#284

Using Relative Supersaturation to Support "Urinary Tract Health" Claims for Adult Maintenance Cat Food

Guidance for Industry

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For further information regarding this document, contact AskCVM@fda.hhs.gov.

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine (CVM)
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Contains Nonbinding Recommendations

Using Relative Supersaturation to Support "Urinary Tract Health" Claims for Adult Maintenance Cat Food

Guidance for Industry

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I. Introduction

In response to requests from pet food manufacturers, the FDA's Center for Veterinary Medicine (CVM) has evaluated the use of relative supersaturation (RSS) methodology to support urinary tract health (UTH) claims for certain adult maintenance cat food. RSS is a measurement that estimates the potential for crystal formation and bladder stone (urolith) growth, which is a common affliction in cats. This guidance provides recommendations for how pet food manufacturers can use RSS methodology to substantiate general structure or function claims that an adult maintenance cat food supports UTH by promoting a healthy mineral content in the urinary tract.

The recommendations we provide in this guidance include: (1) the wording of a UTH claim based on RSS methodology for use on adult maintenance cat food labeling; (2) the RSS criteria to substantiate a UTH claim; and (3) the study data CVM requests from pet food manufacturers that demonstrate the utility and target animal safety of the cat food.

For purposes of this guidance, "you" means the manufacturer of adult maintenance cat food with UTH claims (UTH cat food) based on RSS methodology.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. Background

A. Introduction to Urolithiasis in Cats

Urolithiasis (the presence of uroliths in the bladder) is a fairly common affliction in cats, with prevalence estimates from clinical studies ranging from 9-23% (Forester *et al*. 2010). The two most common types of uroliths in cats are magnesium ammonium phosphate (struvite), and calcium oxalate (CaOx), accounting for 49% and 38% of analyzed uroliths, respectively (Minnesota Urolith Center Global Data 2023).

In response to concerns about urolithiasis, pet food manufacturers use various formulation strategies to make UTH cat food. Historically, manufacturers of UTH cat food restricted the magnesium content and/or formulated their cat food to produce slightly acidic urine (pH of 5.9 to 6.4). The slight acidity and low magnesium content create a urinary environment that is unfavorable for struvite crystallization and urolith growth but may be favorable for CaOx urolith growth in predisposed cats (Lekcharoensuk *et al.* 2001). Formulating cat food based on RSS methodology is a more recent dietary strategy that some pet food manufacturers use to create UTH cat food. The principles of RSS apply to all urolith types, so UTH cat food based on RSS methodology has the potential to help protect cats from both struvite and CaOx urolithiasis.

B. Relative Supersaturation (RSS) Methodology

RSS methodology originated in human medicine in the 1960's and is widely used for measuring the potential for urolith formation in humans. Researchers have also validated RSS methodology for use in dogs and cats (Robertson *et al.* 2002).

One of the primary conditions for urolith formation in any species is oversaturation of the urine with dissolved substances (solutes) that have the potential to precipitate out of solution and form crystals. These crystals can eventually grow into uroliths. RSS provides a numerical measurement of the degree of saturation of a specific urolith-forming substance, and thus a quantitative method to evaluate the risk of urolith formation. There is a unique RSS value for each possible urolith type in a urine sample. The RSS value for a specific urolith is determined by first measuring the concentration of multiple solutes, including magnesium, ammonium, phosphate, calcium, and oxalate, as well as the pH in a urine sample collected over at least 24 hours. These data are entered into a computer program that calculates the concentrations of the solutes that remain free to interact and form crystals, also known as the activity product (AP). An RSS value is obtained by dividing the AP for a given urolith-forming compound by its known thermodynamic solubility product (SP), which represents the maximum concentration at which a solute can be completely dissolved in solution.

$$RSS = AP_{urolith} \text{ of the urine}/SP_{urolith}$$

There are three zones of urine saturation that describe the activity of a given crystal in a urine sample:

(1) *Undersaturation* – crystals will not spontaneously form, and if present, will dissolve.

(2) *Metastable supersaturation* – crystals will not spontaneously form but may form in the presence of solid particles (i.e., a nucleus) such as cells, an existing urolith, or other particulate matter. Existing crystals and uroliths will not dissolve and may grow.

