

Effects of new sampling protocols on procaine penicillin G withdrawal intervals for cattle

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Analytical precision and accuracy in residue chemistry are constantly improving toward the goal of a safe food supply. On July 6, 2012, the USDA FSIS announced a restructuring of the US NRP with respect to sampling of compounds in meat, poultry, and egg products and the scheduling of animal production classes.¹ The FSIS has also implemented several new MRMs for analyzing tissue samples from harvested animals for violative residues. These MRMs allow for several compounds to be tested simultaneously. As a result, compounds that have not been previously tested in certain animal production classes are now included in standard testing procedures.

These modernized and sensitive MRMs allow for the simultaneous screening of more chemical compounds than was previously possible and are predicted to provide reliable results. As a result, animal production classes that do not have specific tolerances established for residues of new animal drugs are under increased scrutiny. The absence of an established tolerance for residues of a particular compound in Title 21 of the Code of Federal Regulations part 556 (21 CFR 556) equates to a tolerance of zero (or no detection with an analytic assay) for residues for that compound in tissue or milk samples.² For example, dexamethasone is approved for use in both beef and dairy cattle but does not have a residue tolerance listed in 21 CFR 556. Therefore, any detection of dexamethasone found in tissue or milk samples is a violation. As another example, enrofloxacin is approved for use in beef cattle but not approved for use in lactating dairy cattle. The detection of any level of desethylene ciprofloxacin,

ABBREVIATIONS

ELDU	Extralabel drug use
FARAD	Food Animal Residue Avoidance and Depletion Program
FSIS	Food Safety Inspection Service
MRM	Multiresidue method
NRP	National Residue Program
PPG	Procaine penicillin G
WDI	Withdrawal interval
WDT	Withdrawal time

the marker residue for enrofloxacin, in milk or tissue from a dairy cow is a residue violation. Consequently, FARAD has made alterations to WDI recommendations for some products that are commonly used in an extralabel manner in food animal production medicine in an effort to minimize the occurrence of violative residues.

Additionally, the FSIS modified the scheduled sampling approach so that it will be analyzing fewer samples; however, the newer MRMs will allow more compounds to be assessed per sample. The sampling scheme for the NRP is now divided into 3 tiers. Tier 1 is similar to the old scheduled sampling program. In the past, the NRP scheduled sampling was performed in each production class. In the new program, scheduled sampling will rotate among production classes. Tier 2 resembles the traditional inspector-generated sampling program, a targeted testing program in which the field public health veterinarian performs in-plant tests on suspect carcasses. Tier 3 is targeted testing of an entire herd on the basis of suspicion of chemical exposure involving more than 1 animal. This part of the NRP will allow public health veterinarians to test entire groups of animals when there is suspected misuse of veterinary drugs or exposure to an environmental contaminant that involves an entire herd.

The MRM provides the FSIS with a more sensitive method to quantify compounds in animal products. This new testing method has a lower limit of detection for many compounds, compared with that of previously used testing methods. Recently, FARAD has had an increase in the number of inquiries regarding residue violations that have occurred in production systems in which the same treatment protocol had not previously caused violative residues. Many of these inquiries have involved the use of PPG in cattle. Potential reasons for the increased concern over PPG WDI information, compared with that for other compounds, are its widespread use,

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over-the-counter availability, and anecdotal evidence that it is often administered at a dose many fold higher and for a longer duration than those listed on the FDA-approved label. The purpose of this FARAD Digest is to discuss the issues that could potentially lead to violative PPG residues and changes in the WDI for PPG in cattle.

WDI versus WDT

An important distinction exists between the WDI and WDT. The WDI is a scientifically derived, recommended withholding time following ELDU in a food animal. The WDT is the withholding time established by the FDA following an approved (labeled) use of a drug in a food animal.

The most common causes of violative residues for any drug are failure to adhere to recommended WDTs, poor record keeping, improper identification of treated animals, or inadvertent administration of the wrong drug, formulation, or dose.³ Additionally, ELDU (a change in the dose, frequency, duration, or route of administration) can result in dramatic changes in a drug's elimination kinetics within the treated animal, which in turn may lead to violative residues if the WDT is not extended to accommodate those changes.

