Glomerular filtration rate estimation by use of a correction formula for slope-intercept plasma iohexol clearance in cats

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Objective—To develop a formula for correcting slope-intercept plasma iohexol clearance in cats and to compare clearance of total iohexol (TIOx), endo-iohexol (EnIox), and exo-iohexol (ExIox).

Animals—20 client-owned, healthy adult and geriatric cats.

Procedures—Plasma clearance of TIOx was determined via multisample and slope-intercept methods. A multisample method was used to determine clearance for EnIox and ExIox. A second-order correction polynomial factor was derived by performing regression analysis of the multisample data with the slope-intercept data and forcing the regression line through the origin. Clearance corrected by use of the derived formula was compared with clearance corrected by use of Brochner-Mortensen human and Heiene canine formulae. Statistical testing was applied, and Bland-Altman plots were created to assess the degree of agreement between TIOx, EnIox, and ExIox clearance.

Results—Mean ± SD iohexol clearance estimated via multisample and corrected slope-intercept methods was 2.16 ± 0.35 mL/min/kg and 2.14 ± 0.34 mL/min/kg, respectively. The derived feline correction formula was Cl_corrected = (1.036 × Cl_uncorrected) − (0.062 × Cl_uncorrected²), in which Cl represents clearance. Results obtained by use of the 2 methods were in excellent agreement. Clearance corrected by use of the Heiene formula had a linear relationship with clearance corrected by use of the feline formula; however, the relationship of the feline formula with the Brochner-Mortensen formula was nonlinear. Agreement between TIOx, EnIox, and ExIox clearance was excellent.

Conclusions and Clinical Relevance—The derived feline correction formula applied to slope-intercept plasma iohexol clearance accurately predicted multisample clearance in cats. Use of this technique offers an important advantage by reducing stress to cats associated with repeated blood sample collection and decreasing the costs of analysis. (Am J Vet Res 2011;72:1652–1659)
tions, urinary catheterization, or nuclear medicine facilities or because of lack of an available medical-grade injectable formulation. The nonionic radiographic contrast agent iohexol can be administered exogenously as a single bolus dose, and its plasma clearance can be used to estimate GFR. Iohexol is useful as a clearance marker because it is freely filtered by the kidneys, is not secreted into the renal tubules, has negligible protein binding, and is not metabolized within the body.3–5 Several studies6–17 have involved the use of this marker for estimation of GFR in cats.

Iohexol exhibits endoisomerism and exoisomerism.18 Analysis of iohexol concentrations in samples by use of high-performance liquid chromatography allows detection of the 2 types of isomers (EnIox and ExIox). The predominant isomer is quantitatively known to be ExIox. Previous studies12,13,16,17 have revealed a difference in the clearance of the 2 isomers in cats. Measurement of T1ox may therefore lead to inaccuracies in the estimation of clearance. To determine the doses of EnIox and ExIox that have been administered, the correct ratio of each isomer must be known. The stereoselective disposition of the isomers may change in different solutions as a result of their physicochemical properties, the solution temperature,18 or the storage duration.19 The ratio of plasma concentrations may therefore change after administration when compared with the ratio in the administered formulation.

Accurate methods of determining plasma clearance rely on the collection of blood samples at multiple points to create a plasma concentration-versus-time curve. Development of techniques that involve collection of a limited number of samples has become an important goal. It was first proposed by Brochner-Mortensen20 that the initial rate of redistribution of a marker is not only independent of GFR but is also relatively constant from subject to subject and that an estimate of GFR may, without any important loss of accuracy, be based on the second elimination exponential. Errors may nevertheless arise when calculating clearance from a limited number of samples; therefore, inclusion of both exponentials (redistribution and elimination exponentials) is recommended. Exclusion of the redistribution exponential leads to an overestimation of the GFR because the AUC is underestimated.21 The error becomes particularly important in patients with clinically normal or near-normal renal function, in which the initial rapid redistribution exponential contributes to a relatively larger proportion of the AUC, compared with patients with a low GFR.

