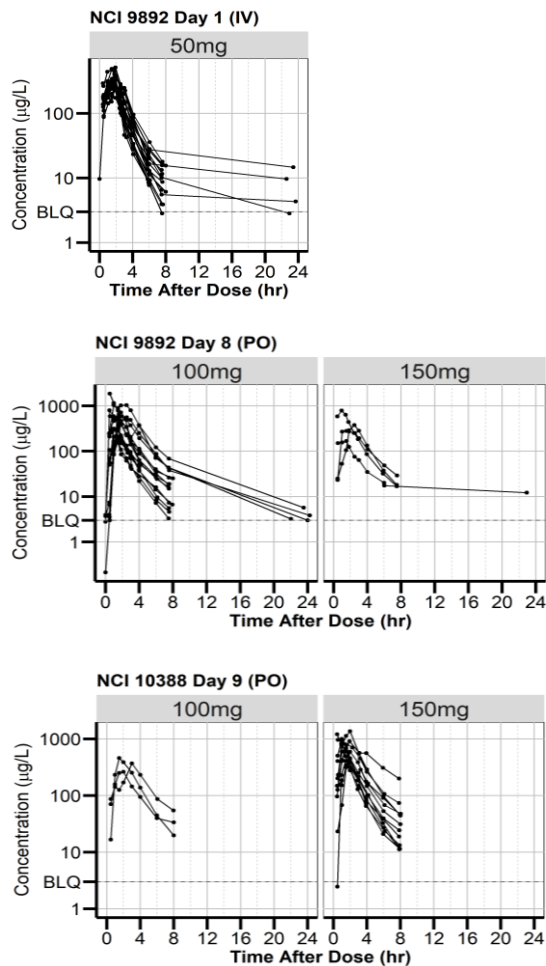


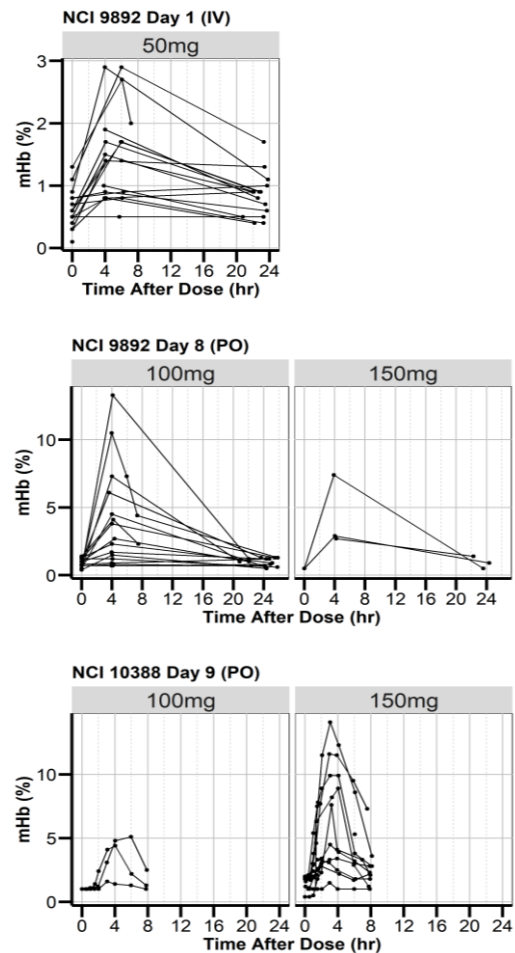
Supplementary Material:

Figure S1. Individual concentration-time curves.