Many different PPG products with similar names and label designs are available over the counter; therefore, the FDA-approved product label should always be carefully inspected before treatment is initiated. Currently, 17 different registered products with PPG as the active ingredient filed under 4 separate new animal drug application numbers are available, with varying FDA-approved treatment durations and WDTs for meat (Table 1). The WDTs for meat for these products range from 4 to 14 days and can differ within each group of equivalent generic products that originate from the same pioneer product. For example, Norocillin and Penicillin G Procaine have equivalent generic products with meat WDTs that range from 10 to 14 days, depending on the product label referenced. Reasons for the differences in meat WDTs between and within products are not easily discerned but could include differing excipients, propensities for injection site reactions, and formulations among the products.

Effect of Dose on WDI

In the United States, the label dose for PPG is 6,600 U/kg (3,000 U/lb) of body weight, and any deviation from the label dose, duration, frequency, site, or route of administration is considered ELDU. When the dose

administered is increased, the time required for tissue concentrations to deplete below tolerance concentrations for residues also increases. Familiarity with the concept of elimination half-life makes this relationship intuitive. In the simplest of circumstances (ie, linear, first-order kinetics), the elimination half-life is the time required for 50% of a drug to be eliminated from an animal or depleted from the tissues. For example, if it takes 5 half-lives for 10 g of an administered substance to decay to below some hypothetical tolerance concentration, it would take 1 additional half-life (ie, 6 half-lives) for 20 g of the same administered substance to decay to below the same hypothetical tolerance concentration. Thus, doubling the amount of drug administered will effectively increase the time needed to eliminate the drug from the animal's body by 1 half-life.⁴

Alteration of the dose of a drug administered is often a deliberate decision made on the basis of the medical needs of the patient, but the dose can be inadvertently altered because of inappropriate handling of a product. Procaine penicillin G is a suspension, an undissolved drug in a liquid vehicle, which needs to be shaken vigorously to ensure that the entire amount of the active ingredient is evenly dispersed throughout the storage container and the volume administered contains the appropriate concentration of the active ingredient. Hypothetically, a bottle with a label that indicates that the PPG concentration of the suspension is 300,000 U/mL is opened and 20 mL of the suspension is drawn into a syringe without the bottle being agitated such that the concentration of PPG in the syringe is 50,000 U/mL. The suspension remaining in the bottle now contains 362,000 U/mL, a 21% increase from the label concentration. In another hypothetical example, a suspension of PPG is incompletely agitated and the calculated volume is withdrawn from the concentrated drug slurry near the stopper; however, the concentration in that volume is much higher than the label concentration, resulting in the administration of a higher-than-expected dose. Consequently, the WDT should be extended to account for the higher effective dose (concentration) administered. The WDT should also be extended when large volumes of a suspension, especially those with a higher-than-label drug concentration, are injected per injection site; failure to do so will almost certainly result in violative residues.

Effect of Dose Frequency and Duration of Treatment on WDI

Few pharmacokinetic studies for PPG in cattle have been published, and results for many that have

Table 1—A nonexhaustive list of PPG products commercially available for use in cattle with varying FDA-approved treatment durations and meat WDTs.

Trade name	NADA number	Route of administration	Dose (U/kg)	Treatment duration (d)	Meat WDT (d)	Milk WDT (h)
Norocillin	065-010*	IM	6,600	4	10-14	48
Penicillin G Procaine	065-493*	IM	6,600	4	10-14	48
Microcillin-AG	065-505*	IM	6,600	5-7	4	48
Pen-G-Max	065-110*	IM	6,600	4	10	48

*Within this NADA, numerous lines of generic PPG formulations are marketed under different trade names. NADA = New animal drug application.

been published are confounded because of the inclusion of benzathine penicillin G, sodium or potassium penicillin G, or dihydrostreptomycin in the study design.⁵ Therefore, data on which recommendations for WDTs following administration of PPG in an extralabel manner in cattle are based are limited. An additional complication in the determination of the WDI for PPG in cattle is the fact that the rate-limiting step in the metabolism of PPG is the hydrolysis of penicillin from the procaine moiety. As a result, PPG has lower maximum plasma concentration that is prolonged, compared with that of potassium penicillin.⁶ This phenomenon, often termed flip-flop pharmacokinetics, occurs when the absorption process at the administration site is slower than the rate of total body elimination. In other words, it takes longer for the drug to be absorbed from the injection site than it does for the drug to be eliminated from the body once it is absorbed.

