Pharmacokinetics of butorphanol tartrate in a long-acting poloxamer 407 gel formulation administered to Hispaniolan Amazon parrots (Amazona ventralis)

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OBJECTIVE
To determine pharmacokinetics of butorphanol tartrate incorporated into poloxamer 407 (P407) after SC administration to Hispaniolan Amazon parrots (Amazona ventralis).

ANIMALS
11 adult Hispaniolan Amazon parrots (6 males and 5 females; 11 to 27 years old).

PROCEDURES
A sterile formulation of butorphanol in P407 (But-P407) 25% (percentage determined as [weight of P407/weight of diluent] X 100) was created (8.3 mg/mL). Five preliminary experiments (2 birds/experiment) were performed to determine the ideal dose for this species. The formulation then was administered (12.5 mg/kg, SC) to 2 birds. Blood samples were collected before (time 0) and 0.08, 0.5, 1, 2, 4, 8, 12, and 24 hours after drug administration. Some birds were used more than once, with a washout period of ≥ 3 months between subsequent treatments. Butorphanol concentrations were quantitated by use of liquid chromatography–tandem mass spectrometry. Pharmacokinetic analysis was performed by use of noncompartmental analysis.

RESULTS
Maximal plasma butorphanol concentration was reached at 1.31 hours. Plasma concentrations of butorphanol remained > 100 ng/mL for > 3 hours (all birds) or > 4 hours (5/8 birds) but < 8 hours (all birds). Half-life of the terminal slope was 3.41 hours. No adverse effects were detected.

CONCLUSIONS AND CLINICAL RELEVANCE
Butorphanol was absorbed well from the But-P407 25% by Hispaniolan Amazon parrots, and absorption followed a pharmacokinetic profile compatible with a sustained-release drug. A dose of 12.5 mg/kg, SC, would theoretically provide analgesia for 4 to 8 hours. No adverse effects were detected. Studies on the pharmacodynamics of this formulation are necessary to confirm the degree and duration of analgesia. (Am J Vet Res 2017;78:688–694)

Opioids are the most efficacious analgesics for acute and postoperative pain in a wide variety of species.1 They provide analgesia through actions on specific cell membrane opiate receptors, which mimic the effects of endogenous opioids (endorphins, enkephalins, and dynorphins). Butorphanol, a κ-opioid receptor agonist and µ-opioid receptor antagonist, is the drug of choice in Psittacidae because it is affordable and readily available and provides antinociception in Hispaniolan Amazon parrots (Amazona ventralis).2,3 African grey parrots (Psittacus erithacus erithacus),4 and green-checked conures (Pyrrhura molinae).5 However, analgesic effects of butorphanol could not be confirmed in American kestrels (Falco sparverius)6 or cockatiels (Nymphicus hollandicus; unpublished data). Butorphanol also causes a reduction in the minimum alveolar concentration for isoflurane in cockatoos (Cacatua spp)7 and sevoflurane in guinea fowl (Numida meleagris)8 and Hispaniolan Amazon parrots.9 Fentanyl administration to white cockatoos (Cacatua alba)10 and hydromorphone11 or buprenorphinea administration to cockatiels did not appear to decrease nociception at the dosages tested and with the analgesimetry methods used.

The most substantial limitation to the use of butorphanol tartrate for the provision of analgesia for avian patients is a short duration of action. (eg, 1 to 3 hours in Hispaniolan Amazon parrots1,12). Moreover, butorphanol has poor oral bioavailability and hence is usually administered SC, IM, or IV as a continuous rate infusion.12 Repeated handling and injections

ABBREVIATIONS
But-P407 Butorphanol in poloxamer 407
Cmax Maximum measured plasma concentration
P407 Poloxamer 407
Tmax Time to maximum measured plasma concentration
are stressful to birds and may induce muscle pain and tissue necrosis, which may exacerbate postoperative pain and discomfort. Conversely, appropriate analgesia can speed recovery, improve prognosis, and decrease complication rates because it improves healing, comfort, appetite, and attitude. Therefore, there is a need for a sustained-released opioid analgesic for use in birds. Reports for such a product describe the use of long-acting liposome-encapsulated butorphanol in parrots (but this formulation is not commercially available and must be compounded before each use) and use of an osmotic pump to deliver butorphanol slowly over time to common peafowl (Pavo cristatus); however, these pumps need to be surgically implanted and may be too large for small birds.

