Shi et al.:

A New Approach Methodology (NAM) for the Prediction of (Nor)ibogaine-Induced Cardiotoxicity in Humans

Supplementary Data

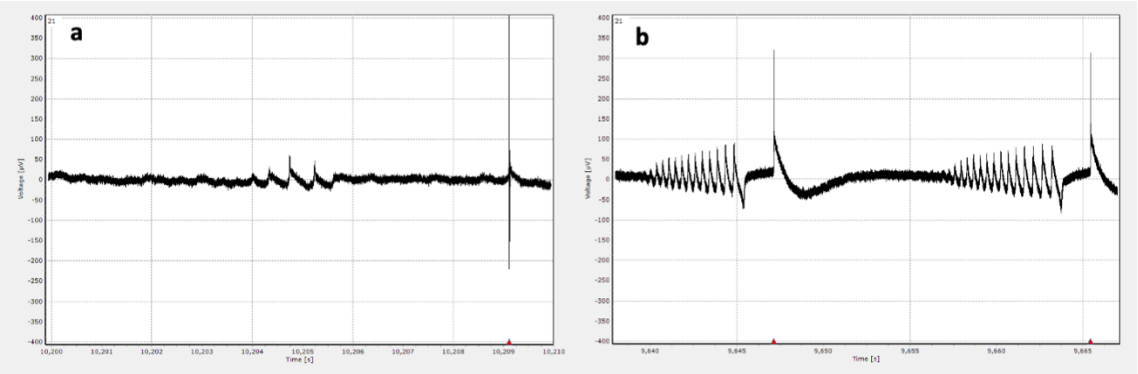

Fig. S1: Irregular waveforms of field potential observed in human induced pluripotent stem cell-derived cardiomyocytes using the multiple-electrode array
a, arrhythmia-type waveform induced by 1 µM ibogaine. **b**, arrhythmia-type waveform induced by 3 µM noribogaine. Waveforms present in a and b were not used for defining the *in vitro* concentration-response curves for FPDc effects.

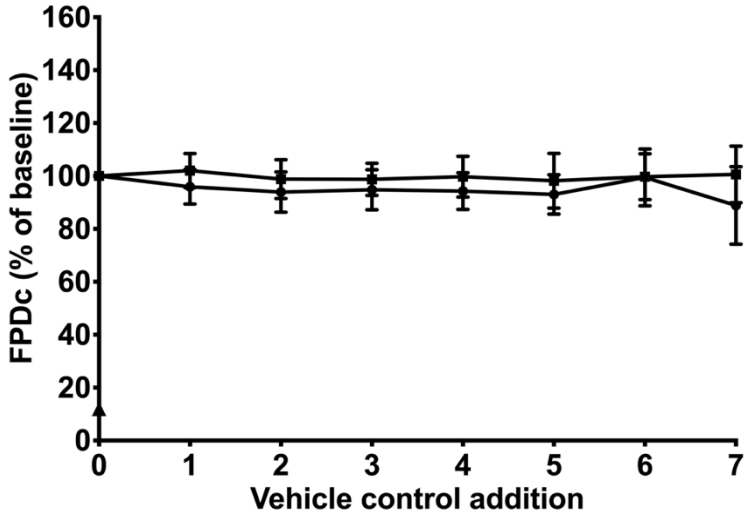

Fig. S2: Effects of repeated addition of 0.05% (v/v) acetonitrile (squares) and 0.1% (v/v) DMSO (circles) on the FPDc relative to baseline conditions in the vehicle control well set at 100%
Vehicle control addition 0 on the X axis represents the response of the baseline control set at 100%. 1-7 represent the 1st to 7th addition of vehicle controls corresponding to the 1st to 7th addition. Each data point represents the mean ± SD of three independent experiments.

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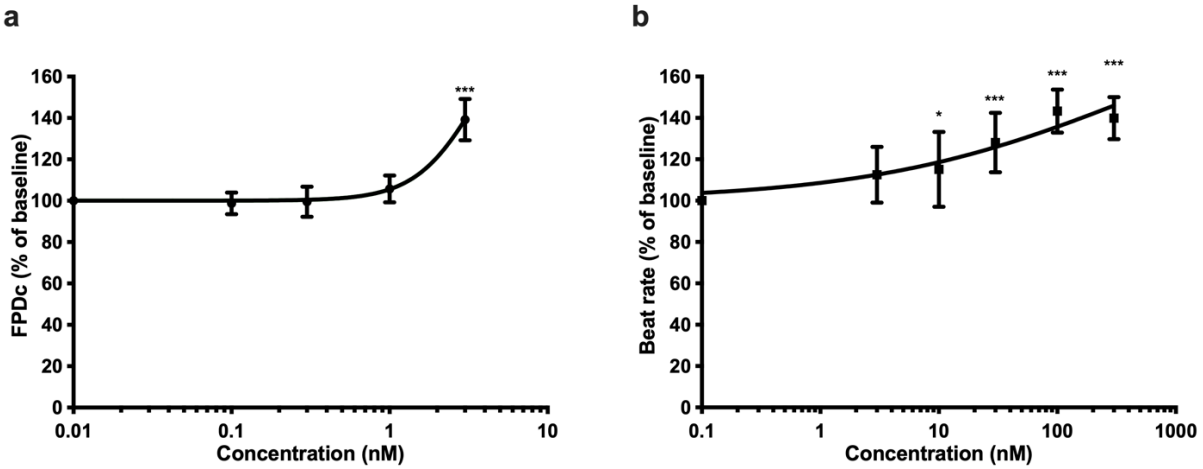