Clearance calculated from a limited number of samples collected during the elimination phase is referred to as the slope-intercept technique. Formulas that correct for the distribution exponential when a slope-intercept method is used have been developed for human patients, the most commonly used being the Chantler22 and Brochner-Mortensen 20,23 formulae. The final purpose was to compare the plasma clearance of EnIox, ExIox, and T1ox.

Materials and Methods

Cats—Twenty client-owned healthy cats were enrolled in the study, which was conducted at the Referral Clinic De Kompaan, Ommen, The Netherlands. Informed consent was obtained from owners, and the study was conducted in accordance with national animal welfare regulations. Physical examinations and serum biochemical analyses were performed to confirm whether the cats were clinically normal. Cats with any concurrent medical disorder were excluded. Plasma iohexol clearance measurements performed in this study were components of breeding, rescue shelter rehoming programs, or geriatric screening programs.

Plasma clearance of iohexol—Food was withheld from cats for 12 hours prior to clearance measurement, although free access to water was allowed. In each cat, an IV catheter was placed in a cephalic vein. A bolus dose of iohexol (647 mg/kg [300 mg of iodine/kg]) was administered IV followed by 1 mL of saline (0.9% NaCl) solution. Completion of the iohexol was defined as injection time 0. At 5, 15, 30, 60, 120, 180, 240, and 360 minutes after iohexol injection, blood samples (1 mL) were collected via jugular venipuncture or from an IV catheter other than that used for iohexol administration and then transferred to serum separation tubes. The exact sample collection time was recorded for each cat and used in the iohexol clearance calculations. The serum was separated, harvested, and stored at −80°C until analysis.

For practical purposes, analysis was performed at 2 reference laboratories6 by use of a high-performance liquid chromatography method. At the first laboratory6 (Belgium), EnIox and ExIox concentrations were determined, whereas at the second laboratory6 (United Kingdom), only T1ox concentrations were measured. Duplicate analysis of samples at both laboratories was performed for 12 cats. Clearance was calculated by use of identical samples analyzed at both laboratories (T1ox_cal and T1ox_ex), and agreement of results between the 2 laboratories was assessed.

Pharmacokinetic analysis—The multisample method was defined as estimation of GFR from the full-clearance curve, whereas the slope-intercept method was defined as estimation of GFR from samples collected at 120, 180, and 240 minutes after iohexol injection. Pharmacokinetic analysis was performed by use of a commercially available pharmacokinetic computer program.6 Clearance was calculated as dose/AUC. Doses of the endoisomers and exoisomers were calculated for each cat from the total volume of iohexol administered and the mean plasma ratio. The plasma ratio was chosen rather than the ratio in the iohexol formulation because it is the plasma ratio of the isomers that is available for clearance by the kidneys. Dose of T1ox was determined from the total volume of iohexol administered.

The AUC was determined by use of noncompartmental and compartmental approaches. The noncompartmental approach involved the trapezoidal rule with
extrapolation to infinity.\textsuperscript{25} Clearance of EnIox and ExIox was determined by use of a noncompartmental model only. Compartmental modelling involved the application of a 1-compartment model to the slope-intercept method. The AUC was calculated as $A/\alpha$, in which $A$ is the iohexol concentration at the time 0 intercept and $\alpha$ is the elimination rate constant (slope) of the curve. For the multisample method, a 2-compartment model was applied and the AUC was calculated as $A/\alpha + B/\beta$, in which $A$ is the iohexol concentration at the time 0 intercept of the first (redistribution) exponential, $\alpha$ is the slope of this exponential, $B$ is the iohexol concentration at the time 0 intercept of the second (elimination) exponential, and $\beta$ is the slope of this exponential.\textsuperscript{26}

Assessment of the best fit of a model was performed by application of the Akaike information criterion.\textsuperscript{27} In addition, calculation of clearance manually (without the use of computer software) was undertaken for the multisample data by resolving the exponential decay curve by use of a 2-stage, curve-stripping procedure. The slow elimination exponential ($\beta$) was constructed from the log-transformed iohexol concentrations in blood samples collected after the 60-minute point and extrapolated back to obtain the time 0 intercept. The antilog of the time 0 intercept was denoted as $B$. The fast redistribution exponential ($\alpha$) was fitted by use of the equation $y = B \times e^{\beta t}$ applied to the early time points. These values were then subtracted from the raw values, and the logarithmically transformed data were plotted to obtain $\alpha$. The fast redistribution exponential was extrapolated back to obtain the time 0 intercept. The antilog of the time 0 intercept was denoted $A$. Clearance was calculated manually for the slope-intercept data by graphically plotting the logarithmically transformed values and extrapolating back to the time 0 intercept to derive $B$ and $\alpha$. The clearance values were standardized to body weight in kilograms.

