

RMTC Scientific Advisory Committee  
Phenylbutazone Review  
Lawrence R Soma, VMD  
University of Pennsylvania  
School of Veterinary Medicine  
New Bolton Center Campus

**Initial opinions on the effects of phenylbutazone (PBZ) and historical prospective.**

Phenylbutazone was introduced into veterinary medical practice in the 1950s (Tobin et al., 1986) and still remains one of the more commonly used non-steroidal anti-inflammatory drugs (NSAID) in the horse. In the late 1970s, the use of PBZ came under scrutiny which resulted in the publication of the book “The Misuse of Drugs in Horse Racing: a Survey of Authoritative Information on Medication of Race Horses” by the Illinois Hooved Humane Society. This publication stirred controversy on the use of PBZ especially on race day. In 1977 the National Association of State Racing Commissioners Veterinary-Chemist Advisory Committee concluded that “phenylbutazone is a safe, effective nonsteroidal anti-inflammatory drug (NSAID) with antipyretic and analgesic activity. In recommended doses, there is no evidence that it changes a horse’s innate ability to race, except to make a horse perform more nearly normal with pain due to inflammation of part of the musculoskeletal system” (Gabel et al., 1977).

It was also the opinion of many veterinarians, at that time, that PBZ would allow a horse to compete with mild chronic arthritic changes, but did not possess sufficient analgesic activity to allow a horse with a serious injury to compete. The NSAIDs can be used to restore normal performance in a horse affected by some minor injury to joints, tendons, or muscle by providing relief of inflammatory pain. Some consider the use of NSAIDs justified in jumpers, and exceptions are made in racing by some jurisdictions. Many veterinarians agreed that the use of anti-inflammatory drugs could mask unsoundness in horses being examined in a pre-purchase examination for soundness (Sanford, 1983).

Results from performance studies suggested that PBZ had no clear effect on the performance of normal, healthy horses (Sanford, 1974). This impression was confirmed by exercise studies performed in the equine. Plasma concentration of prostaglandins are increased in man (Demers et al., 1981) and equine during exercise (Birks et al., 1991; Mitten et al., 1995). These exercise-induced increases in cyclooxygenase activity was inhibited by the administration of PBZ, but PBZ did not produce detectable changes in systemic hemodynamic or acid-base variables in either standing or running horses (Hinchcliff et al., 1994). In a exercising horses the effect of inhibition of cyclooxygenase activity on the hemodynamic response was examined. Administration of PBZ abolished the exertion-induced increases in plasma 6-ketoprostaglandin F<sub>1</sub> alpha and thromboxane B<sub>2</sub>. Phenylbutazone treatment resulted in significantly higher heart rates and right atrial pressures than control.

There was no effect of PBZ on carotid or pulmonary arterial pressures, oxygen consumption, carbon dioxide production, blood lactate concentrations, or plasma volume during exertion. These results suggest that cyclooxygenase products likely mediate or modulate some of the systemic hemodynamic responses to exertion in horses (Mitten et al., 1996), but there is no evidence that the administration of PBZ and/or the suppression of cyclooxygenase products alters performance.

Based on these opinions and observations, investigators conducted a number of studies to determine the plasma PBZ concentrations 24 hours following various dosing schedules, formulations, and dosages (Soma et al., 1983; Chay et al., 1984; Houston et al., 1985; Soma et al., 1985). Following completion of these studies the recommended dosing schedule was as follows: oral administration of 4.4 mg/kg (2 g) for 3 - 4 days followed by a single IV dose of 4.4mg/kg 24 h prior to racing. If these dosing recommendations are followed, plasma PBZ concentrations on race day should not exceed 5 µg/ml. However, these studies did not attempt to determine the pharmacological effect of PBZ at 5 µg/mL or the contribution by its pharmacologically-active metabolite, oxyphenbutazone (OPB).

