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Solubility Criteria for Veterinary Drugs

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ABSTRACT

This *Stimuli* article is the first step toward the development of a general chapter addressing solubility criteria for veterinary drug products. The current criteria for classifying drug solubility are based on human gastrointestinal (GI) physiology. These criteria may not be appropriate to the unique conditions encountered within the GI tract of veterinary species. Thus, this article discusses the relationship between the species-specific GI characteristics and the criteria appropriate for describing drug solubility in veterinary species. Initially the discussion focuses on dogs and cattle, the most common veterinary patients in small- and food-animal practices, respectively. Later the discussion will include various other veterinary species of interest.

INTRODUCTION

The determination of drug solubility is important to facilitate an appreciation of the formulation variables that can influence drug absorption. Highly water-soluble compounds designed for immediate release tend to be far more forgiving with regard to the effect of formulation changes on oral product bioavailability compared to drugs that exhibit poor aqueous solubility (*1*). Furthermore, within veterinary medicine, drug solubility is one of the essential pieces of information needed to support the biowaiver of oral soluble powders and Type A medicated articles (premixes) (*2*).

The Biopharmaceutics Classification System (BCS) provides a foundation for the consideration of biowaivers and for predicting formulation variables that can influence human oral drug absorption (*3*). Initially proposed by Amidon et al. in 1995, the BCS is founded on an understanding of the solubility and intestinal permeability characteristics of the drug substance. Subsequently, the classification has been proposed for a wide range of compounds considered by the World Health Organization to be essential for human therapeutics (*4*).

FDA's Center for Drug Evaluation and Research (CDER) guidance on granting BCS-based biowaivers states the following solubility criteria:

An objective of the BCS approach is to determine the equilibrium solubility of a drug substance under physiological pH conditions. The pH–solubility profile of the test drug substance should be determined at 37 ± 1 °C in aqueous media with a pH in the range of 1–7.5. A sufficient number of pH conditions should be evaluated to accurately define the pH–solubility profile. The number of pH conditions for a solubility determination can be based on the ionization characteristics of the test drug substance. For example, when the pK_a of a drug is in the range of 3–5, solubility should be determined at pH = pK_a, pH = pK_a + 1, pH = pK_a – 1, and at pH = 1 and 7.5. A minimum of three replicate determinations of solubility in each pH condition is recommended. Depending on study variability, additional replication may be necessary to provide a reliable estimate of solubility. Standard buffer solutions described in the *USP* are considered appropriate for use in solubility studies. If these buffers are not suitable for physical or chemical reasons, other buffer solutions can be used. Solution pH should be verified after addition of the drug substance to a buffer. Methods other than the traditional shake-flask method, such as acid or base titration methods, can also be used with justification to support the ability of such methods to predict equilibrium solubility of the test drug substance. The solubility class should be determined by calculating

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the volume of an aqueous medium sufficient to dissolve the highest dose strength in the pH range of 1–7.5. A drug substance should be classified as highly soluble when the highest dose strength is soluble in ≤250 mL of aqueous media over the pH range of 1–7.5 (*5*).

The challenge in veterinary medicine is that the current criteria for classifying drug solubility are based on human gastrointestinal (GI) physiology. Because of markedly different GI characteristics between humans and animals, these criteria may not be appropriate for the unique conditions encountered within the GI tract of veterinary species (*1, 6, 7*). For this reason, USP convened an Expert Panel (within the USP Dosage Forms Expert Committee) to consider the relationship between species-specific GI characteristics and the criteria appropriate for describing drug solubility in veterinary species. At this time, discussions will focus on the dog (monogastric, carnivore) and cattle (ruminant, herbivore) as representative monogastric and ruminant species that also represent the most common veterinary patients in small- and food-animal practices, respectively. If agreement is reached regarding the solubility criteria for dogs and cattle, a new *USP* informational chapter will be developed. The next step in identifying appropriate solubility criteria would be to extend the development of criteria in various other veterinary species of interest (which then would be published as a revision to the new chapter on solubility).

At this juncture, species-specific permeability criteria are not considered because of the complexities of making these determinations. For example, although many lipophilic compounds absorbed via transcellular pathways are likely to have similar membrane permeability characteristics across animal species, paracellular pathways can be markedly different. For small hydrophilic molecules absorbed via the paracellular route, pore size, density, and intestinal morphology can have important effects on the permeability (*6*). Moreover, use of absolute bioavailability (*F*) as a permeability indicator is not appropriate because of the potential bias introduced by the effects of gut wall and hepatic metabolism and species or breed differences in the activity of membrane influx and efflux transporters. Lastly, unlike the systems that are available to support permeability assessments in human medicine, there are no validated in vitro systems that can be used to assess drug permeability in animals. Therefore, determining the permeability component of the BCS for animals will be a long-term goal of this USP initiative.

SOLUBILITY SCIENCE

Thermodynamic (equilibrium) solubility often is regarded as the true solubility of a compound and therefore serves as the gold standard for product development needs. It represents the saturation solubility of a compound in equilibrium with an excess of undissolved material at the end of the dissolution process. The thermodynamic solubility value is not an absolute number but rather depends on a multitude of compound properties and experimental factors, including: polymorphism; compound purity; particle size and shape; buffer composition, including pH and common ion effect; stability in solution; potential for molecular aggregation; time for attaining equilibrium; temperature; mixing conditions; and adsorption onto filter or vessel surfaces.

The solubilization of a drug in an aqueous medium is controlled by interactions of the solute molecules with itself, the solvent molecules within themselves, and the interaction between the solute and the solvent. The strength of the interaction between a molecule of the drug substance and the molecules of the solvent favors drug substance solubilization. The stronger the interaction between the solute and the solvent, the greater the likelihood that the drug will go into solution. Counteracting this solubilization process is the strength of the affinity of the solute for itself or how tightly bound the compound is to its own solid-state form. Thus, solubility can be considered to be a function of three types of interactions: solvent–solvent interaction (A–A), solute–solute interaction (B–B), and solvent–solute interaction (A–B). Consequently, solubility is not a universal value but rather should be considered from the perspective of the interactions between the drug substance in its solid form and solution conditions such as: pH, primary solvent (e.g., water), cosolvents (e.g., DMSO), additives (e.g., albumin, lipids, surfactants, cyclodextrin, or bile salts), ionic strength, incubation time, testing volume, and temperature.