Several techniques have been developed over the past 30 years to provide sustained-release formulations for drug delivery by use of hydrogels. Poloxamer 407 is a thermosensitive hydrogel (a compound that exhibits a property known as reverse gelation). Poloxamer 407 is liquid at room temperature, which allows it to be easily mixed with therapeutic agents and routinely handled for injection. Once injected into homeothermic animals, micellar packing occurs at body temperature, and the compound becomes a gel. This micellar packing is responsible for the high viscosity, partial rigidity, and slow dissolution of the gel, which makes it a highly effective sustained-release system for both hydrophilic and hydrophobic drugs. Sustained-release properties of P407 formulations have been characterized for lidocaine, hirudin, urease, vancomycin, insulin, and interleukin-2 in rats; deslorelin and gonadotropin-releasing hormone in cows; difloxacin in goats; recombinant human growth hormone, naproxen, and simvastatin in dogs; moxifloxacin, quinine, piroxicam, and atenolol in rabbits; and doxycycline in broiler chickens (Gallus gallus). Poloxamer 407 is commercially available and is considered innocuous when administered parenterally. However, adverse effects were reported after parenteral administration of extremely high doses of P407 to rats

The objective of the study reported here was to determine the pharmacokinetic profile of a sustained-release butorphanol formulation, But-P407 25%, after parenteral administration to Hispaniolan Amazon parrots. Our hypothesis was that plasma concentrations of butorphanol considered to be therapeutic would be reached after administration of the But-P407 hydrogel and that concentrations would remain above a therapeutic threshold for a longer duration than after injection of butorphanol tartrate solution.

Materials and Methods

Animals

Eleven adult Hispaniolan Amazon parrots (6 males and 5 females) were used in the study. Mean ± SD age was 14.6 ± 4.8 years (range, 11 to 27 years), and mean body weight was 305.5 ± 18 g. All birds were part of a research colony and were housed individually in wire cages in a room maintained at 23°C with a photoperiod of 12 hours of light and 12 hours of darkness. They were fed a commercially available pelleted diet ad libitum and had constant access to water. The parrots had not received any therapeutic agents for at least 6 months before the study. Birds were assessed as healthy on the basis of results of a physical examination. The study was approved by the University of Guelph Animal Care Committee and by the University of California-Davis Institutional Animal Care and Use Committee.

Preparation of But-P407

The But-P407 formulation was prepared by use of the cold method described in another study. The amount of P407 sufficient to yield a 25% gel (percentage determined as [weight of P407/weight of diluent] X 100) was added to a plastic syringe case that contained cold (5°C) butorphanol tartrate. Ice packs were placed around the syringe case, and contents of the case were mixed by use of a vortex device (touch mode at 3,200 rpm) for 5 minutes until a visually homogenous solution was obtained. The syringe case then was placed in a refrigerator at 4°C for 5 to 10 minutes until the solution was clear of bubbles. The solution subsequently was sterilized by filtration through a 0.22-μm microfilter and injected directly into a sterile rubber-topped glass tube. The solution was kept on ice and used within 2 hours after preparation. Theoretical concentration of butorphanol in the formulation was 8.3 mg/mL.

Preliminary experiments

Five preliminary experiments (2 birds/experiment) were conducted to determine the optimal dose of butorphanol and route of administration. In 1 study, plasma concentrations > 55 ng/mL (butorphanol and metabolites) were considered therapeutic for Hispaniolan Amazon parrots when birds received an electric stimulus, and concentrations > 80 ng/mL were considered therapeutic when birds received a thermal stimulus. For the purpose of the study re-
ported here, we conservatively set the therapeutic threshold at 100 ng/mL, although lower concentrations of butorphanol would most likely be sufficient to cause analgesia.