Fig. S3: Concentration-response curves for cardiotoxicity in hiPSC-CMs of the reference compounds (a) dofetilide and (b) isoproterenol

The response of the baseline at 0.1% (v/v) DMSO was set at 100%. Data represent the mean of results obtained from three independent experiments each containing six well replicates. Each data point represents the mean ± SD. Statistically significant changes in response compared to the solvent control are marked with * with $p < 0.05$: *, $p < 0.01$: ** and $p < 0.001$: ***.

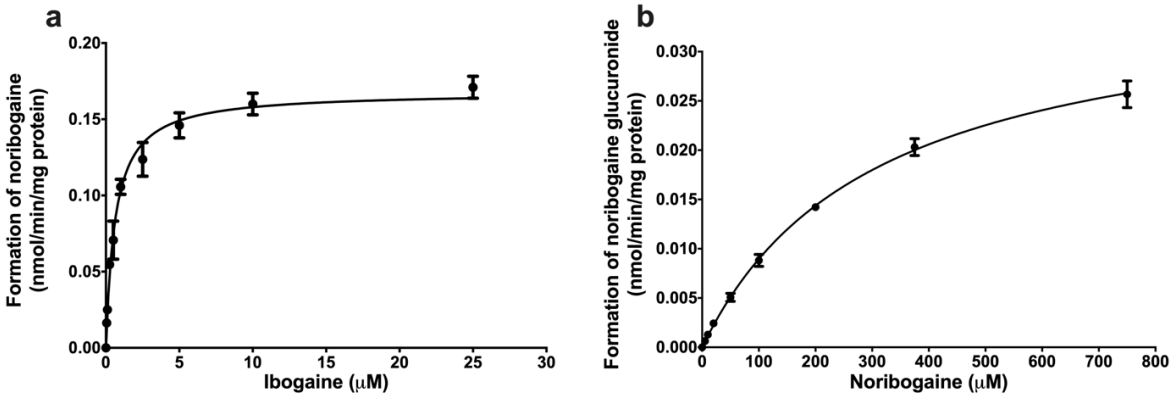

Fig. S4: Concentration-dependent formation of (a) noribogaine from ibogaine and (b) noribogaine glucuronide from noribogaine using *in vitro* incubations with human liver microsomes

Data represent the mean of three independent experiments. Each data point represents the mean ± SD.

Tab. S1: Summary of case reports of QT prolongation upon oral administration of ibogaine

Reference [a]	Reason for ibogaine use	Sex	Dose (mg/day) [a]	Baseline QTc (ms) [b]	Post QTc (ms)	QTc (% to baseline)
Asua (2013)	Heroin addiction	Male	7000	405	600	148.1
Grogan et al. (2019)	Cocaine and heroin addiction	Female	2000	411	788	191.7
Henstra et al. (2017)	Heroin addiction	Female	1400	411	647	130.2
Hildyard et al. (2016)	Heroin addiction	Male	7000	405	730	182.5
Hoelen et al. (2009)	Alcohol addiction	Female	500 [c]	411	616	149.9
Meisner et al. (2016)	Heroin addiction	Male	4000	405	588	145.2
Pleskovic et al. (2012)	Not reported	Male	600	405	460	113.6
Steinberg et al. (2018)	Opioid addiction	Male	5600	405	714	176.3
Vlaanderen et al. (2014)	Not reported	Male	2400	405	663	163.7

[a] internet-purchased ibogaine with unknown purity. [b] Baseline was assumed to be 405 ms and 411 ms for male and female, respectively, given that no baseline information was reported (Wedam et al., 2007). [c] a dose of 3500 mg ibogaine was corrected for the reported purity of 15%.

Text S1

Development of the PBK models

A PBK model consisting of multiple organ compartments was developed to describe the ADME of ibogaine and its metabolite noribogaine upon oral administration. Noribogaine has also been reported to cause the prolongation effects on the QTc interval in human (Glue et al., 2016). Therefore, an oral administration route was included in the submodel of noribogaine, which enables modeling of noribogaine kinetics and prediction of its cardiotoxicity upon oral administration. Human physiological parameters reported in Brown et al. (1997) were used in the PBK model (Table S2).

For the absorption parameters, the ka values of ibogaine and noribogaine were extrapolated from *in vitro*-derived P_{app} values obtained in the present study as described in the "*in vitro* intestinal transport studies" section. Due to limited pharmacokinetic data of both ibogaine and noribogaine, the experimental fractions absorbed (Fa) were not available. However, many studies demonstrated a positive correlation between P_{app} values and human Fa, and also indicated that Fa values can be estimated to be 1 when the P_{app} value is higher than 10^{-5} (cm/s) (Lozoya-Agullo et al., 2015; Lüpfert and Reichel, 2005; Skolnik et al., 2010). Considering the relatively high P_{app} values measured for ibogaine and noribogaine (see Results), the Fa values for both compounds were assumed to be 1.

