Impact of Omission or Replacement of Data below the Limit of Quantification on Parameter Estimates in a Two-Compartment Model

Vincent Duval[1,3] and Mats O. Karlsson[2]

Received May 2, 2002; accepted August 21, 2002

Purpose. To evaluate the influence of omission and replacement approaches for data below the limit of quantification (LOQ) on the estimation of pharmacokinetic parameters for two-compartment models when using nonlinear mixed-effect models.

Method. Nine data sets were simulated according to a two-compartment intravenous bolus model with interindividual and residual variabilities, and a sparse sampling strategy was adopted. The data sets differed with respect to area-under-the-curve (AUC) ratio (0.1, 0.2, 0.3) and half-life ratio (0.03, 0.1, 0.3) between the distribution and elimination phases. For each of the nine data sets, six reduced data sets were created by omitting 5%, 10%, 20%, 30%, 40%, or 50% of the lowest concentration values. For each of the reduced data sets only one simple correction procedure to handle observations below LOQ was applied. All the values below the LOQ were deleted, and the first one was replaced by half of the LOQ value. Population parameters were estimated for each of the 117 resulting data sets (one initial, six reduced, and six "corrected" data sets for each of the nine cases). This approach was also applied on a real data set of patients administered multiple IV bolus doses.

Results. For many of the data sets, particularly when a large fraction of the data was omitted, one or several population parameters were biased. When there was bias, clearance (CL) usually was underestimated, whereas peripheral volume was overestimated. The parameters related to the distribution phase (central volume and intercompartmental clearance) were less affected, and changes were not systematic. The correction procedure markedly decreased overall bias on the fixed effect of the parameters. Results for the real data were similar.

Conclusion. Omission of data below the LOQ value may induce a not negligible bias on fixed-effect parameter estimates. The influence of the omission of values below LOQ was related to the underlying shape of the concentration–time profile and fraction of omitted observations. The use of a simple replacement rule seems to reduce this bias in estimates but needs further investigation.

KEY WORDS: NONMEM; pharmacokinetics; limit of quantification; parameter estimates; bias.

INTRODUCTION

Drug concentration measurements are based on analytic methods that display imprecision and to some extent are dependent on the underlying concentration. For most analytic methods, limits of quantification (LOQ) are defined. The lower LOQ is the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision (1). According to International Conference on Harmonisation (ICH) guidelines (2), it is defined as 10 σ/s, where σ is the standard deviation (SD) of the response and s is the slope of the calibration curve. According to the Eurachem guide, "LOQ is an indicative value and should not normally be used in decision making" (3). However, in practice, measurements below the LOQ are often not available for pharmacokinetic analysis.

One pharmacokinetic analysis approach is nonlinear mixed-effects modeling, also called "the population approach" (4). Mixed-effect models can appropriately handle varying amounts of data from different individuals when the amount of data is not linked to the individual's parameter values. When this is not true, and individual-specific characteristics influence the amount of data available from an individual, so-called *informative missingness* (5), the estimation procedure is not guaranteed to produce unbiased estimates. In that situation, a specific case of left-censored data (6), the information carried by the last point is biased because of the lack of information for subjects with measurements below LOQ. Omission of data below LOQ will generally result in informative missingness, as subjects for which concentrations are lower or decline faster will have a higher probability of having data below LOQ. Normally, in population analyses with programs such as NONMEM (7), it is assumed that the loss of information as a result of the omission of values below LOQ will not bias parameter estimates. At least, most population analysis publications that contain omission of data below LOQ do not mention this potential problem. Two recently published simulation studies (8,9) evaluate the influence of the LOQ in the field of population pharmacokinetic analysis. In a one-compartment model, both showed that omission of the data below LOQ does create a bias in parameter estimates and that correction procedures help in correcting the bias. However, the simulation study investigating the more common situation, more than one PK measurement per subject (9), demonstrated that the bias in fixed effects parameter estimates was minor with omission of data below LOQ. It is possible that this result is a consequence of the simple structure of the one-compartment model.

