Pharmacokinetic Assay

Midazolam, 1-OH-midazolam, 4-OH-midazolam, 1-OH-midazolam-glucuronide were analysed using LC-MS/MS in the positive ionisation mode on a Shimadzu LC-30 (Nishinokyo-Kuwabaracho, Japan) system coupled to an ABSciex (Framingham, MA, USA) 5500 Qtrap MS. To 10 µl of patients' plasma, 75 µl acetonitril/methanol 84:16 (v%/v%) containing the internal standards midazolam-d5, 1-OH-midazolam-d5 and 4-OH-midazolam-d5, was added to precipitate proteins. Samples were vortexed, stored at -20°C for 30 minutes, vortexed again and centrifuged. For the determination of midazolam, 1-OH-midazolam and 4-OH-midazolam, 3 µl was injected onto a Thermo Scientific Hypersil Gold RP (50 x 2.1 mm, 1.9 µm) column. A stepwise chromatographic gradient was applied using acetonitril and water with a constant 5% addition of 1% ammonium formate / 2% formic acid in water. The flow was 400 µl/min and the column-oven temperature was 40°C. Midazolam, 1-OH-midazolam, 4-OH-midazolam, 1-OH-midazolam-glucuronide were measured as [M+H]+, using the mass transitions 326.1/291.1, 342.1/168.1, 342.1/234.1 and 518.1/324.1, respectively. The method was validated over a range of 4 – 1000 ng/mL for midazolam, 2 – 500 ng/mL for 1-OH-Midazolam, 4-OH-midazolam and 8 – 2000 ng/mL for 1-OH-midazolam-glucuronide. The accuracies ranged from 93.5% to 105.5%, the intra-day precisions were below 9.6% and the inter-day precisions were below 12.9%.

Pharmacokinetic analysis

Goodness of fit plots including observations vs individual predictions, observations vs population predictions, conditional weighted residuals vs time and conditional weighted residuals vs population predictions were used for diagnostic purposes. Furthermore, the confidence interval of the parameter estimates, the correlation matrix and visual

improvement of the individual plots were used to evaluate the model. Midazolam and metabolites were modelled simultaneously, and concentrations were expressed as μmol/L. The volumes of 1-OH-midazolam were fixed to 0.9 times the volume of the central compartment of midazolam.(7) Furthermore, it was assumed that the volume of distribution of 1-OH-midazolam equaled the volume of distribution of 4-OH-midazolam and that the central volume of distribution of 1-OH-midazolamglucuronide equaled the peripheral volume of distribution of 1-OH-midazolamglucuronide. Inter-individual variability was assumed to follow a log normal distribution. Residual variability was tested using proportional, additive or a combined proportional and additive error models for midazolam and metabolites. The M6 method was used to deal with data below the limit of quantification (LLQ).(8)

Pharmacodynamic analysis

The COMFORT-B scores were described using a logistic regression model for the probability (P) of observing a particular COMFORT-B sedation level by use of the Laplacian estimation method with the LIKELIHOOD option and Method =1. For this purpose, the COMFORT-B scale were categorized based on three clinically significant cut-off points,

1. COMFORT B > 16 in combination with a NRS <4 indicating undersedation
2. COMFORT-B ≥ 11 and ≤ 16 indicating adequate sedation
3. COMFORT-B < 11 indicating oversedation

The cumulative logits L_i were modeled as:

$Logit_1 = \vartheta_1 + \vartheta_3 * C_{1,ij} + \eta$

$Logit_2 = \vartheta_1 + \vartheta_2 + \vartheta_3 * C_{1,ij} + \eta$

$\vartheta_{1\text{-}2}$ describes the sedation level without midazolam. ϑ_3 is the typical value of the increment in the logit relating to midazolam concentration. η is a normally distributed, zero mean random variable with standard deviation ω describing the discrepancy between the typical value of the logit and the individual logit.