The initial regulatory race day plasma concentrations suggested to all Commissions was 2 µg/mL. This conservative concentration was based on the opinion of many analysts that the higher concentrations of PBZ were associated with masking or interfering with drug testing in urine (Gabel et al., 1977). This was subsequently reviewed and it was concluded that this concentration could be increased to 5 µg/mL which is the regulatory limit now accepted by most jurisdictions in the United States (see footnote in reference section).

#### **Effects of Phenylbutazone on nociception (pain perception).**

Pain is difficult to assess in the horse as it is influenced by many factors. Equally difficult to assess is the alteration of pain by analgesic drugs. The latency to onset of movement of a limb in response to a noxious thermal stimulus has been used as a nociceptive end-point for analgesic studies in many species (Kamerling et al., 1985). Thermal-evoked skin-twitch reflex and thermal evoked hoof withdrawal reflex have been used to compare analgesic activity of procaine, mepivacaine and phenylbutazone. Compared to procaine and mepivacaine, phenylbutazone failed to alter pain thresholds over a 36 hours post-administration period (Kimmerling et al., 1985). This type of stimulation produces an acute pain response and can be used to objectively compare the duration of anesthetic agents regionally administered and other drugs used to reduce the perception of pain. In the horse as in other species, PBZ is not an effective analgesic drug when used to block thermal pain and specific nociceptive pain stimuli.

#### **Central nervous system effects and crossing the 'blood brain barrier'.**

Phenylbutazone has no known spinal or central nervous system effects that are involved in the suppression of pain. The effects are primarily thought to be peripheral in action with no central nervous system action or any noticeable sedation. To exert a central effect, NSAIDs have to cross the blood brain barrier

(BBB) and numerous studies have shown that they do cross the BBB (Bannwarth et al., 1989). The presence of NSAIDs in the brain may explain the antipyretic properties and some side effects of the NSAIDs.

Concentrations of oxyphenbutazone (OPB) in spinal fluid are similar to corresponding concentrations of unbound free OPB in plasma, which is approximately 5% of the total concentration of OPB in plasma (Gaucher et al., 1983a). Similarly, cerebral spinal fluid concentrations of ketoprofen reflect the unbound plasma ketoprofen concentrations and were in equilibrium with the plasma concentration from 2 to 13 hours after administration (Netter et al., 1985). Ibuprofen, flurbiprofen, and indomethacin rapidly cross the BBB. Plasma protein binding limits the driving force for uptake of NSAIDs into the brain by reducing the free fraction of NSAIDs in plasma (Parepally et al., 2006). The observation that long-term treatment of patients with ibuprofen results in a reduced risk and delayed onset of Alzheimer's disease suggests that it crosses the BBB, has a central effect, and reduces inflammation in the Alzheimer's diseased brain (Dokmeci and Dokmeci, 2004). Attempts to correlate the CFS concentrations of indomethacin with its regional inflammatory suppression and analgesic activity have not been successful (Bannwarth et al., 1989). The statement that the NSAID do not have central effects and all actions are peripheral in nature is certainly not substantiated by a number of studies.

### **Synovial fluid concentrations of NSAIDs**

There is no barrier to the diffusion of NSAIDs into or out of the joint cavity and the efficacy of NSAIDs in rheumatic diseases may depend on their concentrations within the joint. Piroxicam concentrations in plasma and synovial fluid after a single oral dose of 20 mg, in humans were 2.51 and 1.31 µg/ml, respectively. The elimination from synovial fluid was longer than from plasma (Bannwart et al., 2001). Concentration of OPB in synovial fluid were 57.1% of the corresponding plasma concentration. The OPB concentration was higher in patients with severe inflammation than in those with no or little inflammation (Gaucher et al., 1983a). Concentrations of diclofenac and the diffusion of aspirin and its metabolites through the synovial membrane have also been reported (Gaucher et al., 1983b; Bannwarth et al., 1985). In horses with no joint disease, equilibrium between synovial fluid and plasma concentrations was attained in 8 hours following the IV administration of naproxen; this was followed by a parallel decline in plasma and synovial fluid concentrations for up to 36 hours (Soma et al., 1995). In the horse, ketoprofen was no longer detectable in synovial fluid after 5 h whereas synovial fluid carprofen concentrations did not peak until 12 h and were still detectable at 48 h (Armstrong et al., 1999).