The primary aim of the present work was to assess how the structure of a two-compartment model could influence any potential bias in parameter estimates secondary to omission of data below LOQ. A secondary aim was to evaluate if a simple correction procedure helps in handling data below the LOQ in the particular case of the two-compartment model.

MATERIAL AND METHODS

To investigate the influence of the omission of the values below LOQ, this study was based on simulated data sets; then, as an example, the same approach was used on a real data set.

The Simulated Data Sets

All the data sets used were based on the same structural model, a two-compartmental model with interindividual variability (IIV) on central volume (V_c), clearance (CL), intercompartmental clearance (Q), and peripheral volume (V_p) and a proportional residual error model. Both interindividual and residual variabilities were parameterized as exponential

[1] Department of Clinical Pharmacokinetics, IRIS, F-92415 Courbevoie Cedex, France.

[2] Division of Pharmacokinetics and Drug Therapy, Department of Pharmaceutical Biosciences, Faculty of Pharmacy, Uppsala University, Sweden.

[3] To whom correspondence should be addressed. (e-mail: vincent.duval@fr.netgrs.com)

0724-8741/02/1200-1835/0 © 2002 Plenum Publishing Corporation

distributions with a coefficient of variation of 10% for the residual error, 25% IIV on CL and V_c, and 30% on Q and V_p.

The differentiation between the data sets was based on two ratios: (a) the ratio of the area under the curve (AUC) of the distribution phase to the total AUC and (b) the ratio of the half-life of the distribution phase to the half-life of the elimination phase. For both the AUC and half-life ratios, three values were defined: 0.1, 0.2, and 0.3 and 0.03, 0.1, and 0.3, respectively. The terminal half-life in all cases was set to 24 h for the typical individual and CL to 10, 20, or 30 L/h. The pharmacokinetic parameters of the drug were then calculated given the different ratio value combinations and the clearance value (Table I).

For all data sets, the same number of patients and the same sampling time schedules were used. One hundred patients were simulated, and a sparse sampling strategy with four samples per subject was applied. Sampling times were extracted from a uniform distribution over four sampling time windows (0–24 h, 24–48 h, 48–72 h, and 72–96 h) with the restriction of at least a 12-h delay between two samples for the same subject. Thus, nine initial data sets of 400 points were obtained. The nine originals data sets were simulated using NONMEM version VI β.

Creation of the Reduced and Reduced Corrected Data Sets

For each of the nine initial data sets, in order to mimic the omission of observations below the LOQ, various fixed percentages of the lowest data set observations were omitted in order to obtain reduced data sets. Six percentage values were used: 5%, 10%, 20%, 30%, 40%, and 50%.

For each reduced data set, a corresponding "corrected" data set was created. A correction procedure was applied in order to prevent or limit any potential bias created by the omission of the values below LOQ. For each subject, all the observations but the first one below the LOQ were omitted. The first observation below the LOQ was given the value of half of the LOQ value. This approach is the one used as method M6 by Beal and Sheiner (9). Its rationale is to provide the model with some information for the measurements below LOQ by addition of a point below the LOQ. This value of half LOQ was chosen assuming a normal distribution of the measurements in the interval [0,LOQ]. It is assumed that the variabilities estimated around the typical values of the parameters as well as the residual error can deal with the *a priori* uncertainty of this assigned value below LOQ. The reduced corrected data sets were analyzed with and without addition of an additional additive residual error component devoted to these values below LOQ.

Thus, nine groups of data sets with one original data set, six reduced data sets, and six "corrected" data sets were created.

Table I. Primary Parameters Used for Each Set of Simulations Depending on Both AUC and Half-Life Ratios

Half-life ratio		AUC ratio 0.1	0.2	0.3
0.3	CL	10	20	30
	V_c	270	454	588
	Q	3.22	8.1	11.9
	V_p	29	119	202
0.1	CL	10	20	30
	V_c	175	238	270
	Q	20	33	37
	V_p	128	308	460
0.03	CL	10	20	30
	V_c	79	89	93
	Q	52.5	60	57
	V_p	222	448	615

Real Data Set

A real data set with data from 97 subjects and 270 observations, obtained after multiple intravenous bolus doses, was also used to assess the impact of informative missingness caused by LOQ. In the same manner as for the simulated data sets, six reduced and six corrected data sets were generated.