(3) *Labile supersaturation* – crystals will spontaneously form and grow.

The calculated RSS value for a given crystal indicates which zone its concentration is in for that urine sample. An RSS value below the solubility product for a given crystal, i.e., RSS less than 1.0, puts it in the undersaturation zone. The concentration at which dissolved urolith material will spontaneously precipitate out of solution to form crystals is called the formation product, and the formation product defines the limit between the metastable and labile supersaturation zones. Studies suggest that the formation product for struvite uroliths is an RSS of 2.5, and for CaOx uroliths is an RSS of 12 (Houston and Elliot 2008; Bartges 2011), and these values are generally accepted and used by the veterinary community. The degree to which an RSS value falls above the solubility product and approaches or exceeds the formation product indicates the level of risk for urolith formation.

C. Support for Using RSS Methodology in UTH Cat Food

We received requests to assess the use of RSS methodology to substantiate a UTH claim on adult maintenance cat food not restricted to sale through or under the guidance of veterinarians. We therefore reviewed the available literature regarding RSS in cats, and we summarize our findings from that literature review below.

1. RSS and Dietary Factors

Many studies investigate the relationship between RSS values and various dietary factors that possibly influence UTH in cats, such as ingredients that alter urine pH (Bartges 2013; Queau *et al.* 2013; Stevenson *et al.* 2000; Markwell *et al.* 1999; Smith *et al.* 1998), dietary moisture (Buckley *et al.* 2011), and dietary sodium content (Queau *et al.* 2020; Hawthorne and Markwell 2004). These studies help pet food manufacturers, nutritionists, and diet formulators to make cat food that produce the desired RSS values.

2. RSS and Urolith Activity

There are numerous studies that evaluate the utility of RSS methodology that specifically assess the relationship between RSS values and struvite urolithiasis in cats. Several studies demonstrated that cat urine undersaturated for struvite ($RSS_{Struvite} < 1$) successfully dissolved struvite uroliths *in-vitro*, and the smaller the RSS value, the faster the dissolution occurred (Tournier *et al.* 2008; Tournier and Biourge 2008; Sagols *et al.* 2015). Studies also show that RSS is better than pH for predicting struvite urolith activity *in-vitro* (Van Hoek *et al.* 2009, Sagols *et al.* 2015). Published *in-vivo* studies demonstrated that cats that consumed diets formulated to induce urine undersaturated for struvite experienced complete struvite urolith dissolution after 2 to 10 weeks of feeding (Houston *et al.* 2004, 2011; Tefft *et al*. 2021; Torres-Henderson *et al.* 2017).

Although researchers cannot perform dissolution studies for CaOx uroliths because this urolith is not able to be dissolved, several studies document a positive association between greater RSS_{CaOx} values and CaOx urolith formation in the bladder and/or kidney in humans (Ferraro *et al.* 2018; Prochaska *et al.* 2018; Parks *et al.* 1997; Thun

and Schober 1991), rats (Bushinsky *et al.* 1995), and dogs (Stevenson *et al.* 2003, 2004). We conclude that this information about the relationship between RSS and CaOx uroliths in other species, and the results from the struvite dissolution studies, support the use of RSS methodology to substantiate claims that a cat food supports UTH in cats.

III. Recommended Information to Substantiate RSS-Based UTH Claim

If you use RSS methodology to substantiate general structure or function claims that an adult maintenance cat food supports UTH, we recommend that prior to marketing the product, you submit for our review:

- Empirical data demonstrating the utility and safety of the product's mechanism(s) for supporting UTH (See sections IV. *Utility* and V. *Target Animal Safety*),
- A complete quantitative ingredient formulation of the product,
- A complete quantitative nutrient composition of the product, and
- A complete product label, which can be a pdf-file, printer's proof, or an unused label.

If you include an RSS-supported UTH claim on the product label of the adult maintenance cat food, you also should include a mechanism of action statement on the product label and any additional labeling. We recommend you use the mechanism of action statement: "Promotes a healthy mineral content in the urinary tract." We may also accept variations of that phrase and other statements that reflect the RSS basis for support of the UTH claim.

The submitted information helps us ensure the UTH claim is substantiated and helps CVM review the claim in the context of the entire product label and labeling as a whole. You may contact CVM's Division of Animal Food Ingredients (DAFI) if you have any questions about the recommended submission information.