Many authors have suggested that the plasma concentrations of NSAIDs do not correlate well with assessments of therapeutic response. This may reflect weaknesses in experimental design, capability of determining the changes in pain levels and inflammation, and in clinical studies the variability in the diseases being studied. It may be that concentrations in plasma bear only a distant relationship to those in the inflamed tissues where NSAID presumably act (Famaey, 1985; Grennan et al., 1985; Simkin, 1988). Compared to the

CNS, NSAIDs readily penetrate into the joint and the concentration is not limited to the unbound fraction. The concentrations reported have been lower than in plasma. The peak concentrations and elimination from the joint are related to the pharmacokinetics and characteristics of the drug, dose and route of administration.

### **Post-operative pain and phenylbutazone**

Post-operative pain can be considered primarily nociceptive pain produced by trauma to tissues by direct intervention and disruption of these tissues. Inflammation is a part of the pain response due to surgical intervention and the use of NSAIDs has been promoted for this purpose post-operatively. Minimal differences were noted between PBZ and placebo administrations in a group of horses undergoing arthroscopic surgery (Raekallio et al., 1997).

In a similar post-operative study, flunixin, phenylbutazone or carprofen were administered intra-operatively just prior to the end of anesthesia. The time following surgery when additional analgesic drugs were required post-operatively were; 8.4 hours, 11.7 hours and 12.8 hours for the PBZ, carprofen, and flunixin, respectively. Horses receiving the opioid, butorphanol, during surgery needed significantly fewer analgesic agents post-operatively (Johnson et al., 1993).

In a double-blind, randomized, prospective study of human patients undergoing arthroscopic surgery, those receiving a prostaglandin inhibitor (naproxen sodium) had significantly less pain, less synovitis, less effusion and faster recovery (Ogilvie-Harris et al., 1985; Rasmussen et al., 1993) than those without. In equally as large a prospective study, no advantages were observed over control group of patients when compared to physical therapy and administration of the NSAID, diclofenac (Birch et al., 1993).

### **Effects of Phenylbutazone on naturally occurring osteoarthritis.**

In a randomized controlled clinical trial, efficacy and safety of paste formulations of firocoxib (Equioxx<sup>®</sup>) and PBZ in horses with naturally occurring osteoarthritis were compared. Horses were treated with firocoxib (0.1 mg/kg, orally every 24 h) or phenylbutazone (4.4 mg/kg, orally every 24 h) for 14 days. Clinical improvement was defined as a reduction of at least 1 lameness score grade or a combined reduction of at least 3 points in scores for pain during manipulation or palpation, joint swelling, joint circumference, and range of motion. Results suggested some greater improvement in some categories tested following firocoxib, but overall clinical efficacy of firocoxib and PBZ in horses were comparable (Doucet et al., 2008).

Horses with naturally occurring forelimb and hind limb lameness were exercised on a treadmill and the degree of lameness evaluated by the use of kinematic analysis while horses were trotting on the treadmill. Horses entered into the study were judged to have AAEP lameness scores of 1 to 3 based on a scale of upper severity score of 5. In a cross-over study, PBZ paste was administered at 2.2 mg/kg (orally every 12 h for 5 days), alone or in combination with flunixin meglumine administered at 1.1 mg/kg, (IV every 12 h for 5 days). Lameness evaluations were performed before and 12 hours after administration of two NSAID treatment

regimens. Administration of a combination of the two NSAIDs alleviated lameness more effectively than did oral administration of phenylbutazone alone. Based on the authors' conclusion when evaluating all 28 horses, there was a significant clinical improvement after the administration of both drugs in all horses except 5 with forelimb lameness. PBZ alone did not result in significant clinical improvement in all horses. This study suggested that the use of combinations of NSAIDs (stacking) did have a better effect at 12 hours and would have a greater effect at 24 hours and the "stacking of drugs" should be a real concern. (Keegan et al., 2008).