A two-compartment model (ADVAN3 TRANS4) with IIV in CL and central volume was used to describe the data. After a logarithmic transformation of the data, an additive residual error model was applied.

Parameter Estimation

For both simulated and real data sets, we estimated the pharmacokinetic parameters using NONMEM VI β with a first-order (FO) estimation method as well as the FO conditional with (FOCE-INTERACTION) and without (FOCE) interaction estimate methods. The difference between these estimation methods is based on the estimation of the individual parameters. All three methods apply linearization approximations, and the difference between FO and FOCE is that in the former linearization occurs around the population estimate, whereas in the latter it occurs around the individual parameter estimate. The difference between FOCE with and without interaction is that individual and population parameter estimates, respectively, are used in the residual error model; in the case this includes a prediction based on parameter values. The results were processed in Splus 2000 (10). For all the simulated data sets, the results were expressed as a percentage of deviation of the fixed-effect parameter estimates from the theoretic typical values.

$$\frac{\text{(Estimated value} - \text{Theoretical typical value)}}{\text{Theoretical typical value}}$$

Regarding the real data set, fixed-effect results were expressed as a percentage of deviation from the estimated value with the whole data set.

RESULTS

Simulated Data Sets

The nine data sets were simulated, and based on them the "reduced" data sets and the "reduced corrected" data sets were created. This study aimed to investigate the impact of the omission of values below LOQ on a structural bias. Therefore, the influence on the IIVs was not extensively investigated. As consequence, the runs were considered as successful when the minimization step succeeded. Successful terminations including estimation of standard errors (SEs) were obtained for the nine full data sets (Table II) and for the majority of the data sets with omission or replacement of data below LOQ. Some terminated data sets with rounding errors

Table II. Fixed Effect of the Parameter Estimates and the Related Standard Error (*in Italics*) of the Estimates for the Nine Full Data Sets

		AUC ratio		
Half-life ratio		*0.10*	*0.20*	*0.30*
0.30	CI	9.41	20.20	28.40
		0.03	*0.03*	*0.03*
	V_c	274.00	461.00	595.00
		0.05	*0.05*	*0.06*
	Q	1.34	6.38	11.50
		0.72	*0.24*	*0.17*
	V_p	17.20	110.00	176.00
		0.70	*0.14*	*0.15*
0.10	CI	9.43	18.90	28.50
		0.03	*0.05*	*0.04*
	V_c	190.00	265.00	302.00
		0.08	*0.09*	*0.11*
	Q	16.50	28.90	34.70
		0.27	*0.15*	*0.13*
	V_p	106.00	274.00	425.00
		0.17	*0.21*	*0.16*
0.03	CI	10.50	19.10	31.60
		0.04	*0.03*	*0.04*
	V_c	88.20	100.00	108.00
		0.34	*0.21*	*0.16*
	W	55.00	68.30	67.90
		0.38	*0.08*	*0.15*
	V_p	236.00	452.00	636.00
		0.17	*0.05*	*0.06*

were considered successful if the minimization step succeeded and the rounding error was related to the IIV. The IIVs on the distribution parameters were difficult to assess when values below LOQ were omitted, even with the correction procedure, although the random effect on CL could be estimated in most of the cases. It became difficult to assess when the percentage of omitted values was over 40% and when the distribution and the elimination phases were hardly differentiated (i.e., the following structures: half-life ratio of 0.3 and half-life ratio of 0.1; AUC ratio of 0.1) (Table III).

When the values below the LOQ were omitted, the estimated parameters were shifted. The estimated clearance was lower than the theoretical one, shown as a decrease in the percentage of deviation (Fig. 1). This difference increased when the percentage of omitted values below LOQ increased. This shift of the estimated clearance seems to be amplified when the half-life ratio value increases. A similar decrease in the estimated clearance appeared with the three estimation methods implemented in NONMEM: FO, FOCE (not shown), and FOCE interaction (not shown). Regarding the IIV on CL, the same trends appeared with a decrease of the estimated coefficient of variation. This influence of the LOQ seems to be related to the half-life ratio as well (Table III).