Because β -lactam antimicrobials such as PPG are considered time-dependent drugs, there are therapeutic advantages of prolonged plasma drug concentrations; however, that prolonged plasma drug concentration becomes a disadvantage in the calculation of WDTs that are sufficient to avoid violative meat or milk residues, especially when an inappropriate dose has been administered. Additional confounders in the calculation of WDTs for PPG in cattle include the fact that the absorption of PPG from the administration site is the rate-limiting factor for its elimination from the body and cattle are frequently administered multiple doses of PPG during a course of treatment to resolve severe local or systemic infections. In many instances, PPG is administered once every 24 hours such that each subsequent dose is administered before the previous dose has been eliminated from the body, resulting in what is termed dose stacking or dose accumulation. The tissue and plasma drug concentrations achieved during dose accumulation are dependent on the elimination half-life of the drug and the dosing interval chosen by the clinician. For PPG, the duration of treatment recommended on the label varies from 4 to 7 days (Table 1).

Hypothetical pharmacokinetic simulations were generated by use of data (PPG volume of distribution, elimination, and absorption rate constants) from FARAD to illustrate the effects of dose accumulation and dosing interval on plasma PPG concentration. These simulations represent the expected fluctuation of plasma PPG concentrations over time in an average animal that was administered the respective described treatment regimens and were created to help explain basic concepts, not provide an estimate of the WDI for each hypothetical dosing protocol.

Dose accumulation should be considered when drugs are administered in an extralabel manner. For example, when PPG is administered in accordance with the label directions (6,600 U/kg, IM, q 24 h for 5 days), plasma drug levels achieve a steady state (PPG entering the system is equal to that exiting the system) by the administration of the second dose (Figure 1). When the dose is increased, the time required for a drug to reach a steady state in plasma is unchanged, but the mean concentration at the steady state in plasma increases as the dose is increased from the labeled dose to

20,000 U/kg (9,091 U/lb) or 45,000 U/kg (20,454 U/lb). Although these data are simulated and should not be used to predict WDTs, the time needed for the plasma concentration of PPG to deplete to 0 increased with the dose and was dependent on the PPG concentration achieved in each scenario. Furthermore, it is important to note that the plasma elimination half-life of a drug may not be indicative of the tissue elimination half-life of that drug.

The prescribed dosing interval also affects the estimated WDI because as the dosing interval becomes shorter, the steady-state plasma drug concentration increases (Figure 2). Consequently, it will take longer for the body to excrete the excess drug that accumulates and an extended WDI will be needed for the tissue drug concentration to decay below the allowable residue tolerance.

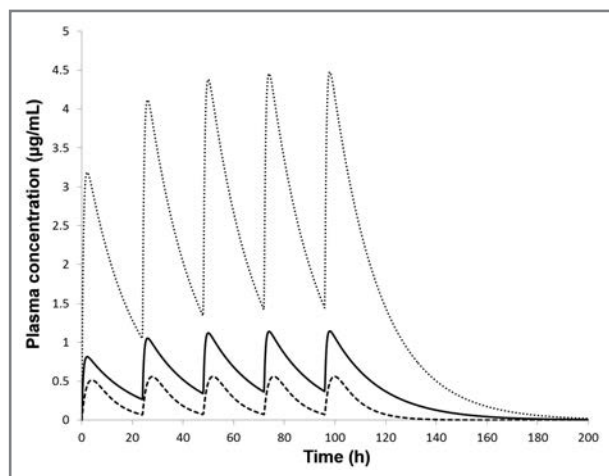


Figure 1—Effect of dose accumulation on plasma PPG concentration following IM administration of 6,600 U/kg (3,000 U/lb; dashed line), 20,000 U/kg (9,091 U/lb; black line), or 45,000 U/kg (20,454 U/lb; dotted line) of PPG to cattle. Drug concentration curves are hypothetical and were generated by use of data for PPG volume of distribution and elimination and absorption rate constants from FARAD. Notice that the steady-state plasma concentration and time required for plasma concentration to approach 0 increase as the dose increases.