To describe how ibogaine and noribogaine distribute in organs and the systemic blood circulation upon absorption, tissue: blood partition coefficients (P) of ibogaine and noribogaine were obtained by converting tissue: plasma partition coefficients using the corresponding blood/plasma ratio (BPr) as previously described (Shi, 2020). The tissue: plasma partition coefficients were predicted using the QIVIVE tool (version 1.0) from Wageningen Food Safety Research (WFSR, 2020) in which the algorithm of Berezhkovskiy (2004) was applied for ibogaine and the algorithm of Rodgers and Rowland (2006) was used for noribogaine given it generally shows better prediction for zwitterions (Graham et al., 2012; Utsey et al., 2020). Other input parameters including acid-base properties (pKa), lipophilicity (logP) and fraction unbound in plasma ($f_{u,p}$). The logP and pKa values were predicted using Chemicalize (ChemAxon, Hungary). The logP and pKa of ibogaine were 3.53 and 8.97, respectively. The log P and pKa of noribogaine were 3.0 and 8.87 (basic) and 9.66 (acidic). The $f_{u,p}$ values were determined using pooled human plasma in the present study. A BPr value of 2.5 for noribogaine in human was reported by Mash et al. (2016) while no published BPr value was available for ibogaine. Given that also for ibogaine the concentration was reported to be higher in the blood compared to plasma (Alper, 2001; Maciulaitis et al., 2008) the BPr value of ibogaine was assumed to be the same as that for noribogaine. Tissue: blood partition coefficients for ibogaine and noribogaine are summarized in Table S3.

Based on *in vitro* metabolism and *in vivo* pharmacokinetic studies, liver was considered as the major organ for the metabolism of ibogaine and noribogaine (Glue et al., 2015b, 2016; Obach et al., 1998). The kinetic parameters obtained in the current study were used to define the conversion of ibogaine to noribogaine and the glucuronidation of noribogaine by applying Michaelis-Menten kinetics. To extrapolate the *in vitro* V_{max} to an *in vivo* V_{max}, a total microsomal protein per gram of liver (MPL) value of 32 mg/g was applied in the PBK model (Barter et al., 2007). The *in vivo* K_m was assumed to be similar to the *in vitro* K_m.

Hepatic metabolism was reported to be the major elimination route for ibogaine (Mash et al., 2016). For noribogaine, Glue et al. (2015a) found that only a small amount of the dose administered (1.4-3.9 %) was detected in urine as noribogaine and its glucuronide after a single oral dose of noribogaine in human, indicating the negligible contribution of urinary excretion to the elimination of noribogaine. For this reason, renal excretion was not considered in the PBK model of noribogaine. Given the higher molecular weight of ibogaine and noribogaine than the cut-off value of 275 Da for biliary excretion in human, the compounds could be excreted via bile instead of via urine (Haddad and Nong, 2020). This is supported by the fact that both ibogaine and noribogaine were detected in human bile (Kontrimavičiūtė et al. 2006; Maciulaitis et al. 2008) and were excreted via the gastrointestinal tract (Alper, 2001) and present in faeces in rat (Jeffcoat et al., 1993). Therefore, biliary excretion was assumed to be the major elimination route for ibogaine and noribogaine and was included in the PBK model. The biliary excretion rate constant (kb) of noribogaine was obtained by the curve fitting option in Berkeley Madonna (version 8.3.18, UC Berkeley, CA, USA) in which the predicted blood maximum concentration (C_{max}) of noribogaine was fitted to the C_{max} of noribogaine in the blood that was reported in clinical studies (Glue et al., 2015a,b, 2016). The averaged fitted kb for noribogaine was 0.575 (/h). Due to the limited pharmacokinetic data on ibogaine and little influence of biliary excretion on ibogaine blood kinetics (see the results of the sensitivity analysis), the same kb value was assumed for ibogaine. Kinetic model calculations and curve fitting were performed with Berkeley Madonna, applying Rosenbrock's algorithms for solving stiff systems. Model equations are shown in Text S2.

Tab. S2: Physiological parameters used in the PBK models

Parameters	Symbol in model code	Value [a]
Body weight (kg)	BW	70
Tissue volume (% body weight)		
Liver	VLc	2.57
Fat	VFc	21.4
Lung	VLuc	0.76
Arterial blood	VAc	1.98
Venous blood	VVc	5.93
Kidney	VKc	0.4

Heart	VHc	0.47
Slowly perfused tissue	VSc	58
Rapidly perfused tissue	VRc	3.7
Cardiac output (l/h)	Qc	347.9
Blood flow to tissue (% cardiac output)		
Liver	QLc	22.7
Fat	QFc	5.2
Kidney	QKc	17.5
Heart	QHc	4
Slowly perfused tissue	QSc	29.1
Rapidly perfused tissue	QRc	21.5

[a] Reported in Brown et al. (1997)

Tab. S3: Tissue: blood partition coefficients for ibogaine and noribogaine

Compound	Tissue: blood partition coefficients [a]						
	liver	fat	slowly perfused tissue	rapidly perfused tissue	lung	kidney	heart
Ibogaine	1.62	0.18	2.73	1.62	0.32	1.02	0.7
Noribogaine	15.3	1.38	2.33	15.3	13.1	16.9	7.6

[a] Obtained by dividing tissue: plasma partition coefficients by the BPr value.