The estimated CL for the corrected reduced data sets were similar to the theoretical values as shown with a small percentage of deviation, whatever the percentage of omitted values was (Fig. 1). Similarly, the correction procedure allowed a better estimation of the IIV for clearance.

The percentage of deviation of the typical values for V_c and Q (Table IV) did not show any major trends on the influence of both the omission of values below LOQ and the correction procedure for those two parameters.

Table III. Estimates of the Interindividual Variability on Clearance Depending of the Percentage of Omitted Values below LOQ and the AUC and Half-Life Ratios (Simulated IIV = 0.25)

		AUC ratio					
		0.10		0.20		0.30	
	Below LOQ (%)	Deletion	Correction	Deletion	Correction	Deletion	Correction
0.30	0	0.23		0.23		0.23	
	5	0.23	0.24	0.22	0.21	0.23	0.21
	10	0.23	0.24	0.21	0.21	0.21	0.20
	20	0.22	0.24	0.10	0.21	0.18	0.21
	30	0.19	0.23	0.12	0.19	0.15	0.19
	40	0.00*	0.23	0.00*	0.17	0.12	0.15
	50	0.00*	0.20	0.00*	0.14	0.00*	0.17
0.10	0	0.24		0.24		0.24	
	5	0.22	0.22	0.23	0.23	0.23	0.23
	10	0.17	0.22	0.20	0.22	0.21	0.22
	20	0.13	0.21	0.17	0.21	0.18	0.21
	30	0.08	0.20	0.11	0.20	0.12	0.21
	40	0.04	0.15	0.08	0.16	0.09	0.17
	50	0.00[a]	0.12	0.04	0.16	0.07	0.16
0.03	0	0.30		0.30		0.30	
	5	0.31	0.31	0.29	0.29	0.23	0.22
	10	0.30	0.30	0.29	0.29	0.22	0.22
	20	0.27	0.31	0.29	0.29	0.20	0.23
	30	0.27	0.32	0.31	0.28	0.19	0.21
	40	0.23	0.29	0.13	0.27	0.19	0.20
	50	0.26	0.27	0.09	0.26	0.21	0.20

[a] For these estimations, no IIV could be estimated on clearance.

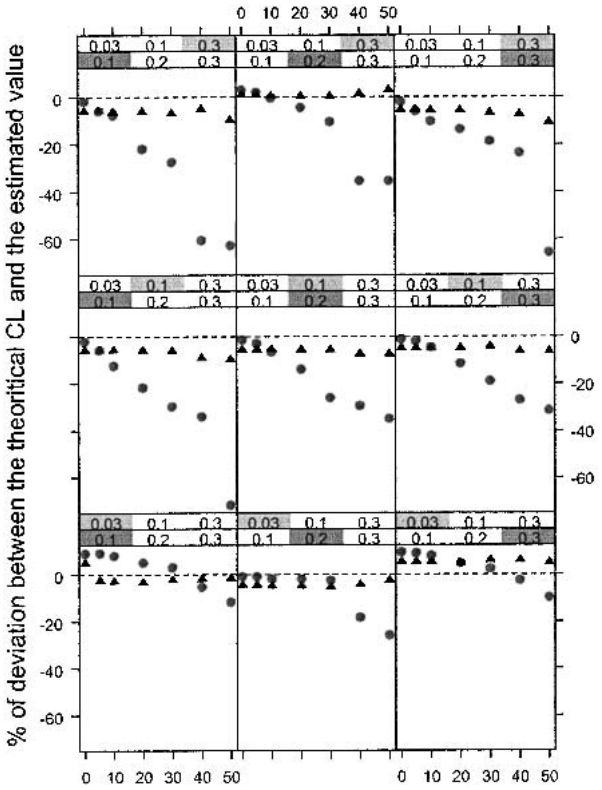

Fig. 1. Simulated data sets. Deviation from the typical value of CL vs. the percentage of observations below LOQ for the reduced data set (●) and the corrected data set (▲).

The estimated peripheral volume (Fig. 2) was overestimated when the values below LOQ were omitted. The greater the half-life ratio, the greater the influence of omitted values. As for CL, once the correction procedure was applied, a decrease in the percentage of deviation was observed. The terminal half-life, calculated from the other parameters, consistently showed a positive bias (Fig. 3).