IV. Utility

A. Target RSS Values

To demonstrate utility of a UTH cat food based on RSS methodology, you should submit results to CVM from at least one feeding study that demonstrates cats eating the food achieve RSS values below the formation products for struvite and CaOx (2.5 and 12, respectively). The study should evaluate RSS for both struvite and CaOx since they are the two most common types of uroliths found in cats and preventing one urolith type while potentially promoting formation of the other is not supportive of a general UTH claim. To ensure that cats consuming the food are less likely to develop urinary crystals or uroliths, we recommend the mean RSS for each urolith type at each measured timepoint be no greater than the midpoint of that urolith's metastable supersaturation range. In addition, the upper bound of the 95% confidence interval (CI) around the mean

for each timepoint should be no greater than the value of the formation product for the specific urolith. The values meeting the recommended mean RSS and 95% CI limits are:

- Struvite: Mean RSS ≤ 1.8; Upper bound of 95% CI ≤ 2.5
- CaOx: Mean RSS ≤ 6.0; Upper bound of 95% CI ≤ 12

With these target values, it is possible some cats in the study group may produce urine in the labile supersaturation range, but most cats will produce urine in the metastable or undersaturated range. Thus, these values allow for some spread in individual cat RSS values and still provide an acceptable margin of safety against urolith formation.

If the RSS analysis provides separate RSS values for each of the two forms of CaOx crystals – monohydrate and dihydrate – rather than providing an RSS value for total CaOx, then the values for recommended mean RSS and CI limit apply to each of those forms.

We also recommend you conduct RSS measurements on urine collected over at least 24 hours, which would likely provide more accurate results because it accounts for possible diurnal variation in mineral excretion and other parameters.

B. Study Duration and RSS Assessment Schedule

Although we do not define a specific timepoint when a study group of cats should achieve the target RSS values, the study should demonstrate that once the cats do achieve the RSS targets, the cats continue to meet the RSS targets from that point of the study onward. Data from industry studies measuring RSS submitted to CVM demonstrate that RSS is stable from 5 to 26 weeks in cats. Therefore, we recommend you conduct utility studies for a minimum of 40 days with RSS for struvite and CaOx measured at prescribed intervals no less than every 10 days during the study. The first RSS measurement should occur between day 10 and 21 of feeding the product. There should be at least four separate RSS measurements, but they need not occur at regularly spaced intervals. Study durations longer than 40 days and other RSS measurement schedules are possible, but we recommend you use the guidelines we have described in this paragraph when designing your study.

It is important to note that although we request a minimum of four RSS measurements that meet the criteria listed in section IV.A. *Target RSS Values* to demonstrate utility, this does not mean that meeting the utility criteria at any four times out of the total number of measurements performed would be acceptable. The study should demonstrate that cats can *consistently* produce urine that meets the RSS targets, and that consistency should be demonstrated by at least four consecutive measurements. Although it is your decision to determine how long a study and how many RSS measurements you wish to perform to meet that goal, you should make that decision prior to study initiation and adhere to that schedule regardless of the RSS results obtained.

V. Target Animal Safety

A. Safety Concerns

There may be several methods for formulating a cat food that will produce the desired RSS parameters, so safety concerns will vary with the formulation method(s) used. One safety concern is the degree to which the cat food acidifies the urine, because creation of an overly acidic urine can increase the risk of metabolic acidosis. Although acidification may not be the primary mechanism pet food manufacturers employ when formulating UTH cat food based on RSS methodology, urinary pH is one of the variables that affects RSS values, so adjusting a dietary formula to affect RSS may inherently affect urinary pH as well. Thus, urinary pH measurements are an important safety component for manufacturers of UTH cat food when relying on RSS methodology. Data from studies submitted to CVM by pet food manufacturers show a correlation between very low urine pH and negative balance of magnesium (Mg) and potassium (K). Thus, if urinary pH is low enough to cause metabolic acidosis, mineral balance data (calcium (Ca), phosphorus (P), Mg, and K) are needed to evaluate product safety. This is addressed in more detail in section V.B. *RSS Study Design*.