Birds were manually restrained and injected by use of a Luer-lock 1-mL syringe and 23-gauge needle. Injections were given via 2 routes of administration and consisted of P407 25% with various concentrations of butorphanol (4 mg/kg, IM; 8.3 mg/kg, IM; 8.3 mg/kg, SC; 12.5 mg/kg, SC; and 16.6 mg/kg, SC). Intramuscular injections were administered into the pectoral muscles, and SC injections were administered between the scapulae. Blood samples were collected 1 week before (time 0) and 0.08, 0.5, 1, 2, 4, 8, and 12 hours after drug administration. For each sample, 0.3 mL of blood was collected from the right jugular vein by use of a 26-gauge needle mounted on a 0.3-mL insulin syringe. Digital pressure was applied to the vein for approximately 3 minutes after sample collection to prevent hematoma formation. Blood samples were transferred to heparin-containing microtainer tubes; tubes were kept on ice for a maximum of 2.5 hours until centrifugation (3,500 × g for 8 minutes). Plasma was immediately transferred to cryotubes and stored at −20°C until butorphanol concentrations were measured.

Some birds were used more than once in the experiments. For those birds, a washout period of at least 3 months was provided between subsequent treatments. Birds were monitored during the experiments for adverse effects, including sedation, regurgitation, vomiting, and diarrhea.

Study design

Eight birds (5 females and 3 males) were injected SC with butorphanol (12.5 mg/kg) in P407 25%, as described for the preliminary experiments. This dose and route of administration were determined on the basis of results of the preliminary experiments. Blood samples were collected and processed as for the preliminary experiments, except an additional blood sample was collected at 24 hours after drug administration.

Measurement of plasma butorphanol concentrations

Butorphanol concentrations were quantitated in plasma with liquid chromatography–tandem mass spectrometry analysis of protein-precipitated samples by use of modifications of a previously published method and butorphanol-d6 as the internal standard. A partial validation was performed with Hispaniolan Amazon parrot plasma as a matrix. The response for butorphanol was linear (R2, 0.99). Intraday, interday, and analyst-to-analyst precision and accuracy of the assay were determined by assaying the butorphanol concentration in quality control samples (n = 6 replicates). Accuracy was reported as the percentage of the nominal concentration, and precision was reported as the percentage relative SD. Intraday accuracy was 104%, 115%, and 99% and intraday precision was 6%, 4%, and 4% for butorphanol concentrations of 0.3, 5.0, and 80 ng/mL, respectively. Interday accuracy was 107%, 111%, and 105% and interday precision was 6%, 5%, and 3% for butorphanol concentrations of 0.3, 5.0, and 80 ng/mL, respectively. Accuracy and precision for assays were considered acceptable on the basis of FDA guidelines for bioanalytic method validation.9 The assay was optimized to provide a limit of quantitation of 0.1 ng/mL and a limit of detection of 0.01 ng/mL.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed for plasma butorphanol concentrations by use of non-compartmental analysis and a commercially available software program. Values for Cmax and Tmax were obtained directly from the plasma concentration data. The area under the concentration-time curve from 0 to 24 hours was calculated by use of the log-linear trapezoidal method. Pharmacokinetic parameters for butorphanol were reported as individual and mean ± SD values. Differences between these values and values determined in another study by use of a standard butorphanol tartrate solution were assessed on the basis of the 95% confidence intervals of the means. Values were considered significant at P < 0.05.

Results

Results of the 5 preliminary experiments were graphed (Figure 1). For both routes of administration, at all time points at which plasma butorphanol concentrations were measurable, mean concentrations differed on the basis of the butorphanol dose administered. For example, at 4 hours after administration, the mean plasma concentration of the birds receiving 16.6 mg/kg SC was 1.5 times as great as that of the birds receiving 12.5 mg/kg SC, which in turn was 1.5 times as great as that of the birds receiving 8.3 mg/kg SC.

Mean plasma butorphanol concentrations were >100 ng/mL by 30 minutes after administration for all birds. Mean plasma concentrations decreased to <100 ng/mL between 1.5 and 2 hours after administration for the birds receiving 4 mg/kg IM, between 2 and 4 hours after administration for the birds receiving 8.3 mg/kg IM, and between 4 and 8 hours after administration for all other birds. At 4 hours after administration, the mean plasma butorphanol concentration for the birds receiving 8.3 mg/kg SC was twice that of the birds receiving the same dose IM.

The only adverse effects detected were for the 2 birds receiving 16.6 mg/kg SC. Both birds regurgitated or vomited approximately 1.31 hours after injection, which corresponded to Cmax.