The real data set also showed a decrease of the estimated CL with an increase of the percentage of omitted values below LOQ. However, the bias induced by the omission did not appear so clearly for the peripheral volume (Fig. 4).

DISCUSSION

This study demonstrates that bias on typical values of the parameters may result from omitting data below the LOQ in a two-compartment model. Previous modeling exercises have shown comparable results for the one-compartment model (8,9). However, the magnitude of the bias can be considerably higher with two-compartment models. The magnitude of the bias depends on the pharmacokinetic characteristics of the drug (AUCr and half-life ratio), the value of the LOQ (percentage of omitted values), the parameter in question, and, although not investigated here, most likely the dosing and sampling schedules. As can be expected, bias increases with increased percentage of omitted values. For CL, a bias >20% could be observed for some data sets with omission of 20% of all observations. This is no guarantee that a marked bias may not occur at a lower fraction omitted for other situations. Regarding the sign of the bias, some general conclusions

Table IV. The Percentage of Deviation for Typical Values of Vc and Q Are Presented Depending of the Percentage of Omitted Values and the Method Used to Deal with the BLQ Values

			AUC ratio											
			0.10				0.20				0.30			
			Δ Vc		Δ Q		Δ Vc		Δ Q		Δ Vc		Δ Q	
Half-lives ratio		BLQ (%)	Deletion	Correction	Deletion	Correction	Deletion	Correction	Deletion	Correction	Deletion	Correction	Deletion	Correction
0.30		0	0.01		−0.58		0.02		−0.21		0.01		−0.03	
		5	−0.01	0.01	−0.59	−0.58	0.03	0.01	−0.27	−0.16	0.02	0.02	−0.37	−0.03
		10	−0.13	0.01	0.18	−0.56	0.06	0.04	−0.40	−0.26	−0.01	0.02	−0.28	−0.01
		20	−0.10	0.02	0.38	−0.51	0.02	0.04	−0.18	−0.29	−0.06	0.00	0.00	−0.02
		30	−0.11	−0.01	0.70	−0.14	−0.02	−0.02	0.22	0.13	−0.09	−0.01	0.31	0.11
		40	−0.08	−0.07	1.76	0.13	−0.04	−0.02	0.96	0.07	−0.07	−0.04	0.50	0.39
		50	−0.07	0.00	1.90	−0.02	−0.04	−0.04	0.96	0.23	−0.04	0.05	1.59	−0.13
0.10		0	0.09		−0.18		0.11		−0.12		0.12		−0.06	
		5	0.23	0.09	−0.73	−0.16	0.19	0.12	−0.39	−0.12	0.13	0.12	−0.19	−0.06
		10	0.28	0.09	−0.72	−0.17	0.24	0.12	−0.49	−0.12	0.19	0.12	−0.30	−0.05
		20	0.20	0.10	−0.65	−0.16	0.29	0.13	−0.52	−0.11	0.26	0.14	−0.35	−0.06
		30	0.17	0.11	−0.58	−0.16	0.28	0.12	−0.45	−0.07	0.21	0.14	−0.25	−0.02
		40	0.13	0.24	−0.49	−0.56	0.15	0.23	−0.28	−0.27	0.16	0.22	−0.16	−0.14
		50	0.x23	0.22	−0.33	−0.53	0.14	0.22	−0.22	−0.25	0.11	0.22	−0.07	−0.12
0.03		0	0.12		0.05		0.12		0.14		0.16		0.19	
		5	0.16	−0.12	0.06	−0.16	0.22	0.12	0.06	0.14	0.13	0.17	0.16	0.19
		10	0.18	−0.11	0.06	−0.17	0.12	0.13	0.01	0.14	0.13	0.16	0.11	0.18
		20	0.35	−0.09	−0.01	−0.16	0.27	0.12	0.02	0.14	0.13	0.17	0.08	0.13
		30	0.35	−0.09	−0.10	−0.13	0.31	0.05	0.10	0.14	0.16	0.23	0.08	0.15
		40	0.30	−0.13	−0.14	−0.12	1.25	0.04	0.07	0.10	0.24	0.22	0.11	0.14
		50	0.32	−0.14	−0.06	−0.18	1.07	0.06	0.14	0.12	0.32	0.16	0.16	0.11