Text S2

```
;PBK model code human model
;=============================================================================
; physiological parameters
;=============================================================================

; Tissue volumes (L or Kg)

  BW = 70                       ; body weight human in kg

; All fractions are taken from Brown et al. (1997)
 VLc = 0.0257                   ; fraction of liver tissue
 VFc = 0.2142                   ; fraction of fat tissue
 VLuc =0.0076                   ; fraction of lung tissue
 VAc = 0.0198                   ; fraction of arterial blood: 0.074*1/4
 VVc = 0.0593                   ; fraction of venous blood: 0.074*3/4
 VKc = 0.004                    ; fraction of kidney tissue
 VHc = 0.0047                   ; fraction of heart tissue
 VRc = 0.037                    ; fraction of rapidly perfused tissue
 VSc = 0.58                             ; fraction of blood flow to slowly perfused tissue
                                        ; total of fractions = 0.9527

VL = VLc * BW                   ; volume of liver
VF = VFc * BW                   ; volume of fat
VLu = VLuc * BW                 ; volume of lung
VK = VKc * BW                   ; volume of kidney
VH = VHc * BW                   ; volume of heart
VR = VRc * BW                   ; volume of rapidly perfused tissue
VS = VSc * BW                   ; volume of slowly perfused tissue
VA = VAc * BW                   ; volume of arterial blood
VV = VVc * BW                   ; volume of venous blood
;-----------------------------------------------------------------------------------------------------------------------
; Blood flow rates (L/h)
  QC = 15 * BW^0.74                     ; QC = 15 * BW^0.74 (Brown et al., 1997)
  QLc = 0.227                           ; fraction of blood flow to liver
  QFc = 0.052                           ; fraction of blood flow to fat
  QKc = 0.175                           ; fraction of blood flow to kidney
  QHc = 0.04                            ; fraction of blood flow to heart
  QSc = 0.291                           ; fraction of blood flow to slowly perfused tissue
  QRc = 0.215                           ; fraction of blood flow to rapidly perfused tissue
                                                ; total of fractions = 1
; all fractions are taken from Brown et al. (1997)
QL = QLc*QC                     ; blood flow rate to liver in L/h
```

```
QF = QFc*QC                     ; blood flow rate to fat
QK = QKc * QC                   ; blood flow rate to kidney
QH = QHc*QC                     ; blood flow rate to heart
QR = QRc*QC                     ; blood flow rate to rapidly perfused tissue
QS = QSc*QC                     ; blood flow rate to slowly perfused tissue

;====================================================================
; Partition coefficients
;====================================================================
PFibo = 0.18                    ; fat/blood partition coefficient ibogaine
PSibo = 2.73                    ; slowly perfused tissues/blood partition coefficient ibogaine
PHibo = 0.7                             ; heart/blood partition coefficient ibogaine
PKibo = 1.02                    ; kidney/blood partition coefficient ibogaine
PLibo = 1.62                    ; liver/blood partition coefficient ibogaine
PRibo = 1.62                   ; rapidly perfused tissues/blood partition coefficient ibogaine
PLuibo = 0.32                   ; lung/blood partition coefficient ibogaine

PFnor = 1.38                    ; fat/blood partition coefficient noribogaine
PSnor= 2.33                             ; slowly perfused tissues/blood partition coefficient noribogaine
PHnor =7.6                              ; heart/blood partition coefficient noribogaine
PKnor = 16.9                    ; kidney/blood partition coefficient noribogaine
PLnor = 15.3                    ; liver/blood partition coefficient noribogaine
PRnor = 15.3                   ; rapidly perfused tissues/blood partition coefficient noribogaine
PLunor = 13.1                   ; lung/blood partition coefficient noribogaine

;====================================================================
; Biochemical parameters
;====================================================================
; Linear uptake rate (/h)       ; calculated based on P_app values obtained from the current study using
methadone as a reference compound.

kaibo = 0.79
kanor = 1.23

; Fraction absorbed
Faibo = 1
Fanor = 1

; Biliary excretion
kbibo=0.575                             ; the kb of noribogaine was assumed to be same for ibogaine
kbnor=0.575                             ; biliary excretion rate constant (/h) of noribogaine was obtained by fitting
CVBnor to reported in vivo data (Glue et al., 2016; Glue et al., 2015a; Glue et al., 2015b).

;-------------------------------------------------------------------------------------------------------------------------
; Metabolism of ibogaine in the liver
; Scaling factors;
MPL=32                          ; liver microsomal protein yield (mg/gram liver) (Barter et al., 2007)
L=VLc*1000                              ; liver =25.7 (gram/kg BW)

; Metabolites of ibogaine, unscaled maximum rate of metabolism (nmol/mg protein/min)
Vmaxc1 = 0.17                   ; obtained from in vitro microsomal incubation in the current study.
; Metabolites of ibogaine, scaled maximum rate of metabolism (µmol/h)
Vmax1 = Vmaxc1 / 1000 * 60 * MPL * L * BW

; Metabolites of ibogaine, affinity constants (µmol/L)
Km1 = 0.63                      ; obtained from in vitro microsomal incubation in the current study.

; metabolism of noribogaine in the liver

; Metabolites of noribogaine, unscaled maximum rate of metabolism (nmol/mg protein/min)
Vmaxc2 = 0.036                  ; obtained from in vitro microsomal incubation in the current study.
; Metabolites of noribogaine, scaled maximum rate of metabolism (µmol/h)
