Assay.

Ropivacaine and levobupivacaine were measured using gas chromatography (23, 24). In brief, 100 µl internal standard solution (mepivacaine 4 $mg.l^{-1}$), 100 µl NaOH 0.1 N and 200 µl pentane were added to 0.5 ml plasma. The usual procedure includes rapid vortex agitation during 45 seconds and centrifugation at 3500 g during 5 minutes. Because recovery was not complete in the presence of Intralipid®, the vortex time was increased to 90 seconds in the presence of 200 µl 4 M NaOH in order to induce partial saponification of the lipids. Two µl of the supernatant were injected on-column. The chromatograph (Varian model 3400, Varian Les Ulis, France) equipped with a nitrogen-phosphorus detector was fitted with a megabore J&W DB-1701 column (30 m X 0.53 mm, film thickness 1 µm). Helium was used as carrier gas at 30 $ml.min^{-1}$, air and hydrogen were set at 150 and 4.5 $ml.min^{-1}$, respectively. The temperatures were as follows: injector 250°C, detector 290°C, oven 230°C. The standard curve was linear in the range 0.01-8 $mg.l^{-1}$. The limit of quantification at four times the basal noise was less than 0.01 $mg.l^{-1}$ for the two drugs. The intra- and inter-day coefficients of variation were 6 and 8 % at 0.2 $mg.l^{-1}$ in the absence of Intralipid® and between 10 and 16 % in the presence of Intralipid® depending of the emulsion concentration. In the absence of Intralipid® the fraction extracted (recovery) is 97-102 % in plasma. However, because Intralipid® may have decreased the efficacy of extraction, we also measured ropivacaine and levobupivacaine (0.5 and 4 $mg.l^{-1}$) *in-vitro* in plasma in the presence of various Intralipid® dilutions (1/10, 1/25 and 1/100). Three conditions of extraction were tested: 1) immediate assay after mixing, 2) after rapid centrifugation at 20800 g for 10 minutes (17), 3) rapid freeze of the mixed solution at -80°C and assay of the thawed samples (Table A1 and Figure A1).

Table S1. Recovery of ropivacaine and levobupivacaine from human plasma containing different concentrations of Intralipid®. Three conditions have been tested: 1) the three constituents (human plasma, Intralipid® and ropivacaine or levobupivacaine) are mixed and immediately extracted, 2) the same solution is rapidly centrifuged, the undernatant (plasma layer) is recovered and extracted according to Litonius et al, reference 17, 3) the solution is frozen (-80 °C) and extracted after thawing. Data are mean (coefficient of variation %).

| **IL solution** | **Ropivacaine** | | **Levobupivacaine** | |
|---|---|---|---|---|
| | 0.5 mg/L | 4 mg/L | 0.5 mg/L | 4 mg/L |
| **Immediate assay** | | | | |
| 1/10 | 1.0 ( 9.4) | 0.97 (4.0) | 0.85 (5.7) | 0.64 (11.8) |
| 1/25 | 0.88 (5.7) | 0.82 (10.4) | 0.83 (5.4) | 0.58 (8.1) |
| 1/100 | 0.76 (7.8) | 0.74 (3.8) | 0.67 (12.4) | 0.56 (2.6) |
| **Undernatant** | | | | |
| 1/10 | 0.42 (4.8) | 0.55 (4.7) | 0.51 (6.0) | 0.33 (8.6) |
| 1/25 | 0.67 (2.2) | 0.56 (3.7) | 0.61 (2.4) | 0.54 (3.1) |
| 1/100 | 0.56 (5.0) | 0.52 (11.7) | 0.69 (9.2) | 0.50 (9.2) |
| **Assay after freezing** | | | | |
| 1/10 | 0.96 (13.8) | 0.93 (3.6) | 1.02 (9.2) | 0.88 (13.5) |
| 1/25 | 0.93 (4.7) | 1.02 (8.3) | 1.12 (6.8) | 0.93 (14.5) |
| 1/100 | 0.87 (2.5) | 0.81 (2.7) | 0.9 (3.1) | 0.87 (4.5) |

