; Fox et al., 2002). There are no mucous glands in the rabbit esophagus. Rabbits are a true non-ruminant herbivore, meaning that they have a single stomach (cardia, fundus, and pylorus), and they do not regurgitate or re-chew their food (Fox et al., 1984; Suckow, 2002; Fox et al., 2002). Rabbits do, however, produce dry and moist feces. During the daytime, dry feces consisting largely of indigestible fiber are produced, while at night, a moist soft feces is produced consisting primarily of cecal fermentation products termed “night feces” (Fox et al., 1984; Suckow, 2002; Fox et al., 2002). The night feces (cecotrophs) are a major source of nitrogen

containing compounds and B-complex vitamins, and are generally consumed by the rabbit (Suckow, 2002; Fox et al., 2002).

The rabbit stomach is a thin walled, J-shaped organ that holds roughly 15% of the volume of the entire gut and is never entirely empty in a healthy rabbit. The pH in the stomach is similar to that of a human. The rabbit's small intestine is short relative to other common species, and is smallest by volume of the entire GI tract, at roughly 12%. A minimal amount of fermentation occurs in the small intestine during the GI transit time of four to five hours. Peyer's patches, pale foci of lymphoid tissue observed along the ileum, especially near the cecal junction, are relatively large in rabbits compared to other species (Suckow, 2002). The sacculus rotundus is a large bulb of lymphoid tissue located at the cecal junction. The last and main section of the rabbit gut is the hindgut consisting of the cecum and colon (ascending, transverse, and descending colon) (Kararli, 1995; Fox et al., 2002). This is where a majority of the fermentation occurs (Kararli, 1995; Fox et al., 2002). The cecum is the largest section of the gut containing approximately 40% of the intestinal content by volume, and has ten times the capacity of the stomach (Kararli, 1995; Fox et al., 2002). The pH in the hindgut, particularly in the cecum, can change as the microorganisms fluctuate from alkaline to acidic (Kararli, 1995; Fox et al., 2002).

Rat

Rats are similar to mice for research, having a large historical data base, reduced cost, short life span, and availability of pure, inbred lines or outbred lines (NRC, 1988; Fox et al., 1984; Suckow, 2002; Rollin & Kesel, 1995; Fox et al., 2002). However, variability in multiple parameters can be seen in the stocks and strains between vendors. The rat has continuous growing incisors, lacks water taste receptors, lacks tonsils, and has three pair of salivary glands (parotid-serous secretion; submaxillary-serous and mucous secretion; and sublingual-mucous secretion) (Suckow, 2002). The saliva protein concentration is approximately 2% and is unique to rats. The esophagus enters the lesser curvature of the stomach through a fold in the limiting ridge of the stomach which prevents the rat from vomiting (Fox et al., 1984; Suckow, 2002). The esophagus is lined with keratinized epithelium, which extends into the nonglandular

portion of the stomach. The limiting ridge separates the nonglandular from the glandular portion. The transit time and lengths of the small intestine vary with the age of the rat. An average length of the duodenum for the adult rat is 8 to 10 cm, jejunum is 80 to 100 cm, and ileum is 3 cm. The large intestine is comprised of the: 1) cecum—a thin-walled, comma-shaped pouch with prominent lymphoid tissue (analogous to vermiform appendix of humans) lateral and on apex portion of cecum. Unlike other rodents with an inner septa, the rat has an inner constriction separating the cecum into apical and basilar regions; 2) colon—consists of ascending (oblique mucosal ridges), transverse (longitudinal mucosal ridges), and descending (longitudinal mucosal ridges) segments; and 3) rectum (Fox et al., 1984; Suckow, 2002; Fox et al., 2002). The transit time of the GI tract is 12 to 24 hours in an adult rat. The liver has four lobes: median, right lateral, left, and caudate. A deep fissure for the hepatic ligament is located in the median lobe, and the caudate lobe partially surrounds the esophagus. Bile ducts from each lobe form the common bile duct and enters the duodenum about 25 mm distal to the pyloric sphincter. The rat has no gall bladder. The pancreas is darker and firmer than surrounding adipose tissue and extends from the duodenal loop to the gastrosplenic omentum as a lobulated and diffuse organ. Numerous excretory ducts fuse into two to eight large ducts that empty into the common bile duct.

Mouse

Mice have been used in scientific research since the 1600s (Foster et al., 1981; NRC, 1988; Fox et al., 1984; Suckow, 2002; Rollin & Kesel, 1995; Fox et al., 2002). The development of a laboratory mouse as a research model began with genetic experiments in the 1900s (Foster et al., 1981; NRC, 1988). Other types of research uses include but are not limited to behavioral, genetic, cancer (spontaneous and genetically engineered), immunology, toxicology, pharmacology, bioassay, metabolism, developmental biology, embryology, diabetes, obesity, aging, convulsive disorders, ophthalmology, infectious disease, monoclonal antibody production, and cardiovascular research (Foster et al., 1981; NRC, 1988; Fox et al., 1984; Suckow, 2002; Rollin & Kesel, 1995; Fox et al., 2002).

