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Considerations in the Extrapolation Drug Toxicity Between Humans and Dogs

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Abstract

The dog is an important species used in preclinical studies in support of human drug product development. Likewise, because of the many active pharmaceutical ingredients (APIs) with therapeutic relevance to both humans and dogs, extrapolation can also occur in the reverse, from human to dog. In either situation, it is important to appreciate species-specific factors influencing drug pharmacokinetics (absorption, metabolism, disposition, and elimination) and the potential impact of disease on the applicability of these extrapolations. Furthermore, tools such as physiologically based pharmacokinetic (PBPK) models not only enable investigators to extrapolate species-specific data on systemic or organ exposure to the parent compound and metabolite(s) but also facilitates an interrogation of factors that can lead to species-specific differences in drug effectiveness and toxicity. In this review, we explore the factors and tools that comprise our current arsenal for understanding and predicting human-canine comparative toxicity.

Keywords

Dog-Human Toxicology, Dog-Human Shared Diseases, Interspecies Extrapolation, PBPK Models

Disciplines

Medical Toxicology | Small or Companion Animal Medicine | Veterinary Toxicology and Pharmacology

Comments

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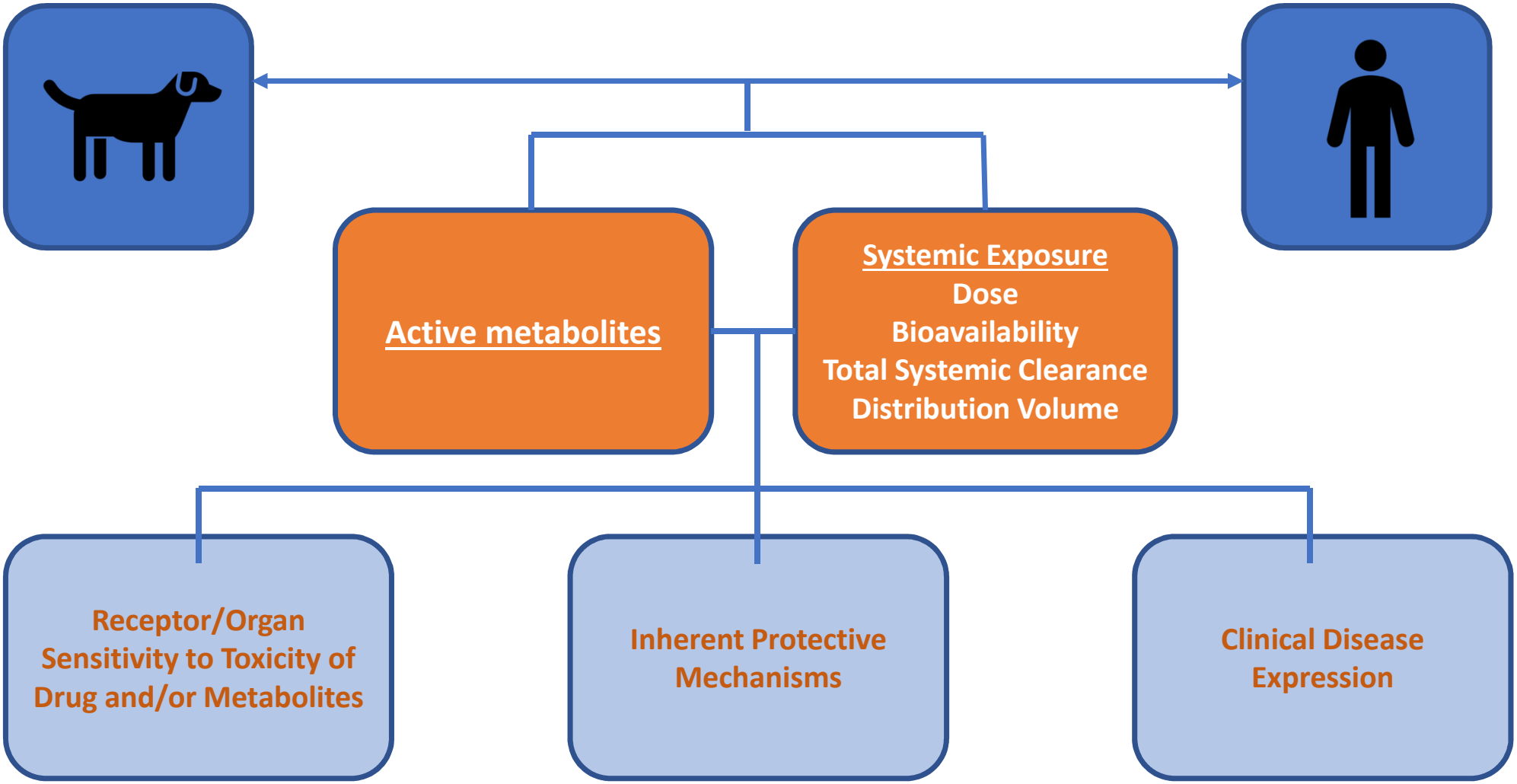
Factors Influencing Comparative Toxicology in Dogs and Humans