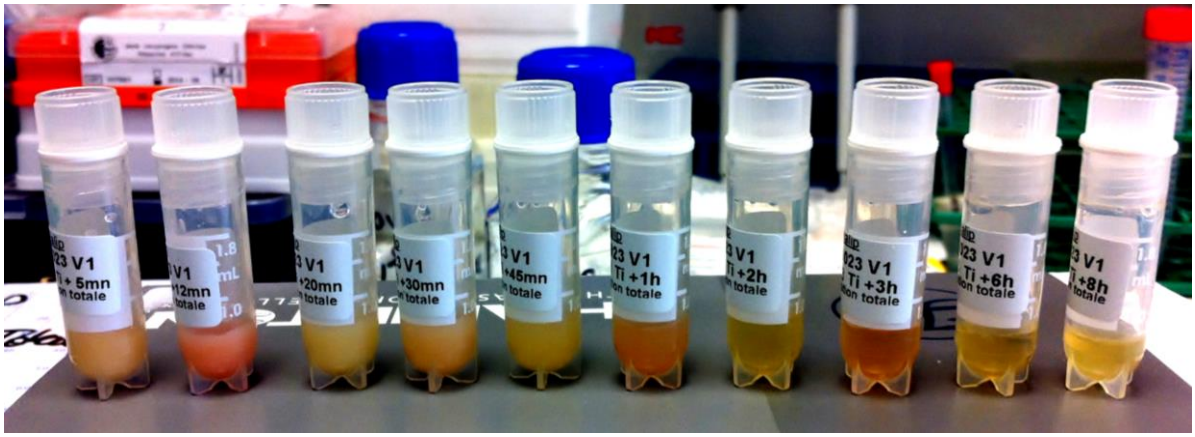

Figure S1. Aspect of plasma in a sequence with Intralipid® from time+5 min to time+8 hours. The high plasma turbidity is clearly visible during the first 2-3 hours.

Pharmacokinetic analysis.

A two-and three-compartment open models were tested. In order to model the effect of Intralipid® (lipid sink effect), we added a compartment ($V_{IL}$) to the central compartment ($V_1$). Then $V_1$, the volume of the central compartment was equal to $V_1$ in the placebo sequences or to $V_1 + V_{IL}$ in the Intralipid® sequences. The three compartment model was overparameterized likely because of the lack of sampling during infusion. Then, only two-compartment models were tested. Proportional random effect intervariability parameters were added to each structural fixed effect parameters. A combined multiplicative and additive error model was used.

The two and three compartment models were first compared with the Akaike criterion and the pertinence of parameters within models was compared with the Log Likelihood (LL) Ratio test. In addition, goodness of fit was always checked by visual inspection of the observed concentrations vs. predicted population and Bayesian posthoc concentrations. Likelihood profiling and the NPDE add-on for R with 1024 replications were used to assess goodness of fit. Bootstrap was not performed because of the complex stratification (Placebo/Intralipid® and levobupivacaine/ropivacaine) of the data set, but $\omega^2$, the variance of the interindividial random parameter with mean zero was reported instead.

Considering the principle of superposition, Cmax normalized for dose was analyzed with the procedure lme: `Cmax<-lme(fixed=CMNORM~RL*IL, random=~1|C.ID,Cmax)`. Similarly, clearance calculated by the trapezoidal rule was compared between groups using lme: `LMCL<-lme(fixed=CL~IL+ROPI,random=~1|ID,CL)`

Control stream of the final model.

```
$PROBLEM TOXALIP
$INPUT ID TIME AMT RATE DV LDV IL RL MDV EVID
$DATA Tox.dat IGNORE=C
$SUBROUTINES ADVAN3 TRANS4

$PK
; Ropi = 1
RCL  = THETA(1)*EXP(ETA(1))
RVC  = THETA(2)*EXP(ETA(2))
RQ   = THETA(3)*EXP(ETA(3))
RV2  = THETA(4)*EXP(ETA(4))
RVL  = THETA(5)*EXP(ETA(5))
RV1  = RVC + IL*RVL

; Levo = 0
LCL  = THETA(6)*EXP(ETA(6))
LVC  = THETA(7)*EXP(ETA(7))
LQ   = THETA(8)*EXP(ETA(8))
LV2  = THETA(9)*EXP(ETA(9))
LVL  = THETA(10)*EXP(ETA(10))
LV1  = LVC + IL*LVL

LR = 1-RL
CL   = (RCL*RL + LCL*LR)
V1   = (RV1*RL + LV1*LR)
Q    = (RQ*RL  + LQ*LR)
V2   = (RV2*RL + LV2*LR)
ALAG1= THETA(11)
S1   = V1

$ERROR
IPRED = F
Y   = IPRED*(1+ERR(1)) + ERR(2)

$THETA NOABORT
(0,.5,1)
(0,40,80)
(0,.5,100000)
(0,80,200)
(.5,8,20)

(0,.6,2)
(0,40,80)
(0,.5,100000)
(0,80,200)
(.5,15,30)
(0,5,15)

$OMEGA  .5 .5 .5 .5 .5 .5 .5 .5 .5 .5

$SIGMA 5 5

$ESTIM METHOD=1 INTER SIGDIGITS=3 MAXEVAL=9900 POSTHOC NOABORT
$COV COMPRESS
$TABLE ID RL IL TIME DV IPRED FILE=TOXF.TAB NOPRINT
$SCATTER PRED VS DV UNIT
$SCATTER IPRED VS DV UNIT
```