Because manufacturers of UTH cat food based on RSS methodology may use various, potentially novel, formulation methods to achieve the RSS goals, nutritional adequacy of the cat food is another safety concern. UTH cat food based on RSS methodology should therefore meet at least one, and preferably both, of the methods for substantiation of nutritional adequacy as described in the Association of American Feed Control Officials (AAFCO) *Official Publication* (AAFCO, 2024). These methods are: (1) formulate the cat food to meet the AAFCO Cat Food Nutrient Profile for Adult Maintenance, and/or (2) successfully complete and pass an appropriate AAFCO Protocol Feeding Study that demonstrates the cat food to be adequate for maintaining the nutritional status of adult cats.

B. Study Design

1. Duration

If the cat food is adequate for maintaining the nutritional status of adult cats because you formulated the cat food to meet the AAFCO Cat Food Nutrient Profile for Adult Maintenance or completed an appropriate AAFCO Protocol Feeding Study, we recommend you conduct at least a 40-day feeding study to evaluate product safety. Although an AAFCO Protocol Feeding Study runs for at least 26 weeks, the required analyses are minimal, so we do not consider completion of such a study to provide sufficient data on its own to demonstrate safety of UTH cat foods. However, if you conduct a study that includes the safety data described in sections V.B.2. *Urinary pH Measurements and Mineral Balance Data* and V.B.3. *Additional Clinical Data*, 40 days is likely a sufficient study duration for any safety concerns to manifest. This 40-day duration also matches the minimum study length recommended to demonstrate utility, so you may choose to assess both utility and safety in the same study. A study of longer duration may be necessary if mineral balance data is needed (see section

V.B.2. *Urinary pH Measurements and Mineral Balance Data*). If the nutritional adequacy of the cat food has not been substantiated using either of the AAFCO methods, then you should conduct a 26-week adult maintenance feeding study as described in the AAFCO *Official Publication* (AAFCO, 2024) and collect safety data during that time.

2. Urinary pH Measurements and Mineral Balance Data

We recommend safety studies include measurements of each cat's urinary pH at a minimum of four timepoints no less than 10 days apart. Thus, a 40-day study should include urine pH measurements on days 10, 20, 30, and 40, but other schedules could be acceptable if the study is intended to run longer than 40 days. You should perform urine pH measurements on 24-hour urine samples (rather than on single point-in-time urine collections), and then calculate the mean pH and 95% CI for each time point. The CI values will indicate if the study needs to include collection of mineral balance data (Ca, P, Mg, K), which would necessitate a study of longer duration, or if the study can end after 40 days. We recommend the following guidelines:

a. If the lower bound of the 95% CI for the mean pH is *equal to or greater than* 6.0 for at least three time points, the study can end after a minimum of 40 days.

b. If the lower bound of the 95% CI for the mean pH is *less than* 6.0 for two or more of the time points, the study should continue with mineral balance collections.

c. You should start mineral balance collections as soon as they are determined to be needed and you should collect mineral balance data at a minimum of three timepoints no less than 30 days apart. A baseline collection prior to feeding the test diet is optional. For this collection to serve as one of the three needed timepoints, it should be performed only after all cats have been consuming the same complete and balanced diet (e.g., standard colony diet) for a minimum of 7 days.

Additional urinary pH and/or RSS measurements could be made if the study is extended to allow for mineral balance collections. We do not consider these additional measurements to be necessary if the study already included the recommended four RSS measurements, but the additional pH and RSS data should be included in the final study report if they are collected.

3. Additional Clinical Data

Regardless of the duration, studies that adequately address target animal safety should also include:

a. Veterinary physical exams performed at the beginning and end of the study, and at the midpoint if the study duration is longer than 40 days,

b. Serum chemistries,[1] hematologies,[2] and urinalyses[3] performed at the beginning and end of the study, and at the midpoint if the study is longer than 40 days,

c. Body condition scores determined at least monthly,

d. Body weights measured weekly,

e. Food consumption measured daily,

f. Stool observations daily ± periodic fecal scoring, and

g. Morbidity and mortality observations, with any animal that dies being necropsied and a cause of death determined.