Considerations in the Extrapolation Drug Toxicity Between Humans and Dogs

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Abstract:

The dog is an important species used in preclinical studies in support of human drug product development. Likewise, because of the many active pharmaceutical ingredients (APIs) with therapeutic relevance to both humans and dogs, extrapolation can also occur in the reverse, from human to dog. In either situation, it is important to appreciate species-specific factors influencing drug pharmacokinetics (absorption, metabolism, disposition, and elimination) and the potential impact of disease on the applicability of these extrapolations. Furthermore, tools such as physiologically based pharmacokinetic (PBPK) models not only enable investigators to extrapolate species-specific data on systemic or organ exposure to the parent compound and metabolite(s) but also facilitates an interrogation of factors that can lead to species-specific differences in drug effectiveness and toxicity. In this review, we explore the factors and tools that comprise our current arsenal for understanding and predicting human-canine comparative toxicity.

Comparative Toxicity:

In 2013, it was estimated that 90,000 dogs/annum were used in scientific research in the United States (US) + European Union (EU), with about 80% of this use being related to preclinical studies (as the non-rodent mammalian species) for evaluation of drug toxicity and effectiveness [1]. Although the occurrence of toxicity in dogs was associated with a high likelihood of the presence of human drug toxicity (based upon the FDA Adverse

Event Reporting System for 2366 FDA-approved human compounds), the expression/organ system associated with that toxicity was not highly correlated [1]. Conversely, the finding of no toxicity in the dog did not assure the absence of human drug toxicity. Interestingly, another study based on preclinical data generated in rodent and non-rodent species noted that of the 93 human adverse drug reactions (ADRs) associated with 43 small molecule drugs in the EU, only 19% of the toxicities were identified during the preclinical studies [2]. Although the latter is not limited to dogs, experience with prior failures to identify human ADRs underscores potential challenges associated with efforts to employ interspecies extrapolation for prediction of drug toxicity.

With regard to the latter conclusion, an important point to note is that these published assessments exclude those drugs not progressing to market may lead to an over-estimation of the error associated with the prediction of human ADRs from animal model data [3]. Nevertheless, these assessments are consistent with drugs that pass preclinical tests and later move on to fail in clinical trials for which an estimated 92-94% are largely due to unforeseen toxicities. These kinds of statistics lead some investigators [4] to remain consistent in their conclusion that public information does not support claims that canine toxicity studies can reliably predict human drug safety, toxicity or effectiveness.

Clark [5] also comments on the importance of considering false negatives. Using a likelihood ratio, he observed that while the precipitation of cardiac arrhythmias, liver damage and renal failure in animals were highly indicative of the likelihood of a similar event in humans, the lack of canine toxicity did not share the same prognostic value. In fact, most compounds for which there were post approval clinical findings (leading to either relabeling or product withdrawal) did not exhibit a corresponding toxicity in animals. Of note was the low animal vs human frequency of QT prolongation, the onset of jaundice, abnormal hepatic function, renal failure and impairment, or hepatitis. The question is whether this reflects a lack of toxicity in the animal model or rather the very small number of animals challenged relative to the thousands of human patients that will be exposed to the drug.