The corresponding probabilities (P) are given by:

$$F_i = \frac{\exp(L_i)}{(1+\exp(L_i))}$$

$P_1 = 1\text{-}F_1$

$P_2 = F_1\text{-}F_2$

$P_3 = F_2$

For diagnostic purposes predicted variables as midazolam concentrations and time were rank-ordered independent from which individual the data were obtained and divided in bins. The observed probabilities were calculated based on the number of observations of COMFORT scores 1, 2 and 3 in each bin and the total number of observations for that bin. The predicted probabilities in each bin were the mean probabilities of the individual projections from P1, P2 and P3 of the observations in that bin. Observed and predicted probabilities were plotted versus the median values of the time or midazolam concentrations for each group and inspected for signs of model misspecifications as suggested by Ette et al.

Reference:

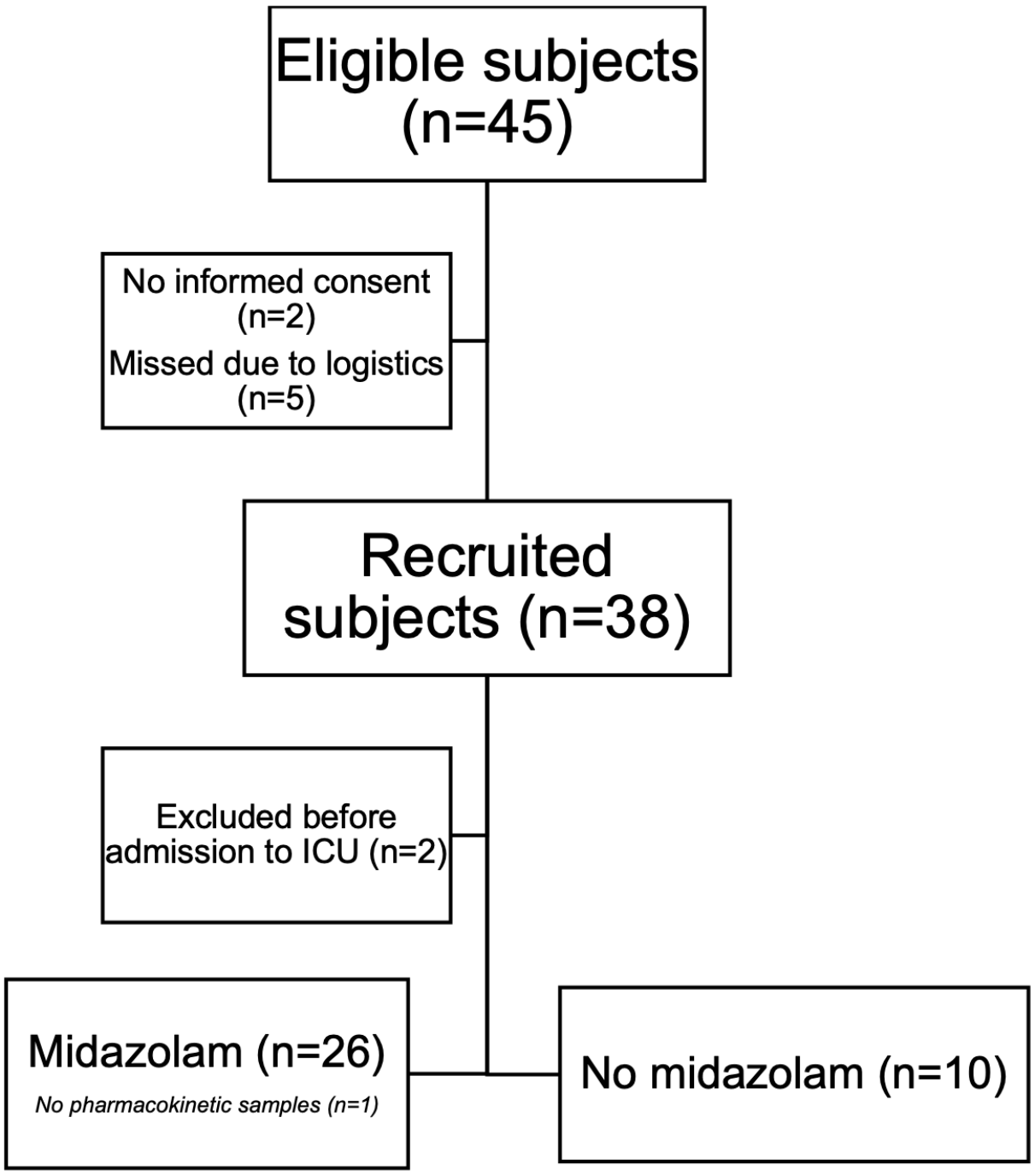
Ette, Williams. Pharmacometrics, The Science of Quantitative Pharmacology 2007, chapter 24, 25

