Prediction of paraquat exposure and toxicity in clinically ill poisoned patients: a model based approach.

Journal:	*British Journal of Clinical Pharmacology*
Manuscript ID:	MP-00661-13
Manuscript Type:	PK-PD relationships
Date Submitted by the Author:	06-Dec-2013
Complete List of Authors:	Wunnapuk, Klintean; The University of Queensland, Therapeutics Research Centre, School of Medicine, mohamed, Fahim; South Asian Clinical Toxicology Research Collaboration, ; Faculty of Allied Health Scienes, Pharmacy Gawarammana, Indika; South Asian Clinical Toxicology Research Collaboration, Department of Medicine, Faculty of Medicine, University of Peradeniya Liu, Xin; University of Queensland, Princess Alexandra Hospital, Therapeutics Research Centre Verbeeck, Roger; Université catholique de Louvain, Buckley, Nicholas; University of NSW, Prince of Wales Clinical School Roberts, Michael; University of Queensland, Department of Medicine; University of South Australia, Pharmacy & Medical Science Musuamba, Flora; Université Catholique de Louvain, Louvain Drug Research Institute; Université Catholique de Louvain, Louvain centre for toxicology and applied pharmacology
Key Words:	Paraquat, toxicokinetics, toxicodynamics, kidney function, creatinine clearance, immunosuppressive
Abstract:	Context. Paraquat poisoning is a medical problem in many parts of Asia and the Pacific. The mortality rate is extremely high as there is no effective treatment. Objective.We analysed data collected during an ongoing cohort study on self-poisoning and from a randomized controlled trial assessing the efficacy of immunosuppressive therapy in hospitalized paraquat-intoxicated patients. The aim of this analysis was to characterise the toxicokinetics and toxicodynamics of paraquat in this population. Methods.A nonlinear mixed effects approach was used to perform toxicokinetic/toxicodynamic population analysis in a cohort of 78 patients. Results. The paraquat plasma concentrations were best fitted by a two-compartment toxicokinetic structural model with first-order absorption and first-order elimination. Changes in renal function were used for the assessment of paraquat toxicodynamics. The estimates of toxicokinetic parameters for the apparent clearance, the apparent volume of distribution and elimination half-life were 1.17 L/h, 2.4 L/kg and 87 h, respectively. Renal function, namely creatinine clearance, was the most significant covariate to explain between patient variability in paraquat clearance.This

model suggested that a reduction in paraquat clearance occurred within 24 to 48 h after poison ingestion, and afterward the clearance was constant over time.The model estimated that a paraquat concentration of 429 µg/L caused 50% of maximum renal toxicity. The immunosuppressive therapy tested during this study was associated with only 8% improvement of renal function.

Conclusion. The developed models may be useful as prognostic tools to predict patient outcome based on patient characteristics on admission and to assess drug effectiveness during antidote drug development.

Dear Editor,

We are hereby submitting our manuscript entitled:" Prediction of paraquat exposure and toxicity in clinically ill poisoned patients: a model based approach".

It is an original work conducted by the authors and is not submitted elsewhere. These results was presented as a poster at the 22th population approach group in Europe (PAGE) meeting in June 2013 in Glasgow. Miss Klintean Wunnapuk was the principal investigator for this work and she is listed as the first author.

We hope this work will be of interest for the readers of British Journal of Clinical Pharmacology.

Sincerely,

Flora Musuamba

Prediction of paraquat exposure and toxicity in clinically ill poisoned patients: a model based approach.

Klintean Wunnapuk[1,2], Fahim Mohammed[3,4,5], Indika Gawarammana[3,4,6], Xin Liu[1], Roger K. Verbeeck[7,8], Nicholas A. Buckley[4,5,9], Michael S. Roberts[1,10], Flora T. Musuamba[7]

[1]Therapeutics Research Centre, School of Medicine, The University of Queensland, Brisbane, QLD, Australia

[2]Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

[3]Department of Pharmacy, Faculty of Allied Health Science, University of Peradeniya, Sri Lanka.

[4]South Asian Clinical Toxicology Research Collaboration, Faculty of Medicine, University of Peradeniya, Sri Lanka

[5]Professorial Medicine Unit, The Prince of Wales Clinical School, University of New South Wales, NSW, Australia

[6] Department of Medicine, Faculty of Medicine, University of Peradeniya, Sri Lanka

[7]Louvain Drug Research Institute, Louvain Centre for Toxicology and Applied Pharmacology, Catholic University of Louvain, Brussels, Belgium

[8]Faculty of Pharmacy, Rhodes University, Grahamstown, South Africa

[9] Australian National University, Canberra, Australia

[10]School of Pharmacy & Medical Science, University of South Australia, Adelaide, SA, Australia

1

2

Address correspondence to Flora T. Musuamba, Louvain Drug Research Institute, Pharmacokinetics/Drug Metabolism, Catholic University of Louvain, Brussels, Belgium, Telephone: + 3227649470, Email: flora.musuamba@uclouvain.be

Words count: 7158

Structured summary

Context. Paraquat poisoning is a medical problem in many parts of Asia and the Pacific. The mortality rate is extremely high as there is no effective treatment.

*Objective.*We analysed data collected during an ongoing cohort study on self-poisoning and from a randomized controlled trial assessing the efficacy of immunosuppressive therapy in hospitalized paraquat-intoxicated patients. The aim of this analysis was to characterise the toxicokinetics and toxicodynamics of paraquat in this population.

*Methods.*A nonlinear mixed effects approach was used to perform toxicokinetic/toxicodynamic population analysis in a cohort of 78 patients.

Results. The paraquat plasma concentrations were best fitted by a two-compartment toxicokinetic structural model with first-order absorption and first-order elimination. Changes in renal function were used for the assessment of paraquat toxicodynamics. The estimates of toxicokinetic parameters for the apparent clearance, the apparent volume of distribution and elimination half-life were 1.17 L/h, 2.4 L/kg and 87 h, respectively. Renal function, namely creatinine clearance, was the most significant covariate to explain between patient variability in paraquat clearance.This model suggested that a reduction in paraquat clearance occurred within 24 to 48 h after poison ingestion, and afterward the clearance was constant over time.The model estimated that a paraquat concentration of 429 µg/L caused 50% of maximum renal toxicity. The immunosuppressive therapy tested during this study was associated with only 8% improvement of renal function.

Conclusion. The developed models may be useful as prognostic tools to predict patient outcome based on patient characteristics on admission and to assess drug effectiveness during antidote drug development.

What was already known about this subject:

- Although heavily restricted, paraquat remains widely used in the developing world.

- There is currently limited information on paraquat pharmacokinetics and pharmacodynamics in humans

 - Available prognostic tools for patients outcome after paraquat poisoning were not developed based on pharmacology and do not account for differences in treatment effects.

What this study adds?

- This is the first report on population pharmacokinetic and pharmacokinetic/pharmacodynamic analyses of paraquat in humans .

- The developed models can be used to quantitatively characterize treatment effects and therefore optimize prognostic tools dedicated to predict patient outcome after poisoning.

4

Introduction

Paraquat is a commonly used herbicide that causes many deaths from accidental or intentional ingestion. Although heavily restricted, it remains widely used in the developing world, especially in Asia. Ingestion of more than 15-30 ml of a 20% (w/v) paraquat can result in death from multiple organ failure or respiratory failure within a month of intoxication. Due to the lack of effective treatment, [1-5] the mortality rate after paraquat ingestion is around 60%, which is much higher than that of other commonly used herbicides such as glyphosate and chlorophenoxy herbicides (both around 5-30%). [6, 7]

Understanding the disposition of paraquat in humans is important for evaluating treatments that aim to reduce paraquat concentrations and/or effects. Animal studies indicate that paraquat, a cation of a strong base, is rapidly but poorly absorbed from the gastrointestinal tract. Only 5-15% reaches the blood stream where the peak level is obtained within 2-6 hours. [3] Protein binding of paraquat is very low and paraquat is not metabolized. [8] Small amounts of paraquat have been found in bile (post-mortem), indicating that some excretion via bile occurs. [8] However, up to 98%of paraquat is excreted unchanged via urine indicating that renal function is a key factor in the elimination of paraquat. [9-11] Paraquat toxicokinetics have been studied in several animal species including dogs, rats, and rabbits. [11-15] To date, only a few studies have focused on paraquat toxicokinetics in humans. [16,17] Moreover, the relationship between human toxicokinetics (TK) and toxicodynamics(TD) has not been studied. Over decades, many procedures and treatments have been used to modify toxicity of paraquat without any great success. [1, 18-20] The best predictors of outcome are volume of ingestion, kidney function and age.