Similarly, although canine Torsade de Pointe is rare, there are examples of similar human-dog response to drug-induced QT prolongation. However, the human vs canine mechanism of this effect appears to differ. QTc changes are potentially induced by changes in the potassium channel or the sodium-calcium currents. However, the ion channel responsible for that change appears to differ in humans vs dogs. This conclusion was reached upon examining the ECG changes associated with the administration of several compounds known to induce ion channel inhibition and ECG changes in humans [6], including dofetilide, sotalol, verapamil, and cisapride.

The IQ consortium evaluated the first-in-human safety translation of safety data from preclinical species to humans [7]. Considering 182 molecules, they evaluated the positive predict value (PPV: the proportion of positive nonclinical findings that had positive clinical findings), negative predict value (NPV: the proportion of negative nonclinical findings that had negative clinical findings), sensitivity (the proportion of positive clinical findings that had positive nonclinical finding) and the reduction in uncertainty in human drug safety as a function of data generated in the specific preclinical species (or combination of preclinical species). The drugs used in this evaluation were known to exhibit adverse outcomes in humans. The proportion of PPV, NPV and the sensitivity of the human-canine comparison as a function of organ systems is provided in **Figure 1**.

INSERT FIGURE 1

As a general rule, a test is considered “diagnostic” in predicting a positive outcome when the LR+ is ≥10 or for predicting a negative outcome when the iLR− is ≥10. For dogs, there was a 40% reduction in uncertainty in human drug safety for adverse reactions as it pertains to the CNS and a 28% reduction for GI toxicity. For hepatotoxicity, the dog had approximately 40% false positives (i.e., demonstrated toxicity when none occurred in the humans). The corresponding drug exposure for false positive reactions occurred largely at doses >5-fold that of the human, although some were at lower total drug exposure. Of course, the possibility of differences in active metabolites were not considered but could

have influenced this outcome. Furthermore, within any species, there are polymorphisms (particularly as it pertains to metabolism and transporter activity) that can affect drug effectiveness or safety [e.g., 8, 9, 10].

Potential reasons for these interspecies differences are depicted in **Figure 2**.

FIGURE 2:

The challenges associated with interspecies extrapolations apply not only to extrapolating dog to human but also human to dog. For instance, there are foods typically innocuous in humans that are potentially toxic in dogs (Table 1).

Table 1: Example of foods that are non-toxic in humans but toxic to dogs

Food	Toxicity	Reference
Chocolate (theobromides and caffeine)	Clinical signs of chocolate toxicosis usually occur within 6–12 hr of ingestion. Initial signs may include polydipsia, vomiting, diarrhea, abdominal distention, and restlessness. Signs may progress to hyperactivity, polyuria, ataxia, rigidity, tremors, and seizures.	11,12,13,14
Citrus fruits	Due to the psoralen compounds and the aromatic oils, oranges, lemons, limes, etc. can lead to irritation of the canine digestive track and cause central nervous system (CNS) depression if consumed in large quantities.	13,15
Coconut	Contains high levels of potassium and medium chain triglycerides that can lead to GI upset and diarrhea.	13
Grapes and raisins	Although specific toxic substance has not been identified, consumption of grapes and raisons can lead to kidney failure if consumed in large quantities.	13,14,16
Nuts (especially macadamia nuts)	Some nuts are less toxic than others, and some (e.g., peanuts and small quantities of peanut butter) are typically safe. However, digestibility of nuts can be an issue, leading to vomiting, diarrhea and pancreatitis.	13,14,17
Xylitol	Liver failure and by inducing high levels of insulin release, hypoglycemia and seizures.	13,14
Milk and dairy	Because many dogs are lactose intolerant, digestive upset, and diarrhea can occur if consumed in large quantities.	13,18
Onions and garlic	Onions, garlic, leeks and chives contain N-propyl disulfide which reduces the activity of glucose-6-phosphate dehydrogenase in red blood cells; thereby interfering with regeneration of reduced glutathione needed to prevent oxidative denaturation of hemoglobin. Interestingly, it is not without impact in humans and if consumed raw in large quantities can lead to clotting issues.	13,14,19

In addition, some human medications can be highly toxic (e.g., acetaminophen, ibuprofen, naproxen and as well as several other pharmaceutical agents) or lead to unexpected paradoxical effects (e.g., alprazolam or zolpidem) when used in dogs. [20].