The objective function (equal to - 2 LL) was -2342.816 for the full two-compartment model, i.e. with all structural parameters including the additional $V_{IL}$ compartment and all random effect parameters, -2271.552 for the model with equal $V_{IL}$ for levobupivacaine and ropivacaine (one THETA and one ETA less), and -2166.498 for the model without $V_{IL}$. The objective function

for the basic model with one ETA common for each structural parameter (CL, V1, V2, Q) without VIL was -2108.819.

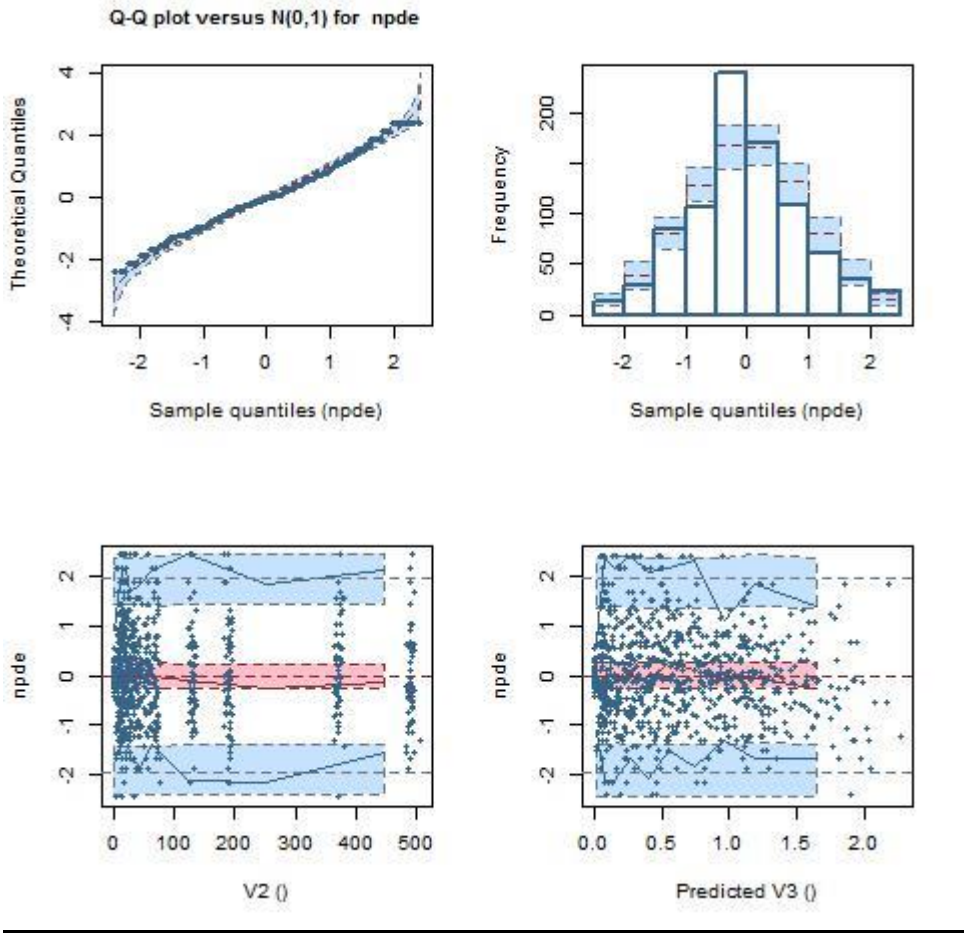


Figure S2. Goodness-of-fit. On this figure are represented the goodness-of-fit measures for the total data set. From left to right and from top to bottom are depicted the QQ plot of npde (normalized prediction distribution errors), the frequency of the npde quantiles, the npde vs. time (V2) and npde vs. predicted (IPRED) values (V3).