The analgesic effects of phenylbutazone in 9 horses with chronic forelimb lameness were studied. The horses were administered saline control or PBZ at doses of 4.4 and 8.8 mg/kg IV daily for 4 days. Peak vertical force (force plate) was measured and AAEP clinical lameness scores were assigned before initiation of each treatment. All horses were evaluated 6, 12, and 24 hours after the final dose. The vertical force was significantly increased at all post-treatment evaluation times after PBZ compared to control horses. Clinical lameness scores were significantly decreased 6 and 12 hours at both doses but were only significantly decreased 24 hours after treatment with the higher dose (Hu et al., 2005).

Force plate analysis and the AAEP lameness scoring system were used to evaluate the analgesic efficacies of flunixin (1.1 mg/kg), PBZ (4.4 mg/kg), or physiologic saline solution administered IV in 12 horses with navicular syndrome. Medications were administered once daily for 4 days with a 14-day washout period between treatments. At 6, 12, and 24 hours after the fourth treatment, AAEP lameness evaluations and force plate data indicated significant improvement in lameness from baseline values in horses treated with flunixin or phenylbutazone, compared with saline controls. The effect of flunixin or phenylbutazone was maintained for at least 24 hours but no differences from control were noted at 30 hours. Flunixin meglumine and PBZ appear to have similar analgesic effects in horses with navicular syndrome (Erkert et al., 2005).

The analgesic effects of the nonsteroidal anti-inflammatory drugs, ketoprofen at 2.2 and 3.63 mg/kg and PBZ at 4.4 mg/kg were compared in 7 horses with bilateral forelimb chronic laminitis. Hoof pain was quantified objectively by means of an electronic hoof tester and lameness was subjectively graded on a modified Obel scale. Ketoprofen administered at a dose of 3.63 mg/kg (equimolar to a dose of 4.4 mg/kg of phenylbutazone) reduced hoof pain and lameness score to a greater extent than the 2.2 mg/kg dose of ketoprofen or the 4.4 mg/kg dose of PBZ. These data suggested that ketoprofen, at the dose of 3.63 mg/kg, was more potent than phenylbutazone in alleviating chronic pain and lameness in horses. PBZ and both doses of ketoprofen were still effective at 24 h (Owens et al., 1995).

#### **Effects of phenylbutazone on an induced lameness model.**

The objective was to test the hypothesis that PBZ alleviates lameness in an adjustable heart bar shoe model of equine foot pain following a single IV dose of 4.4 mg/kg. Heart rate and lameness score (1-5) were assessed every 20 min for 2 h and then hourly through 9 h. A lameness grade of 4 was produced for the study

and no lameness was observed following the study when the set screw was removed. In the PBZ-treated horses the lameness score was lowest between 4-5 h post-treatment when the score was reduced from 4 to 1.5 compared to control horses. PBZ was efficacious in alleviating lameness in this model. The PBZ plasma concentrations were approximately 15 and 7 µg/ml at 4 and 8 hours, respectively. The study period did not include observations beyond 9 hours, but the lameness score had not recovered to baseline values at that time (Foreman et al., 2008).

### **Indirect assessment of duration of NSAID effects**

Vane in 1971 suggested that the mechanism of the action of aspirin-like compounds was a direct inhibition of prostaglandin synthetase, thereby preventing prostaglandin biosynthesis (Vane, 1971; Moncada et al., 1974; Moncada et al., 1975; Vane and Botting, 1987).