Extrapolation failure may also reflect dog-human differences in the formation of a toxic metabolite. For example, the plasticizer, Di(2-ethylhexyl) phthalate (DEHP) is rapidly

metabolized to mono(2-ethylhexyl)phthalate (MEHP), an active metabolite. While neither human nor dogs hydrolyzed DEHP to MEHP in the intestine, substantial conversion occurred in the liver. In that regard, this conversion was 4.6-fold greater in dogs than humans. This markedly higher efficiency of conversion of DEHP to MEHP in dogs than humans is probably a reason for the greater toxicity of this plasticizer in dogs than humans [21].

Another example of extrapolation failure reflects differences in transporter activity. For example, dogs are particularly susceptible to the toxicity of the herbicides, phenoxyacetic acids and related organic acids, relative to that observed in other species [22]. This greater sensitivity has been linked with the dog's lower capacity to secrete organic acids from the kidney as compared to humans. This translates to a T½ difference for two phenoxyacetic acids, 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) of 92–106 and 63 h, respectively, in dogs versus 12 and 11 h, respectively, in humans.

Thus, from a pharmacokinetic perspective, variables that can contribute to human-canine PK and toxicity differences include:

- Enzymatic conversion (Phase 1 and 2) [23,24,25,26,27,28,29]
- Hepatic transporters [8,30,31,32]
- Renal transporters [22,33,34,35,36]
- Gut microbiome [37,38,39]
- Protein binding/volume of distribution [31,40,41,42,43]

The Value of Spontaneous Animal Disease Models to Characterize Drug Safety and Activity

Failure of accurately describing product safety and effectiveness in human patients [1] is particularly problematic in the neurosciences [44] and oncology [45] where these estimates are closer to 95%. These high attrition rates in Phase II/III clinical programs

stem partly from toxicity characterization being performed in ***healthy*** animals, thereby ignoring the impact of ***disease state*** on drug pharmacokinetics and pharmacodynamics [44,45,46,47,48,49,50,51,52,53]. There is, therefore, a critical need to incorporate the effect of disease on candidate drug safety and effectiveness early in the drug research and development lifecycle. That objective can be achieved by using spontaneous animal disease models. In that regard, pet dogs and humans share numerous analogous clinical diseases, including cancer, inflammatory bowel disease (IBD), diabetes and cognitive dysfunction. For instance, canine cognitive dysfunction (CCD) is the only naturally occurring mammalian dementia to mimic Alzheimer's Disease, with striking clinical similarities to the human analog [54,55]. Similarly, as opposed to genetically-modified murine models, cancers develop spontaneously in dogs [56,57]

In an effort to characterize the oral absorption and gastrointestinal safety of orally administered drugs, 3-dimenstional (3D) intestinal organoids have been developed from intestinal crypts obtained via endoscopic biopsies [58]. Recent findings show that 3D intestinal organoids can be successfully maintained from healthy dogs and dogs with naturally occurring IBD. These *in vitro* systems can potentially serve as a relevant preclinical model for drug testing prior to live animal studies [59,60,61]

Use of Mechanistic PBPK Models to Support Interspecies Extrapolation.

It is well known that for orally administered drugs, bioavailability studies in animals cannot quantitatively predict human drug bioavailability [62]. Some of the factors that may be confounding this correlation between animals (single or combined species) and human are the differences in (i) drug metabolizing enzymes, (ii) drug transporters, (iii) anatomy and physiology of the GI tract, (iv) plasma protein binding, etc. Therefore, animal models that take into consideration species specific factors affecting drug bioavailability are recommended to enable quantitative predictions [62, 63]. PBPK models take into consideration species specific anatomy and physiology in combination with drug data (physicochemical, solubility, permeability, distribution and elimination). These are linked with an *in vitro-in vivo* extrapolation (IVIVE) to quantitatively predict

drug pharmacokinetics in that animal species [64]. During early drug development, from discovery to first – in – human, *in vitro* drug data are limited. However, the amount of available information increases as the product progresses through the different stages of development. PBPK platforms, combined with IVIVE techniques, are positioned to incorporate and utilize these experimental data as they become available [65]. In so doing, the PBPK models can greatly enhance the information derived from that early data.