Vmax2 = Vmaxc2 / 1000 * 60 * MPL * L * BW
```

```
; Metabolites of noribogaine, affinity constants (µmol/L)
Km2 = 305                       ; obtained from in vitro microsomal incubation in the current study.

;=============================================================================
; Run settings
;=============================================================================

; Molecular weight (g/mol)
        MWibo= 310.4                    ; molecular weight of ibogaine
        MWnor = 296.4                   ; molecular weight of noribogaine

; Given dose (mg/kg bw) and oral dose in µmol/kg bw for ibogaine

        TDOSEibo = 0.0000001                    ; whole body total dose (mg)
        GDOSEibo = TDOSEibo / BW                        ; given dose (mg/kg bw)
        ODOSEibo = GDOSEibo * 1e-3 / MWibo*1e6; determine odose (µmol/kg bw)
        DOSEibo = ODOSEibo * BW                 ; determine dose (µmol)

        TDOSEnor = 30                           ; whole body total dose (mg)
        GDOSEnor = TDOSEnor  / BW               ; given dose (mg/kg bw)
        ODOSEnor  = GDOSEnor  * 1e-3 / MWnor *1e6       ; determine odose (µmol/kg bw)
        DOSEnor  = ODOSEnor  * BW               ; determine dose (µmol)

        doseibo_int = 2400                              ; dosing interval in hours, long dosing interval indicates
a single dose
        dosenor_int = 2400

; Time (h)
        Starttime = 0                   ; in h (days * hours in a day)
        Stoptime = 1*24                 ; in h (days * hours in a day)
        DTMIN = 1e-6
        DTMAX = 1
        DTOUT = 0
        TOLERANCE = 0.00001

;=============================================================================
; Knetics ibogaine
;=============================================================================
; Slowly perfused tissue compartment

; ASibo = Amount ibogaine in slowly perfused tissue (µmol)

ASibo' = QS * (CAibo - CVSibo)
        Init ASibo = 0
        CSibo = ASibo / VS
        CVSibo = CSibo / PSibo

;-----------------------------------------------------------------------------------------------------------------------
; Rapidly perfused tissue compartment

;ARibo = Amount ibogaine in rapidly perfused tissue (µmol)

ARibo' = QR * (CAibo - CVRibo)
        Init ARibo = 0
        CRibo = ARibo / VR
        CVRibo = CRibo / PRibo

;-----------------------------------------------------------------------------------------------------------------------
; Fat compartment

;AFibo = Amount ibogaine in fat tissue (µmol)

AFibo' = QF * (CAibo - CVFibo)
        Init AFibo = 0
```

```
        CFibo = AFibo / VF
        CVFibo = CFibo/ PFibo
;-----------------------------------------------------------------------------------------------------------------------
; Uptake ibogaine from GI tract

;AGIibo= Amount ibogaine remaining in GI tract (µmol)

Init AGIibo = 0
AGIibo' = pulse (DOSEibo* Faibo, 0, doseibo_int) -kaibo * AGIibo

;-----------------------------------------------------------------------------------------------------------------------
; Liver compartment
;ALibo = Amount ibogaine in liver tissue (µmol)

ALibo' = QL * (CAibo - CVLibo )+ (kaibo * AGIibo) - AMLibo' -ABibo'
                Init ALibo= 0
        CLibo = ALibo/ VL
                CVLibo= CLibo / PLibo

;AMLibo=Amount ibogaine metabolized in liver to noribogaine

                AMLibo' = (Vmax1*CVLibo) / (Km1 + CVLibo)
        init AMLibo = 0

; ABibo= amount of biliary excretion of ibogaine

                ABibo'=kbibo*ALibo
                init ABibo = 0


;-----------------------------------------------------------------------------------------------------------------------
; Kidney compartment

;AKibo = Amount ibogaine in kidney tissue (µmol)

AKibo' = QK * (CAibo - CVKibo)
                Init AKibo = 0
                CKibo = AKibo / VK
        CVKibo= CKibo/ PKibo
;-----------------------------------------------------------------------------------------------------------------------
;Heart compartment

;AHibo = Amount ibogaine in heart tissue (µmol)

        AHibo' = QH * (CAibo - CVHibo)
        Init AHibo = 0
        CHibo = AHibo / VH
        CVHibo= CHibo / PHibo

;-----------------------------------------------------------------------------------------------------------------------
;Lung compartment

;ALuibo = Amount ibogaine in lung tissue (µmol)

ALuibo' = QC * (CVibo - CALuibo)
Init ALuibo = 0
CLuibo= ALuibo / VLu
CALuibo = CLuibo / PLuibo

;-----------------------------------------------------------------------------------------------------------------------
; Arterial blood compartment

;CAibo = Concentration arterial blood ibogaine
```

```
AAibo' = QC * (CALuibo- CAibo);
                    Init AAibo = 0
                    CAibo=  AAibo / VA

;-----------------------------------------------------------------------------------------------------------------------
; Venous blood compartment

;AVibo = amount venous blood ibogaine (µmol)

AVibo' = (QF * CVFibo + QR * CVRibo + QS * CVSibo + QL * CVLibo + QK * CVKibo + QH *CVHibo- QC * CVibo)
Init AVibo = 0
        CVibo = (AVibo / VV)

;=======================================================================
; Kinetics noribogaine sub-model
;=======================================================================
;Slowly perfused tissue compartment

; ASnor = Amount noribogaine in slowly perfused tissue (µmol)

ASnor' = QS * (CAnor- CVSnor)
Init ASnor = 0
CSnor = ASnor / VS
CVSnor = CSnor / PSnor

;-----------------------------------------------------------------------------------------------------------------------
; Rapidly perfused tissue compartment

; ARnor = Amount noribogaine in rapidly perfused tissue (µmol)

ARnor' = QR * (CAnor - CVRnor)
                    Init ARnor = 0
                    CRnor = ARnor / VR
                    CVRnor= CRnor/ PRnor