To assess whether drug solubilization will occur, one needs to consider the enthalpy change (i.e., the increase or loss of energy) during solution formation (ΔH_{soln}) and the entropy change (ΔS_{soln}). On the basis of the equation describing the Gibbs free energy of solution (G_{soln}), at some temperature, *T*, enthalpy and entropy changes are combined to describe the free energy change (ΔG_{soln}) for the solution formation:

$$\Delta G_{soln} = \Delta H_{soln} - T\Delta S_{soln}$$

Under conditions of negative values of enthalpy (i.e., exothermic reactions) and positive values of entropy (i.e., a more disordered system), the free energy will always be negative and solubility will readily occur. For cases when solution formation is endothermic (positive enthalpy change), solubilization still can occur as long as the entropy change is sufficiently positive to counteract the endothermic reaction. In other words, the change in entropy must be of a sufficient magnitude to result in a negative value of free energy change. For example, although there is a positive enthalpy change when sodium chloride goes into solution (enthalpy change + 3.9 kJ mol^{-1}), the concomitant increase in entropy overrides the small cooling effect, resulting in a net negative free energy change and therefore solution formation. In this case, the increase in entropy can be understood by considering the arrangement of ions (sodium and chloride in this example) in the crystal lattice. When the ions are arranged in a crystal lattice, their entropy (disorder) is low. However, as the salt gets dissolved in water, the ions are released from the crystal lattice, leave their previous arrangement, and are freely surrounded by water molecules. Hence, there is a higher degree of disorder (increase in entropy) that promotes the formation of a sodium chloride solution.

Polymorphism is another factor that should be considered when analysts assess the solubility of any drug substance. This may include solvation or hydration products (also known as pseudopolymorphs) and amorphous forms. Solvation involves a chemical or physical interaction between a solute and a solvent molecule. When this interaction occurs in an aqueous medium, it is called hydration. Hydration can be considered a mechanism that results in the stabilization of a solute in the solution. In contrast, solubility reflects a dynamic equilibrium between the rate at which the drug goes into solution versus the rate at which it precipitates.

The solid-state properties of any API can have considerable influence on the apparent solubility. Polymorphic forms differ in their internal solid-state structure (and therefore differ in the energy needed to break the crystalline lattice structure). Consequently, a drug substance that exists in various polymorphic forms can have different aqueous solubility and dissolution rates. For this reason, additional attention should be given to the potential effect of polymorphism on drug product bioavailability (BA) and drug product bioequivalence (BE) and whether or not a change in the polymorph can affect performance for the drug substance and drug.

A rule of thumb used for predicting solubility is "like dissolves like." This statement indicates that a solute will dissolve best in a solvent that has a similar polarity. Two substances with similar intermolecular forces are likely to be soluble in each other. Nonpolar molecules are soluble in nonpolar solvents (e.g., CCl_4 is soluble in C_6H_6). Polar molecules (C_2H_5OH) are soluble in polar solvents (H_2O), and ionic compounds (NaCl) are more soluble in polar solvents (H_2O or NH_3).

Once the solute molecules are surrounded by the solvent, a new stabilizing interaction is formed between the solvent and the solute. This is known as the solvation energy. The lower the solid-state energy stabilization that needs to be overcome (i.e., breaking the energy of interaction between solute molecules, also known as the cavitation energy), the greater the number of molecules that can be accommodated in the solution. Thus, the most stable crystalline forms also have the lowest aqueous solubility (*1*).

Common-Ion Effects

Solubility depends on the excess or deficiency of a common ion in the solution, a phenomenon known as the common-ion effect. This term describes the effect on a solution of two dissolved solutes that contain the same ion or ions. The presence of a common ion suppresses the ionization of a weak acid or a weak base. Accordingly, to some extent solubility also depends on the ionic strength. The ionic strength of a solution is a measure of the concentration of ions in that solution.

The total electrolyte concentration in solution affects important properties such as the dissociation or the solubility of different salts. Thus, when discussing thermodynamic solubility, analysts must define whether the assessments are based on the drug substance itself or a salt form of the drug substance. Analysts also should describe the relationship between the drug substance's solubility and the characteristics of the dissolving medium. For this reason, solubility can be defined as unbuffered, buffered, and intrinsic solubility.

Unbuffered solubility, usually in water, pertains to the solubility of a saturated solution of the compound at the final pH of the solution (which may be far from pH 7 because of self-buffering).

Buffered (apparent) solubility refers to solubility at a given pH (e.g., 2 or 7.5) measured in a defined pH-buffered system. This solubility estimate usually neglects the influence on the measured solubility value of salt

formation with counterions of the buffering system. Thus, at any given pH, buffered solubility may vary as a function of the type of buffer system employed (e.g., acetate vs. phosphate buffers).

Intrinsic solubility means the solubility of the neutral form of an ionizable compound.

For neutral (non-ionizable) compounds, all three definitions coincide. Salts of organic acids and bases usually are soluble in water because they are ionized in this medium and because the resulting charged group is highly hydrophilic and is capable of bringing large hydrophobic groups into solution. On the other hand, the undissociated weak acid or base is a weak electrolyte and is therefore only slightly ionized in water. The resulting uncharged group is only weakly hydrophilic so that only those acids and bases with small hydrophobic groups are able to dissolve to any extent in water. Another property of weak acids and bases is that their aqueous solubility is sensitive to pH because an uncharged, water-insoluble molecule can be changed to a charged, water-soluble species when analysts change the hydrogen ion concentration. As an example, sodium salicylate is considerably more soluble in water than salicylic acid because of the different hydrophilic powers of COO– and COOH. If a strong acid is added to an aqueous solution of sodium salicylate, there will be a notable drop in solubility and salicylic acid will precipitate out of solution. A strong base has the same effect on weak bases.