An approach towards using PBPK models for interspecies extrapolation is shown in Figure 3.

FIGURE 3:

A successful cross species translation to human for the pharmacokinetics of a drug cannot be guaranteed and largely depends on the type of drug being investigated. The probability of a successful translation (pharmacokinetic parameters within 2-fold of observed) can be maximized using reliable species-specific drug parameters (such as fraction unbound in plasma, blood to plasma ratio, fraction unbound in the microsomes, etc) in the relevant PBPK models. Drug input parameters that are intrinsic in nature should be identified (such as intrinsic solubility, intrinsic transcellular permeability, bile partitioning coefficient, etc.) and verified using *in vitro* experiments [66,67,68]. In certain cases, allometric scaling can be coupled with animal PBPK models to predict the pharmacokinetics in human [69].

Closing Comment:

Given the frequency of parallel drug development and increasing availability of *in vitro* tools to support PK understanding, and the advancements in *in silico* systems to have the dog and human physiological (population) models for prediction of in vivo parent and metabolite exposure, there is the potential to expect tremendous progress in our ability to extrapolate drug toxicity between humans and dogs. A realization of this potential

depends upon the willingness of the scientific communities to utilize these tools and dialogue on shared opportunities for filling existing holes. Ultimately, it will be through that effort that we can greatly increase the efficiency of our predictions.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Dr. Martinez is a Senior Scientist with the US Food and Drug Administration has no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Dr. Mochel is a co-founder of 3D Health Solutions, a startup company that develops 3D canine organoids for drug testing purposes.
Dr. Pade is a scientific expert employed by Certara UK, for the Simcyp PBPK Modeling and Simulation Software

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Captions:

Figure 1: Assessment of the extrapolation between human and dog toxicity across various organ systems. Based upon information from [7]. Sen (%) = sensitive percent (the proportion of positive clinical findings that had positive non findings; PPV = predictive positive value (the proportion of positive nonclinical findings that had positive clinical findings), NPV = negative predictive value (the proportion of negative clinical findings that had negative clinical values), LR = likelihood positive ratio (the increase in odds of a positive clinical finding that is due to the knowledge of a positive nonclinical finding); iLR- = Inverse likelihood ratio negative (the increase in odds of a negative clinical finding that is due to the knowledge of a negative nonclinical finding). Values estimated as a ratio of the True Positive and True Negative number of observations divided the total number of observations associated with positive or negative outcomes. All parameter values are expressed within a range of 0 to 100.

Figure 2: Potential mechanisms underlying dog-human differences in drug toxicity.

Figure 3: General workflow of inter species extrapolation to human using PBPK models