Supplementary Table 1 Results of the analysis for the pharmacodynamic model.

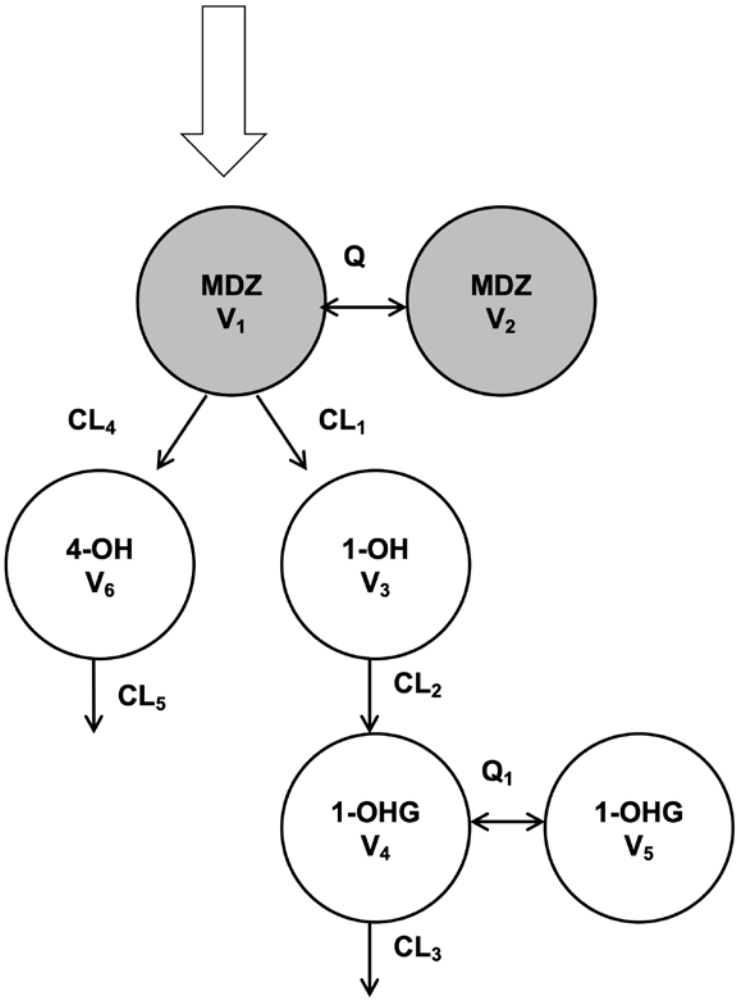
Model	-2LL	Description
E1=C1= θ_1 + ETA(1) E2=C2= θ_1 + θ_2 + ETA(1)	567	
E1=C1 + θ_3*CP E2=C2 + θ_3*CP	560.69	Basic model (midazolam effect)
E1=C1 + θ_3*CP+ θ_4*DOWN E2=C2 + θ_3*CP+ θ_4*DOWN	560.653	Effect of Down syndrome
E1=C1 + θ_3*NRS E2=C2 + θ_3*NRS	545.848	NRS effect, with 0=NRS<4 and 1=NRS ≥4))
E1=C1 + θ_3*CP*(1-NRS) + θ_4*NRS E1=C1 + θ_3*CP*(1-NRS) + θ_4*NRS	543.43	At NRS≥4, no midazolam effect
E1=C1 + θ_3*CP+ θ_4*NRS E2=C2 + θ_3*CP+ θ_4*NRS	540.411	Final model (Midazolam effect + NRS effect)