Acute kidney injury (as measured by a change in creatinine) is very common and very strongly predicts death after paraquat poisoning. [21, 22] However, a rise in creatinine is a good predictor because it is both an indicator of the extent of ongoing toxicity and of the ability to eliminate paraquat. It would be useful to determine the relative contribution of these two factors as they have different implications for improving management.

We report on a nonlinear mixed effects approach to better characterise paraquat kinetics and toxicity in a paraquat intoxicated population (population TK/TD or pop TK/TD), the uncertainty around TK and TD and the potential covariates that could explain variability in paraquat disposition in intoxicated patients.The aims of the present study were: (1) to predict the time course of paraquat exposure based on information recorded on admission such as the initial kidney function and the ingested volume, (2) to better understand the relationship between paraquat exposure and toxicity in humans and (3) to assess the influence of patient characteristics on paraquat exposure and toxicity, including the effects of commonly used treatment approaches.

Materials and methods

Patients

The subjects (n = 78) included in the present analysis were from two different sources: (1) an ongoing cohort study on self-poisoning and (2) a nested randomized controlled trial (RCT) assessing the efficacy ofimmunosuppressive therapy on paraquat poisoning (ISRCTN85372848) in Sri Lankan tertiary hospitals. Demographic and clinical data were collected prospectively from all consenting patients. The studies were approved by the Ethical Review Committee of the Faculty of Medicine, University of Peradeniya, Sri Lanka, and the Human Research Ethics

6

Committee of the Australian National University. Informed written consent was obtained from the patients or relatives where this was not possible.

Paraquat ingestion was initially diagnosed based on patient's or relative's history and/or by examination of the bottle or label brought to the hospital. Paraquat ingestion was then confirmed via these mi-quantitative urine dithionate test (done at least 4 hours after ingestion). The amount of paraquat ingested was estimated from the volume described by the patients or their relatives. A "little" or " a teaspoon" was interpreted as equivalent to 5 ml, a "mouthful" to 25 ml, a "small cup" to 100 ml, a "glass" to 300 ml, and a "bottle" to 400 ml of a commercial product containing 20% paraquat. If the patient reported a range of volumes of ingestion, the mean volume was used. [23]

Sixty-eight patients were included in the TD study. The patients were grouped by treatment regimen as: standard care (n = 19), standard care plus placebo (n = 26), and standard care plus immunosuppressive therapy (comprising pulse therapy with methylprednisolone and cyclophosphamide/MESNA, and dexamethasone) (n = 23) as described elsewhere.[20] Standard care consisted of resuscitation (assessment and management of airway, breathing and circulation), decontamination using charcoal or Fuller's earth, and intravenous fluids. Haemoperfusion/haemodialysis were not used in any patients.

Blood and urine sampling

Serial blood and urine samples were collected for the quantification of paraquat levels at admission (t=0), 4, 8, 16, and 24 hour (post admission) and then daily until discharge. After discharge, all patients were followed up at one month and three months at the clinic or their home. At follow-up, some clinical data, blood and urine samples were collected. Around 6-8 ml

7

blood were withdrawn at each time point and were transferred to two EDTA tubes and mixed thoroughly. Then soon after collection,blood samples were centrifuged for 10 minutes at 3000 rpm and plasma was separated. Urine volumes were not recorded, but where possible 20 ml of urine was also collected at these time points, and centrifuged at 2000 rpm for 10 minutes. Clear supernatant was then transferred into small tubes. Plasma and urine samples were then carefully labeled and immediately transferred to a -20°C freezer, and later shipped to Australia for further analysis.

Paraquat analysis

Paraquat concentrations in plasma and urine were determined using the LC–MS/MS system consisting of an SLC-10AVP system controller, two LC-10AD pumps, an SIL-20AC-HT autosampler (Shimadzu, Kyoto, Japan) and an API2000 triple quadrupole(Applied Biosystems Inc., Foster City, CA, USA) a mass spectrometer coupled with an electrospray ionization (ESI) source and a divert valve.[24] Briefly, plasma and urine samples were carried out by one-step protein precipitation using cold acetonitrile (−20 to −10 ℃). After centrifugation, an aliquot of 10 µl of supernatant was injected into a KinetexTM hydrophilic interaction chromatography (HILIC) column with a KrudKatcherTM Ultra in-line filter. The chromatographic separation was achieved using the mobile phase mixture of 250 mM ammonium formate (with 0.8% aqueous formic acid) in water and acetonitrile at a flow rate of 0.3 ml/min. The calibration curve was linear over the concentration range of 10–5000µg/L, with an LLOQ of 10 µg/L. The inter- and intra-day precision (% R.S.D.) was<8.5% with accuracy within the range of 95.1–102.8%. Paraquat in plasma and urine samples was stable when stored at -20°C for 3 freeze–thaw cycles.

8

Descriptive statistics and graphic generation

Graphics and descriptive statistics were generated using GraphPad Prism version 6.01 (GraphPad Solfware, San Diego, USA).The relationship between paraquat plasma and urine concentration over time was also plotted to provide a rough model-independent assessment of the expected change over time in renal paraquat clearance.

Population toxicokinetic-toxicodynamic analysis

Pop TK: Structural model

The concentration-time data for paraquat in plasma were analysed by a non-linear mixed-effect modeling approach using Phoenix NLME version 1.2 Build 6.3.0.395 (Pharsight Corporation, Mountain View, CA, USA). Initial toxicokinetic model selection was performed using graphical analysis. Plots of paraquat plasma concentration versus time were generated for each individual and examined to determine the appropriate descriptive model. During this analysis, it was assumed that the renal function modified by paraquat had already reached a steady state at the time most of plasma paraquat concentrations were measured . This was based on the fact that renal function has been shown to decrease exponentially after paraquat ingestion before reaching a steady state impaired renal function within the first 48 hours. [13] Based on the graphical analysis and the known physicochemical and TK properties of paraquat, a two-compartment model with first-order absorption and first-order elimination was used as the toxicokinetic structural model. Data were fitted using the extended least squared first order conditional estimation method (FOCE ELS) as implemented in Phoenix software. The model was parameterised in terms of paraquat apparent oral clearance (CL_{PQ}/F), apparent volume of distribution of central compartment after oral administration (V_1/F), apparent volume of

9

distribution of peripheral compartment after oral administration (V_2/F), inter-compartmental clearanceafter oral administration (Q/F), absorption rate constant (K_a) and bioavailability factor (F). K_a was fixed to 1 h^{-1}given the lack of data during the absorption phase and given that the reported mean T_{max} in human was 3 h.[3] As the ingested dose was estimated from volume of ingestion, the varying doses were imputed as covariates on the bioavailability factor and the median dose of paraquat (10 g) was given as the amount administered to each patient. In order to avoid numerical issues due to boundaries of F between 0 and 1, the logit of bioavailability factor (XF) was first estimated, and the bioavailability factor was subsequently regenerated using the following formula:

$$F=XF/(1+XF) \tag{1}$$

Pop TK: Stochastic model

Inter-individual variability (IIV) in TK parameters were modeled using an exponential model as illustrated below:

$$P_i = P*exp(\eta i) \tag{2}$$

where P_i is the parameter estimate of the ith individual, P is the typical value for the population, and η_i is the random effect for individual i, $\eta$was assumed to be normally distributed with a mean value of 0 and a variance of ω^2.[25]IIV terms were added on all the TK parameters.