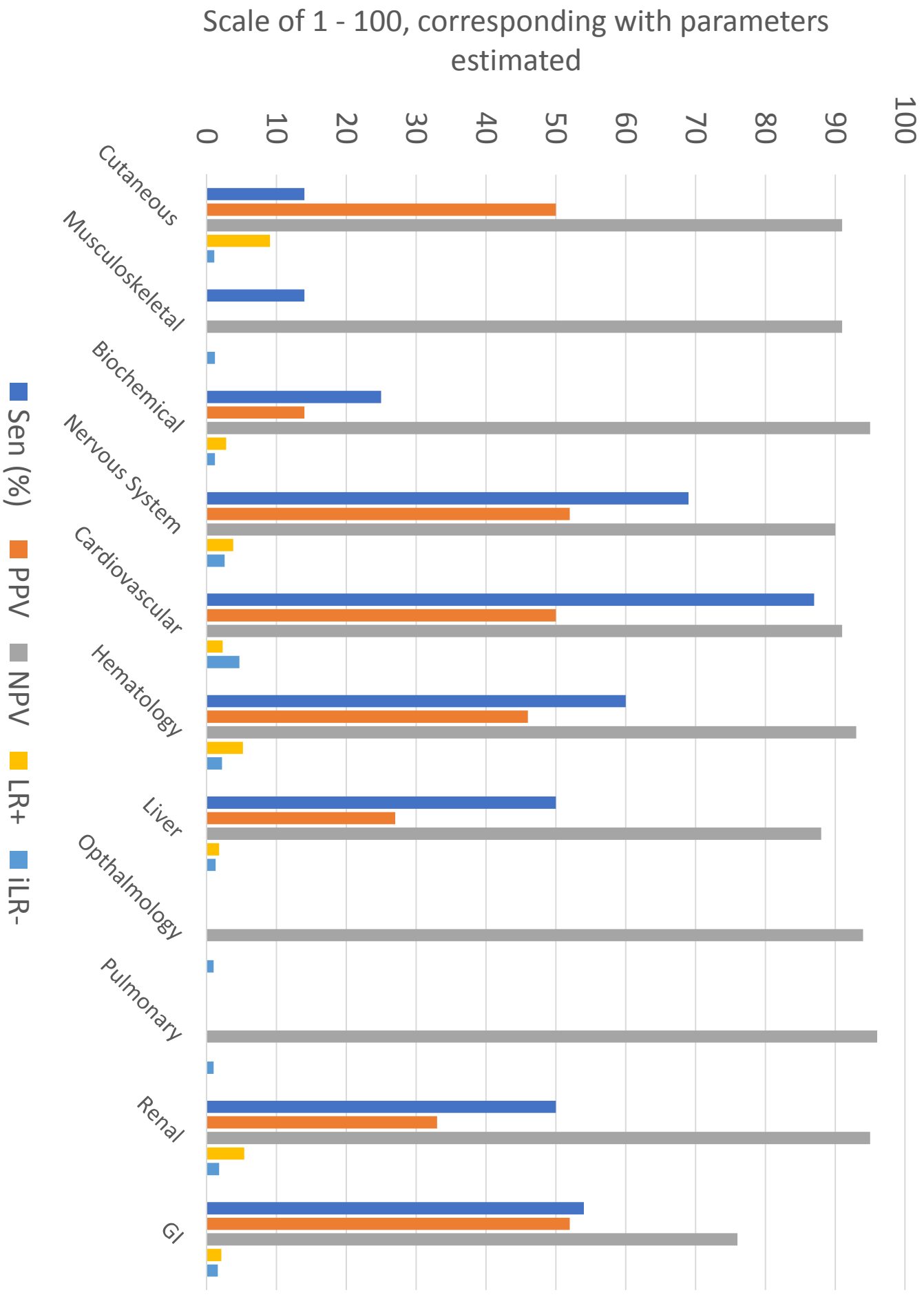
Scale of 1 - 100, corresponding with parameters estimated
0
10
20
30
40
50
60
70
80
90
100
Cutaneous
Musculoskeletal
Biochemical
Nervous System
Cardiovascular
Hematology
Liver
Opthalmology
Pulmonary
Renal
GI
Sen (%)
PPV
NPV
LR+
iLR-

Figure 1: Assessment of the extrapolation between human and dog toxicity across various organ systems. Based upon information from [7]. Sen (%) = sensitive percent (the proportion of positive clinical findings that had positive non findings; PPV = predictive positive value (the proportion of positive nonclinical findings that had positive clinical findings), NPV = negative predictive value (the proportion of negative clinical findings that had negative clinical values), LR = likelihood positive ratio (the increase in odds of a positive clinical finding that is due to the knowledge of a positive nonclinical finding); iLR- = Inverse likelihood ratio negative (the increase in odds of a negative clinical finding that is due to the knowledge of a negative nonclinical finding). Values estimated as a ratio of the True Positive and True Negative number of observations divided the total number of observations associated with positive or negative outcomes. All parameter values are expressed within a range of 0 to 100.