Analysts distinguish the intrinsic solubility from the solubility measured at a given pH value in a defined medium. Intrinsic solubility is relatively independent of the nature of the medium used because it involves only the neutral compound. In contrast, when one deals with acids and bases the solubility measured at a fixed pH value may be highly dependent on the nature and concentration of the counter ions present in the medium. This is especially critical for poorly soluble compounds that are strongly ionized at the pH of the measurement. The solubility of weak bases tends to decrease as the pH increases (approaching pK_a), and for weak acids solubility increases as pH increases (moving farther from the pK_a). However, although the Henderson–Hasselbalch equation would suggest no obvious plateau in potential solubility, counteracting factors must be considered. These include the pH_{max}, which is defined as the region where the ionizing portion of the curve meets the salt plateau on the pH–solubility profile. At pH_{max} the equilibrium solid state is a salt, and the limiting factor is the relationship between the completely ionized drug vs. the oppositely charged counter ion. For both acids and bases, there is a region within which pH has a large effect on the solubility of ionizable compounds. For this reason, the pH of the region of the GI to which the drug will be exposed is a pivotal consideration when analysts evaluate biologically relevant drug solubility (*1*).

PHYSIOLOGICALLY BASED DEFINITION OF SOLUBILITY

Whether considering biowaivers as described in FDA/CVM Guidance 171 (*2*) or examining the applicability of the FDA/CDER biowaiver guidance in the assessment of immediate-release oral dosage forms for companion and food animals (*5*), analysts must clarify the criteria used for classifying a drug as highly soluble. Further this assessment must be firmly grounded in the GI physiology of the target animal species. When developing species-specific solubility criteria, analysts must consider the following five critical questions:

- The regions in the body that must be considered during evaluation of the solubility criteria for that particular species,
- The pH of the GI fluids to which the drug will be exposed,
- The rate at which materials move through the segments responsible for drug dissolution,
- The fluid volume(s) to which the drug will be exposed,
- The relationship between dose, volume of "solvent" to which the drug will be exposed, and body weight (i.e., defining the highest label dose).

Measurements of thermodynamic solubility typically do not involve a time component. Rather, time is taken into consideration only when one is dealing with pharmaceutical dosage forms, and therefore the rate at which the drug in the dosage form goes into solution is evaluated during the in vitro dissolution study. Accordingly, as seen in the FDA/CDER biowaiver guidance, a time criterion for product dissolution is included when FDA evaluates requests for biowaivers. However, within veterinary medicine, some drug products, specifically some Type A medicated articles, may be difficult to test using typical in vitro dissolution methods (e.g., because of the insoluble nature of food substances that are included in these medicated articles). In these situations, a time component within the framework of the solubility test should be an additional consideration (*1*).

In a dosage-adjusted approach, solubility is based on the volume of the GI tract of the target species and the maximum amount of drug that will be administered. To

this end, analysts must consider three parameters when predicting the bioavailability of a drug and drug product:

The *dose number* (*Do*) is the ratio of the dose to the amount of drug that will dissolve in 250 mL of test solution at the lowest solubility within the pH range from 1 to 8. Ideally, this ratio should be below 1 if full dissolution is possible in principle. Obviously, higher doses will raise the ratio and make good absorption less likely. The simulated 250-mL fluid volume reflects the standardized volume of water ingested with an oral dosage form in people. Thus, measurement of *Do* assumes negligible residual gastric volumes in the human. The question is whether other monogastric species such as dogs likewise have a negligible residual gastric volume so that the *Do* should be based on the amount of water likely to be ingested at around the time of oral drug administration.

The *absorption number* (*An*) is the ratio of the GI transit time to the absorption time (1/absorption rate constant). Ideally, this should exceed 1. Longer absorption times resulting from lower permeability will reduce this ratio.

The *dissolution number* (*Dn*) is the ratio of the transit time to the dissolution time (1/dissolution rate constant). Ideally, it should exceed 1. In the case of solid dosage forms, a combination of inadequate solubility or diffusivity or excessive particle size or density can increase the time needed for full dissolution and therefore can reduce this ratio.

With regard to solubility, *Do* is the parameter of interest. An integral component of estimating *Do* is determining the pH range and the solvent volume that are reflective of the specific target animal species. Therefore, if the conditions under which *Do* is defined are modified to reflect the GI tract of different veterinary species, it is likely that what constitutes a highly soluble compound in one species may not be similarly categorized in another animal species. Furthermore, because of the marked differences in GI transit time and the corresponding duration of product residence within any particular GI segment, analysts also are likely to see different values of *Dn* across animal species (*1*).

To avoid confusion with human BCS terminology, the classification of *highly soluble* will be avoided. Rather, we will explore appropriate criteria for ascertaining if the highest approved dose will be *fully soluble* in the gastric fluids of the veterinary species of interest.

DEFINING SOLUBILITY IN DOGS

Although the beagle dog is frequently used as a preclinical species for evaluating human oral formulations, there are important differences between the GI tract of numerous dog breeds and those of humans. These GI differences can render it inappropriate to apply the human criteria for drug solubility classification to dogs (*8*).

Volume

The stomach acts as a repository for storing food and fluid. Dogs evolved with the capacity for intermittent large-volume meals. Therefore, relative to body size, their stomach capacity is larger than that of humans and cats, which eat smaller meals more frequently. According to a 1943 study, the stomach capacity in dogs is 100–250 mL/kg, with a range of 0.5 to 8 L per dog. However, stomach capacity does not reflect the fluid volume. The residual fluid volume in a fasted canine stomach is much smaller. Dogs do not voluntarily drink water after receiving an oral medication, and pet owners do not typically *flush* an oral dose with water. This is different from what is assumed to occur with humans. Therefore, in vivo dissolution of an oral medication in dogs must rely on residual stomach and intestinal water (*8*).

Solubility is considered in terms of the volume of fluid available to support in vivo drug dissolution. As with humans, canine fasted gastric volume is likely to contain negligible fluid volume. The question is whether the dog's fluid volume in the fasted state can be linked to some minimum amount of fluid intake likely to occur at the time of treatment and throughout the day and whether that volume varies as a function of body weight. For example, if we assume that 15–20 mL of fluid is administered to fasted beagle dogs (approximately 10 kg body weight) under laboratory conditions and if we can assume that water intake in nonlaboratory dogs is likely to result in similar residual gastric fluid volume, then can we also expect similar volumes of fluid, regardless of dog size? If not, does gastric volume scale linearly with body weight or in a manner consistent with body surface area (*8*)?