Residual errors were best described using a combined model (additive and proportional) as depicted below:

$$C_{ji} = C_{0ji}*(1+\varepsilon_{1ji}) + \varepsilon_{2ji} \tag{3}$$

where C_{ji} and C_{0ji} are the ith measured (observed) and model predicted paraquat plasma concentrations for the jth patient, respectively. $\varepsilon_{1ji}$and ε_{2ji} denote the proportional and the additive

10

terms for random residual error, respectively. They were assumed to be normally distributed with a mean of 0 and variances of σ_1^2 and σ_2^2.

Pop TK: Covariate model

A stepwise approach was used for toxicokinetic covariate model building with forward inclusion followed by backward exclusion. The following covariates were assessed for their effects on paraquat disposition: body weight (BW, kg), gender, age (years), amount ingested (g) and renal function markers: serum creatinine concentration (sCr, mg/dL) and estimated creatinine clearance (eCL$_{cr}$, L/h). eCL$_{cr}$ was estimated using the Cockcroft-Gault equation. [26] Covariates first tested separately according to their biological plausibility and where considered to be significant when their addition to the base model led to a decrease of at least 3.84 points in the objective function value (OFV) (P-value < 0.05 in the approximate χ^2 distribution with 1 degree of freedom). [27, 28] The continuous covariates were normalized to their corresponding medians and introduced into the model as shown by Equation 4:

$$P_k = \theta_{k1}*[(\text{Cov}/\text{Cov}_{\text{median}})^{\theta k2}] \tag{4}$$

where P_k is the TK parameter, θ_{k1} is the typical value of the TK parameter in the population, θ_{k2} is the effect coefficient of the covariate, Cov is the value of the covariate and Cov$_{\text{median}}$ is the median of the covariate in the population under investigation.

Categorical covariates were entered into the model using an exponential model. For example, the following model was used to assess gender effect on PD parameters:

$$P_j = \theta_0*\exp(\theta_{1j}*X_{1j}) \tag{5}$$

where $X_{1j} = 0$ and 1 for males and females respectively, θ_0 is the typical value of the TD parameter in the population and θ_{1j} is the effect coefficient for females.

11

TK model evaluation

Criteria for selecting the final model included change in the OFV, precision of parameter estimation (coefficient of variation (CV) estimates smaller than 50%), [29] graphical analysis and quality of goodness-of-fit plots. All of these criteria were taken into account when evaluating alternate models. Lead TK models were evaluated with regards to their accuracy and their stability using nonparametric bootstrapping and visual predictive check (VPC) methods. [30] A nonparametric bootstrapping method was allowed to assess the stability and uncertainty of the final model and estimate the confidence intervals (CI) around parameter estimates in order to characterise the precision of their estimation. [31, 32] One thousand replicates of the data sets were generated by randomly sampling the patient data, and the final model was fitted individually to each of them. All of the model parameters were estimated, and their median and 95% CI were generated. The VPCs were performed using the final model parameters to simulate TK data for 1000 virtual subjects. The 95% prediction interval of simulated concentrations or effects was computed and plotted against the observed values. Bayesian estimates of parameters for individual patients were also computed from the final model. The final TK model was used to generate individual predicted concentrations at times of TD measurements. These individual predicted concentration values were used as input for the TD model. TD parameters and their associated variability were estimated in a subsequent step as described below.

Population toxicodynamic model development

TD: Structural model

Acute renal failure is a common and important acute toxic effect, and the extent of injury predicts death in severe paraquat poisoning. [3, 21] Estimated CL_{cr} (eCL_{cr}) was used as the

marker of paraquat renal toxicity. Initial Pop TD model selection was carried out using graphical analysis. Plots of paraquat plasma concentration and TD effect (eCL_{cr}) was constructed for each individual and examined to determine the appropriate descriptive model. Based on this graphical analysis, the inhibitory fractional sigmoid E_{max} model including the baseline was chosen and is described as follows:

$$E_{(t)} = E_0 * (1 - E_{max} * C^\gamma / (IC_{50}^\gamma + C^\gamma)) \tag{6}$$

where $E_{(t)}$ is the TD effect (eCL_{cr}) at time t, E_0 is the baseline eCL_{cr} of each patient at admission, E_{max} is the maximal fractional decrease of eCL_{cr} caused by paraquat, IC_{50} is the concentration of paraquat causing a 50% of maximum paraquat induced eCL_{cr} decrease, and γ is a shape factor characterizing the slope of the response.[25]

TD: Stochastic model

Inter-individual variability in TD parameters was also assumed to be log-normally distributed and was also modeled using an exponential model. IIV terms were imputed on all TD parameters.

The residual errors were best described using a log-additive error model as depicted below:

$$\text{Log } Eo_{ij} = \text{Log } E_{ji} + \varepsilon_{ji} \tag{7}$$

Where Eo_{ij} is the observed effect (eCL_{cr}) for the ith individual at concentration j, E_{ji} is the individual predicted effect (eCL_{cr}) for the ith individual at concentration j, ε_{ji} is the residual error term. ε_{ji} is assumed to be normally distributed with a mean of 0 and a variance equal to σ^2.

TD: Covariate model

The following covariates were tested on the baseline parameter given that they plausibly affected baseline CL_{cr}: age, gender, body weight. The effects of different treatments were tested

13

as covariate on E_{max}, IC_{50} and γ. Continuous covariates were entered using power models and categorical covariates were entered into the model using exponential models as previously described.

When the covariate had more than 2 categories, such as treatment groups, the following equations were used:

$P_j = \quad \theta_0$ if treatment group = placebo

$P_j = \quad \theta_0 * \exp\theta_{1j}$ if treatment group = immunosuppressive

$P_j = \quad \theta_0 * \exp\theta_{2j}$ if treatment group = control   (8)

θ_{1j} and θ_{2j} are the effect coefficients of the covariate

TD model evaluation

Lead TD models were also evaluated by a bootstrapping approach and visual predictive checks (VPC) as previously described. Bayesian estimates of parametersfor individual patients were also computed from the final model.

Results

Patient demographics and plasma: urine paraquat ratios

A total of 698 plasma concentrations from 78 paraquat poisoned patients were included in the TK analysis. The demographics of the patients in this study are shown in Table 1. Most paraquat plasma concentrations were proportional to urine concentrations with a median ratio (plasma:urine) of 0.17 (Fig. 1) in this population. The plasma:urine paraquat ratio was variable but overall did not change significantly over time.

Population toxicokinetic model

14

As described in the methods section, a two-compartment toxicokinetic structural model with first-order absorption and first-order elimination was fitted to the data. Parameter estimates for the TK base model are presented in Table 2. Given the lack of data in the early distribution phase, V_2/F value was fixed to 0.17 L/kg based on a sensitivity analysis and taking into account previously reported values of paraquat volume of distribution and patient body weights.

Parameter estimates for the final model are also shown in Table 2. Ingested dose and renal function markers were found to be significant covariates on the paraquat bioavailability (F) and apparent paraquat clearance (CL_{PQ}), respectively. Estimated CL_{cr} or sCr were tested to assess which marker performed better in reflecting renal contribution to CL_{PQ}. Even though smaller standard error estimates were obtained when sCr was used (data not shown) as a renal function marker, the OFV was significantly higher than when CL_{cr} was used (Table 2). Inclusion of body weight in the model also significantly reduced the OFV. The goodness-of-fit plots obtained from the final toxicokinetic model are shown in Fig. 2, which indicate the model satisfactorily fitted the data.

Population toxicodynamic model

The final TK model was used to predict individual paraquat concentrations at times of TD measurement. Based on graphical analysis, an inhibitory fractional sigmoid E_{max} model including the baseline as shown in Equation (5) was chosen as structural TD model. Parameter estimates for the base TD model are presented in Table 3. Parameter estimates for the final model are also included in Table 3.