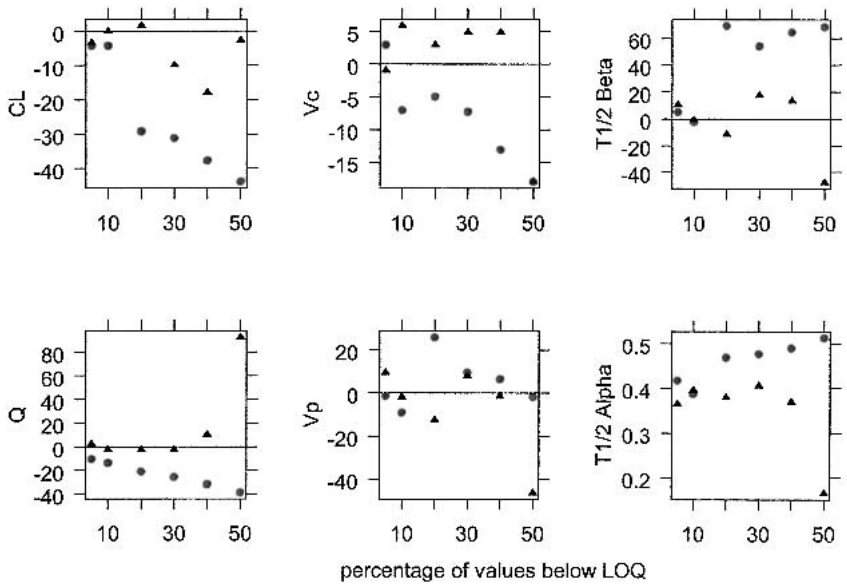

Fig. 2. Simulated data sets. Deviation from the typical value of V_p vs. the percentage of observations below LOQ for the reduced data set (●) and the corrected dataset (▲).

across the studied situations can be made. When biased, CL is generally underestimated, as omission is mainly of data from subjects having a high CL. For peripheral volume of distribution, the opposite is true. Subjects with a small peripheral volume of distribution tend to have a shorter terminal half-life and therefore have concentrations declining below LOQ earlier than others. The peripheral volume is therefore generally overestimated when biased. As a consequence of the bias in CL and peripheral volume, terminal half-life is often overestimated. For one data set, the terminal half-life estimate was twice the true value with only 10% of data omitted as below LOQ. For central volume and intercompartmental

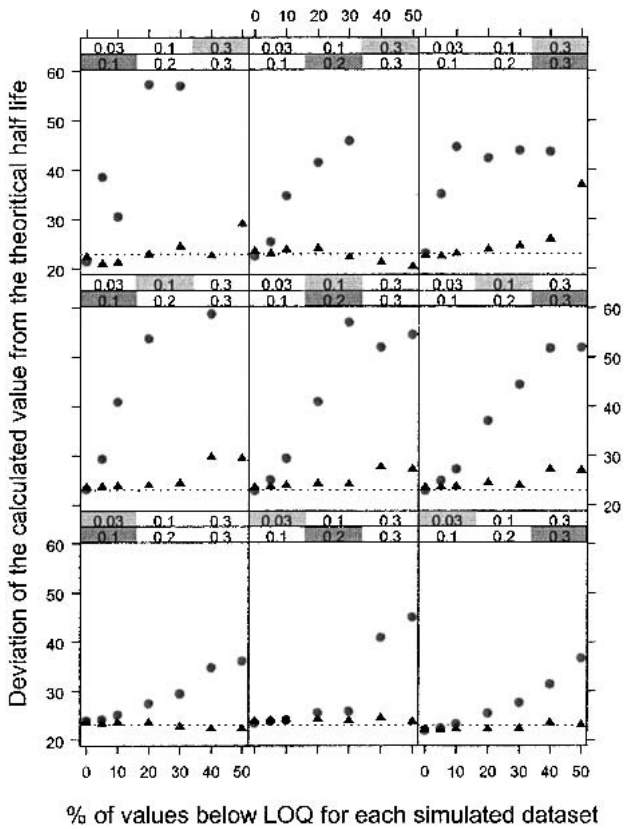

Fig. 3. Simulated data sets. Deviation from the theoretical value of elimination half-life vs. the percentage of observations below LOQ for the reduced data set (●) and the corrected data set (▲).