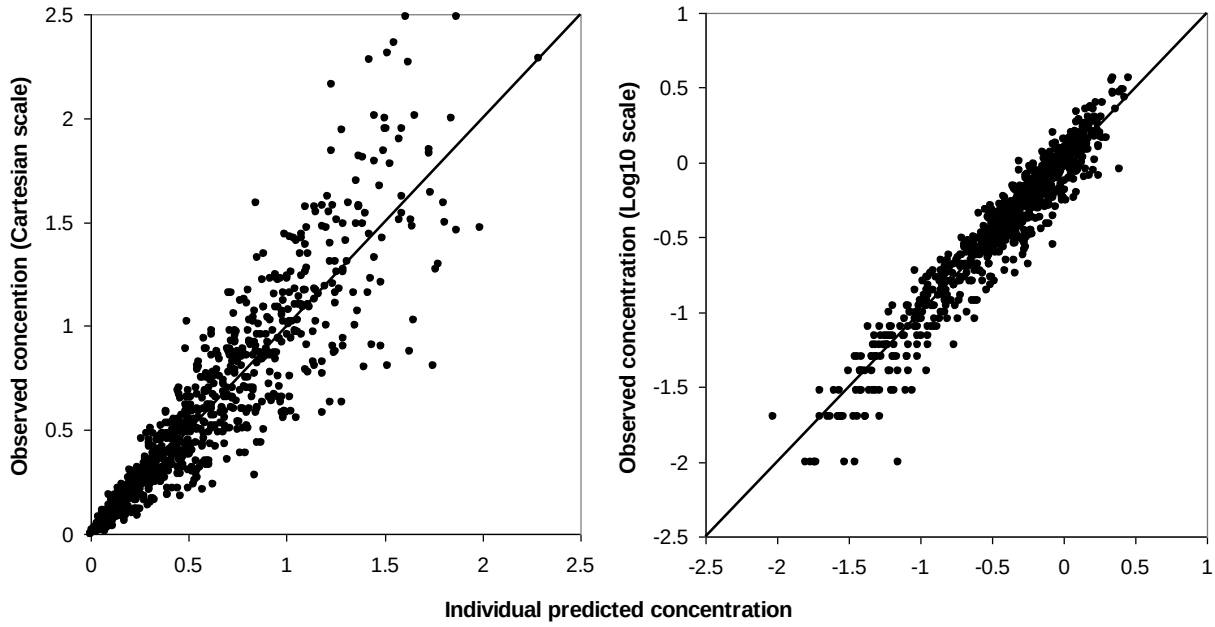


Figure S3. Plot of Observed Concentration vs. Individual Predicted (IPRED) data on a Cartesian scale (left) and on a log10 scale (right). Obviously as in figure A2, fiting is not perfect because of the model over simplification. However, more complicated model led to overparameterization.

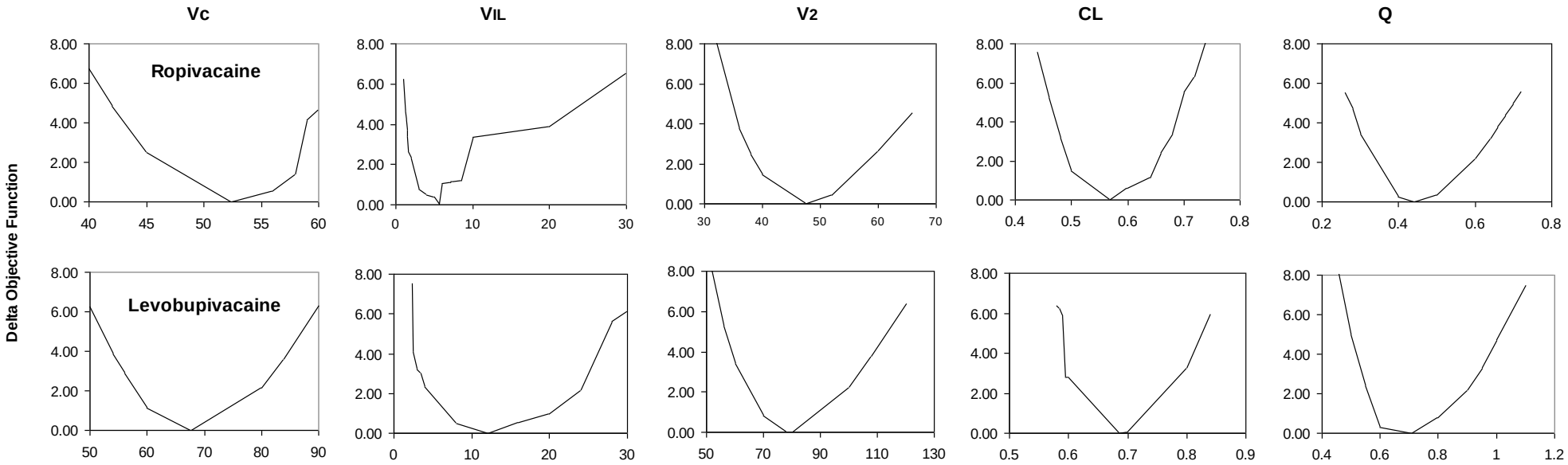


Figure S4. Likelihood profile obtained for the structural parameters.