;-----------------------------------------------------------------------------------------------------------------------
; Fat compartment

; AFnor= Amount noribogaine in fat tissue (µmol)

                    AFnor' = QF * (CAnor - CVFnor)
                    Init AFnor= 0
CFnor= AFnor/ VF
CVFnor = CFnor/ PFnor
;-----------------------------------------------------------------------------------------------------------------------

;-----------------------------------------------------------------------------------------------------------------------
; Uptake noribogaine from GI tract

;AGInor= Amount noribogaine remaining in GI tract (µmol)

Init AGInor = 0
AGInor' = pulse (DOSEnor* Fanor, 0, dosenor_int) + -kanor * AGInor

;-----------------------------------------------------------------------------------------------------------------------

; Liver compartment

; ALnor= Amount noribogaine in liver tissue (µmol)

ALnor' = QL * (CAnor - CVLnor) + (kanor * AGInor) +AMLibo' - ABnor'- AMLnor'
                    Init ALnor = 0
                    CLnor = ALnor / VL
                    CVLnor = CLnor / PLnor
```

```
;AMLnor=Amount noribogaine metabolized in liver to noribogaine glucuronide

                    AMLnor' = (Vmax2*CVLnor) / (Km2 + CVLnor)
          init AMLnor = 0

; ABnor= amount of biliary excretion of noribogaine

                    ABnor'=kbnor*ALnor
                    init ABnor = 0
;--------------------------------------------------------------------------------------------------------------------------------
; Kidney compartment

; AKnor = Amount noribogaine in kidney tissue (µmol)

AKnor' =QK * (CAnor - CVKnor)
                    Init AKnor = 0
                    CKnor = AKnor / VK
                    CVKnor= CKnor / PKnor
;--------------------------------------------------------------------------------------------------------------------------------
; Heart compartment

; AHnor = Amount noribogaine in heart tissue (µmol)

                    AHnor' = QH * (CAnor- CVHnor)
                    Init AHnor = 0
                    CHnor = AHnor / VH
                    CVHnor = CHnor / PHnor

;--------------------------------------------------------------------------------------------------------------------------------
;Lung compartment

; ALunor = Amount noribogaine in lung tissue (µmol)

                    ALunor' = QC * (CVnor - CALunor)
                    Init ALunor = 0
                    CLunor = ALunor / VLu
                    CALunor = CLunor / PLunor

;--------------------------------------------------------------------------------------------------------------------------------
; Arterial blood compartment

; CAnor= Concentration arterial blood noribogaine(µmol)

AAnor' = QC * (CALunor- CAnor)
Init AAnor = 0
CAnor =  AAnor / VA
;--------------------------------------------------------------------------------------------------------------------------------
; Venous blood compartment

; AVnor = Amount venous blood noribogaine (µmol)

AVnor' = (QF * CVFnor + QR * CVRnor+ QS * CVSnor+ QL * CVLnor + QK * CVKnor + QH *CVHnor- QC * CVnor)
                    Init AVnor = 0
                    CVnor= (AVnor/ VV)


;=====================================================================
; Mass balance calculations of ibogaine
;=====================================================================
Totalibo' = pulse (DOSEibo *Faibo, 0, doseibo_int)
init Totalibo = 1E-50

Calculatedibo = AFibo + ASibo+ ARibo + ALibo+ AVibo+ AAibo + AGIibo + AMLibo + ALuibo + AKibo + AHibo+ABibo
```

```
ERRORibo = ((Totalibo - Calculatedibo) / (Totalibo + 1E-30)) * 100
MASSBALibo = Totalibo - Calculatedibo + 1

;=====================================================================
; Mass balance calculations of noribogaine
;=====================================================================
Totalnor' = AMLibo'+pulse (DOSEnor *Fanor, 0, dosenor_int)
init Totalnor = 1E-50+AMLibo

Calculatednor = AFnor + ASnor+ ARnor + ALnor + AVnor+ AAnor+ ALunor + AKnor + AHnor +ABnor+ AMLnor +
AGInor

ERRORnor= ((Totalnor - Calculatednor) / (Totalnor + 1E-30)) * 100
MASSBALnor = Totalnor - Calculatednor + 1

;=====================================================================
; Calculation with model
;=====================================================================
; Calculations to evaluate the model performance of ibogaine

CViboB = CVibo* MWibo                    ; Concentration of ibogaine in venous blood (µg/l)

AUCibo' = CViboB                         ; Calculate AUC for ibogaine
init AUCibo = 0

CVheartibo= CVHibo*MWibo                 ; Concentration of ibogaine in heart venous blood (µg/l)

;---------------------------------------------------------------------------------------------------------------------
; Calculations to evaluate the model performance of noribogaine

CVnorB = CVnor * MWnor                   ; Concentration of noribogaine in venous blood (µg/l)

AUCnor' = CVnorB                         ; Calculate AUC for noribogaine
init AUCnor = 0

CVheartnor= CVHnor*MWnor                 ; Concentration of noribogaine in heart venous blood (ug/l)

BPribo=2.5                                   ;blood to plasma ratio of ibogaine, assumed to be same as
noribogaine
BPrnor=2.5                                    ;blood to plasma ratio of noribogaine (Mash et al. 2016)
fupibo=0.04                                  ;fraction unbound in plasma of ibogaine obtained from the
current study
fupnor=0.26                              ;fraction unbound in plasma of ibogaine obtained from the current study

; toxic equivalency factor based on in vitro cardiotoxic potency (unbound BMCL10 of ibogaine =0.078 µM; unbound
BMCL10 of noribogaine= 0.12µM) obtained in the hiPSC-CM MEA assay in the current study.
TEFibo=1
TEFnor=0.65

; toxic equivalency concentration upon the oral exposure of ibogaine
fCVheartTEQ=CVheartibo* (fupibo/BPribo) *TEFibo+CVheartnor* (fupnor/BPrnor)  *TEFnor
```