VI. Combined Safety and Utility Study

Because we recommend you conduct both target animal safety and utility studies for a minimum of 40 days, you may opt to combine both studies in one feeding trial to maximize efficiency of time and use of resources. Appendix 1 contains a flowchart that depicts the recommended timeline for a minimum 40-day combined safety and utility study. Please note that this flowchart applies only to cat food for which you have substantiated the nutritional adequacy for adult maintenance, as recommended in V. *Target Animal Safety*. UTH cat food based on RSS methodology that has not been substantiated for nutritional adequacy to maintain adult cats should undergo a 6-month safety study, during which nutritional adequacy for adult maintenance can be substantiated according to AAFCO protocols along with the other parameters discussed in this guidance.

VII. Other Study Considerations

We recommend you conduct appropriate statistical comparisons, using suitable methodology and numbers of animals to confidently (95%) detect statistically significant differences (10%) in the serum chemistry and hematology data between baseline and later timepoints, should they exist. You may also conduct statistical comparisons on additional safety data,[4] but we do not consider statistical comparisons to be relevant for the utility (i.e., RSS) evaluation.

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[1] A serum chemistry profile should include glucose, albumin, total protein, total bilirubin, blood urea nitrogen, creatinine, alkaline phosphatase, alanine transaminase, aspartate aminotransferase, gamma-glutamyltransferase, calcium, magnesium, phosphorus, potassium, sodium, chloride, and tCO_2 or pCO_2.

[2] Hematology profile should include a complete blood count and differential white cell count.

[3] Urinalysis should include color, protein, glucose, ketones, bilirubin, specific gravity (by refractometer), and microscopic sediment examination to look for crystals, casts, red and white blood cells, and epithelial cells.

[4] All data other than RSS values, such as food consumption, body weight, and mineral balance data, pertain to the safety evaluation.

Successful studies should have fewer than 25% of the cats starting the study removed for non-nutritional reasons or poor food intake, and cats may be removed for poor food consumption and their data excluded from the final analysis only within the first 14 days of the study. The reason for any cat's removal should be recorded and included in the final study report. A minimum of 10 cats should complete the study, so we recommend at least 12 cats start the study to ensure the minimum number of cats complete the study.

Any medical treatment provided to cats on study and the reason for it should be recorded and included in the final study report.

The final study report should include all data generated from individual animals as well as summary statistics for each day of measurement. The study report should also include a full discussion of the diet formulation, experimental methodology, statistical methods used to analyze the data, and interpretation of findings.

This guidance document may not address all specifics important for the design of the study. We encourage you to submit protocols to CVM's DAFI for review and comment prior to study execution. You should address our questions on details of the protocol before the study begins.