CP = individual predicted midazolam concentration (microg/L)
NRS= numerical rating score with 0=NRS<4 and 1=NRS ≥4
DOWN, children with and without Down syndrome
-2LL, objective function

Supplementary Figure 1 Flowchart of recruited subjects

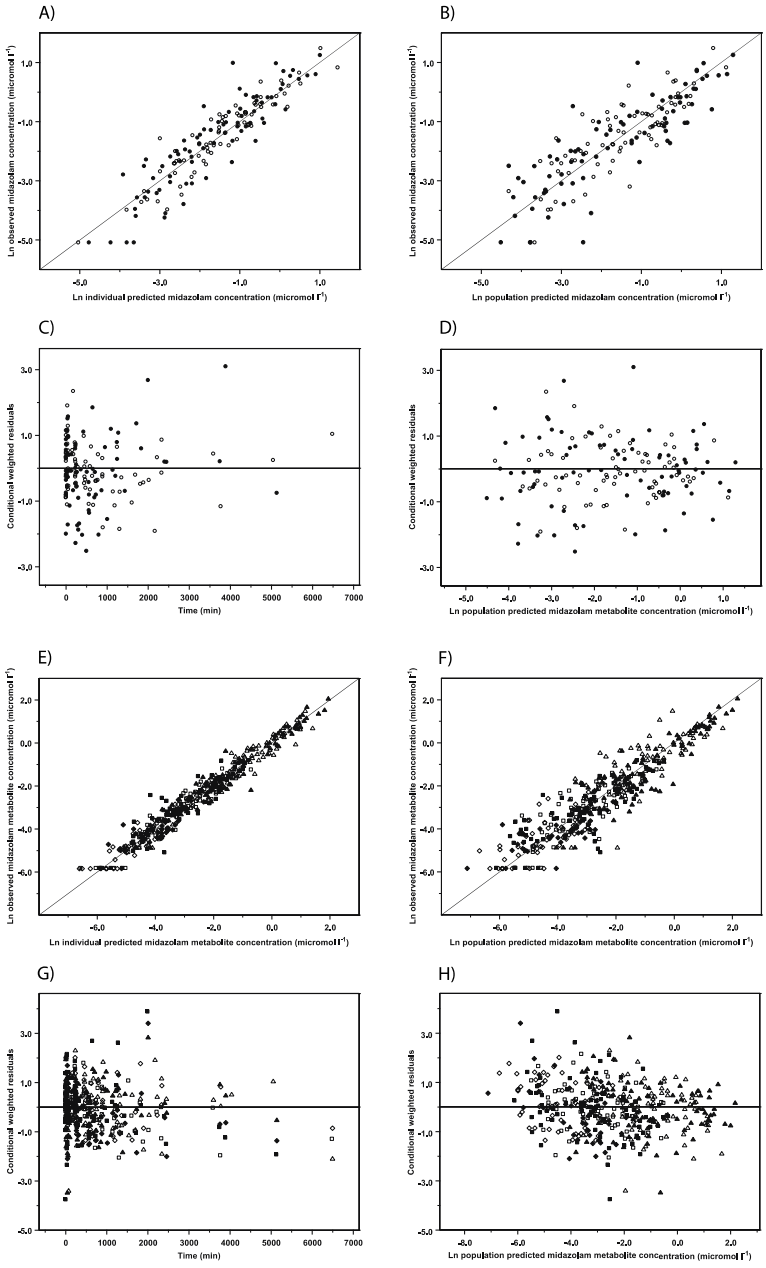


Supplementary Figure 2 Schematic representation of the pharmacokinetic model of midazolam (MDZ) and its metabolites 1-OH-midazolam (1-OH), 4-OH-midazolam (4-OH) and 1-OH-midazolamglucuronide (1-OH-G) in plasma.