Whether we assume that the gastric fluid volume is based on an administered amount of water or on some other mechanism by which the dog achieves its fasted gastric water content, we need to ascertain the relationship between the fluid volume used in our solubility estimation versus the body size of the dog. Applying a linear scaling approach and assuming approximately 15–20 mL of water is administered to a 10-kg beagle dog under laboratory

conditions, the volume/body weight relationship would be approximately 2 mL/kg. However, with this method of estimation the fluid volume for toy breeds (e.g., a 3-kg dog) would be approximately 6 mL and that in giant breeds (e.g., 75 kg) would be 150 mL. Because this volume represents predose fluid intake, these values (especially for the 75-kg animal) do not seem reasonable or practical. Therefore, a range of potential relationships was explored from the perspective of considering their implications for an estimate of *Do*.

In trying to scale fluid volume to kg body weight, our first approach was to consider the allometric relationship between organ mass and body weight. Based on information reported by Boxenbaum in 1982, heart, lung, and kidney mass scales proportionately to body weight across animal species in a manner consistent with the kg body weight (see discussion, reference *9*). In addition, we considered three other approaches: scaling as a function of body weight to the 2/3 power (i.e., body weight to the power of 0.66), scaling as a function of body weight to the ¾ power (i.e., body weight to the power of 0.75), and scaling based upon a simple V_{max} model of the form:

$$V = \frac{V_{max} \times BW}{BW + V_{50}}$$

where V is gastric volume, V_{max} is the maximum volume achievable, regardless of body weight, V_{50} is the body weight associated with 50% V_{max}, and BW is the body weight of the dog (kg). As an example, we developed a hypothetical model using the parameter values V_{max} = 53 mL and V_{50} = 31 kg (*8*). Because the necessary actual data are lacking with respect to body size and gastric volume, these values were selected simply on the basis of volumes that were considered to be reasonable estimates. Clearly, should this algorithm be applied, experimental data will be needed to select an appropriate V_{max} value that will depend on the model selected in the study. Nevertheless, for this exploratory exercise these values and the corresponding V_{max} model adequately reflect the diversity in mg/mL solubility outcomes that can be achieved as a function of the relationship between body weight and gastric fluid volume.

Table 1 provides the resulting predictions of gastric volume across the four methods of volume estimation.

Table 1. Hypothetical Relationships between Gastric Volume and Body Weight (BW) Using Various Functions.

Gastic Volume (mL)				
BW (kg)	Scaled to 2/3 BW	Scaled to ¾ BW	Scaled Directly with BW	V_{max} Relationship
3	4	5	6	9
10	9	11	20	13
50	26	38	100	33
75	35	51	150	38

We considered the most physiologically plausible of these model relationships to be reflected either by BW scaled to the 2/3 power or by some form of a V_{max} model. In the latter two situations, if gastric volume scaling is applied and if the dose is administered on a mg/kg basis, then the mg dose/mL fluid relationship will vary as a function of body weight. This point is seen in Table 2 and in Figure 1 (where BW is scaled to the 2/3 power) and Figure 2 (where the V_{max} model is applied) (*2*). For the sake of this exercise, the assumed targeted dose was 2 mg/kg BW.

Table 2. Potential Relationships between Estimated Gastric Drug Concentration and Body Weight (BW) as a Function of Estimated Relationship between Volume and BW.

BW	Dose (if 2 mg /kg)	V_{max} Model		BW Scaled to the 2/3 Power	
		Fluid Volume		Fluid Volume	
		(mL)	mg/mL	(mL)	mg/mL
3	6	5	1.28	4	1.45
5	10	7	1.36	6	1.73
10	20	13	1.55	9	2.19
15	30	17	1.74	12	2.51
20	40	21	1.92	14	2.77
25	50	24	2.11	17	2.99
30	60	26	2.30	19	3.18
35	70	28	2.49	21	3.35
40	80	30	2.68	23	3.51
45	90	31	2.87	25	3.65
50	100	33	3.06	26	3.78
55	110	34	3.25	28	3.91
60	120	35	3.43	30	4.02
65	130	36	3.62	31	4.13
70	140	37	3.81	33	4.24
75	150	38	4.00	35	4.34
80	160	38	4.19	36	4.44
90	180	39	4.57	39	4.62
100	200	40	4.94	42	4.79

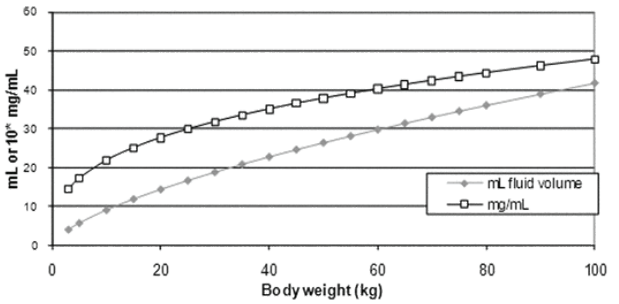

Figure 1. Relationship between mg/mL for solubility testing and mL fluid volume as a function of canine body weight scaled to the 2/3 power.

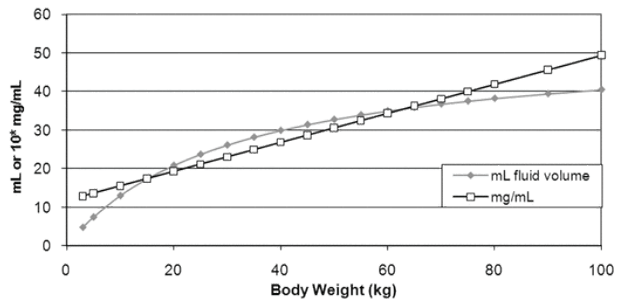

Figure 2. Relationship between mg/mL for solubility testing and mL fluid volume as a function of canine body weight: V_{max} model.

As seen in Figure 3, of the two methods scaling BW to the 2/3 power results in higher estimated concentrations at the lower body weights but approximately the same concentration at the upper body weights (based upon the constants for the V_{max} equations used in this example). In this comparison, both methods likely would result in similar highest concentrations. This is discussed in more detail below.

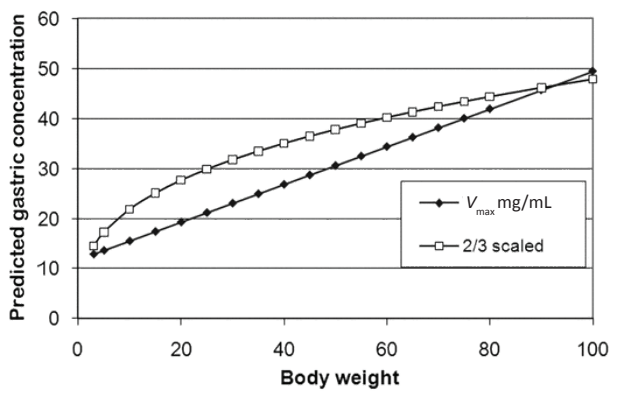

Figure 3. Relationship between concentration of volume estimated by V_{max} or to the 2/3 power (allometric approach).