Factors Influencing Comparative Toxicology in Dogs and Humans

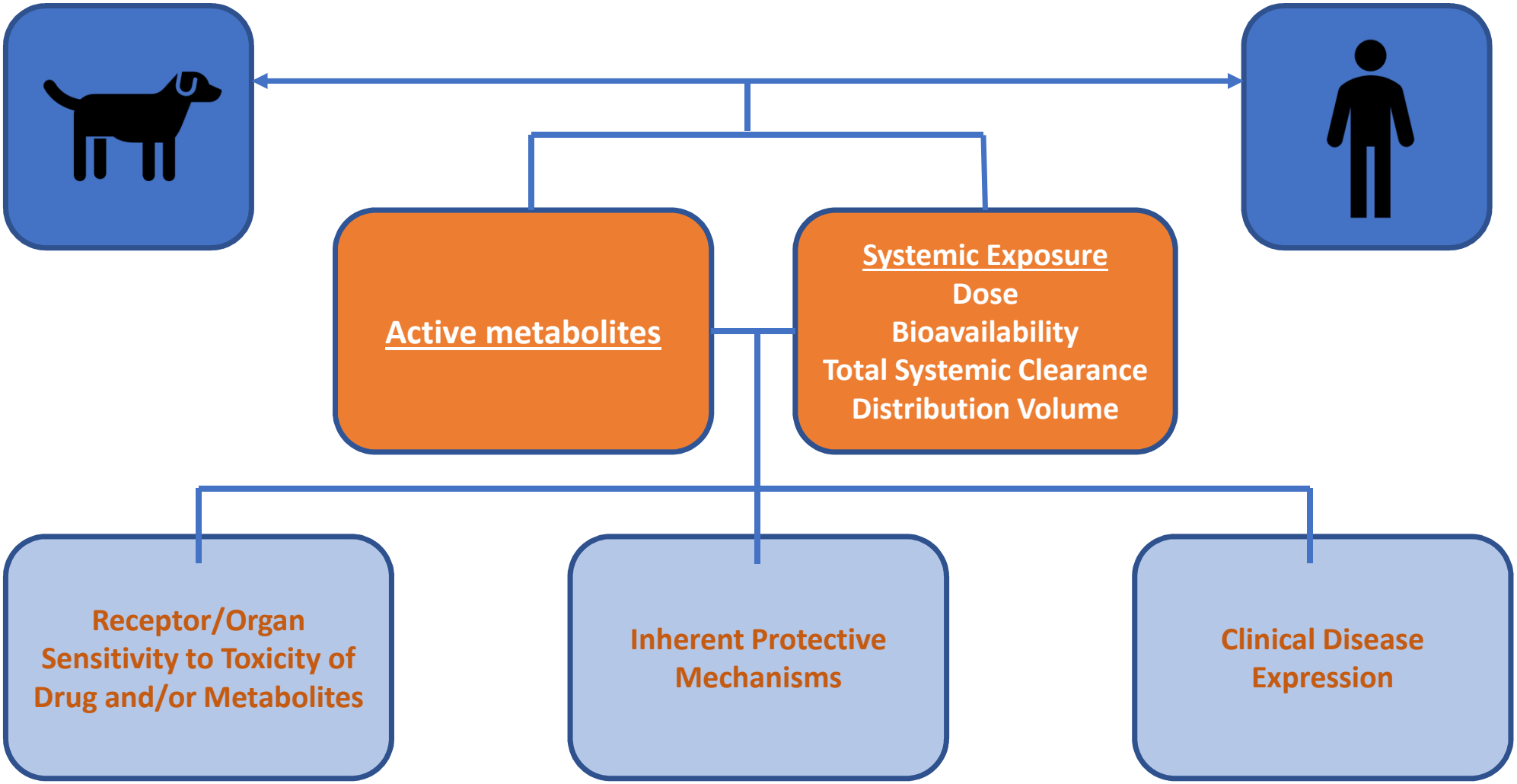

Figure 2: Potential mechanisms underlying dog-human differences in drug toxicity.