On the basis of results of the preliminary experiments, butorphanol (12.5 mg/kg) in P407 25% was administered SC to 8 Hispaniolan Amazon parrots. No adverse effects were detected in any of the birds. Pharmacokinetic parameters were determined.
Plasma concentrations of butorphanol remained >100 ng/mL for >2 hours in all 8 birds, and an estimation determined by examination of the concentration-time curve indicated that they would have been above this threshold for all 8 birds at 3 hours after administration. Finally, plasma concentrations of butorphanol remained >100 ng/mL for >4 hours but <8 hours in 5 birds. In comparison, IM injection of butorphanol tartrate solution (5 mg/kg) to the same species resulted in a plasma concentration >100 ng/mL in 12 of 12 birds at 1.5 hours after administration but in none of the birds at 3 hours after administration.12

The Tmax was 1.31 hours, which was significantly greater than the value for administration of butorphanol tartrate solution (5 mg/kg, IM) in this species in another study (Tmax = 0.25 hours).12 Terminal half-life (3.41 hours) for the present study was also greater than the terminal half-life of butorphanol tartrate solution in this species in that other study (0.51 hours).12

### Discussion

Incorporation of butorphanol into a poloxamer base has shown promise for the development of a long-acting analgesic formulation for avian patients. Pharmacokinetic parameters associated with SC administration of butorphanol (12.5 mg/kg) in P407 25% to Hispaniolan Amazon parrots suggested that analgesia could be provided for at least 4 to 8 hours, which is a period longer than that provided by administration of butorphanol solution alone. To the authors' knowledge, the study reported here was the first in which the pharmacokinetics of butorphanol incorporated into a P407 base has been evaluated in any species.

Pharmacokinetics after administration of single doses of butorphanol has been evaluated in red-tailed hawks (Buteo jamaicensis; 0.5 mg/kg, IM and IV),40 great horned owls (Bubo virginianus; 0.5 mg/kg, IM and IV),40 broiler chickens (2 mg/kg, IV),41 American kestrels (6 mg/kg, IM),6 and Hispaniolan Amazon parrots (5 mg/kg, IV, IM, and PO).12 For a pharmacodynamic study3 performed by use of Hispaniolan Amazon parrots, investigators reported that plasma concentrations of butorphanol required for analgesia were >55 ng/mL (thermal threshold) and 80 ng/mL (electric threshold), as estimated from the concentration-time curve. By use of 100 ng/mL as the threshold plasma concentration above which analgesia is likely provided, analysis of the pharmacokinetic parameters suggested that butorphanol tartrate would have to be administered to Hispaniolan Amazon parrots at a dose of 5 mg/kg IV every 2 hours or 5 mg/kg IM every 3 hours to achieve analgesia.12 Oral administration was not recommended because oral bioavailabil-

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**Table 1**—Pharmacokinetic parameters after SC administration of But-P407 25% that contained butorphanol at a dose of 12.5 mg/kg to 8 Hispaniolan Amazon parrots (Amazona ventralis).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>452.3 ± 78.0</td>
<td>310.8–571.0</td>
<td>398.3–506.3</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.31 ± 0.26</td>
<td>1.00–1.50</td>
<td>1.13–1.49</td>
</tr>
<tr>
<td>λz (1/h)</td>
<td>0.203 ± 0.083</td>
<td>0.055–0.293</td>
<td>0.146–0.260</td>
</tr>
<tr>
<td>Half-life λz (h)</td>
<td>3.41 ± 3.44</td>
<td>2.37–12.50</td>
<td>1.03–5.79</td>
</tr>
<tr>
<td>AUClast (h·ng/mL)</td>
<td>1,318 ± 220</td>
<td>96–1,510</td>
<td>1,165–1,461</td>
</tr>
<tr>
<td>CL/F (mL/h/kg)</td>
<td>193.9 ± 34.5</td>
<td>154.6–258.0</td>
<td>170.0–217.8</td>
</tr>
</tbody>
</table>

All parameters were determined by use of noncompartmental analysis for plasma concentrations.

*The percentage of P407 was determined as (weight of P407/weight of diluent) X 100. | Harmonic mean.