The final PopTD model (model 7) included age, sex and body weight as covariates on baseline eCL_{cr} and method of treatment as covariate on maximum reduction of eCL_{cr} (E_{max})

15

(Table 3). Immunosuppression treatment slightly lowered the E_{max}, by 8% compared to the placebo group. The E_{max} was 24% lower in the patients treated with standard treatments outside the RCT compared to the placebo group (suggesting these two standard treatment only groups may differ due to the inclusion criteria for the RCT, in a way not accounted for by measured covariates). Goodness-of-fit plots were generated for the final model and the weighted residual showed no apparent visual bias for the prediction (Fig. 3).

Model evaluation

The accuracy and stability of these models were assessed by nonparametric bootstrap and VPC. As shown in Table 4, the mean population parameters estimated from the bootstrapping were stable and comparable to the estimates from the final model. The estimates of the parameters from the final model all fell within the 95% CIs of the corresponding parameters obtained with the 1,000 bootstraps, indicating that the final model was fairly robust. The VPCs showed that approximately 95% of the observed data appear to fall within the 95% confidence interval (Fig. 4), suggesting that the final model accurately described the observed data. The estimated TK and TK/TD parameters of the individual patients are listed in Table 5.

Clinical usefulness of the model

The change of estimated CL_{PQ} over time is shown in Fig.5 showing that after an initial rapid decrease the CL_{PQ} was stable over time. The resulting median paraquat concentrations and CL_{cr} were simulated for three different values CL_{cr} at admission: the minimum, the median and the maximum values (0.3, 6 and 13L/h, respectively) in this cohort of patient. The results displayed in Fig. 6 show that the model is able to predict paraquat exposure and toxicity given

16

patient characteristics on admission. It could therefore serve to optimize prognostic tools dedicated to predict patients outcome on admission and could serve as a tool to evaluate treatment options and candidate antidotes.

Discussion

We used a population approach   to describe paraquat disposition and its effects on renal function in poisoned patients. A two compartment toxicokinetic model with rapid absorption fitted the data well. Renal function was the most important factor influencing paraquat clearance. As a function of paraquat concentration, there was a variable but rapid reduction in paraquat clearance within 24 to 48 hours, which then was constant and low (around 10ml/min). The time at which most of paraquat samples were collected is estimated to be more than 48 hours after ingestion. Whilst paraquat does induce an immediate and variable change in renal function, this renal function appears to reach an impaired new steady state renal function within 48 hours. [13] Accordingly, the PK model used in this work used the simpler constant impaired renal function after paraquat ingestion and did not consider the time variant changes in renal function at early times. Renal injury occurred with relatively low concentrations of paraquat (IC_{50} =429 µg/L). This analysis has implications for the development of better prognostic tools, for evaluation of candidate antidotes and for the design of the optimal methods to enhance elimination.

In humans, there is limited data on absorption of paraquat. Any uncertainty on the ingestion dose and time of ingestion in our study would be expected to propagate into uncertainty on TK and TD parameter estimates. There is only one study that has reported the complete recovery of paraquat after oral dosing, [16] and they estimated a V of 1.4 L/kg. If this value is substituted into our V/F estimated, the bioavailability(F) is estimated to be around 0.58.

17

Other studies have estimated values for V/F ranging from 1.2 to 2.75 L/kg. [33, 34] and thus the V/F of about 2.4 L/kg estimated from our TK model is in the range of estimates. Several factors are known to influence paraquat distribution. The extent to which the herbicide is actively taken up by lung, liver, kidney and other tissues determines V/F. Therefore, if tissue binding of paraquat increased over time, V/F will increase as well. However, a high V/F estimation could be due to the over-estimation of the ingested doses. A limitation of this study is the lack of any way to accurately quantify ingestion volume, which impacts on estimated TK and TK/TD parameters (in both our and all other human studies). Moreover, the uncertainty in the estimation of some key covariates (e.g. renal function) also affects the degree of precision in these models.

The deep compartment consists of tissues where the toxic effects are manifested (in particular lungs and kidneys) and other tissues which act as a reservoir. Paraquat is actively taken up into type II pneumocytes resulting in slow elimination of paraquat from lung compared to other tissues. [1] Active renal uptake and excretion of paraquat is also concentration dependent and saturable; [35] consequently, high levels of paraquat are seen in kidney. Impairment of kidney function in turn leads to higher concentrations of paraquat in the plasma. [12] Muscle is an important paraquat reservoir explaining the persistence of paraquat in plasma and urine for several weeks after exposure. [16] The long elimination half-life ($t_{1/2\,\beta}$) of 3-4 days in our study reflects the combined effects of reduced clearance from the decline in renal function and the slow release of paraquat from tissues into the circulation.

An obvious large change in paraquat renal clearance in these patients was not observed and the plasma/urine paraquat ratio changed little over time (Fig. 1) which could wrongly suggest a simple and static first-order elimination process. Modeling individual patient concentrations did demonstrate a progressive decline in clearance. Several previous reports also indicate that

18

paraquat renal clearance (and total clearance) is a nonlinear function of time. A large decrease over time in CL_{PQ} occurs with nephrotoxicity.[8, 13] At low doses, CL_{PQ} may exceed 12 L/h in humans with normal kidney function.[36] The mean estimate for CL_{PQ}/F (1.17 L/h) obtained from our PopTK model is considerably lower than that reported in some previous human case reports (mean = 4.39 L/h), [34] and they showed a rapid change in CL_{PQ}/F from 8.77 to 2.72 L/h within 12 h. Substituting F = 0.58 into CL_{PQ}/F yields an estimated mean CL_{PQ} of 0.68L/h (11.33 ml/min) in our study. This CL_{PQ} is similar to those reported by Houze et al which range from 0.47 to 0.59 L/h (7.9 to 9.9 ml/min). [16] It seems likely we and Houze et al have missed a very short early phase of high CL_{PQ} reported by others. [34, 36] The low CL_{PQ} reflects the very rapid onset of paraquat induced renal impairment. [12, 13, 35, 37-39] Therefore, the low estimated CL_{PQ}/F in the present study reflects that most blood samples were collected 6 or more hours post-paraquat ingestion when renal damage was already established. While a further modest decline in estimated CL_{PQ}/Fwasfound over 24 to 48 h (Fig. 5), thereafter the estimated clearances were constant over time.

In our analysis, the effect of the eCL_{cr}, (reflecting GFR), on CL_{PQ} was lower than one would expect. This probably reflects errors in the estimate of GFR rather than the model. [40] The Cockcroft-Gault equation has at least a 30% variance around actual renal function in chronic kidney disease population with sCr≤ 1.5 mg/dl. [41] It also performs very poorly when Cr is changing (i.e. it assumes steady-state). However, other estimation methods perform equally poorly (when compared with gold standard methods such as iohexol clearance). Future studies should ideally use more accurate direct measurements of GFR or creatinine clearance.

The model suggested a very small effect of immunosuppressive treatment (8% lower E_{max} compared to placebo group).This is consistent with the clinical outcomes reported in this trial

[20] which was a very small favourable treatment effect that was not statistically significant. However, this difference was less than that seen in the non-RCT patients (24% lower E_{max});which might be a result of the RCT inclusion criteria aiming to select people who had significant poisoning but also who would survive long enough to measure renal injury. Further, anotherRCT has also reported that immunosuppressive therapy did not improve renal function.2, [42] So, these results should not be used to imply that immunosuppressive treatment has substantial effects on renal injury.

Model predictions may serve a number of purposes. From a clinical perspective, they can identify patients on admission who are very likely to develop kidney dysfunction. Using the TK model developed in this study, a paraquat plasma concentration-time profile can be predicted for individual patients on admission. Thereafter, a combination of the TK and the TD model could predict kidney function, namely creatinine clearance, changes over time. Prediction of toxicokinetics and acute kidney injury might in turn be used to identify patients most likely to benefit from enhanced elimination or who are most suitable for inclusion into clinical trials of strategies to prevent kidney injury and other manifestations of paraquat toxicity.

Conclusion

Renal function was the most significant covariate to explain between patient variability in paraquat clearance. Renal injury occurred with relatively low concentrations of paraquat (IC_{50} =429 µg/L). A reduction in paraquat clearance occurred over 24 to 48 h after paraquat ingestion, and afterward the clearance was constant and low. The low clearance (around 10ml/min) and long half-life (3-4 days) in these cases suggest further studies of extracorporeal elimination would be worth exploring, as it is clear much greater clearance can be obtained by such methods.