**Statistical analysis**—Statistical analyses were performed by use of a statistical software package.\textsuperscript{28} The data were assessed for normality through graphical plots and use of the Kolmogorov-Smirnov test. Because the data met the assumptions of Gaussian distribution, parametric testing was used. Correlations between the clearance of EnIox, ExIox, Ti oxBelg, and Ti oxUK were explored by calculating Pearson used. Correlations between the clearance of EnIox, ExIox, and ExUK were assessed for normality through graphical plots and use of the Kolmogorov-Smirnov test. Because the data met the assumptions of Gaussian distribution, parametric testing was used. Agreement between the 2 clearance methods was assessed by use of the Bland-Altman plots as previously described. Percentage error was calculated as follows:

$$\frac{Cl_{\text{slope-intercept}} - Cl_{\text{multisample}}}{Cl_{\text{multisample}}} \times 100$$

in which $Cl$ represents clearance. In addition, multisample data were compared with slope-intercept clearance corrected by use of the Heiene canine\textsuperscript{24} and Brochner-Mortensen human (adult and child)\textsuperscript{20,23} formulae.

All data are reported as mean ± SD. Values of $P < 0.05$ were considered significant.

**Results**

Cats—The median age of the 20 enrolled cats was 4.6 years (range, 1.5 to 18.8 years), and the median weight of the cats was 4.18 kg (range, 2.10 to 6.25 kg). Breeds represented included the following: domestic shorthair (n = 14), domestic longhair (2), and Bengal (4). Physical examination and serum biochemical analysis performed before the study began indicated no abnormalities in any cat. The mean ± SD plasma creatinine concentration before iohexol administration was $1.30 ± 0.29$ mg/dL. No adverse clinical signs were noticed in any of the cats during the study period.

For the multisample data, the plasma concentration-versus-time curve could not be fitted to a 2-compartment model in 1 cat. In addition, the curve for that cat could not be resolved into 2 exponentials when attempted manually. There were therefore 20 clearance measurements available for noncompartmental analysis and 19 available for compartmental analysis. For the slope-intercept data, the goodness of fit of the data was not considered acceptable for 1 cat; therefore, only 19 sets of clearance measurements were used for analysis. Samples obtained at all 8 collection points (5, 15, 30, 60, 120, 180, 240, and 360 minutes).

The correlation between results of the 2 methods was evaluated by calculating the Pearson correlation coefficient. Agreement was assessed by use of Bland-Altman plots as previously described. Percentage error was calculated as follows:

$$\frac{Cl_{\text{slope-intercept}} - Cl_{\text{multisample}}}{Cl_{\text{multisample}}} \times 100$$

Figure 1—Bland-Altman plot of agreement between clearance of EnIox and ExIox in healthy cats (n = 20) after IV administration of a bolus dose of iohexol (647 mg/kg). The solid line represents the bias, and dashed lines represent upper and lower 95% limits of agreement. Agreement was considered excellent.
360 minutes after iohexol administration) were not available for 9 cats for the EnIox and ExIox evaluations as a result of insufficient sample volume. Data obtained at 6 to 8 time points were therefore used in the clearance calculations for EnIox and ExIox. In addition, the ExIox:EnIox ratio was not available at all time points for each cat; therefore, data for 17 cats at 7 time points were included in the repeated-measures ANOVA. The assumptions of the polynomial model were not met for data for 2 cats; therefore, the correction formula was derived by use of the clearance data for 17 cats. However, the formula was validated in 19 cats.