Text S3

Method of sensitivity analysis

A local parameter sensitivity analysis was conducted to estimate to what extent the model parameters can influence the model output, which refers to C_{max} of ibogaine and noribogaine in the heart venous blood upon the oral administration of ibogaine or noribogaine. Furthermore, given that the *in vivo* cardiotoxicity of ibogaine is dependent on the unbound concentration of both ibogaine and noribogaine, the sensitivity analysis was also performed for the unbound toxic equivalence (TEQ) concentration (details see in "QIVIVE using PBK modeling-based reverse dosimetry" section). The sensitivity coefficient (SC) was calculated according to the Eq. S1:

$$SC = \frac{(C'-C)}{(P'-P)} \times \frac{P}{C} \qquad (S1)$$

where P and C represent the initial value of the model parameter and output, respectively. P' and C' stand for the model parameter and model output after a 1% increase in an individual model parameter value, respectively. Only parameters with an absolute SC > 0.1 are considered to be influential on the model output (Rietjens et al. 2011). The sensitivity analysis was carried out for a subject with a body weight of 70 kg (Brown et al., 1997) and for a single oral dose of 20 and 500 mg ibogaine, representing a safe and well tolerated dose for healthy people (Glue et al., 2015b) and a clinically relevant dose for the treatment of drug addiction (Maciulaitis et al., 2008), respectively. For the sensitivity analysis of the noribogaine model, a single oral dose of 20 mg and 200 mg was chosen, respectively representing a safe dose for healthy people and a dose level associated with prolonged QTc in human (Glue et al., 2016).

Results of sensitivity analysis

Figure S5 shows the results of the sensitivity analysis presenting the influential model parameters for the prediction of C_{max} of ibogaine and noribogaine in the heart venous blood and of the C_{max} expressed in unbound ibogaine equivalents using a TEQ approach, upon exposure to an oral dose of ibogaine or noribogaine. For the oral administration of ibogaine (Figure S5a), results reveal that C_{max} of ibogaine in the heart venous is most sensitive to the body weight, fraction absorbed of ibogaine, fraction of liver, percentage of blood to liver and metabolic parameters for conversion of ibogaine to noribogaine (MPL, V_{maxc1} and K_{m1}). When the oral dose increased from 20 mg to 500 mg, the normalized SC values of body weight, fraction of liver, absorption related parameters (Faibo and kaibo) and metabolic parameters (MPL and V_{maxc1}) increased 2- to 3-fold while the normalized SC values of K_{m1} shows a 3.6-fold decrease.