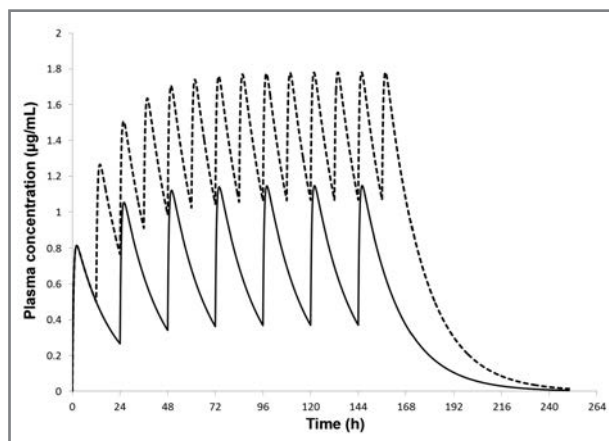


Figure 2—Effect of dosing interval on plasma PPG concentration following IM administration of the same dose of PPG to cattle every 12 (dashed line) or 24 (black line) hours. Notice the steady-state plasma concentration and time required for plasma concentration to approach 0 increase as the dosing interval is shortened. See Figure 1 for remainder of key.

Effect of Injection Site and Injection Volume on WDI

The rate of absorption of a drug following IM or SC injection is dependent on the concentration of the drug administered, volume of drug injected per site, vascularity of the injection site, drug diffusion into surrounding tissues, and permeability of blood and lymphatic vessels near the injection site.⁷ From a food safety perspective, a concern regarding delayed absorption of PPG at the site of injection is that tissue from that injection site could be consumed by a person with penicillin hypersensitivity. The greater the volume of drug injected at each site, the greater the risk for drug residues to persist at the injection site as well as systemically. Also, as injection volume increases, the likelihood of tissue penetration following IM or SC injection increases, and these sites can be difficult to identify on routine postmortem examination and may not be trimmed out at slaughter.⁸

In cattle, PPG is labeled for deep IM injection in the neck region. A WDT for SC administration of PPG has not been established. Subcutaneous administration of PPG can result in hematoma formation, extensive local inflammation, and scar tissue, which can result in delayed and incomplete absorption of PPG and a prolonged elimination half-life.⁸ Investigators of that study⁸ found that calves administered PPG SC had tissue residues longer than did calves administered the same dose of PPG IM.

Results of multiple studies^{5,9,10} that involved cattle indicate that injection site and route affect the disposition and elimination of PPG (Table 2). The peak plasma concentration was higher for cattle administered PPG IM in the gluteal ($2.63 \pm 0.27 \mu\text{g/mL}$) or neck ($4.24 \pm 1.08 \mu\text{g/mL}$) region than that for cattle administered the same dose of PPG SC in the neck region ($1.85 \pm 0.27 \mu\text{g/mL}$).⁵ Cattle administered PPG IM in the neck region had a higher peak plasma concentration than did cattle administered the same dose of PPG IM in the gluteal region.⁵ The elimination half-life for PPG in cattle was longer when it was administered SC in the neck region (18.08 hours) or IM in the gluteal region (15.96 hours) than when the same dose was administered IM in the neck region (8.85 hours).⁵ Although the reasons for differences in PPG absorption among various routes and sites of injection are not fully understood, other studies have yielded similar findings for PPG absorption in cattle⁹ and horses.¹¹

Conclusions

Extralabel drug use by US veterinarians is permitted under AMDUCA, which allows practitioners, especially those that treat food-producing species, to customize treatment protocols for the needs of individual patients provided that a specific set of conditions or requirements are met. Briefly, these requirements include the establishment of a valid veterinary-client-patient relationship, verification that no other medication approved for the treatment of the condition in question exists or that such a medication is ineffective, and proper labeling of and record keeping for drugs prescribed to be used in an extralabel manner.¹² Whenever ELDU occurs, an appropriately extended WDI needs to be extrapolated and observed to ensure food safety.

Drug residues might remain above the established tolerance for that drug in tissues or milk following a WDI for several reasons. Clinicians considering treatment of a patient with a drug in an extralabel manner or concerned about treatments resulting in violative residues should contact FARAD for assistance in determining appropriate WDIs. As analytic techniques used to screen tissue and milk samples become more sophisticated and sensitive, lower concentrations of drugs can be detected in those samples. Thus, the frequency of violative drug residues will likely increase until animal management practices evolve to keep pace with these increasingly sensitive analytic techniques. Although the new MRMs adopted by the NRP will undoubtedly make our food supply safer, the fact that they allow for the simultaneous screening of samples for several compounds will likely increase the incidence of violative residues.