Dose Definition in Dogs

In human medicine, solubility is based on the highest approved dose in mg. However, in the case of veterinary medicine, the dose is administered on a mg/kg basis. Therefore, we need to consider the highest approved mg/kg dose. This leads to three factors that analysts must consider when assessing drug solubility:

- The labeled or target mg/kg dose.
- The dose band: particularly for solid oral dosage forms for administration to dogs, tablet sizes are approved for administration within a weight range. This implies that the exact mg/kg dose will vary as a function of tablet strength and the range of weights for which that dose band is approved. So, for example, if a 10-mg tablet is approved for dogs ranging from 10 to 20 kg, then the actual mg/kg administered dose will range from 1 mg/kg to 0.5 mg/kg. These wide bands are possible for drugs that have a high therapeutic index, e.g., antibiotics.
- The method used to estimate gastric volume: as seen in the previous three figures, if the gastric volume is described as a function of the V_{max} or BW to the 2/3 power, the estimated concentration of drug within the gastric fluids will likewise vary. Assume, for example, that using the V_{max} scaling method and having a targeted dose of 1 mg/kg, in 4 tablet strengths approved for the dose bands shown in Table 3.

Table 3. Relationship among Body Weight (BW), Estimated Gastric Volume (Based on the V_{max} Scaling Method), and Drug Concentration in the Stomach Fluids

Dose Band	BW	Tablet (mg)	mg/kg Dose	Estimated Gastric Volume (mL)	Gastric Drug Concentration (mg/mL)
1	3	6.5	2.17	4.68	1.39
	10	6.5	0.65	12.93	0.50
2	11	20	1.82	13.88	1.44
	30	20	0.67	26.07	0.77
3	31	40	1.29	26.50	1.51
	50	40	0.80	32.72	1.22
4	51	62	1.22	32.96	1.88
	75	62	0.83	37.50	1.65

As Table 3 indicates, the highest concentration that would require testing to ensure solubility is 1.88 mg/mL. Thus, a new paradigm for highest dose would be required relative to the dose band and the scaled gastric volume (*2*).

Finally, we must consider the potential implications of errors in gastric volume estimates on the classification of a compound as being fully soluble (highly soluble). To this end, we considered the estimation of solubility by estimating *Do* (see previous discussion). *Do* can be calculated according to the formula: *Do* = (M/V)/C (*4*), where *Do* is the dose number, *M* is the dose strength of the tablet or capsule, *V* is the volume administered [defined as 250 mL in people, but both 6 mL and 35 mL were used in this analysis (*8*)], and *C* is the drug's solubility (mg/mL). A *Do* ≥ 1 has been used as a definition of a low-solubility drug whereas a *Do* < 1 defines a highly soluble drug.

To support our estimation of *Do*, the drug's solubility estimates were derived from a Web site: http://www.tsrlinc.com/resources/services/. [NOTE—This Web site is mentioned here solely for informational purposes. The reported values provided by this Web site have not been verified and validated.] This site provides an estimate of a drug's aqueous solubility (in mg/mL), pK_a, dose size in humans, and human BCS classification. A search was performed on 46 orally administered drugs that have been used in dogs (not all of these drugs are FDA approved). For each drug, the largest dose size used in the pharmacokinetic study was recorded, as well as the animal's weight, to obtain a mg/kg oral dose (*2*).

To explore the degree to which estimates of solubility may be in error because of an incorrect assumption about gastric fluid volume, we estimated *Do* using volumes of 6 mL and 35 mL. This exploratory exercise was based upon the body weight of beagle dogs (10–11 kg) because this is the breed most frequently used in published experimental studies. Although fluid volumes other than 6 mL and 35 mL could have been selected, these choices were considered to have the greatest physiological relevance. The value of 6 mL was selected as the extreme lower value because some canine practitioners suggest that 5–6 mL may in fact reflect the residual fluid volume in the fasted dog stomach. An upper value of 35 mL was selected because it is the scaled equivalent (for a 10-kg beagle dog) of the 250 mL (approximately one cup) volume used to estimate *Do* for the average human (250 mL = approximately 3.6 mL/kg) (*9*).

The results of this assessment are shown in Figure 4. The area delineated by the coordinates 0:0, 0:1, 1:1, and 1:0 represents those compounds that are classified as "highly soluble" based on their solubility in water.

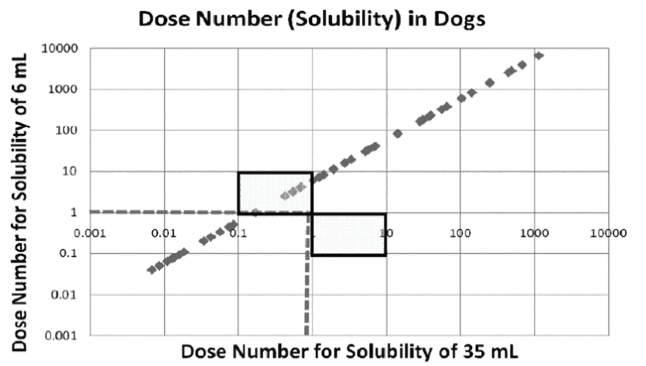

Figure 4. Do as a function of estimated solvent volume (6 mL versus 35 mL). The two shaded rectangular boxes delineate the region within which there is potential for volume-related differences in the calculation of Do.

As seen in Figure 4, the use of a gastric volume of 6 mL versus 35 mL changed the solubility classification (that is, whether or not *Do* is above or below a value of 1.0) for only 6 out of 46 drugs. In other words, although the various approaches for estimating gastric volume may affect our estimate of a maximum soluble dose, relatively few molecules would be incorrectly classified (or not classified) as highly soluble (fully soluble). Thus, when one uses a calculation of *Do* as defined by Kasim et al. (*4*), for the majority of compounds the solubility classification for drugs in dogs is not very sensitive to changes in volume between 6 mL and 35 mL (*8*).