As illustrated in Figure S5b similar SC values were obtained for the prediction of the C_{max} of noribogaine in the heart venous blood at two oral doses of 20 mg and 200 mg noribogaine. The predicted C_{max} of noribogaine in the heart venous blood is most affected by the fraction absorbed of noribogaine and the body weight with the normalized SC values being 1. Parameters related to percentage of blood to tissues also influence the prediction especially the percentage of blood to liver, rapidly perfused tissue, slowly perfused tissue and kidney with the normalized SC values above 0.5. Other model parameters show less influence with the normalized SC values ranging from 0.14 to 0.37 (Figure S5b).

Figure S5c shows that the unbound TEQ concentration expressed in ibogaine equivalents is most sensitive to the percentage of blood flow to slowly perfused tissue, followed by the percentage of blood flow to liver, and to rapidly perfused tissue with normalized SC values above 1. Besides, body weight, fraction absorbed of ibogaine, blood to plasma ratio of noribogaine, unbound fraction of noribogaine in plasma and TEF of noribogaine show a high influence on the prediction with the normalized SC values being 1. Figure S5c also indicates that parameters related to percentage of blood flow to tissues (QSc, QLc, QRc, QKc, QFc and QHc) show a dose-dependent influence on the prediction with the normalized SC values being higher at 500 mg compared to those at 20 mg. While the SC of other model parameters generally are not dose-dependent at the two doses of ibogaine.

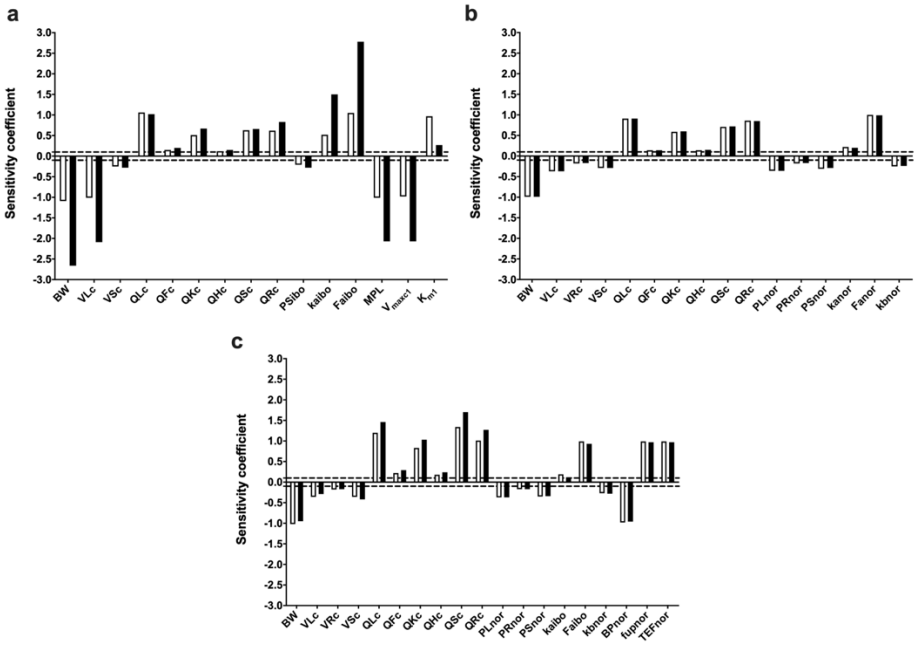

Fig. S5: Sensitivity coefficients of PBK model parameters for the prediction of (a) C_{max} of ibogaine in the heart venous blood upon an oral single ibogaine dose of 20 mg (white bars) and 500 mg (black bars), (b) C_{max} of noribogaine in the heart venous blood upon an oral single noribogaine dose of 20 mg (white bars) and 200 mg (black bars) and (c) C_{max} expressed in unbound ibogaine equivalents upon an oral single ibogaine dose of 20 mg (white bars) and 500 mg (black bars)

Dotted lines indicate the normalized SC with an absolute value higher than 0.1. BW, body weight; VLc, fraction of liver; VRs, fraction of rapidly perfused tissue; VSc, fraction of slowly perfused tissue; QLc, percentage of blood flow to liver; QKc, percentage of blood flow to kidney; QHc, percentage of blood flow to heart; QRc, percentage of blood flow to rapidly perfused tissue; QSc, percentage of blood flow to slowly perfused tissue; PSibo, partition coefficient slowly perfused tissue: blood of ibogaine; MPL, microsomal protein per gram of liver; V_{maxc1}, unscaled maximum rate of ibogaine metabolism in liver; K_{m1}, Michaelis–Menten constant for ibogaine metabolism in liver; PLnor, partition coefficient liver: blood of noribogaine; PSnor, partition coefficient slowly perfused tissue: blood of noribogaine; kaibo, absorption rate constant of ibogaine; kanor, absorption rate constant of noribogaine; Faibo, fraction absorbed of ibogaine; Fanor, fraction absorbed of noribogaine; Kbnor, biliary excretion constant of noribogaine; BPnor, blood to plasma ratio of noribogaine; fupnor, unbound fraction of noribogaine in human plasma; TEFnor, toxic equivalency factor of noribogaine.

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