CL1= clearance of midazolam to 1-OH-midazolam; CL2= clearance of 1-OH-midazolam to 1-OH-midazolam-glucuronide; CL3= clearance of 1-OH-midazolam-glucuronide; CL4= clearance of midazolam to 4-OH-midazolam; CL5= clearance of 4-OH-midazolam; Q= intercompartmental clearance of midazolam; Q2= intercompartmental clearance of 1-OH-midazolam-glucuronide; V1= central volume; V2= peripheral volume of distribution for midazolam; V3 = volume of distribution for 1-OH-midazolam; V4= central volume of distribution for 1-OH-glucuronide; V5= peripheral volume of distribution for 1-OH-glucuronide; V6= volume of distribution for 4-OH-midazolam

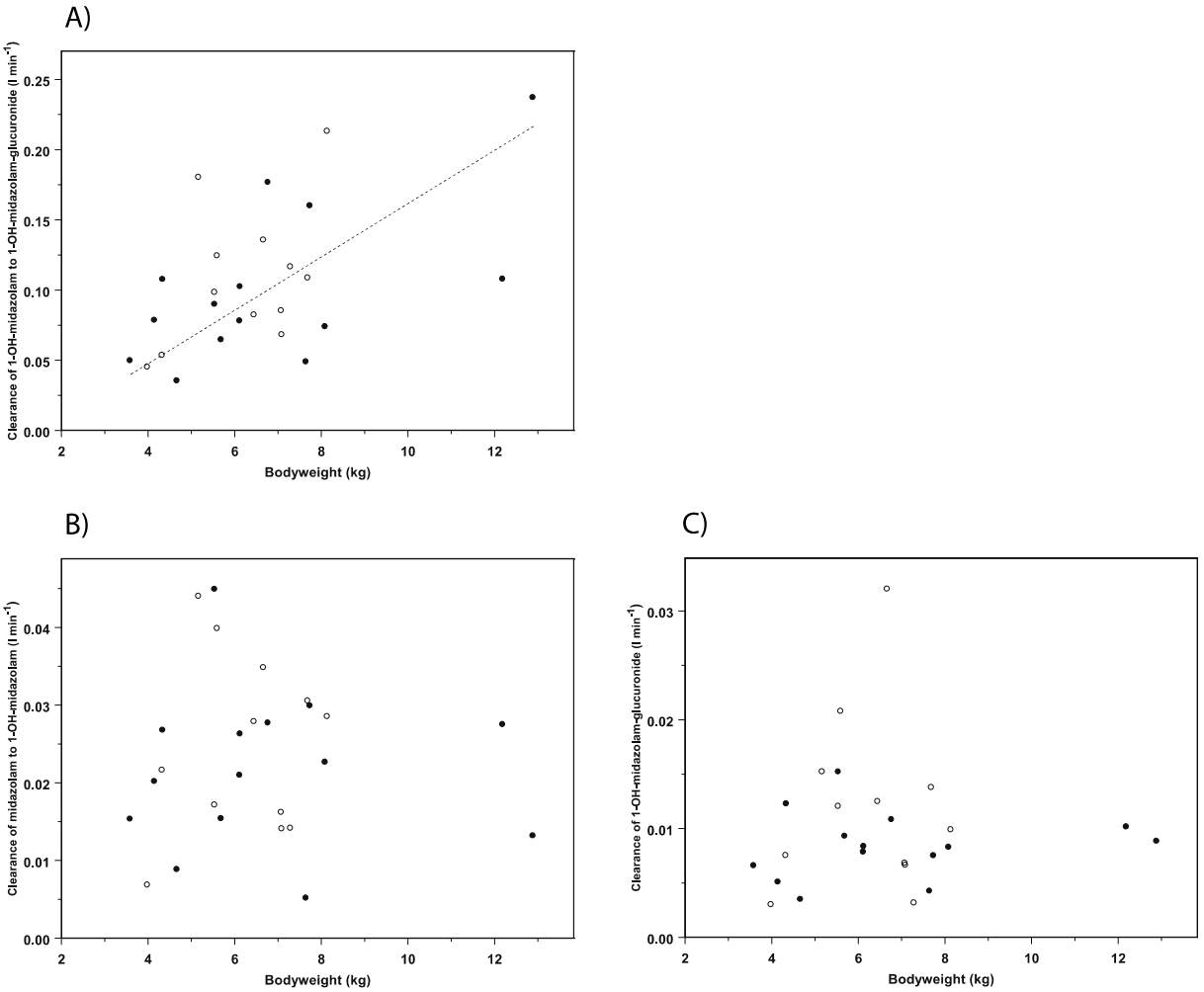
Supplementary Figure 3 Diagnostic plots for the final PK model including observations vs individual predictions, observations vs population predictions, conditional weighted residuals vs time and conditional weighted residuals vs population predictions for log transformed concentrations in plasma of midazolam and metabolites



