**Supplementary Material**

**Statement of Ethics**

The PharmaCool study was approved by the Ethics Committees of all twelve participating NICUs in the Netherlands and Belgium. Parental informed consent was obtained for all included patients.

**Disclosure Statement**

Funding for this study was received from the Netherlands Organization for Health Research and Development (ZonMw) Priority Medicines for Children. Grant number: 40-41500-98-9002. The

authors declare no conflicts of interest.

**Appendix**

*Bioanalyses*

Plasma concentrations of phenobarbital were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The lower limit of quantification (LLQ) was 2.73 mg/L. The calibration curves were linear from 2.73 to 58.4 mg/L. Between-run and within-run coefficients of variation were <9%.

Plasma concentrations of midazolam, OHM and HMG were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The LLQ was 0.02 mg/L for midazolam, OHM and HMG. The calibration curves were linear from 0.02 to 1.5 mg/L for midazolam and OHM and between 0.02 and 10.0 mg/L for HMG. Between-run and within-run coefficients of variation were <2% for midazolam and <5% for OHM and HMG. Samples were stored at -80°C until analyses at the Clinical Pharmaceutical and Toxicological Laboratory of the Department of Clinical Pharmacy of the University Medical Center Utrecht, the Netherlands. Samples with initial results above the range of linearity were diluted and reanalysed. Figure S1 shows the observed OHM and HMG plasma concentrations.

*Population pharmacokinetic analyses*

PK analyses were performed using non-linear mixed effects modelling NONMEM (version 7.3, Icon Development Solutions) with R (version 3.4.1), Xpose (version 4) for data visualization and Piraña for run management. In both models, the exponent defining the relationship of BW and clearance (Cl) was fixed to 0.75 and the exponent defining the relationship of BW and volume of distribution (V) was fixed to 1. In the phenobarbital model, an additive error model was used to model residual unexplained variability.

In order to simultaneously fit the midazolam, OHM and HMG data, all units of dose and concentration for were converted to μmol and μmol/L, respectively. The fractions (F) of midazolam converted to OHM and HMG were unknown; metabolite parameters are therefore estimated relative to F. For midazolam, 48 measurements (12.8%) were below the LLQ; for OHM, 100 measurements (36.1%) and for HMG, 2 measurements (0.53%). To account for below LLQ data, the M3 method was used[35] For each compound, the additive error was fixed on LLQ/2. Separate proportional error models were used to model residual unexplained variability. Because the formation rate for OHM is slower than its elimination rate, OHM volume of distribution could not be estimated and was fixed to 4.18 L based on literature.[8] Model evaluation demonstrated that the final models adequately described the data, although with considerable variability on both clearance and volume of distribution of all compounds. Goodness-of-fit plots of observed versus population and individual predicted concentrations showed no systematic deviation and the weighted residuals were homogeneously scattered for all compounds (Figures S2-S5).

\\storage.karger.intra\ProductionP$\Articles\000\499\330\Original\Appendix_Figure_1.tif

Figure S1: Observed OHM (left) and HMG (right) plasma concentrations versus time after birth.

\\storage.karger.intra\ProductionP$\Articles\000\499\330\Original\Appendix_Figure_2.tif

Figure S2: Phenobarbital goodness-of-fit plots. A = observed vs population predicted plasma concentrations; B = observed vs individual predicted plasma concentrations; C = population conditional weighted residuals vs population predicted plasma concentrations; D = population conditional weighted residuals vs time after birth.

\\storage.karger.intra\ProductionP$\Articles\000\499\330\Original\Appendix_Figure_3.tif

Figure S3: Midazolam goodness-of-fit plots. A = observed vs population predicted plasma concentrations; B = observed vs individual predicted plasma concentrations; C = population conditional weighted residuals vs population predicted plasma concentrations; D = population conditional weighted residuals vs time after birth.

\\storage.karger.intra\ProductionP$\Articles\000\499\330\Original\Appendix_Figure_4.tif

Figure S4: OHM goodness-of-fit plots. A = observed vs population predicted plasma concentrations; B = observed vs individual predicted plasma concentrations; C = population conditional weighted residuals vs population predicted plasma concentrations; D = population conditional weighted residuals vs time after birth; OHM = 1-hydroxymidazolam.

\\storage.karger.intra\ProductionP$\Articles\000\499\330\Original\Appendix_Figure_5.tif

Figure S5: HMG goodness-of-fit plots. A = observed vs population predicted plasma concentrations; B = observed vs individual predicted plasma concentrations; C = population conditional weighted residuals vs population predicted plasma concentrations; D = population conditional weighted residuals vs time after birth; HMG = hydroxymidazolam glucuronide.