[39] After optimal methods for enhancing elimination have been developed, large clinical trials will still be needed to determine if such methods can improve clinical outcomes.

Acknowledgements

References

1.   Gawarammana IB and Buckley NA. Medical management of paraquat ingestion. Br J Clin Pharmacol. 2011; 72: 745-57.

2.   Lin JL, Lin-Tan DT, Chen KH, Huang WH, Hsu CW, Hsu HH, Yen TH.. Improved survival in severe paraquat poisoning with repeated pulse therapy of cyclophosphamide and steroids. Intensive care medicine. 2011; 37: 1006-13.

3.   Dinis-Oliveira RJ, Duarte JA, Sanchez-Navarro A, Remiao F, Bastos ML and Carvalho F. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Crit Rev Toxicol. 2008; 38: 13-71.

4.   Dinis-Oliveira RJ, Sarmento A, Reis P, Amaro A, Remião F, Bastos ML, Carvalho F. Acute paraquat poisoning: report of a survival case following intake of a potential lethal dose. Pediatr Emerg Care. 2006; 22: 537-40.

5.   Zhang Q, Wu WZ, Lu YQ, Wang JZ, Shang AD, Yao F, Chen Y. Successful treatment of patients with paraquat intoxication: three case reports and review of the literature. Journal of Zhejiang University Science B. 2012; 13: 413-8.

6.   Roberts DM, Seneviratne R, Mohammed F, Patel R, Senarathna L, Hittarage A, Buckley NA, Dawson AH, Eddleston M. Intentional self-poisoning with the chlorophenoxy herbicide 4-chloro-2-methylphenoxyacetic acid (MCPA). Ann Emerg Med. 2005; 46: 275-84.

7.   Dawson AH, Eddleston M, Senarathna L, Mohamed F, Gawarammana I, Bowe SJ, Manuweera G, Buckley NA.. Acute human lethal toxicity of agricultural pesticides: a prospective cohort study. PLoS medicine. 2010; 7: e1000357.

8.   Vandenbogaerde J, Schelstraete J, Colardyn F and Heyndrickx A. Paraquat poisoning. Forensic Sci Int. 1984; 26: 103-14.

9.   Hughes RD, Millburn P and Williams RT. Biliary excretion of some diquaternary ammonium cations in the rat, guinea pig and rabbit. The Biochemical journal. 1973; 136: 979-84.

10.   Waddell WJ and Marlowe C. Tissue and cellular disposition of paraquat in mice. Toxicol Appl Pharmacol. 1980; 56: 127-40.

11.   Giri SN, Parker HR, Spangler WL. Pharmacokinetics of [14C]-paraquat and associated biochemical and pathologic changes in Beagle dogs following intravenous administration. Fundam Appl Toxicol. 1982; 2: 261-9.

12.   Hawksworth GM, Bennett PN and Davies DS. Kinetics of paraquat elimination in the dog. Toxicol Appl Pharmacol. 1981; 57: 139-45.

13.   Pond SM, Rivory LP, Hampson EC and Roberts MS. Kinetics of toxic doses of paraquat and the effects of hemoperfusion in the dog. J Toxicol Clin Toxicol. 1993; 31: 229-46.

14.   Murray RE and Gibson JE. Lethality and Pharmacokinetics of Paraquat in Rats. Toxicol Appl Pharmacol. 1971; 19: 405-&.

15.   Murray RE and Gibson JE. Paraquat disposition in rats, guinea pigs and monkeys. Toxicol Appl Pharmacol. 1974; 27: 283-91.

16.   Houze P, Baud FJ, Mouy R, Bismuth C, Bourdon R and Scherrmann JM. Toxicokinetics of paraquat in humans. Hum Exp Toxicol. 1990; 9: 5-12.

17.   Baud FJ, Houze P, Bismuth C, Scherrmann JM, Jaeger A and Keyes C. Toxicokinetics of paraquat through the heart-lung block. Six cases of acute human poisoning. J Toxicol Clin Toxicol. 1988; 26: 35-50.

18.   Hong SY, Yang JO, Lee EY and Kim SH. Effect of haemoperfusion on plasma paraquat concentration in vitro and in vivo. Toxicol Ind Health. 2003; 19: 17-23.

19.   Lin JL, Leu ML, Liu YC and Chen GH. A prospective clinical trial of pulse therapy with glucocorticoid and cyclophosphamide in moderate to severe paraquat-poisoned patients. Am J Respir Crit Care Med. 1999; 159: 357-60.

22

20.   Gawarammana I, Buckley NA, Mohammed F, Naser K , Jeganathan K, Munasinghe A ,

Ariyananada PL , Wannapuk K , Tomenson J, Wilks M, Eddleston M, Dawson AH. Abstracts of the 2012 International Congress of the European Association of Poisons Centres and Clinical Toxicologists, 25 May–1 June 2012, London, UK. Clin Toxicol (Phila). 2012; 50: 273-366.

21.   Roberts DM, Wilks MF, Roberts MS. Changes in the concentrations of creatinine, cystatin C and NGAL in patients with acute paraquat self-poisoning. Toxicol Lett. 2011; 202: 69-74.

22.   Gil HW, Yang JO, Lee EY and Hong SY. Clinical implication of urinary neutrophil gelatinase-associated lipocalin and kidney injury molecule-1 in patients with acute paraquat intoxication. Clin Toxicol (Phila). 2009; 47: 870-5.

23.   Roberts DM, Buckley NA, Mohamed F, Eddleston M, Goldstein DA, Mehrsheikh A, Bleeke MS, Dawson AH. A prospective observational study of the clinical toxicology of glyphosate-containing herbicides in adults with acute self-poisoning. Clin Toxicol (Phila). 2010; 48: 129-36.

24.   Wunnapuk K, Medley GA, Liu X, Grice JE, Jayasinghe S, Gawarammana I, Buckley NA, Roberts MS. Simple and sensitive liquid chromatography-tandem mass spectrometry methods for quantification of paraquat in plasma and urine: application to experimental and clinical toxicological studies. J Chromatogr B Analyt Technol Biomed Life Sci. 2011; 879: 3047-52.

25.   Cox DS, Kleiman NS, Boyle DA, Aluri J, Parchman LG, Holdbrook F, Fossler MJ. Pharmacokinetics and pharmacodynamics of argatroban in combination with a platelet glycoprotein IIB/IIIA receptor antagonist in patients undergoing percutaneous coronary intervention. J Clin Pharmacol. 2004; 44: 981-90.

26.   Cockcroft DW and Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976; 16: 31-41.

27.   Ette EI and Ludden TM. Population pharmacokinetic modeling: the importance of informative graphics. Pharm Res. 1995; 12: 1845-55.

28.   Du X, Li C, Kuti JL, Nightingale CH and Nicolau DP. Population pharmacokinetics and pharmacodynamics of meropenem in pediatric patients. J Clin Pharmacol. 2006; 46: 69-75.

29.   Van Kesteren C, Mathôt RA, Raymond E, Armand JP, Dittrich Ch, Dumez H, Roché H, Droz JP, Punt C, Ravic M, Wanders J, Beijnen JH, Fumoleau P, Schellens JH; Population pharmacokinetics of the novel anticancer agent E7070 during four phase I studies: model building and validation. J Clin Oncol. 2002; 20: 4065-73.

30. Sherwin CM, Saldana SN, Bies RR, Aman MG and Vinks AA. Population pharmacokinetic modeling of risperidone and 9-hydroxyrisperidone to estimate CYP2D6 subpopulations in children and adolescents. Ther Drug Monit. 2012; 34: 535-44.

31. Parke J, Holford NH and Charles BG. A procedure for generating bootstrap samples for the validation of nonlinear mixed-effects population models. Comput Methods Programs Biomed. 1999; 59: 19-29.