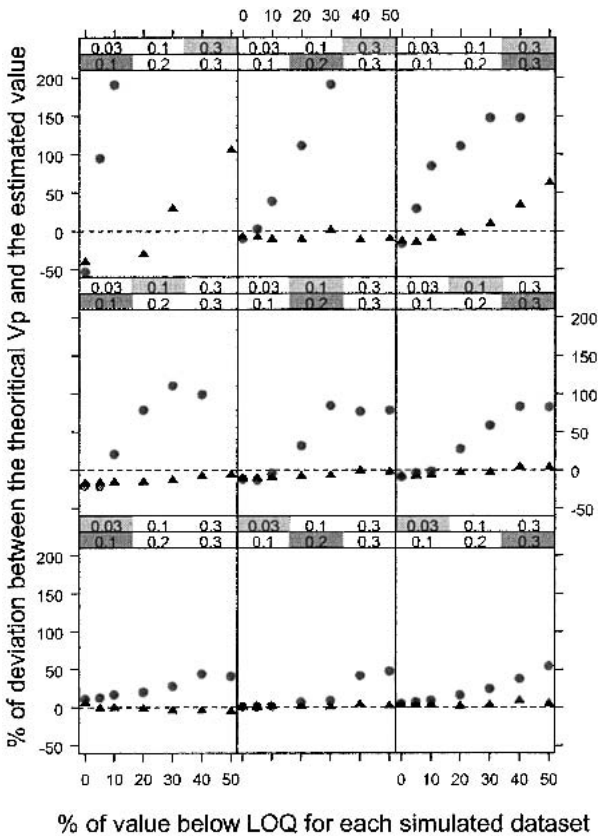

Fig. 4. Real data set. Percentage of deviation from the estimated values regarding the percentage of values below LOQ for the reduced data set (●) and the corrected data set (▲).

CL, parameters for which the majority of the information is contained in the early part of the concentration–time profile, bias is not as pronounced, and the direction of bias varies dependent on pharmacokinetic profiles. Situations in which distribution and elimination phases are not readily distinguishable amplified the bias on parameters. In addition, the sparse sampling strategy adopted with a 12-h delay between the samples was another obstacle to the estimation of the Q and V_c even with the full data set. The distribution half-life is 40 min for a half-life ratio of 0.03 (Table II).

The correction procedure generally performed well with considerably less bias than simple omission of data on typical values of the parameters, although somewhat less well for the real data set than for the simulations. The results on random effects are not as good. IIV in CL seems to be the only variability estimate markedly improved by the correction procedure. These results on IIV could have been anticipated because replacing the different values below LOQ by one specific value does not allow one to take into account any IIV on these latest measurements. So in both cases, omission and correction, there seems to be a loss of information on IIV. Furthermore, the less information in the data set, the more the model is overparameterized in terms of IIV, which often leads to rounding errors related to some IIVs on Q, V_c, or V_p that could not be estimated. It occurred with both reduced and corrected data sets.

This correction procedure was adapted from a suggestion made by Dr. Lewis Sheiner at the nmusers listserver (http://www.phor.com/nonmem/nm/index.html) and had already been applied by Beal and Sheiner (M6 method) (9). In the original suggestion, the addition of the first observation below LOQ was accompanied by the addition of an additive residual error term. The magnitude of this error was set to one-fourth of the LOQ value assuming a normal distribution of the additional point below LOQ around the value of LOQ/2. Such an additional error term did not improve the performance of the correction procedure in the present analysis. Because the main purpose of the investigation was to assess the influence of the structure of a two-compartment model on the bias with sole omission of data below LOQ, we did not explore other types of correction procedures. Correction procedures based on more sophisticated calculations of single replacements or methods based on multiple imputation (11) may well be more robust and take into account the data imputation in the estimates of parameter precision.