To further illustrate this point, Figure 5 shows the *Do* for each of the 45 compounds using a gastric volume of 6 mL or 35 mL.

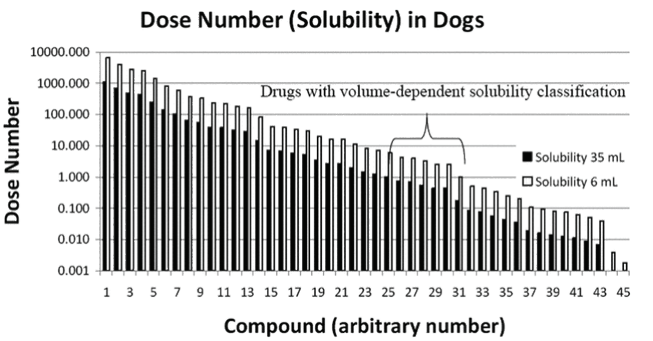

Figure 5. Do in dogs estimated with a water volume of either 6 mL or 35 mL. This figure illustrates the relatively small number of drugs whose solubility classification would differ because of the change in estimated volume.

Although these comparisons are based solely on solubility in pH-neutral water, we expect that despite the potential drug solubility changes as a function of pH a similar relationship will exist among volume, pH, and *Do* at other pH values (*2*).

pH Range in Dogs

The basal pH in the gastric fluid of dogs can be quite variable, and the reported gastric pH value in the dog is highly dependent on the portion of the stomach where the pH is measured. Nevertheless, evidence suggests that the gastric pH of the dog tends to be higher than that in humans because of the lower basal secretory rate of acid in the dog. This lower acid content has been attributed to the evolution of dogs from animals that ate large, infrequent meals (*7*). Because of the lower basal acid output, the pH of the fasted dog stomach is estimated to be approximately 1 pH unit higher than that in humans. After feeding, however, dogs have a higher peak acid output and a lower pH compared to that in people. According to Lui et al. (*10*), the canine postprandial gastric pH is similar to that observed in people, approximately 1.3 and 1.5 in dogs and humans, respectively. Despite species similarities in the amount of bicarbonate secreted, in the fed state the initial pH in the dog's duodenum is lower than that in humans because of the higher canine postprandial output of gastric acid. Within other portions of the small intestine, the intestinal pH of dogs and humans are similar. Considering the range of pH values that affect drug solubility in dogs, it seems appropriate to use the same pH solubility criteria in dogs as is currently used for defining the solubility of human drugs (*2*).

DEFINING SOLUBILITY IN CATTLE

Several important points must be considered when one establishes conditions and criteria for classifying drug solubility in cattle (*9, 11*). These include:

- The gastrointestinal tract of the ruminant is markedly different from that of the human. This influences both the appropriate volume and pH for defining drug solubility.
- The definition of *highest dose* is different in humans and cattle.
- The types of products (i.e., solid oral dosage forms vs. Type A medicated feeds) may require a time factor in the solubility assessment.

The Gastrointestinal Tract in Cattle

The stomach of ruminant animals is composed of four compartments: the rumen, reticulum, omasum, and abomasum. Forage is initially taken into the mouth and swallowed, after which it floats on top of a “hay mat” that is on top of the fluid shared between the rumen and reticulum. More dense solids settle from the hay mat to the bottom of the rumen for microbial digestion. The forage that remains on top of the rumen is regurgitated, remasticated, and then swallowed as a denser bolus. Rather than passing into the rumen, this dense bolus drops to the bottom of the reticulum, whence it eventually passes through the reticul-omasal orifice into the omasum. In the omasum much of the fluid portion of the ingesta is resorbed so that the mass of ingesta entering the abomasum is much smaller. Unlike the forestomachs, which are alkaline, the abomasum is similar to a monogastric stomach where the ingesta encounter an acidic environment before entering the duodenum. The solids concentration in ingesta is increased during passage through the omasum, and fluid is resorbed through the omasal fold epithelium back into systemic circulation.

Grains and solid dosage forms of drugs may fall immediately to the bottom of the reticulum, where they will dissolve in direct fluid contact with the rumen. The hay mat is not present in feedlot cattle fed high-concentrate rations.

As described by Sisson and Grossman, the relative sizes of the 4 stomach compartments change with age (*12*). In the neonatal calf, the rumen and reticulum together account for approximately half the capacity of the abomasum, and this relationship continues while the calf remains on an all-milk diet. At 8 weeks the rumen and reticulum equal the capacity of the abomasum and are twice the capacity by 12 weeks. The final relative capacities of the ruminant stomach are reached at approximately 1 year, when the rumen makes up approximately 80%, the reticulum 5%, the omasum 7%, and the abomasum 8% of the total capacity. The total stomach capacity of an adult cow (all 4 compartments) is estimated to be in the range of 115 to 150 L, and extremes range from 95 to 230 L.

Following is a summary of published data describing the fluid volume, turnover time, and pH of the bovine rumen. These data serve as the basis for proposed solubility test criteria for fully soluble drugs.

Data regarding adult cows' actual stomach volume based on marker data are available. Reynolds et al. evaluated liquid dilution and rumen volume in adult dairy cows during the late dry period and after transition to lactation (*13*). These values are reported in Table 4. The kg values for liquid volume in this study can be considered equal to liters. Fractional clearance of the marker substance from the rumen was assumed to represent the liquid dilution rate. The dry matter volume of the rumen was determined by calculating the dry matter of a composite sample and then extrapolating this to the entire rumen volume. Rumen volume is reported but should be considered as the combined volume of the rumen and reticulum because these two compartments communicate.