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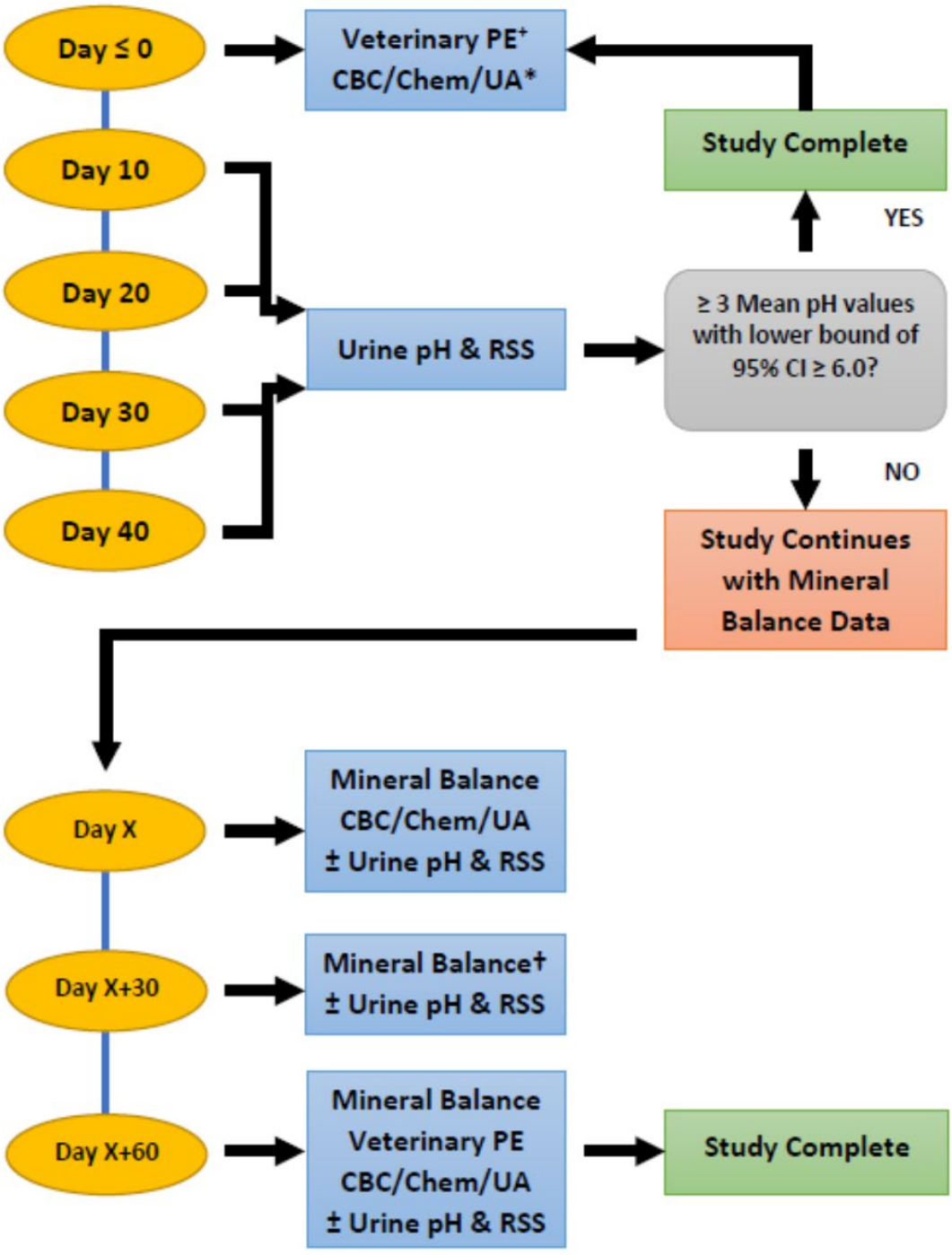
IX. Appendix 1 – Recommended Combined Safety and Utility Study Flowchart



+ PE = Physical Exam

*CBC/Chem/UA = Complete Blood Count, Serum Chemistry, and Urinalysis

† Not needed if baseline collection performed

X. Appendix 2 – Glossary of Terms and Acronyms

AAFCO – Association of American Feed Control Officials

Activity Product – A numerical description for the concentration of a solute remaining free in solution that is free to interact with other solutes and form crystals.

CaOx – Type of urolith composed of calcium and oxalate.

CI – Confidence Interval

CVM – Center for Veterinary Medicine

DAFI – Division of Animal Food Ingredients

FDA – Food and Drug Administration

Formation Product – The concentration at which solute will spontaneously precipitate out of solution to form crystals. Defines the limit between the metastable and labile supersaturation zones.

In-Vitro – Describes an experiment or test that takes place in a test tube, culture dish, or elsewhere outside a living organism.

In-Vivo – Describes an experiment or test that takes place inside a living organism.

Labeling – As defined in section 201(m) of the FD&C Act, "means all labels and other written, printed, or graphic matter (1) upon any article or any of its containers or wrappers, or (2) accompanying such article."

Labile Supersaturation – Describes the urinary zone in which crystals will spontaneously form and grow.

Metabolic acidosis – The condition in which too much acid accumulates in the body, causing serious illness.

Metastable Supersaturation – Describes the urinary zone in which crystals will not spontaneously form but may form in the presence of a nucleus (e.g., an existing urolith or other particulate matter). Existing crystals and uroliths will not dissolve and may grow.

RSS – Relative Supersaturation

Solute – A substance dissolved in solution.

Struvite – Type of urolith composed of magnesium, ammonia, and phosphate.

Thermodynamic Solubility Product – The concentration of a solute at which it completely dissolves into solution. Defines the limit between the undersaturation and metastable supersaturation zones.

Urolith – A bladder stone.

Urolithiasis – The disease condition of having bladder stones.

Undersaturation – Describes the urinary zone in which crystals will not spontaneously form, and if present, will dissolve.

UTH – Urinary tract health

UTH Cat Food – Adult maintenance cat food with UTH claims.