There are many advantages to using the mouse as a model organism, the most important of which is their striking similarity to human anatomy, physiology, and genetics, especially in relation to cyanide mechanism of poisoning. Over 95 percent of the mouse genome is similar to humans, making mouse genetic research particularly applicable to human disease. Many diseases can be modeled through the alteration of a specific gene central to a normal biological process. A large number of disease models have arisen spontaneously in this species and other disease models with manipulation, which are now supplied by various vendors routinely or as part of the services (genetic manipulation) offered by the company. The mouse is the best genetically characterized species of all mammals.

The mouse GI tract consists of the esophagus, stomach, small intestine, cecum, and colon, and is similar to other mammals with the exception of ruminants (Fox et al, 1984; Suckow, 2002; Fox et al., 2002). The submaxillary salivary gland is a mixed gland in most species, but in the mouse it secretes one type of saliva, seromucoid. The esophagus is lined with a thick cornified squamous epithelium allowing for easy gavage. The proximal portion of the stomach is also keratinized (non-glandular) and the distal portion is glandular. Gastric secretions continue whether food is present or not. The GI flora in mice consists of more than 100 species of bacteria selectively colonizing the GI tract shortly after birth. The bacterial flora ecosystem formed throughout the GI tract provides beneficial effects for the animal, such as production of essential vitamins, resistance to certain intestinal pathogens, and maintenance of homeostasis for essential physiological functions.

Mice are small, have short gestation time, ease of breeding, and an accelerated lifespan (one mouse year equals ~ 30 human years), keeping costs, space, time, and labor required to perform research manageable. Besides these clear benefits, some of the greatest advantages to using the mouse are the availability of pure, inbred lines or outbred lines and the ability to genetically engineer new strains. These features provide researchers with uniquely powerful tools for understanding mechanisms of human disease.

Conclusion

An understanding of physiological, anatomical, and biochemical differences between the different animal species and the human can help lead to the selection of the correct animal model. With the advent of the FDA “Animal Rule”, an animal model that closely mimics the human response for the endpoint being evaluated is required. Although no animal model will respond to cyanide and associated treatments in exactly the same fashion as in a human, the better the model, the more likely that a countermeasure tested in that species will prove to be efficacious in the human.

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Table 1. Comparison of median pH Values from Alimentary Tract contents.

Species	Stomach	Small Intestine	Cecum	Colon
Human	1.0-3.5	7.5-8.0	7.5-8.0	
NHP ^a	2.8-4.8	5.6-6.0	5	5.1
Swine ^a	2.2-4.3	6.0-7.5	6.3	6.8
Dog ^a	3.4-5.5	6.2-7.5	6.4	6.5
Rabbit	1.0-2.0 ^a	6.4-7.4 ^c	6.0-6.4 ^c	6.1-6.6 ^c
Rat ^b	2.1-4.7	6.7-7.9	6.6-6.8	7.1-7.5
Mouse	2.7-3.3 ^d	4.5-5.2 ^d	4.2-4.6 ^d	4.1-5.0 ^d

^a Kararli, 1995; ^b Ward et al., 1987; ^c Merchant et al., 2011; ^d McConnell et al., 2008

Table 2. Blood 3-MPST activity comparison

Species	Mean Enzyme Units ($\pm$ S.E.)*	n
Human male (28-36 yr)	113.3 $\pm$ 7.5	8
Human female (28-34 yr)	114.8 $\pm$ 2.6	6
Cynomolgus macaque	40.8 $\pm$ 3.57	6
Pig (Yorkshire, 8 wks to 1 yr)	Not Detected.	13
Pig (Yucatan age > 1 yr)	Not Detected	6
Neutered male beagle dog	18.2 $\pm$ 2.12	6
Intact female beagle dog	15.1 $\pm$ 2.57	6
New Zealand rabbit	62.5 $\pm$ 10.71	4
Old Wistar rat (age 12 mo)	532.2 $\pm$ 22.95	5
Young Wistar rat (age 4 mo)	639.7 $\pm$ 69.47	7
Swiss Webster mouse male	121.8 $\pm$ 7.45	5
C6 Black mice (age 12 mo.)	38.6 $\pm$ 18.12	6

* Enzyme Units of 3-mercaptopyruvate sulfurtransferase are defined as μ moles of pyruvate generated per minute per 10^{10} RBCs at 37°C. Patterson (2012)