A) Triapine Concentration

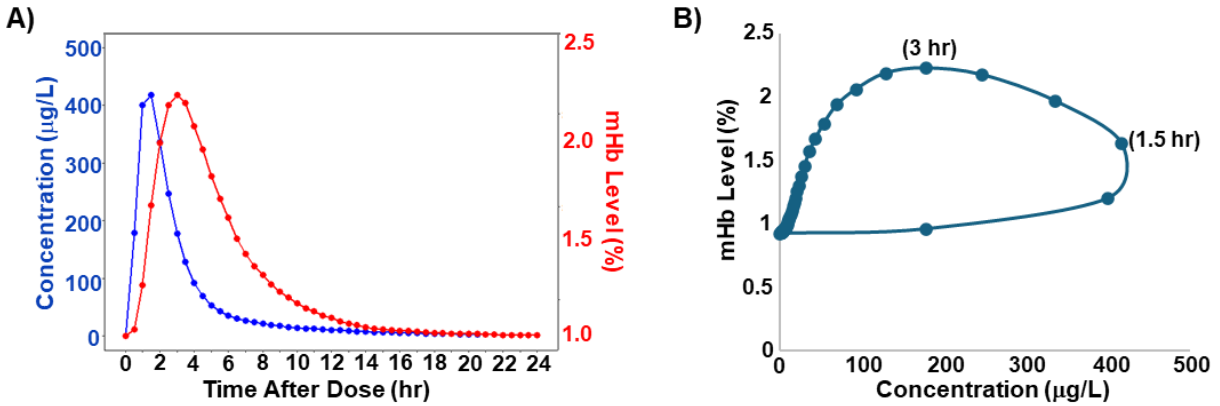


B) mHb Concentration



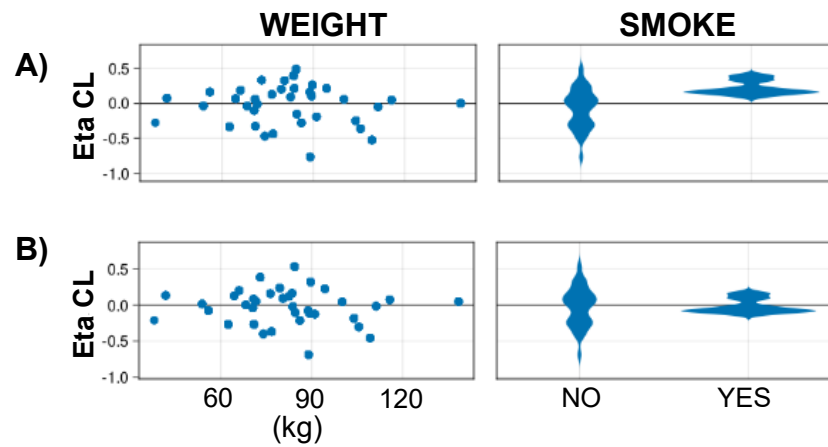
Individual concentration-time curves are plotted by study, dose, and day on a semi-log scale. **A)** Triapine concentrations. For subjects in study NCI 9892, PK samples were collected at the following time points: prior to dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 24 hours after dose. At the 24-hour time point, most individuals had concentrations below the lower limit of quantification (LLOQ); hence LLOQ data point for those individuals are not shown. For subjects in study NCI 10388, PK samples were collected at the following time points: prior to dose, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours after dose. **B)** Methemoglobin (mHb) concentrations. For subjects in study NCI 9892, PD samples were collected at the following time points: prior to dose, 4, and 24 hours after dose. For subjects in study NCI 10388, PD samples were collected at the following time points: prior to dose, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours after dose.

Figure S2. Triapine concentration and mHb relationship shows hysteresis.



The median triapine concentrations are plotted against the median mHb concentrations from 1000 simulated datasets. Each dot represents a timepoint, spaced 30 minutes apart. **(A)** Peak concentration (blue, left Y-axis) occurs at 1.5 hours while peak mHb concentration (red, right Y-axis) occurs at 3 hours, indicating a delay in the mHb response. **(B)** The hysteresis loop is counterclockwise, suggesting that the mHb effect lags behind triapine concentration. Time points indicated in parentheses are time after dose.

Figure S3. Empirical Bayes Estimates on CL versus selected Covariates.