**Plasma iohexol clearance**—The mean ± SD clearance determined with the multisample method for EnIox, ExIox, Tlox_Belg, and Tlox_UK was 2.20 ± 0.34 mL/min/kg, 2.21 ± 0.34 mL/min/kg, and 2.17 ± 0.40 mL/min/kg, respectively. All clearances were in excellent agreement with each other (Figure 1; Table 1). Results of the Mauchly test for the repeated-measures ANOVA indicated that the assumption of sphericity had been violated (P < 0.001); therefore, the df were corrected by use of Greenhouse-Geisser estimates of sphericity (e = 0.36). There was no significant change in the ExIox:EnIox ratio over time (P = 0.347; Figure 2).

Mean clearance for Tlox estimated by use of the multisample method (concentrations determined at the Belgian and United Kingdom laboratories) by use of a noncompartmental model was 2.16 ± 0.35 mL/min/kg (9.23 ± 2.57 mL/min). Multisample clearance determinations were considered the reference method for agreement analysis. Clearance determined by use of the multisample method was determined from 8 plasma samples, and clearance determined with the slope-intercept method was determined from 3 samples. Slope-intercept clearance was corrected by use of the formula derived in the present study.

### Table 1—Limits of agreement and bias (95% CI) and results of correlation analysis for clearances of EnIox, ExIox, Tlox_Belg, and Tlox_UK in healthy cats after IV administration of a bolus dose of iohexol (647 mg/kg).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Lower</th>
<th>Upper</th>
<th>Bias</th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EnIox vs ExIox (n = 20)</td>
<td>-0.061 (-0.081 to -0.041)</td>
<td>0.037 (0.017-0.056)</td>
<td>-0.012 (-0.024 to -0.001)</td>
<td>0.998</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>EnIox vs Tlox (n = 20)</td>
<td>-0.053 (-0.07 to -0.036)</td>
<td>0.031 (0.016-0.048)</td>
<td>-0.011 (-0.021 to -0.001)</td>
<td>0.998</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tlox_Belg vs Tlox_UK (n = 12)</td>
<td>-0.309 (-0.504 to -0.114)</td>
<td>0.401 (0.206-0.596)</td>
<td>0.046 (-0.067 to 0.159)</td>
<td>0.887</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

CI = Confidence interval. Tlox_Belg = Total iohexol as assessed at the Belgian laboratory. Tlox_UK = Total iohexol as assessed at the United Kingdom laboratory. No outliers were identified in any comparison.

### Table 2—Mean ± SD clearance (mL/min/kg) and results of agreement analyses for iohexol clearance in cats determined by use of multisample and corrected slope-intercept methods and calculated by use of noncompartmental and compartmental models.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Clearance</th>
<th>Bias</th>
<th>Limits of agreement</th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multisample NCA (computer calculation; n = 20)</td>
<td>2.16 ± 0.35</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Multisample 2-compartment (computer calculation; n = 19)*</td>
<td>2.25 ± 0.36</td>
<td>-0.076</td>
<td>-0.158 (0.006)</td>
<td>0.955</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Multisample 2-compartment (manual calculation; n = 19)*</td>
<td>2.18 ± 0.38</td>
<td>-0.014</td>
<td>-0.244 (0.216)</td>
<td>0.955</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Slope-intercept 1-compartment corrected (computer calculation; n = 19)*</td>
<td>2.14 ± 0.34</td>
<td>0.019</td>
<td>-0.285 (0.323)</td>
<td>0.955</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Slope-intercept NCA corrected (computer calculation; n = 19)*</td>
<td>1.91 ± 0.24</td>
<td>0.177</td>
<td>-0.311 (0.807)</td>
<td>0.601</td>
<td>0.06</td>
</tr>
<tr>
<td>Slope-intercept 1-compartment corrected (manual calculation; n = 19)</td>
<td>2.17 ± 0.30</td>
<td>-0.012</td>
<td>-0.236 (0.212)</td>
<td>0.955</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Five percent of values obtained with this method were outliers. NA = Not applicable. NCA = Noncompartmental analysis. Clearance determined by use of the multisample method with noncompartmental analysis was considered the reference method for agreement analysis. Clearance determined by use of the multisample method was determined from 8 plasma samples, and clearance determined with the slope-intercept method was determined from 3 samples. Slope-intercept clearance was corrected by use of the formula derived in the present study.
As determined from a 2-compartmental model by use of a computer and by resolving the 2 exponentials manually was in excellent agreement. Results for multisample clearance and corrected slope-intercept clearance determined by application of noncompartmental and compartmental models (calculated by computer program and manually) were summarized (Table 2). Mean clearance estimated by use of the uncorrected slope-intercept method was $2.44 \pm 0.47 \text{mL/min/kg}$. Mean percentage error between multisample clearance and uncorrected slope-intercept clearance was $12.48 \pm 9.32\%$ (range, 5.79% to 29.27%; Figure 3).