The NSAIDs are potent inhibitor of the conversion of arachidonic acid to arachidonic acid-derived mediators of inflammation. The NSAID's site of action is the cyclooxygenase pathway, therefore, blocking the synthesis and release of prostacyclin, thromboxane, PGE<sub>2</sub>, and PGF<sub>2</sub>. All NSAIDs have similar modes of action accounting for both their therapeutic and toxic effects (Lees and Higgins, 1985). There have been considerable advances in the development of pharmacodynamic/pharmacokinetic models in veterinary and human medicine and investigators are studying the effects of the drug and concurrent changes in plasma or tissue concentrations of inflammatory mediators. Modern PK/PD studies link the effect(s) of the drug to its corresponding concentration in plasma. Pharmacokinetic (PK) studies determine the effects of the horse on the drug whereas pharmacodynamic (PD) studies determine the effects of the drug on the horse. (Lees, 2004; Lees et al., 2004a; Lees et al., 2004b; Toutain and Lees, 2004). General PD/PK models have been developed for describing drug actions on various active metabolites and hormones (Krzyzanski and Jusko, 2001; Puchalski et al., 2001).

A number of studies have used the reduction in the metabolic products of inflammation as indirect models of the actions of PBZ and other NSAIDs at the molecular level on the degree and duration of action. Three types of models have been used:

1. Suppression of the release of inflammatory mediators in clotted blood samples. A number of PK/PD models have been developed using this technique (Lees et al., 1987a; Soma et al., 1992; Lees et al., 2004b; Lees et al., 2004c).
2. Suppression of the release of inflammatory mediators in tissue cage and sponge models in which a sterile carrageenan solution is injected into the cage or sterile carrageenin-soaked polyester sponge strips are inserted subcutaneously. Both are based on the creation of a mild, reproducible and reversible inflammatory reaction that causes minimal distress to the experimental animals. The acute inflammatory exudates have been shown to contain proteins, white blood cells, and eicosanoids all as a

result of the inflammatory reaction (Higgins and Lees, 1984; Lees and Higgins, 1984; Higgins et al., 1987a; Lees et al., 1987b).

3. More recently, models in humans have used flow through methods to harvest inflammatory exudates. In-vivo human bioassay can be used to study human volunteers and patients. Samples are collected from pertinent tissue sites such as the skin via aseptically inserted micro dialysis catheters. These experiments measured inflammatory substances in interstitial fluid collected from non-inflamed and experimentally inflamed skin (Angst et al., 2008a; Angst et al., 2008b).

### **Indirect Plasma Models**

This study involved the inhibitory actions of NSAIDs on TXB<sub>2</sub> following a single dose of flunixin (1.1 mg/kg) or PBZ (4.4 mg/kg) to determine the duration of action of these drugs. Flunixin and PBZ produced similar degrees of reversible inhibition of TXB<sub>2</sub> at 4 (98% and 88%), 8 (77% and 76%), and 24 (63 and 50%) hours, respectively. At 48 hours, inhibition of TXB<sub>2</sub> was no longer apparent (Lees et al., 1987a).

In a similar study the concurrent administration of flunixin meglumine (1.1 mg/kg, IV) and PBZ (2.2 mg/kg, IV) on the pharmacokinetics of each drug indicated that the pharmacokinetic variables calculated for each drug when administered alone and in combination were similar. Serum TXB<sub>2</sub> production was significantly suppressed for 8, 12, and 24 hours after administration of flunixin and phenylbutazone in combination. When these drugs were administered alone, the TXB<sub>2</sub> concentrations were not significantly different from control values at 24 h. Note in this study that the dose of PBZ was 2.2 mg/kg. (Semrad et al., 1993).

### **Indirect Tissue Models.**

Distribution of PBZ and its active metabolite OPB into tissue fluids was studied by measuring concentrations in plasma, tissue-cage fluid, peritoneal fluid and acute inflammatory exudate harvested from a polyester sponge model of inflammation in ponies. PBZ and OPB readily penetrated into inflammatory sites. After six hours, the concentration of PBZ was higher in exudate than in plasma and remained so at 24 hours. Mean concentrations of OPB in all fluids were lower than those of PBZ at all times, but OPB readily entered body fluids, especially into inflammatory exudate; suggesting that OPB may contribute to the anti-inflammatory effect. The estimated elimination half-life of PBZ from exudate was 24 h compared to 5 h from plasma. These authors suggested that the persistence of PBZ and OPB in tissues exudates extended the duration of PBZ effectiveness (Lees et al., 1986).