In addition to the effects of drug dose, frequency, duration of treatment, and volume and site of injection on WDI, food animal practitioners should always read FDA-approved labels carefully and be cognizant of differences among brands and formulations of PPG products. The WDIs vary among the many commercially available PPG formulations; therefore, it is important to consult the label prior to initiation of treatment. Furthermore, simple precautions such as thorough mixing of the PPG suspension prior to administration so that the appropriate amount of the active drug is injected will help avoid violative residues and may improve treatment efficacy. Additional handling procedures for PPG should be followed to ensure that the appropriate concentration of the drug is administered to the animal and thus increase the likelihood that the labeled WDI (or a calculated WDI from FARAD) will be sufficient

Table 2—Mean \pm SEM peak plasma concentration and plasma elimination half-life of PPG in cattle following IM or SC injection of various doses of PPG in 3 studies.

Dose (U/kg)	Route of administration	Injection site region	Peak plasma concentration ($\mu\text{g/mL}$)	Plasma elimination half-life (h)*	Reference
24,000	IM	Gluteal	0.99 ± 0.04	15.74	Papich et al ⁵
66,000	IM	Gluteal	2.63 ± 0.27	15.96	Papich et al ⁵
66,000	IM	Neck	4.24 ± 1.08	8.85	Papich et al ⁵
66,000	SC	Neck	1.85 ± 0.27	18.08	Papich et al ⁵
30,000	IM	Neck	2.19 ± 0.33	5.99 ± 0.46	Luthman et al ⁹
3,000	IM	Neck	1.55 ± 0.33	4.84 ± 0.74	Bengtsson et al ¹⁰

*Plasma elimination half-life reported as the harmonic mean \pm SEM when available.

to prevent violative residues. Veterinarians seeking information for determination of a WDI following ELDU should always contact FARAD for assistance.

References

1. USDA, FDA. New analytic methods and sampling procedures for the United States National Residue Program for Meat, Poultry, and Egg Products. Available at: www.federalregister.gov/articles/2012/07/06/2012-16571/new-analytic-methods-and-sampling-procedures-for-the-united-states-national-residue-program-for-meat. Accessed Oct 7, 2013.
2. USDA, FDA. Tolerances for residues of new animal drugs in food. *21 CFR 556* 2012.
3. Paige JCCM, Pell FM. Federal surveillance of veterinary drugs and chemical residues (with recent data). *Vet Clin North Am Food Anim Pract* 1999;15:45–61.
4. Riviere JE, Webb AI, Craigmill AL. Primer on estimating withdrawal times after extralabel drug use. *J Am Vet Med Assoc* 1998;213:966–968.
5. Papich MG, Korsrud GO, Boison JO, et al. A study of the disposition of procaine penicillin G in feedlot steers following intramuscular and subcutaneous injection. *J Vet Pharmacol Ther* 1993;16:317–327.
6. KuKanich B, Gehring R, Webb AI, et al. Effect of formulation and route of administration on tissue residues and withdrawal times. *J Am Vet Med Assoc* 2005;227:1574–1577.
7. Yáñez JA, Remsberg CM, Sayre CL, et al. Flip-flop pharmacokinetics—delivering a reversal of disposition: challenges and opportunities during drug development. *Ther Deliv* 2011;2:643–672.
8. Korsrud GO, Boison JO, Papich MG, et al. Depletion of intramuscularly and subcutaneously injected procaine penicillin G from tissues and plasma of yearling beef steers. *Can J Vet Res* 1993;57:223–230.
9. Luthman J, Jacobsson SO. Distribution of penicillin G in serum and tissue cage fluid in cattle. *Acta Vet Scand* 1986;27:313–325.
10. Bengtsson B, Franklin A, Lutman J, et al. Concentrations of sulphadimidine, oxytetracycline and penicillin G in serum, synovial fluid and tissue cage fluid after parenteral administration to calves. *J Vet Pharmacol Ther* 1989;12:37–45.
11. Firth EC, Nouws JF, Driessens F, et al. Effect of the injection site on the pharmacokinetics of procaine penicillin G in horses. *Am J Vet Res* 1986;47:2380–2384.
12. Payne MA, Baynes RE, Sundlof SE, et al. Drugs prohibited from extralabel use in food animals. *J Am Vet Med Assoc* 1999;215:28–32.