A second-order polynomial regression line was found to provide the best fit to the data. The derived correction formula was as follows:

$$\text{Clearance}_{\text{corrected}} = (1.036 \times \text{Clearance}_{\text{uncorrected}}) - (0.062 \times \text{Clearance}_{\text{uncorrected}}^2)$$

Mean corrected clearance was $2.14 \pm 0.34 \text{mL/min/kg}$. There was excellent agreement between multisample and corrected clearance (Figure 4). Mean clearance estimated via the slope-intercept method and corrected by use of the formula derived in the present study and also the Brochner-Mortensen human and Heiene canine formulae was summarized (Table 3). Mean corrected clearance via the feline-derived correction formula and the Heiene canine formula demonstrated a linear relationship with multisample clearance (Figure 5). However, the relationship between multisample clearance and slope-intercept clearance corrected by use of the Brochner-Mortensen formula was nonlinear. The feline-derived formula had the lowest mean percentage error relative to the multisample data ($0.59 \pm 7.05\%$). The Brochner-Mortensen formula had the highest mean percentage error ($11.13\%$ adult formula and $13.14\%$ child formula). These values were similar to those calculated for uncorrected clearance ($12.48\%$).

**Discussion**

In the present study, GFR in healthy adult and geriatric cats estimated by use of slope-intercept plasma iohexol clearance corrected via the formula derived in the present study was in excellent agreement with GFR estimated with the multisample clearance method. This correction formula was developed and validated in the same sample of cats; however, validation should have ideally been undertaken in a different group of cats, and this remains an area for future studies to examine.

Agreement between corrected slope-intercept clearance and multisample clearance methods was excellent (Figure 4).

<table>
<thead>
<tr>
<th>Method</th>
<th>Formula</th>
<th>Clearance (mL/min/kg)</th>
<th>Percentage error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multisample NCA</td>
<td>—</td>
<td>2.16 $\pm$ 0.35</td>
<td>—</td>
</tr>
<tr>
<td>Slope-intercept (uncorrected)</td>
<td>$(1.036 \times \text{Cl}) - (0.062 \times \text{Cl}^2)$</td>
<td>2.44 $\pm$ 0.47</td>
<td>12.48 $\pm$ 9.32</td>
</tr>
<tr>
<td>Present study</td>
<td>$(1.036 \times \text{Cl}) - (0.062 \times \text{Cl}^2)$</td>
<td>2.14 $\pm$ 0.34</td>
<td>0.59 $\pm$ 0.95</td>
</tr>
<tr>
<td>Heiene and Moe$^{24}$</td>
<td>$-0.08 + (1.06 \times \text{Cl}) - (0.07012 \times \text{Cl}^2)$</td>
<td>2.12 $\pm$ 0.34</td>
<td>1.56 $\pm$ 6.95</td>
</tr>
<tr>
<td>Brochner-Mortensen (adult)$^{23}$</td>
<td>$(0.991 \times \text{Cl}) - (0.00122 \times \text{Cl}^2)$</td>
<td>2.41 $\pm$ 0.46</td>
<td>11.13 $\pm$ 9.17</td>
</tr>
<tr>
<td>Brochner-Mortensen (child)$^{23}$</td>
<td>$(1.01 \times \text{Cl}) - (0.0017 \times \text{Cl}^2)$</td>
<td>2.45 $\pm$ 0.47</td>
<td>13.14 $\pm$ 9.33</td>
</tr>
</tbody>
</table>