Table 4. Mean Rumen Digesta Volume, Liquid Dilution Rate, and Cow Body Weights by Days Precalving (–) or Postcalving (+)

Days Precalving (–) or Postcalving (+)	–17	–8	+10	+20	+31	Mean	SEM
N Rumen Digesta Volume (kg)	10	10	10	10	10		
Dry Matter	7.1	7.2	8.3	9.5	10.3	8.5	0.8
Liquid	51.8	50.5	48.9	54.2	57.7	52.6	4.3
Total	58.9	57.0	57.1	63.1	67.9	60.8	5.1
Liquid Dilution (%/hr)	14.8	15.1	17.7	17.5	16.2	16.3	0.8
Cow Body Weight (kg)	745	749	659	658	651	692	24

Park et al. (*14*) evaluated ruminal dynamics in Holstein dairy cows during the periparturient period. This study also determined the total capacity of the rumen/reticulum but in this case involved filling the entire compartment with water (after emptying of contents and before replacement of contents at each sample time). The volume of fluid represents only part of the total capacity, and the physiological fluid volumes should be used as the basis for solubility studies rather than for predicting the total potential volume of the bovine stomach. Values for total capacity, total fill, liquid fill, and dry matter fill from the Park et al. study are included in Table 5. As in Table 4, the kg values for liquid volume can be considered equivalent to liters. Based on the data by Park, the time required for approximately 94% of the fluid to pass through the rumen is about 19 to 23 h. These two studies are in close agreement for rumen liquid volume in adult dairy cows (52.6 and 55.3 kg) and also liquid passage time (16.3%/h and 12.7%/h).

Islas and Soto-Navarro (*15*) conducted a similar experiment in crossbred beef heifers with average weights of 378 ± 28.4 kg. The heifers grazed small-grain pasture and were supplemented with differing amounts of corn-sourced dried distillers grain with solubles. The small-grains pasture has a lower percent dry matter (higher moisture content) compared to the prepared total mixed ration fed to the dairy cows in the previous two studies. Ruminal volume (again representing both rumen and reticulum), fluid dilution rate, fluid turnover time, and solid particle dilution rate for this study are presented in Table 6.

Table 6. Ruminal Volume, Fluid Dilution Rate, Turnover Time, Forage, and Dried Distillers Grains with Solubles (DDGS) Particle Dilution Rate in Beef Heifers on Small-Grain Pasture

	(DDGS) as % of Body Weight				Mean	SE
	0	0.2	0.4	0.6		
Ruminal Volume (L)	79.5	81.5	88.3	125.5	93.7	18.9
Fluid Dilution Rate (%/h)	12.3	11.0	11.3	10.3	11.2	1.4
Turnover Time (h)	8.6	9.1	9.2	10.9	9.5	1.4
Forage Particle Dilution Rate (%/h)	5.0	5.3	5.4	4.7	5.1	0.5
DDGS Particle Dilution Rate (%/h)	—	6.6	6.4	6.0	6.3	0.6

Table 5. Total Rumen Capacity; Total, Liquid, and Dry Matter Rumen Fill; and Solid and Rumen Liquid Passage Rate in Adult Dairy Cows by Stage of Production

	Day Related to Parturition	Capacity (kg)	Total Fill (kg)	Liquid Fill (kg)	Dry Matter Fill (kg)	Solid Passage Rate (1/hr)	Liquid Passage Rate (1/hr)
Late Lactation	–72	137	57.3	48.8	8.6	0.036	0.123
Far-off	–51	125	60.1	53.9	6.2	0.050	0.126
Close-up	–23	140	53.2	48.3	4.9	0.065	0.120
Early Lactation	–9	143	50.3	44.3	6.1	0.045	0.122
Early Lactation	6	160	63.0	54.2	8.8	0.033	0.132
Early Lactation	20	149	61.1	52.3	8.8	0.038	0.144
Early Lactation	34	170	69.2	59.3	9.8	0.040	0.131
Early Lactation	48	164	66.4	56.4	9.9	0.044	0.141
Early Lactation	62	160	73.9	62.6	11.3	0.040	0.118
Early Lactation	62	160	73.9	62.6	11.3	0.040	0.118
Early Lactation	76	170	73.5	62.2	11.4	0.038	0.127
Early Lactation	90	171	77.4	66.0	11.4	0.030	0.118
Mean		154	64.1	55.3	8.8	0.042	0.127
SEM		9.4	4.3	3.7	0.8	0.005	0.012
Mean expressed as % of total capacity			37.5%	32.3%	5.2%	NA	

A mean total rumen volume of 93.7 L in the Islas study is larger than that of the much heavier cattle on a different ration in the previous two studies [60.8 and 70.3 kg for Reynolds (*12*) and Park (*13*), respectively]. The pH values for the four treatments ranged from 6.05 to 6.21 with an SE of 0.12.

Estell and Galyean (*16*) evaluated the rumen characteristics of steers in seven feeding trials conducted at their research facility. Mean body weight during the trials was 347.1 ± 78.1 kg. Mean rumen fluid volume was 46.1 ± 25.0 L; mean pH was 6.3 ± 0.4 (i.e., 5.1–7.5 = mean ± 3 SD); and the mean fluid dilution rate was 9.2%/h ± 3.0%/h.

Bengochea et al. (*17*) evaluated rumen pH after different degrees of barley and corn processing in medium-concentrate, growing diets. Rumen pH ranged from means of 6.16 to 6.36 in the three treatments.

Enemark et al. (*18*) used two methods for continuous rumen pH monitoring during administration of two rations with increasing energy values at the end of the study. Although the data are presented only graphically, the rumen pH values ranged from 6.3 to 6.8, and the final, higher energy, ad libitum ration drove one cow down to a pH near 5.8. Continuous rumen temperature monitoring during this study (again reported only graphically) demonstrated a relatively constant temperature in the 39 °C range.

Cooper-Prado et al. (*19*) evaluated rumen temperature related to parturition and estrus using intraruminal sensor boluses in Angus cows. Rumen temperature varied from a high of 38.94 ± 0.05 °C before parturition to a low of 38.30 ± 0.09 °C the day after estrus was observed. Ruminal temperature was not influenced by ambient temperature. The cows were on pasture and received a protein supplement during a portion of the study.

These data support the hypothesis that drug entities reside in the rumen/reticulum for an extended period of time that allows for dissolution before passage through the concentrating process of the omassum and into the acidic environment of the abomasum. Therefore, the rumen should be the compartment used for modeling of drug solubility in the ruminant stomach.

Composition of Rumenal Fluids

Because of their extensive fermentation activity, rumenal fluids are complex mixtures. Generally they contain large amounts of glucose, bacteria, volatile fatty acids, cellulose, digestive enzymes, vitamins, proteins, and lipids. However, the relative proportion of these many constituents can vary as a function of diet (*20*).

Unlike monogastric species, ruminants do not fast and therefore a highly complex mixture of materials is present in the rumenal fluids throughout the day. These substances can act as surfactants, thereby affecting the solubility of an API. The question is whether or not the presence of these surfactants must be considered when researchers develop testing conditions for drug substance solubility in ruminants.