32. Sherwin CM, Kiang TK, Spigarelli MG and Ensom MH. Fundamentals of population pharmacokinetic modelling: validation methods. Clin Pharmacokinet. 2012; 51: 573-90.

33. Davies DS. Paraquat poisoning: the rationale for current treatment regimes. Hum Toxicol. 1987; 6: 37-40.

34. Kerr F, Patel AR, Scott PD and Tompsett SL. Paraquat poisoning treated by forced diuresis. British medical journal. 1968; 3: 290-1.

35. Chan BS, Seale JP and Duggin GG. The mechanism of excretion of paraquat in rats. Toxicol Lett. 1997; 90: 1-9.

36. Bismuth C, Scherrmann JM, Garnier R, Baud FJ and Pontal PG. Elimination of paraquat. Hum Toxicol. 1987; 6: 63-7.

37. Lock EA and Ishmael J. The acute toxic effects of paraquat and diquat on the rat kidney. Toxicol Appl Pharmacol. 1979; 50: 67-76.

38. Kim SJ, Gil HW, Yang JO, Lee EY and Hong SY. The clinical features of acute kidney injury in patients with acute paraquat intoxication. Nephrol Dial Transplant. 2009; 24: 1226-32.

39. Kang MS, Gil HW, Yang JO, Lee EY and Hong SY. Comparison between kidney and hemoperfusion for paraquat elimination. J Korean Med Sci. 2009; 24 Suppl: S156-60.

40. Coresh J and Stevens LA. Kidney function estimating equations: where do we stand? Curr Opin Nephrol Hypertens. 2006; 15: 276-84.

41. Bostom AG, Kronenberg F and Ritz E. Predictive performance of renal function equations for patients with chronic kidney disease and normal serum creatinine levels. J Am Soc Nephrol. 2002; 13.

42. Lin JL, Wei MC and Liu YC. Pulse therapy with cyclophosphamide and methylprednisolone in patients with moderate to severe paraquat poisoning: a preliminary report. Thorax. 1996; 51: 661-3.

24

25

Legends to figures

Figure 1. Scatter plot of plasma versus urine paraquat concentrations.

Figure 2. Diagnostic goodness-of-fit plots from the final population toxicokinetic model: Conditional weighted residuals versus time after ingestion (A), Conditional weighted versus population predicted concentration (B), Observed versus individual predicted concentrations (C), Observed versus population predicted concentrations (D). The open circles are the observed data, and the plotted line is the line of identity (y=x)

Figure 3. Diagnostic goodness-of-fit plots from the final population toxicodynamics model: Conditional weighted residuals versus paraquat plasma concentrations (A), Conditional weighted residuals versus population predicted CL_{cr} (B), Observed versus individual predicted CL_{cr} (C), Observed versus population predicted CL_{cr} (D). The open circles are the observed data, and the plotted line is the line of identity (y=x)

Figure 4. Scatter plots for visual predictive check**:** observed and simulated concentrations versus time (A),observed and simulated CL_{cr} versus paraquat plasma concentrations (B). Percentiles (5, 50 and 95) were calculated using the final PopTK and TD model.

Figure 5. Time course of empirical Bayes estimates of CL_{PQ} in PQ-poisoning patients.

Figure 6. Time course of median predicted PQ concentrations for initial CL_{cr} of 0.3, 6 and13L/h.

26

Tables

Table 1. Demographics of the patients enrolled in the population toxicokinetic and toxicodynamic studies

Characteristics	Toxicokinetic study (n= 78) Median (Range)	Toxicodynamic study (n= 68)Median (Range)
Male/female (n)	52/26	45/23
Age (y)	28 (14-76)	30 (14-76)
Weight (kg)	51 (35-66)	51 (35-66)
sCr (mg/dl)	2 (0.3-12.6)	2 (0.3-12.6)
CL_{cr} (L/h)	1.89 (0.29-13.25)	2.05 (0.29-13.25)
Ingestion volume (ml)	50 (5-750)	-
Ingestion dose (g)	10 (1-150)	-

sCr, serum creatinine; CL_{cr}, creatinine clearance

27

Table 2. Toxicokinetic model of paraquat

Description of model	OFV	Parameter (RSE(%))			Covariate effect (RSE(%))			IIV % (RSE(%))		
		V_1/F	CL/F	Q/F	V_1/F	CL/F	XF	V_1/F	CL/F	XF
Base model with mixed error model	9496	16.46 (16)	0.58 (15)	1.74 (8)	-	-	-	91 (20)	125 (44)	142 (38)
Final model with ingestion dose effect on F, CL_{cr} effect on CL_{PQ}, BW on V_1	9449	13.63 (16)	0.15 (31)	0.84 (11)	BW: 1.0	CL_{cr}: 1.57 (10)	Dose: 6.93E-8 (42)	89 (18)	124 (57)	142 (37)

OFV, objective function value; IIV, Inter-individual variability; F, bioavailability; V_1/F, apparent volume of central compartment; CL/F, clearance; Q/F, apparent inter-compartmental clearance; XF, the logit of bioavailability; BW, body weight; %CV, coefficient of variation; %SE, standard error

Table 3. Toxicodynamic model of paraquat

OFV, objective function value;; IIV, Inter-individual variability; IC_{50}, the concentration of PQ causing a 50% of maximum PQ induced-creatinine clearance

Description of model	OFV	Parameter (RSE(%))				Covariate effect (RSE(%))		IIV % (RSE(%))			
		IC_{50}	γ	E_0	E_{max}	E_0	E_{max}	IC_{50}	γ	E_0	E_{max}
Base model with log-additive error model	745	346 (3)	24.02 (2)	3.35 (2)	0.55 (2)	-	-	373 (62)	4 (0.01)	38 (1)	6 (0.02)
Final model with age, sex and BW effect on E_0,Treatment effect on E_{max}	727	207 (1)	5.02 (0.7)	3.23 (0.7)	0.62 (0.7)	Age: -0.07 (0.7) Male: 0.14 (0.7) BW: 1.39 (0.7)	Active: -0.08 (0.7) Control: -0.27 (0.7)	369 (20.2)	31 (0.1)	27 (0.1)	6 (0.01)

reduction; γ, a shape factor characterising the slope of the response; E_0, the baseline creatinine clearance; E_{max}, the maximum fractional decrease of creatinine clearance; BW, body weight; %CV, coefficient of variation; %SE, standard error

Table 4. Results of nonparametric bootstrap analysis of paraquat population toxicokinetics and toxicodynamics

Parameter	Final model				Bootstrap				
	Mean	%CV	2.5%CI	97.5% CI	Mean	%CV	Median	2.5% CI	97.5% CI
Toxicokinetics									
θ_V (L)	13.63	16	9.28	17.98	14.64	36	13.59	6.62	27.32
θ_{CL} (L/h)	0.15	31	0.06	0.24	0.22	86	0.16	0.02	0.73
θ_Q (L/h)	0.84	11	0.67	1.02	0.87	27	0.85	0.48	1.35
Proportional residual error (%)	0.48	39	0.12	0.84	0.80	148	0.44	0.11	4.38
Additive residual error (µg/L)	0.89	2	-	-	1.26	77	0.98	0.1	3.7
Toxicodynamics									
θ_{IC50} (µg/L)	207	1.04	202.91	211.36	286	80	208	126	806
θ_γ	5.02	0.74	4.94	5.09	5.49	15	5.15	3.97	6.84
θ_{E0} (L/h)	3.23	0.73	3.18	3.27	3.17	9	3.17	2.69	3.77
θ_{Emax} (L/h)	0.62	0.72	0.61	0.63	0.62	7	0.62	0.54	0.71
Log additive residual error(L/h)	0.58	-	-	-	0.58	6	0.58	0.51	0.64

θ_V, typical value of the volume distribution; θ_{CL}, typical value of the clearance, θ_Q, typical value of the inter-compartmental clearance; θ_{XF}, typical value of the logit of bioavailability; θ_{IC50}, typical value of the concentration of PQ causing a 50% of maximum PQ induced-creatinine clearance reduction; θ_γ, typical value of a shape factor characterising the slope of the response; θ_{E0}, typical value of the baseline creatinine clearance; θ_{Emax}, typical value of the maximum fractional decrease of creatinine clearance; %CV, coefficient of variation; CI, confidence interval