— = Not available. Cl = Clearance. NCA = Noncompartmental analysis. Clearance was corrected by use of the correction formula derived in the present study, the correction formula for dogs$^{25}$ and the Brochner-Mortensen correction formulae for adults$^{20}$ and children.$^{23}$
Noncompartmental analysis was used as the reference method because unlike compartmental analysis, the noncompartmental method is free of assumptions regarding redistribution and exchange of the filtration marker.29 The advantage of compartmental analysis, however, is that it allows an accurate pharmacokinetic model to be developed. Clearance estimated by use of different modelling methods may yield different results. Nevertheless, we did not find the difference to be clinically important and there was good agreement between methods. Errors can occur in noncompartmental modelling when a limited number of samples are collected,29 and this was clearly demonstrated in the clearance results determined by use of a noncompartmental model applied to the slope-intercept technique (Table 2). Therefore, a noncompartmental model should only be applied to data obtained from a sufficient number of collected samples.

The commercial pharmacokinetic program used in the present study may not be available to all clinicians and researchers. For this reason, clearance was additionally determined manually. Clearance calculated manually by use of 2-compartment and corrected 1-compartment models was in excellent agreement. This demonstrates that clearance can be accurately estimated by use of the corrected slope-intercept technique without the need for expensive pharmacokinetic software.

A 1-compartment simplification overestimates true clearance because the AUC is underestimated. The error introduced by ignoring the initial redistribution exponential in healthy humans is approximately 15%.30 In a study of plasma iohexol clearance in dogs, dogs with clinically normal renal function were found to have a mean overestimation in uncorrected clearance of 20%. The Chantler correction method used in human patients is simply a single multiplying factor. That technique does not take into account the variation in percentage error that can exist at different filtration functions; therefore, polynomial correction factors are generally recognized as being more accurate. The mean overestimation in the present study was 12.48% and ranged from 5.79% to 29.27%, with the error increasing with increasing filtration function (Figure 3).

Forcing the regression line through the origin in our study ensured a constant was not generated in the development of the polynomial equation; therefore, the formula could also be appropriate in cats with very low renal clearance. The clinical indication for GFR estimation is for patients with polyuria or polydipsia or plasma creatinine concentrations at the upper limit of the reference interval and for assessing renal function prior to the use of renally excreted drugs. Such patients may have clinically normal or near normal renal function; therefore, application of the correction formula in this situation would be more important because there would be a larger AUC, which would be underestimated.

The Brochner-Mortensen correction formula was validated in humans by use of chromium isotope (51Cr)--labelled EDTA and has been shown to be valid for iohexol.4 Moreover, good agreement between the plasma clearance of iohexol with the Brochner-Mortensen correction applied and urinary clearance of inulin exists in human patients.31 In a canine study,32

[Graphs showing relationship between clearance and multisample method corrected by use of the derived feline formula, the Heiene canine formula, and the Brochner-Mortensen adult human formula.]

Figure 5—Relationship between clearance for the cats in Figure 4 estimated by use of the multisample method and the slope-intercept method corrected by use of the derived feline formula (A), the Heiene canine formula (B), and the Brochner-Mortensen adult human formula (C). The bold line represents the regression line, and the dashed line is the line of equality.
the Brochner-Mortensen correction formula was accurate for large but not small-breed dogs. The deviation in small-breed dogs suggested the need for the development of a cat-specific correction formula.

The Brochner-Mortensen formula has been used to correct the plasma clearance of iohexol in cats with intact and partially nephrectomized kidneys, and a high degree of agreement with urinary clearance of creatinine has been demonstrated. However, an earlier study revealed only moderate agreement between the 2 methods of estimating GFR. The relationship between plasma iohexol clearance determined by use of the multisample method and slope-intercept method corrected by use of the Brochner-Mortensen adult formula appeared to be nonlinear in the present study, with errors increasing with increasing clearances (Figure 5). This may explain the conflicting results from previous studies, in which the formula was used in cats. The nonlinearity of the relationship was not improved by applying the Brochner-Mortensen correction formula derived for children. Thus, clearance corrected by use of the Brochner-Mortensen formula appears to offer no greater accuracy than uncorrected clearance in cats with healthy renal function. Although the canine correction formula generated results similar to those of the cat-specific correction formula, the mean percentage error was smaller when the cat-specific correction formula was applied. This may have been because the cat formula was developed and validated in the same sample of cats, or it may be that clearance obtained by use of the slope-intercept method requires correction with a species-specific formula to improve accuracy. Additional studies are required to test whether species-specific correction formulae are required.