Defining the Highest Dose

Solid oral dosage forms: Unlike human medicines for which a milligram amount of drug may be administered irrespective of body weight, veterinary drug products generally are administered on a mg/kg basis. Therefore, even for solid oral veterinary dosage forms, the dosage unit with the highest drug content may be exposed to a higher volume of fluid, thereby effectively normalizing the mg/mL concentration of solubilized drug in the GI fluids. For this reason, for solid oral dosage forms the definition of highest milligram dose must be considered from the perspective of the highest approved mg/kg dose.

Type A medicated feeds: Unlike the solid oral dosage form that is administered in a single unit, medicated feed consumption occurs ad libitum. Therefore, depending on whether the product is in a medicated feed that is available throughout the day or is likely to be consumed rapidly (e.g., a medicated top dress), there will be some period of time over which the entire daily dose is consumed. Because the intake is ad libitum, the amount per unit time across a dosing day cannot be ensured.

The intake of the medicated feed over a dosing day must be considered from the perspective of the slow movement of drug out of the rumen. Because the rumen retention time generally exceeds 24 h for solid particles (*20*), we can assume that at some point the rumen will contain the total daily dose consumed by the bovine. For this reason, regardless of the method of feed administration, it seems safe to assume that solubility can be based upon the highest mg/kg amount of drug that will be consumed during a single dosing day.

One can argue that because of its slow transit time through the rumen medicated feed will accumulate there. However, such an accumulation would influence only the amount of a low-solubility drug in the rumen. In other words, if the drug is fully soluble most of the dose will move with the fluids out of the rumen. Accordingly, the amount of residual solubilized drug should be negligible.

Time as a Factor in Solubility Testing

Bearing in mind the point about drug accumulation and

considering the difficulties that can be encountered when conducting in vitro dissolution testing for Type A medicated feeds, analysts must factor time into the solubility test. If we use the conservative turnover time for rumen fluids proposed by Islas and Soto-Navarro (*14*), the duration of fluid transit through the rumen is 8.6–11 h, and solubility must be determined after 8 h of testing. In other words, if a drug is considered *fully soluble* in the bovine rumen based on this conservative time estimate, the total dose should be dissolved within 8.6 h.

Although the solubility test is intended solely to measure a characteristic of the drug substance (i.e., it is not intended to serve as a measure of formulation effects), when in vitro dissolution tests cannot readily be performed, e.g., the situation encountered with some Type A medicated articles (premixes), the solubility test may be required to measure drug substance and solvent interactions and to ensure that the product is fully solubilized before the rumen fluids transit into the abomassum.

TEST CONDITIONS FOR DETERMINING SOLUBILITY

The following proposals are intended to serve as a foundation for future discussions.

Although numerous methods for estimating drug solubility can be found in the literature (*21*), the gold standard is the shake-flask method (*22, 23*). Single-pH measurements (using the shake-flask method, for example) cannot distinguish between soluble monomers and soluble aggregates of the drug molecules (which may range from dimers to micelles) unless more sophisticated experiments are performed (*24, 25*). Some of the physico-chemical factors that influence drug solubility are reviewed elsewhere (*26, 27*).

Thermodynamic solubility is determined by several measurements, generally after 24–48 h of stirring the drug substance in aqueous medium. Equilibrium is considered to be achieved when at least two constant values of solubility are measured over time.

Proposed Criteria for Fully Soluble Drugs

The following proposals are intended to serve as a springboard for discussions in anticipation of an upcoming *USP* general chapter that defines solubility criteria for veterinary species.

DOGS

Test the maximum FDA approved mg/kg dose (based on dose bands of the oral dosage form). The total milligram amount of drug under test should be that maximum mg/kg dose multiplied by the body weight of a typical laboratory beagle dog (10 kg).

Using a midpoint value between the gastric fluid volume scaled to the 2/3 power and to the ¾ power, determine the solubility of that estimated milligram dose in 10 mL of gastric fluid.

Testing should be conducted under the following conditions:

a. 37 °C

b. pH 1.2 (0.1 N HCl), 4.5 (acetate buffer), and 7.5 (phosphate buffer). These conditions are identical to those used when testing the solubility of compounds intended for human use.

c. As with the human BCS, no constraints must be imposed upon the duration of the solubility test. The dissolution of the finished product is an independent study.

CATTLE

The following conditions for assessing drug solubility reflect suggested conditions for testing drug solubility in cattle:

pH: 5.1 to 7.5

Buffer solution: typical phosphate buffer can be used. Although the rumen is rich in surfactants such as fatty acids, we are not recommending their inclusion in the test medium at this time, but this clearly is a point for further discussion.

Volume: to be conservative, the mean ruminal volume of 50 L of fluid should be used during solubility testing based on the Estell and Galyean (1985) report (*16*).

Temperature: 38 °C

Time for solubilization: based on the fluid transit time in the rumen: 8 h appears to be an alternative mechanism for ensuring that the drug in a medicated feed is completely dissolved and delivered to the absorbing portion of the GI tract in a dissolution-independent manner.

When analysts estimate the milligram dose that should be tested when evaluating solubility in the bovine, it should be based on the anticipated mg dose/kg body weight/day that will be consumed by the bovine.

Perspectives on the establishment of bioequivalence for Type A medicated articles (premixes) have been published recently (*28*).

When the drug is a solid oral dosage form intended for administration to ruminating cattle, the time factor associated with the proposed solubility test criteria may be eliminated, and in its place in vitro dissolution testing can be conducted.

NEXT STEPS

Because of the many species for which solubility criteria are needed, the ultimate goal is to expand the *USP* general chapter on veterinary drug solubility to include criteria for such species as cats, swine, and poultry. However, for now our focus will remain on establishing species-specific test conditions and solubility criteria for dogs and cattle. To that end, this *Stimuli* article is intended as a foundation for future discussions.

Controversies, issues, and potential solutions raised in this *Stimuli* article will be discussed during a public USP workshop that will be held at USP Headquarters in Rockville, MD, on 7–8 November 2012. The purpose of that workshop is to discuss and obtain either a resolution or a path forward regarding controversial aspects of the solubility proposals in this *Stimuli* article and to identify areas that need additional research.

The outcome of these discussions will be used in the development of the initial version of the *USP* general chapter on solubility criteria in veterinary species.

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