Table 5. Distribuion of empirical Bayes estimates of population toxicokinetic and toxicodynamic

parameters

Model	Parameter	Mean	SD	2.5%	97.5%CI
Toxicokinetics (n = 78)	V_1 (L/kg)	0.34	0.21	0.28	0.38
	V_2 (L/kg)	2.06	1.73	1.67	2.44
	$K_a(h^{-1})$	0.93	0.40	0.84	1.0
	CL_{PQ} (L/h)	1.17	3.52	0.32	2.01
	$t_{1/2\ \beta}(h)$	86.98	189.2	41.53	132.4
Toxicodynamics (n = 68)	$IC_{50}(\mu g/L)$	429	893	213	645
	$E_{max}(L/h)$	0.62	0.006	0.61	0.62

V_1, volume of distribution of central compartment; V_2, volume of distribution of peripheral compartment; K_a, absorption rate constant; CL_{PQ}, paraquat clearance; $t_{1/2\ \beta}$, elimination half-life; IC_{50}, the concentration of PQ causing a 50% of maximum PQ induced-creatinine clearance reduction; E_{max}, the maximum fractional decrease of creatinine clearance; SD, standard deviation; %CI, confidence interval

List of author contributions

Wunnapuk, K and Musuamba, FT : designed experiments and conducted the analyses; Wunnapuk, K, Roberts, MS , Buckley, NA and Musuamba, FT wrote the paper; Mohammed, F and Gawarammana, I: collected human plasma and urine samples and edited the paper; Gawarammana, I: performed randomized controlled trial study; Liu, X: edited the paper; Buckley, NA, Roberts, MS and Verbeeck, RK: supervised the project.

Conflict of interest

All authors have completed the Unified Competing Interest form at

www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and

declare: no support from any organisation for the submitted   for the submitted work; no financial

relationships with any organisations that might have an interest in the submitted work in the

previous 3 years ; no other relationships or activities that could appear to have influenced the

submitted .
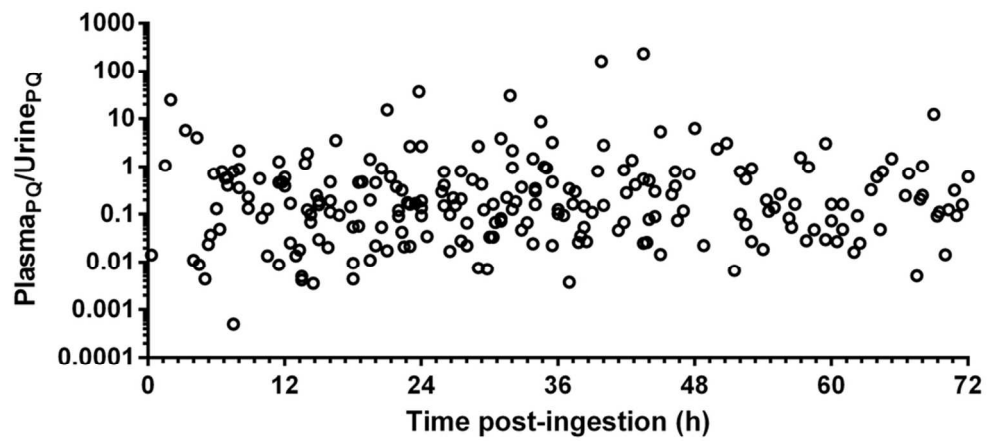
Figure 1. Scatter plot of plasma versus urine paraquat concentrations.
43x20mm (600 x 600 DPI)
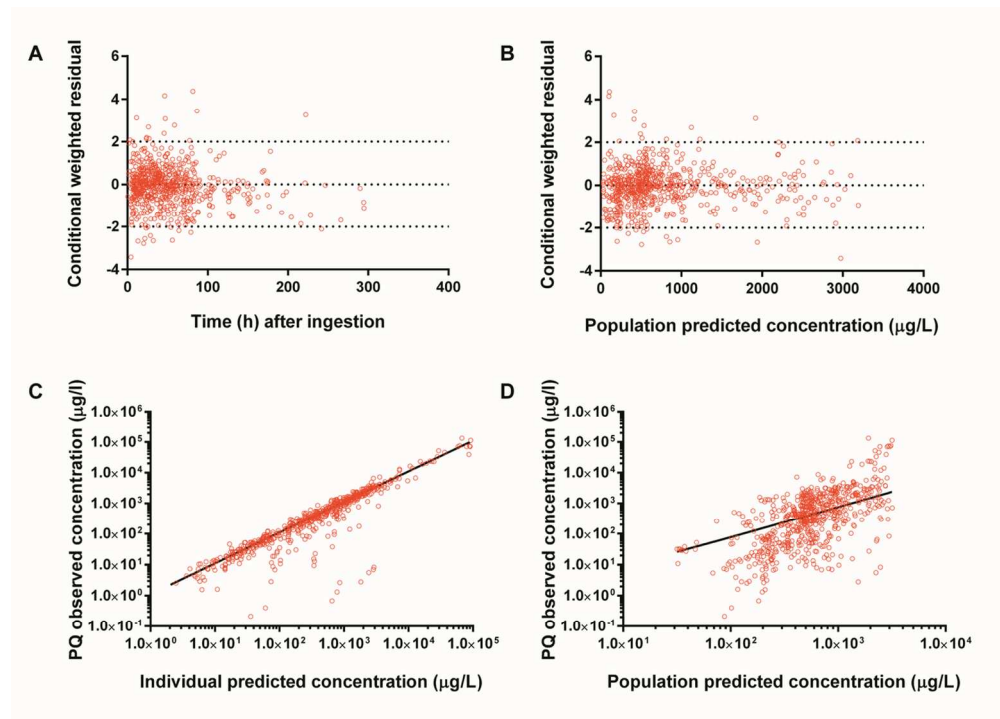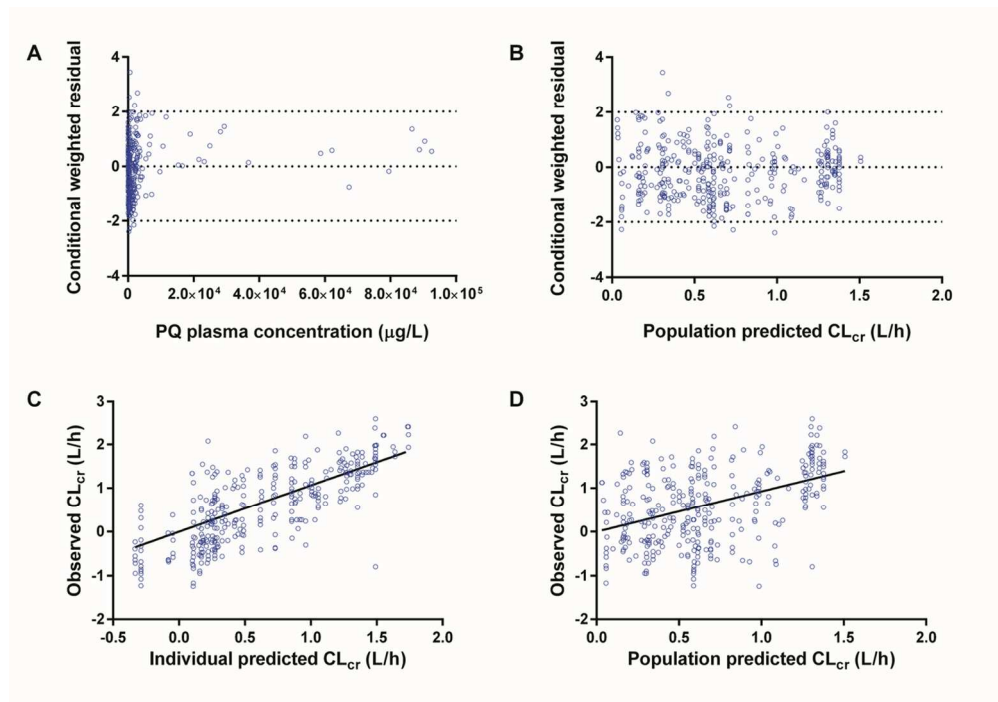
Figure 2. Diagnostic goodness-of-fit plots from the final population toxicokinetic model: Conditional weighted residuals versus time after ingestion (A), Conditional weighted versus population predicted concentration (B), Observed versus individual predicted concentrations (C), Observed versus population predicted concentrations (D). The open circles are the observed data, and the plotted line is the line of identity (y=x)
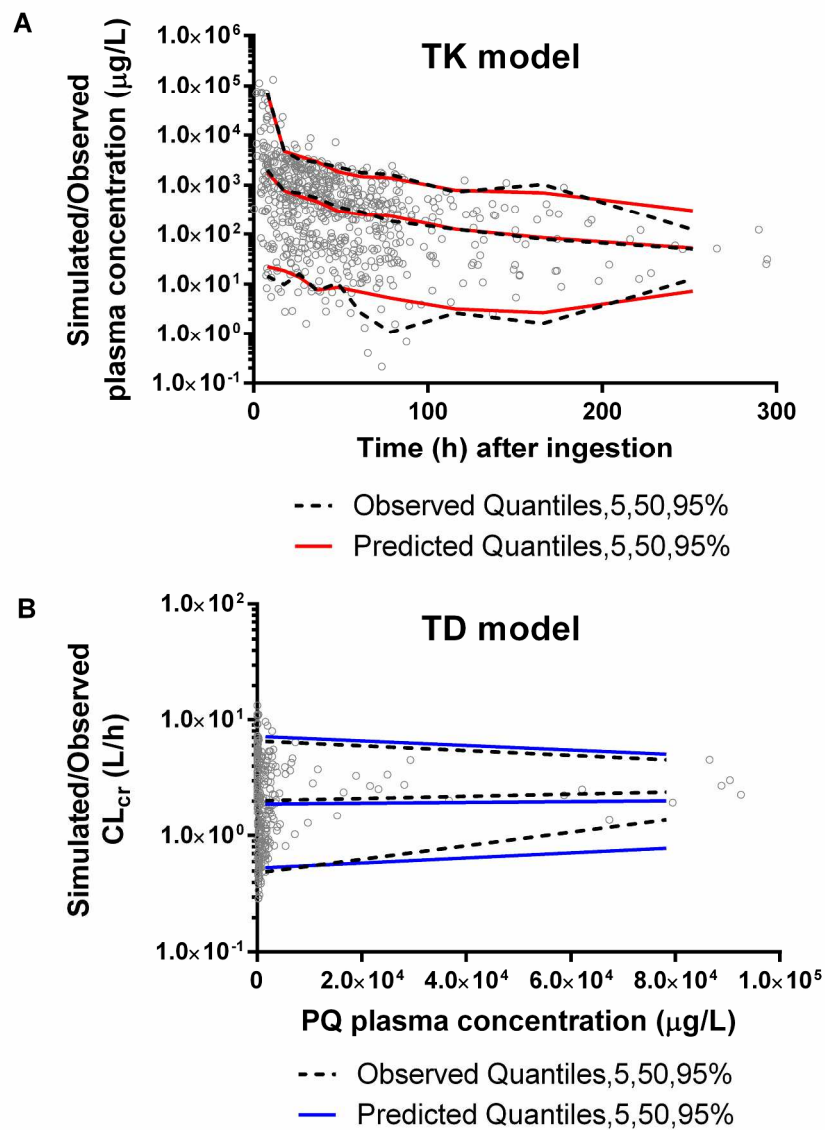59x42mm (600 x 600 DPI)