The mean clearance of Enlox and Exlox was in excellent agreement in the present study. In addition, statistical testing to explore the change in the Exlox:Enlox ratio over time revealed no significant change, suggesting the 2 stereoisomers were cleared at similar rates and that following iohexol administration, no important conversion of one isomer to the other takes place. Taken together, these results provide evidence that the clearance of Enlox and Exlox is identical. A previous study, in which the pharmacokinetics and biotransformation of iohexol in dogs and rats were evaluated, also found that the proportion of isomers remained unchanged.

The present study also revealed that the clearance of the 2 isomers did not differ from the clearance of Tlox. These results agree with those of human and canine studies but differ from results of previous feline studies, in which clearance of the stereoisomers was found to differ. An early study conducted to investigate the isomerism of iohexol found the isomers to be interconvertible and that rotational conversion may be temperature dependent. Investigators in that study concluded that the isomers should be considered a single pharmacological entity. The results of the present study also support this conclusion.

Ratios of Exlox to Enlox have varied in previous studies, with reported ratios of 84:16 (5.25), 78:22 (3.55), 17 and 85:15 (5.68). Furthermore, differences in the clearance of the 2 isomers have been reported. However, it is unclear from these studies how the ratio needed for determination of the dose of administered isomer was derived. In the present study, the mean Exlox:Enlox ratio in plasma was 4.71; however, there was large variation among cats (3.8:1 to 5.6:1) and among batches of product (4.6:1 to 9:1). For these reasons, we determined the administered dose of each isomer for each cat on the basis of its specific plasma ratio. This resulted in identical calculation of Exlox, Enlox, and Tlox clearance, which is biologically more plausible than the glomerular filtration barrier reacting differently depending on the isomer. It is possible the Exlox:Enlox ratio differed in the previous studies because of the combined administration of iohexol and exogenous creatinine. However, that appears unlikely given that creatinine is produced endogenously and interference of creatinine with iohexol measurements in azotemic patients is not known to occur.

Healthy adult and geriatric cats were used in the present study, and exclusion criteria included hyperthyroidism or any other identified disease. The population that this sample was intended to represent is similar to that of previous studies; however, in 2 previous studies, hyperthyroid cats were included. Results from previous studies are inconsistent, with Exlox clearance reportedly higher than Enlox clearance in hyperthyroid cats in 1 study, yet no identified in a different study. Furthermore, there are discrepancies in the clearance of iohexol isomers in healthy adult and geriatric cats, with 2 studies finding Enlox clearance to be higher than that of Exlox and another study finding the opposite. Differences in the relative clearance of Enlox and Exlox between studies suggest inaccuracies in the calculation of clearance. Because GFR is calculated from the dose of the biomarker (in this case, the isomer of iohexol) divided by the area under the plasma clearance curve, inaccurate clearance measurement would result from inaccurate dosage calculation. We propose this is likely to have occurred if a standard Exlox:Enlox ratio was assumed or if the mean ratio determined in a group of cats was used rather than the ratio measured for each cat in the calculation of dose for each cat.

One limitation of the present study is that the repeated-measures ANOVA used to determine the change in the Exlox:Enlox ratio over time did not include the 5-minute point. This was because an Exlox:Enlox ratio was not available at 5 minutes for all of the cats. It could be argued that this may be the most important time point, as it is the time point closest to the point of Enlox and Exlox (iohexol) administration. However, when the analysis was repeated by use of data for 11 cats in which ratios were available at all time points, the results were similar and the change over time was not significant (data not shown; P = 0.604). The same conclusion can therefore be drawn that the ratio of the isomers does not appear to change over time in cats, indicating that both isomers are cleared by the kidneys at the same rate.

Several studies have involved calculation of clearance from the Exlox and Enlox peak only in cats and dogs. The present study revealed that clearance of Exlox, Enlox, and Tlox calculated accurately.

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