AUClast = Area under the concentration-time curve from 0 to 24 hours. CL/F: Clearance divided by bioavailability. Half-life λz = Half-life of the terminal slope. λz = Terminal slope of the concentration-time curve.

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**Figure 1**—Mean ± SD plasma butorphanol concentrations over time for each of 5 preliminary experiments (2 Hispaniolan Amazon parrots [Amazona ventralis]/experiment). Birds received via 2 routes of administration a single dose of P407 25% (percentage determined as [weight of P407/weight of diluent] X 100) that contained butorphanol (4 mg/kg, IM [diamonds and dotted line]; 8.3 mg/kg, IM [black triangles and solid line]; 8.3 mg/kg, SC [squares and dashed line]; 12.5 mg/kg, SC [circles and dashed line]; and 16.6 mg/kg, SC [inverted white triangles and dashed line]).
It has been reported in a previous in vitro diffusion study\(^3\) that butorphanol diffuses more slowly across a biological membrane when in But-P407 25% than does a standard butorphanol tartrate solution. For the present study, multiple preliminary experiments were performed to determine an optimal dose and to compare the IM and SC routes of administration, with the goal of providing plasma concentrations >100 ng/mL for >4 hours. A higher Cmax was reached with IM administration, but the plasma concentration decreased quickly. In comparison, Cmax was lower with SC administration, but the concentration decreased at a slower rate, which resulted in higher plasma concentrations over time. Because the pharmacokinetics of butorphanol after SC administration to avian species have not been described previously and were not part of the study reported here, effects for the SC route versus effects of P407 on the absorption and elimination profile of butorphanol cannot be determined. In dogs, there are no significant differences in absorption, disposition, or clearance of butorphanol after IM or SC administration.\(^1\) If we extrapolate those results to avian patients and assume a similar pharmacokinetic profile between the 2 methods of administration of butorphanol tartrate, the slower rate of absorption, lower Cmax, and greater duration of therapeutic plasma concentrations with SC administration would be a result of slower drug release from the poloxamer base for the SC route of injection, compared with results for the IM route of injection. The authors are aware of no other published studies in which both SC and IM administration for a drug in a P407 base have been compared; therefore, it is difficult to determine whether this finding is typical for this hydrogel. It is possible that dissolution of the gel would be faster in muscle than in subcutaneous tissues. The temperature of muscle is probably close to core body temperature\(^2\) (approx 38° to 41°C) whereas the temperature in subcutaneous tissues (ie, level of the skin) is probably lower. The viscosity of But-P407 25% is significantly higher at 37°C than at 41° or 42°C and significantly higher at 38°C than at 42°C.\(^3\) Thus, one hypothesis would be that the higher viscosity of the gel when injected SC is responsible for the slower absorption of butorphanol. When injected IM, higher shear stress applied on the gel (attributable to muscular contractions) could also explain faster drug absorption, compared with absorption for the SC route. However, it is also possible that in contrast to dogs, there is a difference in the absorption of butorphanol in birds between the 2 routes of administration. The IM and SC routes were compared for administration of spectinomycin\(^3\) and gentamicin\(^4\) to broiler chickens, and differences between the 2 routes similar to those reported for Hispaniolan Amazon parrots in the present study were detected for spectinomycin but not for gentamicin. Pharmacokinetics of butorphanol tartrate administered SC and IM would need to be compared in an avian species to address this issue.

Doses of 8.3, 12.5, and 16.6 mg/kg administered SC to birds in the preliminary experiments achieved plasma concentrations >100 ng/mL for >4 hours; however, both birds that received the 16.6 mg/kg dose regurgitated or vomited at Cmax. Therefore, the lower dose of 12.5 mg/kg SC was used for further investigation. This dose resulted in plasma butorphanol concentrations >100 ng/mL for >3 hours in all 8 birds and >4 hours in 5 of 8 birds but <8 hours in all birds. Comparison with IV administration of But-P407 25% would be necessary to reach a definitive conclusion, but on the basis of published pharmacokinetic parameters for butorphanol tartrate, the
pharmacokinetic profile of the P407 formulation in the present study was highly suggestive of flip-flop pharmacokinetics (ie, absorption is rate limiting and is slower than the rate of elimination). This is typical of a sustained-release drug and has been reported for several poloxamer-based formulations.\textsuperscript{25,29,33} This pharmacokinetic pattern is in contrast to that of non-sustained-release drugs, such as butorphanol tartrate, whereby elimination is the rate-limiting factor. Consequently, the true elimination half-life could not be calculated for But-P407 because the terminal slope was not completely representative of the elimination in that absorption of the drug was probably not complete.