Midazolam=circle, upper panels A, B, C and D) and metabolites (1-OH-midazolam=box, 1-OH-midazolam-glucuronide=triangle and 4-OH-midazolam=diamond, lower panels E, F, G and H),

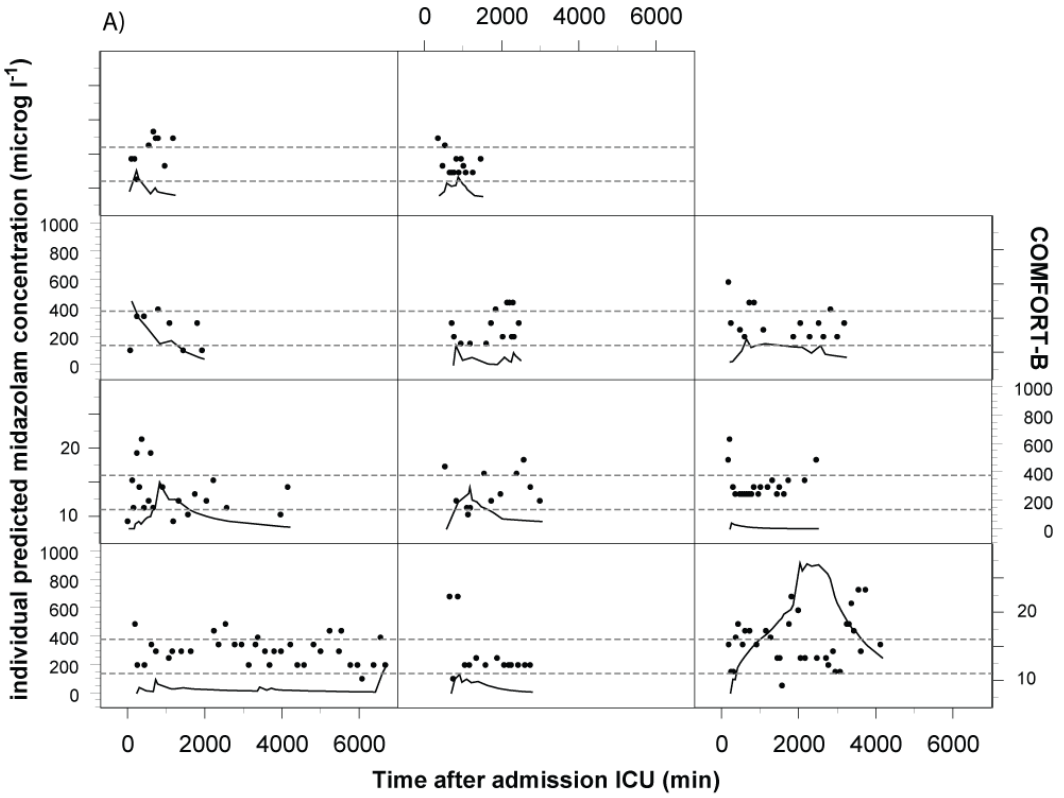
with x=y identity line. The solid symbols indicate children with Down Syndrome, the open symbols children without Down Syndrome.