Figure 3. Diagnostic goodness-of-fit plots from the final population toxicodynamics model: Conditional weighted residuals versus paraquat plasma concentrations (A), Conditional weighted residuals versus population predicted CLcr (B), Observed versus individual predicted CLcr (C), Observed versus population predicted CLcr (D). The open circles are the observed data, and the plotted line is the line of identity (y=x)
58x40mm (600 x 600 DPI)

Figure 4. Scatter plots for visual predictive check: observed and simulated concentrations versus time (A),observed and simulated CLcr versus paraquat plasma concentrations (B). Percentiles (5, 50 and 95) were calculated using the final PopTK and TD model.
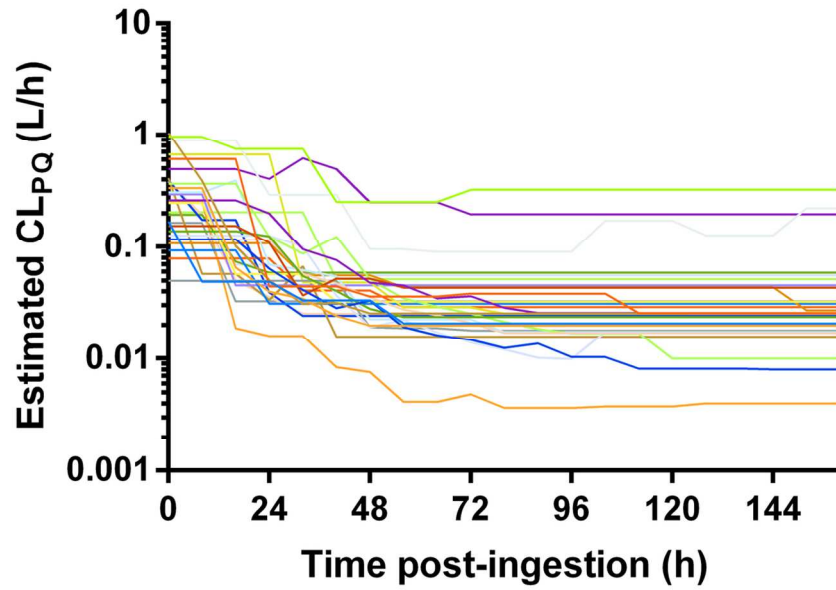121x165mm (600 x 600 DPI)

Figure 5.Time course of empirical Bayes estimates of CLPQ   in PQ-poisoning patients.
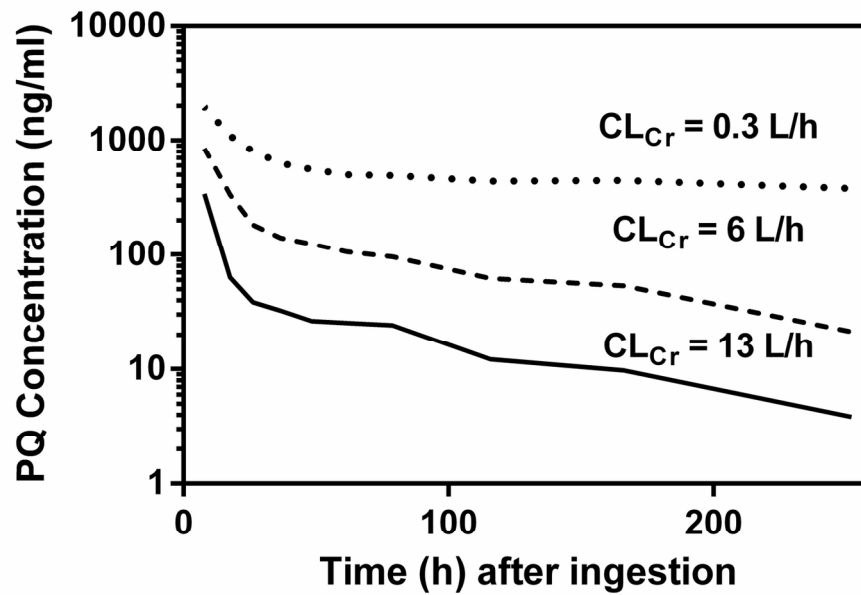61x42mm (600 x 600 DPI)

Figure 6. Time course of median predicted PQ concentrations for initial CLcr of 0.3, 6 and13L/h.
73x48mm (600 x 600 DPI)