Other studies have shown that flunixin was also cleared more slowly from equine tissue inflammatory exudate than from plasma (Higgins et al., 1987b).

Acute inflammation was induced in 7 ponies by subcutaneous implantation of sterile carrageenin-soaked polyester sponge strips. Treatment comprised a single therapeutic dose of 4.4 mg/kg of PBZ administered intravenously at the time of sponge implantation. Exudates were harvested at 6, 12 and 24 hours and examined

for leukocyte and erythrocyte numbers. Leukocyte numbers were significantly increased from 6-hour values at 12 and 24 hours in both control and PBZ-treated animals but differences between control and treated ponies were not significant. The administration of PBZ produced highly significant reductions in exudate concentrations of PGE<sub>2</sub> and 6-keto-PGF<sub>1</sub>α 6 hours. Significant reductions in these eicosanoid concentrations were maintained in treated animals for 12 and 24 hours. Concentrations of TXB<sub>2</sub> were reduced in treated animals at 6 and 12 hours but these changes were not significant. Study results suggested an effect at 24 hours based on the two measured eicosanoids, PGE<sub>2</sub> and 6-keto-PGF<sub>1</sub>α (Higgins et al., 1984).

In a 12-day treatment schedule, 5 ponies were administered an oral paste formulation of PBZ and 5 matched ponies were given equivalent doses of a placebo paste. On day 12, a mild, non-immune inflammatory reaction was induced subcutaneously. Exudate was collected at 4, 8, 12, and 24 hours. There were no significant differences in exudate protein concentration and leukocyte numbers between the treatment groups, but exudate concentrations of 6-keto-PGF<sub>1</sub>α were reduced at 4, 8, and 12 hours and those of TXB<sub>2</sub> at 8, 12, and 24 h in the PBZ treatment group. The increases in surface skin temperature were significantly less in PBZ-treated than in placebo-treated ponies between 4 and 24 hours (Lees and Higgins, 1986).

Leucocyte and erythrocyte accumulation in exudate were not affected in any of the tissue cage and exudate studies. The hypothesis of prostaglandin synthetase inhibition is the most widely accepted mode of action for the NSAIDs, but other actions of NSAIDs including leukocyte migration, neutrophil aggregation, lysosomal enzyme release, and superoxide radical generation have been demonstrated. In-vitro studies have shown that flunixin, PBZ, OPB, and indomethacin suppress leukocyte migration. Flunixin was the most potent of the 4 drugs studied (Dawson et al., 1987).

## **Conclusions**

This review presented an historical prospective and examined the information presented in 4 different models used to determine the effects of NSAIDs, especially PBZ. They included naturally occurring lameness, reversible induced lameness, and indirect plasma and tissue models studying the suppression of the release of arachidonic-derived mediators of inflammation. The majority of studies suggest an effect of PBZ at 24 hours at a dose of 4.4 mg/kg. This reflects and substantiates the opinion of many clinical veterinarians, many of whom will not examine a horse for a pre-purchase lameness examination unless the horse is shown to be free of NSAIDs and glucocorticoids. This remains the opinion of many Commission Veterinarians in that they wish to examine a horse pre-race without the possibility of a NSAID or corticosteroid interfering with the examination and masking a possible musculo-skeletal condition. Based on scientific reports and the impression of clinical veterinarians, residual effects of PBZ remain at 24 hours. The impact of this sustained effect on the health and welfare of the horse remains problematic.

**Footnote:** Memo to Dr Robert Gowen, October 21, 1991 from Tom Tobin; Short overview on phenylbutazone and its regulations.

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