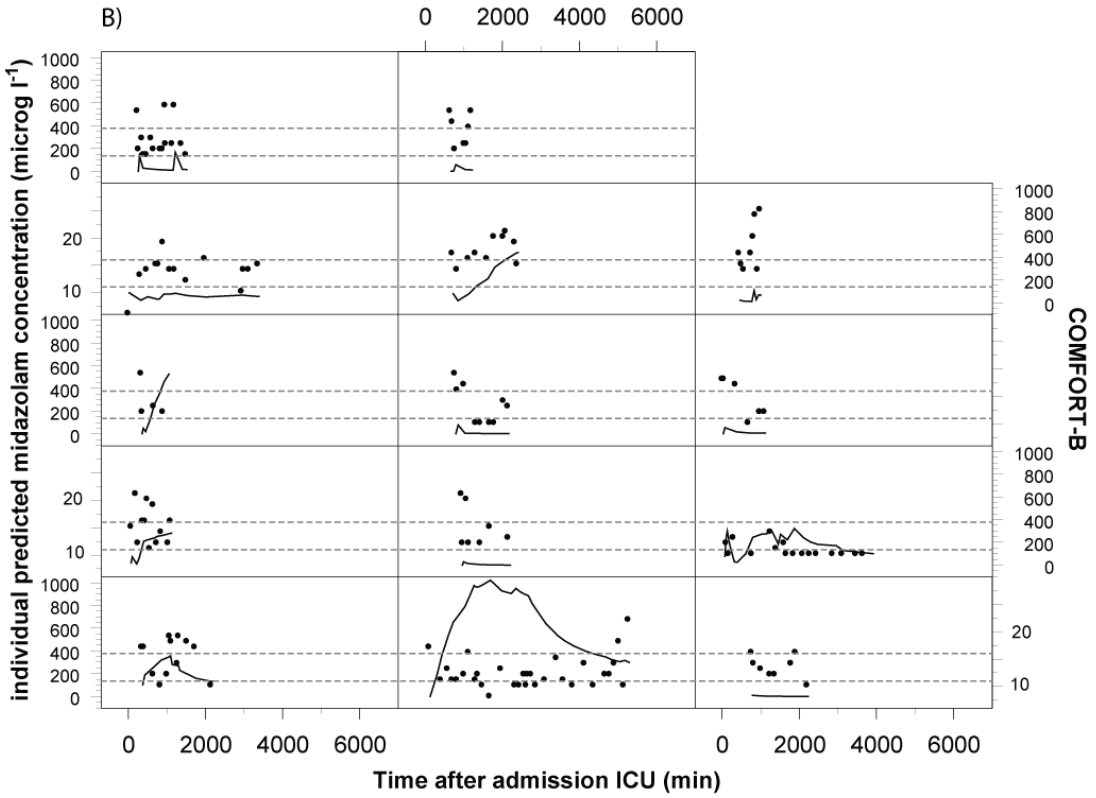
Supplementary Figure 4 Relation between bodyweight and elimination clearances



Individual post hoc estimates of the simple model for A) Clearance of 1-OH-midazolam to 1-OH-glucuronide (Cl2) vs bodyweight (kg) and the estimated relation in the final model according to Table 2, B) Clearance of midazolam to 1-OH-midazolam (CL1) vs bodyweight and C) Clearance of 1-OH-midazolam-glucuronide (CL3) vs bodyweight. The solid symbols indicate children with Down Syndrome, the open symbols children without Down Syndrome.