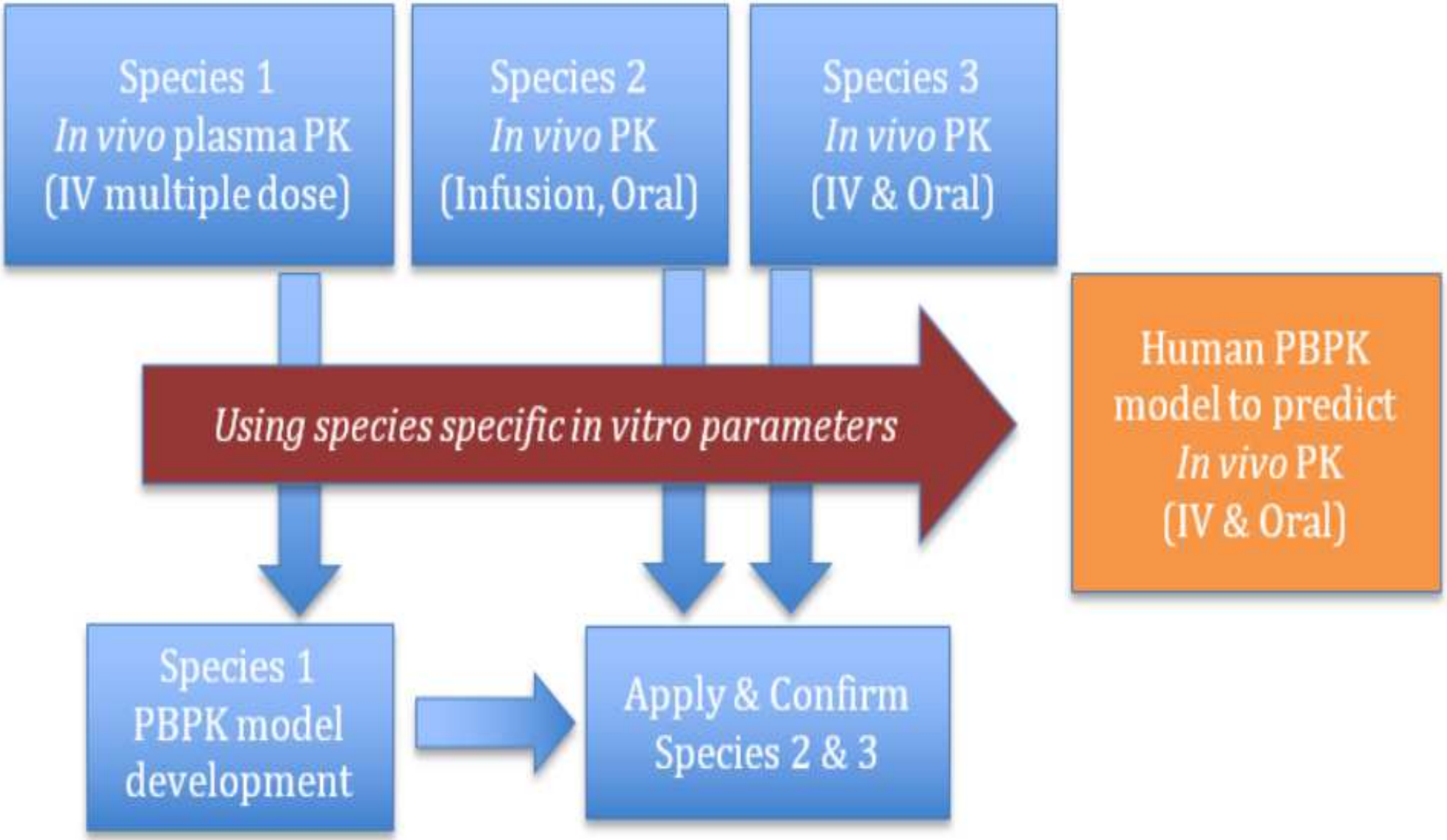
Species 1
In vivo plasma PK
(IV multiple dose)
Species 2
In vivo PK
(Infusion, Oral)
Species 3
In vivo PK
(IV & Oral)
Using species specific in vitro parameters
Human PBPK
model to predict
In vivo PK
(IV & Oral)
Species 1
PBPK model
development
Apply & Confirm
Species 2 & 3

Figure 3: General workflow of inter species extrapolation to human using PBPK models

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Dr. Martinez is a Senior Scientist with the US Food and Drug Administration has no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Dr. Mochel is a co-founder of 3D Health Solutions, a startup company that develops 3D canine organoids for drug testing purposes.
Dr. Pade is a scientific expert employed by Certara UK, for the Simcyp PBPK Modeling and Simulation Software