The duration of antinociceptive effect provided by a single injection was estimated to be between 4 and 8 hours; however, pharmacodynamic studies are required to provide evidence of analgesia. Indeed, it is possible that analgesia occurs at even lower plasma concentrations than the ones mentioned here. Moreover, analgesic thresholds differ with the nociceptive stimulus used, method of evaluation, and species evaluated.\textsuperscript{3} Finally, a high affinity of a given opioid for its CNS receptors may provide a longer duration of action than that predicted by use of plasma concentrations.\textsuperscript{45} Therefore, the duration of effective analgesia for the But-P407 25% formulation might actually be >8 hours in certain patients.

No adverse effects were detected in the 8 birds that received butorphanol (12.5 mg/kg) in a P407 25% base via the SC route of administration. However, during the preliminary experiment, the 2 birds that received butorphanol at a dose of 16.6 mg/kg by the SC route of administration regurgitated or vomited at approximately the time of Cmax, when the plasma concentration reached 505 and 665 ng/mL. Two of the 8 birds receiving 12.5 mg/kg SC had similar plasma concentrations (497 and 570 ng/mL) without such adverse effects. Thus, high doses of butorphanol should be used with caution in birds that have clinical signs of nausea, including regurgitation and vomiting, and such clinical signs should be considered a possible effect of the drug as well as a result of the underlying clinical problem.

Although the birds in the present study were not assessed for degree of sedation, all were subjectively quieter after the administration of butorphanol, but they were still alert and reactive. Mild sedative effects were evident in red-tailed hawks and great horned owls administered a 0.5-mg/kg dose of butorphanol (regardless of route of administration [IM or IV]), but no significant alterations in heart rate or respiratory rate were detected.\textsuperscript{40} In another study\textsuperscript{12} of Hispaniolan Amazon parrots, there was a short period of apnea in 1 parrot after IV administration of a bolus of butorphanol at a dose of 5 mg/kg, but no other clinically relevant adverse effects were detected.

For the present study, absorption of butorphanol from a P407 25% base administered to Hispaniolan Amazon parrots followed a pharmacokinetic profile compatible with that of a sustained-release drug. A dose of 12.5 mg/kg, SC, would theoretically provide analgesia for 4 to 8 hours, with no adverse effects. Studies of the pharmacodynamics of But-P407 25% administered SC at a dose of 12.5 mg of butorphanol/kg are necessary to confirm the degree and duration of analgesia and to provide recommendations for clinical use in parrots and other avian species.

**Acknowledgments**

This manuscript represents part of a dissertation submitted by Dr. Laniesse to the Department of Pathobiology at the University of Guelph as partial fulfillment of the requirements for a Doctor of Veterinary Science degree.

Supported by grants from the Ontario Veterinary College Pet Trust and the Campbell Center for the Study of Animal Welfare and by the Richard M. Schubot Parrot Wellness and Welfare Program. Dr. Laniesse was supported by a stipend provided by the Tasha Award.


The authors thank Dr. Tracy Drazenovich, Dr. Noemie Summa, and Emma Choi for assistance with the birds, and Dan McKemie and Sandy Yim for assistance with the liquid chromatography–tandem mass spectrometry analysis.

**Footnotes**


c. Sigma-Aldrich, Oakville, ON, Canada.

d. Torbugesic, Fort Dodge Animal Health, Fort Dodge, Iowa.

e. BenchMixer, Benchmark, Edison, NJ.

f. Millex GP filter unit 0.22-µm, Millipore Express PES membrane, Merck Millipore Ltd, Cork, Ireland.

g. Toronto Research Chemicals, Toronto, ON, Canada.

h. Phoenix WinNonlin, version 6.2, Pharsight, Cary, NC.

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AJVR • Vol 78 • No. 6 • June 2017 693