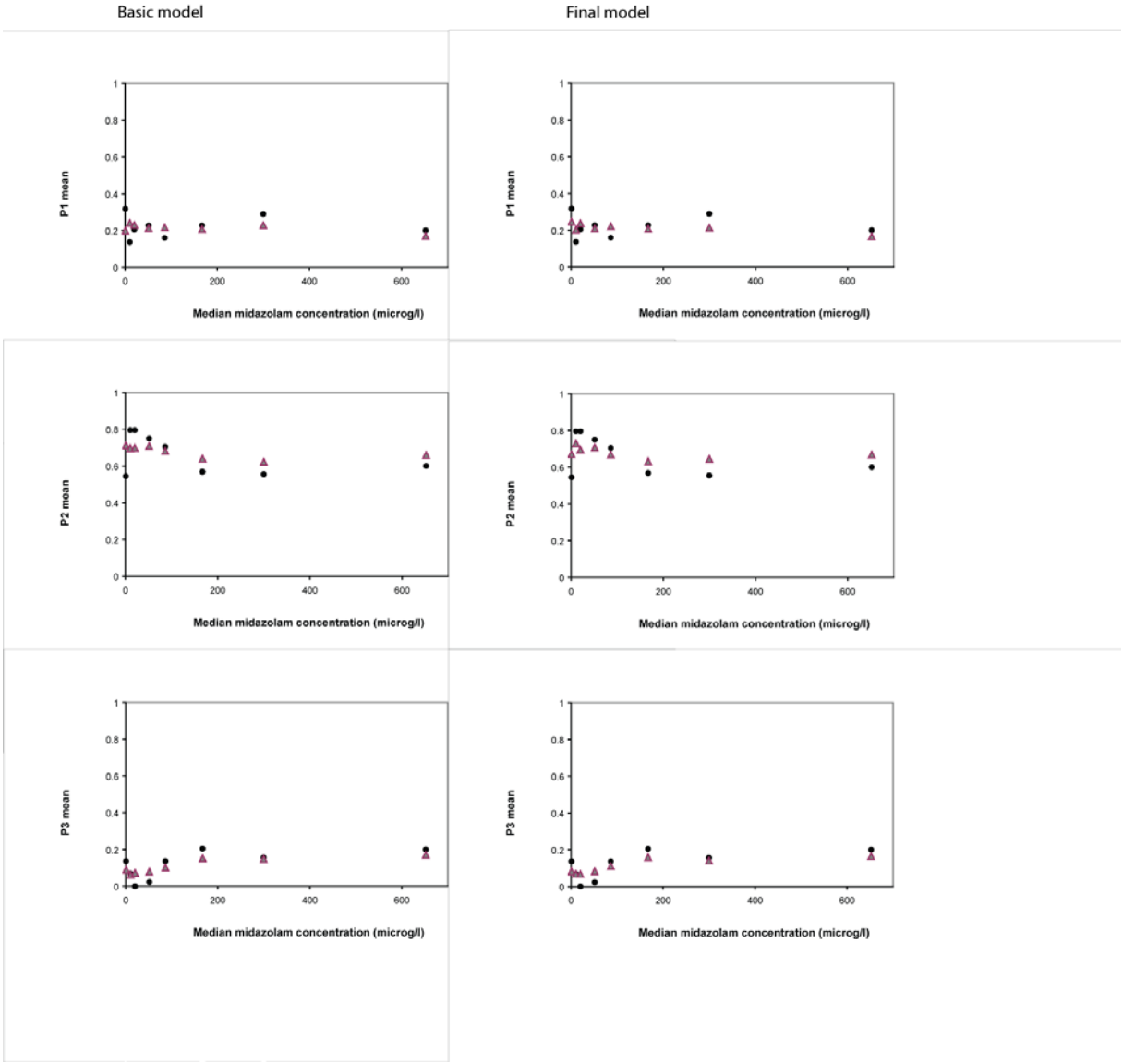
Supplementary Figure 5 COMFORT-B observations and corresponding midazolam concentrations.





Individual predicted midazolam concentrations (line) versus time after admission to the Intensive Care Unit (ICU) and the COMFORT-B scores (circles) for the children without Down syndrome (A) and with Down syndrome (B). The upper dotted line indicates undersedation (COMFORT-B=16), the lower dotted line oversedation (COMFORT-B=11).

Supplementary Figure 6 Pharmacodynamic diagnostics



Diagnostic plots for the basic (left) and final (right) model showing observed probabilities (circles) and predicted probabilities (triangles) on COMFORT-B categorized 1

(undersedation), 2 (adequately sedated) and 3 (oversedation) versus the median of the binned individual predicted midazolam concentrations for each of 8 groups.