The effect of smoking reduces individual variability in clearance (CL). **(A)** When weight is the only covariate considered, the distributions of smoking status do not symmetrically center around 0. **(B)** After adjusting for the smoking status as a covariate, the distributions center around 0, indicating that smoking status explains the observed effect.

Table S1. Comparison of parameter estimates using M1 and M3 methods.

	Laplace Estimation	
Parameters	M1	M3
Ktr	3.16	2.94
CL	50.4	54.1
Vc	66.9	53.9
Q	11.9	22.3
Vp	64.0	32.1
F	0.79	0.79
Effect smoker	0.39	0.38
Ω^2 Ktr	0.21	0.17
Ω^2 CL	0.08	0.08
Ω^2 Vc	0.05	0.08
Ω^2 F	1.34	1.38
σ^2 additive	14.8	21.5
σ^2 proportional	0.10	0.10

Parameter estimates were obtained using the M1 and M3 methods (Beal, 2001) with the Laplace estimation method. The similarity in CL estimates between the M1 and M3 methods suggests that the exclusion of LLOQ observations had minimal influence on the estimation of CL, indicating that the information contained in the censored data was not critical for characterizing drug elimination.

Abbreviations: Ktr, transit rate constant; CL, clearance; Vc, central volume of distribution; Q, intercompartmental clearance; Vp, peripheral volume of distribution; F, bioavailability; Effect_smoker, effect of smoking on CL; σ_{additive} , additive residual error; $\sigma_{\text{proportional}}$, proportional residual error; CI, confidence interval.

Table S2. Non-compartmental analysis results reported as average estimates.

Dose (mg)	C_{max} (µg/L) geomean (geosd)	AUC_(0-∞) (µg/L*h) geomean (geosd)
Inf 50mg		
Smoker (n = 6)	262 (1.2)	656 (1.1)
Nonsmoker (n = 14)	324 (1.3)	903 (1.4)
PO 100mg		
Smoker (n = 4)	286 (1.6)	742 (2.1)
Nonsmoker (n = 15)	544 (1.9)	1321 (1.9)
PO 150mg		
Smoker (n = 2)	252 (1.8)	1067 (1.2)
Nonsmoker (n = 15)	620 (1.8)	1765 (1.7)

Geomean, geometric mean; geosd, geometric standard deviation

Supplemental Methods:

All codes are in Pumas:

Methods S1: Two-compartment pop PK model with two-compartment Erlang absorption

```
mod_erlang_comb_wt_smoke = @model begin
```

```
  @param begin # defining what will be estimating/ fixed effects
```

```
    tvcl ∈ RealDomain(; lower = 0.0001)
    tvvc ∈ RealDomain(; lower = 0.0001)
    tvktr ∈ RealDomain(; lower = 0.0001)
    tvq ∈ RealDomain(lower = 0.0001)
    tvvp ∈ RealDomain(lower = 0.0001)
    tvbio ∈ RealDomain(lower = 0.0001, upper = 1.0)
    tveff_smoker ∈ RealDomain(lower = 0.001, upper=1)
     $\Omega^2_{\text{bio}}$  ∈ RealDomain(lower = 0.0001)
     $\Omega^2_{\text{cl}}$  ∈ RealDomain(lower = 0.0001)
     $\Omega^2_{\text{vc}}$  ∈ RealDomain(lower = 0.0001)
     $\Omega^2_{\text{ktr}}$  ∈ RealDomain(lower = 0.0001)
     $\sigma^2_{\text{add}}$  ∈ RealDomain(lower = 0.0001)
     $\sigma^2_{\text{prop}}$  ∈ RealDomain(lower = 0.0001)
```

```
  end
```

```
  @random begin
```

```
     $\eta_{\text{cl}} \sim \text{Normal}(0, \text{sqrt}(\Omega^2_{\text{cl}}))$ 
     $\eta_{\text{vc}} \sim \text{Normal}(0, \text{sqrt}(\Omega^2_{\text{vc}}))$ 
     $\eta_{\text{ktr}} \sim \text{Normal}(0, \text{sqrt}(\Omega^2_{\text{ktr}}))$ 
     $\eta_{\text{bio}} \sim \text{Normal}(\text{logit}(\text{tvbio}), \Omega^2_{\text{bio}})$ 
```

```
  end
```

```
  @covariates WEIGHT SMOKE_NUM SMOKE TAD
```

```
  @pre begin # how estimates will be used in dynamics block
```

```
    wtcl = (WEIGHT/70)^0.75
    wtv = WEIGHT/70
    CL = tvcl * wtcl * (1+(SMOKE_NUM*tveff_smoker)) * exp( $\eta_{\text{cl}}$ ) # clearance
    Vc = tvvc * wtv * exp( $\eta_{\text{vc}}$ )
    Ktr = tvktr * exp( $\eta_{\text{ktr}}$ )
    Q = tvq * wtcl
    Vp = tvvp * wtv
```

```
  end
```

```
  @dosecontrol begin
```

```
    _bioav = logistic( $\eta_{\text{bio}}$ )
```

```

    bioav = (; Depot = _bioav , )
end

@physics begin
    Depot' = -Ktr * Depot
    Transit1' = Ktr * Depot - Ktr * Transit1
    Transit2' = Ktr * Transit1 - Ktr * Transit2
    Central' = Ktr * Transit2 - (CL+Q)/Vc*Central + (Q/Vp)*Peripheral
    Peripheral' = (Q/Vc)*Central - (Q/Vp)*Peripheral
end

@derived begin
    cp = @. Central/Vc
    TRI ~ @. Normal(cp, sqrt(((abs(cp)^2)*σ²_prop) + σ²_add))
end

end

```

#####

Methods S2: Effect Compartment PD model:

```

mod_erlang_comb_effcmp = @model begin
    @param begin
        tvke0 ∈ RealDomain(lower=0.0001)
        tvebase ∈ RealDomain(lower=0.0001)
        tvemax ∈ RealDomain(lower=0.0001)
        tvhill ∈ RealDomain(lower=0.0001)
        Ω²_ebase ∈ RealDomain(lower = 0.0001)
        Ω²_emax ∈ RealDomain(lower = 0.0001)
        σ²_add_pd ∈ RealDomain(lower = 0.0001)
        σ²_prop_pd ∈ RealDomain(lower = 0.0001)
    end

    @random begin
        ηbase ~ Normal(0, sqrt(Ω²_ebase))
        ηemax ~ Normal(0, sqrt(Ω²_emax))
    end

    @covariates iCL iVc iQ iVp iKtr ibioav_Depot SMOKE_NUM TAD

    @pre begin
        CL = iCL
        Vc = iVc
        Ktr = iKtr
    end
end

```

```

Vp = iVp
Q = iQ
bioav = ibioav_Depot

ke0 = tvke0
ebase = tvebase*exp(ηebase)
ec50 = 900
hill = tvhill
emax = tvemax*exp(ηemax)
end

@dosecontrol begin
    bioav
end

@dynamics begin
    Depot' = -Ktr * Depot
    Transit1' = Ktr * Depot - Ktr * Transit1
    Transit2' = Ktr * Transit1 - Ktr * Transit2
    Central' = Ktr * Transit2 - (CL+Q)/Vc*Central + (Q/Vp)*Peripheral
    Peripheral' = (Q/Vc)*Central - (Q/Vp)*Peripheral
    Ceff' = ke0*(abs(Central/Vc) - Ceff)
end

@vars begin
    edrug = (emax*Ceff^hill)/(ec50^hill + Ceff^hill)
    eff_ = ebase*(1 + edrug)
end

@derived begin
    eff = @. eff_
    mHb ~ @. Normal(eff, sqrt(((abs(eff)^2)*σ²_prop_pd) + σ²_add_pd))
end
end

```