All the data sets have been simulated with a proportional error model. This was done to maximize the impact of omission of the data. For data where residual error is relatively higher at low concentrations, low observations do not contain as much information, and thus, omission of such data will not have the same impact. Using an exponential residual error model will, on the other hand, be maximally unfavorable for the correction procedure that assumes a proportional error structure only. This is because the substitution of the first observation with a value of LOQ/2 is likely to deviate from the predicted concentration by a larger (relative) residual than other observations.

Noncompartmental methods to estimate pharmacokinetic parameters generally handle heteroscedasticity poorly. Because including data below LOQ would increase heteroscedasticity, it is understandable that omission of data below LOQ has become standard for such analyses. However, with nonlinear mixed-effects models, heteroscedasticity can be handled appropriately, and a better correction procedure may well be to make use of all data, including those below the LOQ.

This study is of limited size because its main aim was to investigate the relationship between the structure of a two-compartment model and the bias induced by the omission of values below LOQ. Only one type of pharmacokinetic model was investigated (two-compartment disposition model), and for each combination of pharmacokinetic characteristics, the results of only one simulated data set was reported. However, all the main conclusions based on the simulated data sets were evident when the simulations were replicated twice with different random number seeds (data not shown). This low number of replications does not allow us to obtain some statistics but only to be able to identify some trends on the biases of the typical parameters and the influence of the structure of the two-compartment model on these biases.

CONCLUSION

This study demonstrates that omitting the values below the limit of quantification may create a substantial bias in the estimation of the typical values of the parameters for the case of an IV bolus infusion in a two-compartment model. Parameters do not have the same sensitivity to the omission of the value below LOQ, with the major impact on clearance, peripheral volume, and terminal half-life. The magnitude of the bias depends of the underlying structure of the model. Models with two hardly distinguishable phases were more sensitive to the omission of values below LOQ. It follows that all the values below LOQ could not be simply ignored without taking the risk of model misspecification. Easy to implement, this simple correction procedure considerably decreased the bias on the typical values of the parameter estimates. Regarding the IIV, the procedure seems to improve the estimation of the IIV on CL; nevertheless, its benefits on the other parameters require further investigation.

REFERENCES

1. FDA. Guidance for industry bioanalytical method validation. Available from http://www.fda.gov/cder/guidance/4252fnl.pdf (2001).
2. ICH. Guideline: Validation of analytical procedure: Methodology. Available from http://www.ifpma.org/pdfifpma/q2b.pdf (1996).
3. Eurachem. Guide: The fitness for purpose of analytical method. http://www.eurachem.bam.de/guides/valid.pdf (1998).
4. L. B. Sheiner. Population pharmacokinetics/dynamics. *Annu. Rev. Pharmacol. Toxicol.* **32**:185–209 (1992).
5. L. B. Sheiner and J.-L. Steimer. Pharmacokinetic/pharmacodynamic modelling in drug development. *Annu. Rev. Pharmacol. Toxicol.* **40**:67–95 (2000).
6. J. K. Lindsey, B. Jones, and P. Parvis. Some statistical issues in modelling pharmacokinetic data. *Statist. Med.* **20**:2775–2783 (2001).
7. L. S. Beal. Ways to fit a pharmacokinetic model with some data below the limit the quantification limit. *J. Pharmacokinet. Pharmacodyn.* **28**:481–504 (2001).
8. J. P. Hing, S. G. Woolfrey, D. Greenslade, and P. M. C. Wright. Analysis of toxicokinetic data using NONMEM: impact of quantification limit and replacement strategies for censored data. *J. Pharmacokinet. Pharmacodyn.* **28**:465–479 (2001).
9. S. L. Beal and L. B. Sheiner. *NONMEM Users's Guide.* NONMEM Project Group, University of California, San Francisco, California, 1996
10. *S-plus 2000 Programmer's Guide,* MathSoft, Inc., Surrey, United Kingdom, 1999.
11. J. L. Schafer. Multiple imputation: a primer. *Stat. Methods Med. Res.* **8**:3–15 (1999).