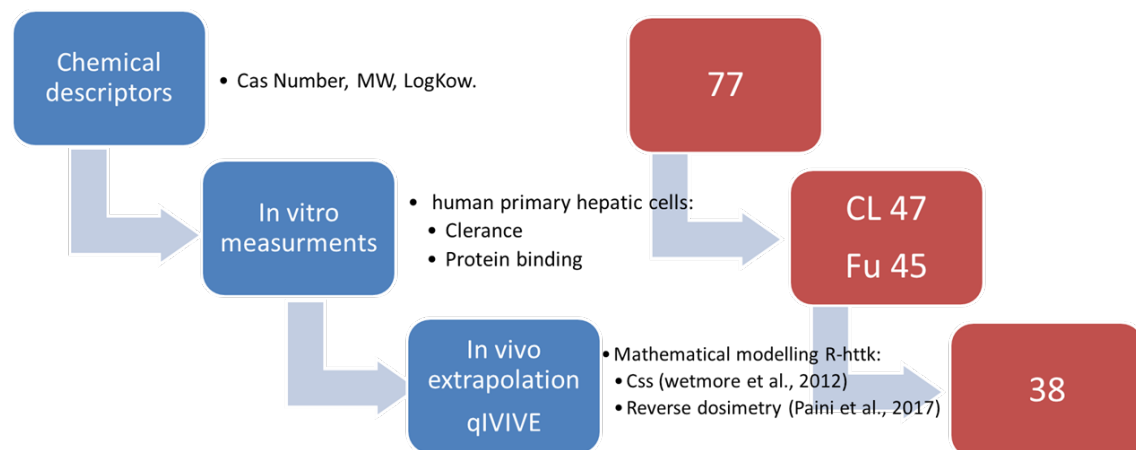# Data on *in vitro* hepatocyte clearance and blood plasma protein binding for a set of 77 chemicals

Including biokinetic modelling for in vitro to in vivo extrapolation

Paini Alicia, Cole Thomas*, Meinero Maria, Fortaner Torrent Salvator, Asturiol David, Carpi Donatella, Deceuninck Pierre, Macko Peter, Palosaari Taina, Sund Jukka, Worth Andrew, Whelan Maurice

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Contents

Abstract

The European Commission's Joint Research Centre (JRC) runs the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) which promotes regulatory acceptance of non-animal tests relevant to chemicals safety evaluation, through research, development and validation. In particular, EURL ECVAM engages with numerous international stakeholders to improve approaches to risk assessment, including the generation, sharing and use of non-animal data. Although not typically a regulatory requirement, information about the toxico-kinetics (TK) or biological fate of a substance, characterised by physiological processes of absorption, distribution, metabolism and excretion (ADME), can be very valuable for predicting toxicological hazard and informing risk assessment. The ADME/TK profiling of chemicals using non-animal methods has advanced considerably in recent years to support prioritisation and screening, read-across, and potentially *ab initio* safety assessment. The aim of this study was to generate *in vitro* data on blood-plasma protein binding (relevant to adsorption) and liver hepatocyte clearance (relevant to metabolism) for a set of 77 (primarily) industrial chemicals and to use the data for TK profiling and quantitative *in vitro* to *in vivo* extrapolation (qIVIVE). The data have been exploited for a 'next generation chemical risk assessment' case study being developed within the context of an international cooperation between regulatory agencies and regulatory science organisations committed to Accelerating the Pace of Chemical Risk Assessment (APCRA).

1   Introduction

The European Union is strongly committed to the Replacement, Reduction and Refinement of testing on animals (the 'Three Rs') as reflected in Directive 2010/63/EU on the protection of animals used for scientific purposes. As mandated by the Directive, the Joint Research Centre (JRC) runs the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) which promotes regulatory acceptance of non-animal tests relevant to chemicals safety evaluation, through research, development, and validation.

Inclusion of toxicokinetics (TK) defined by physiological adsorption, distribution, metabolism and excretion (ADME) improves the quality and relevance of chemical safety assessment, although typically specific data on ADME/TK are not currently mandatory within many regulatory frameworks (Bessems et al., 2015). Moreover, incorporation of TK modelling will advance the 3Rs (replacement, reduction and refinement of animal experiments) alleviating dependence on whole-body *in vivo* studies for characterisation of ADME. Physiologically based kinetic (PBK) models integrate *in silico* and *in vitro* methods, for predictive quantitative *in vitro* to *in vivo* extrapolation (qIVIVE).

A strategy for achieving a 3Rs impact in the area of TK has been defined (EURL ECVAM, 2015) which identifies opportunities for integration of ADME and TK data into biokinetic models, including four overall objectives:

1. ADME methods: Development and standardisation of human in vitro ADME methods.

2. Kinetic modelling: Portals and good kinetic modelling practice.

3. Data collection: Measurements, Analytics and databases to serve kinetic modelling.

4. Regulatory anchoring: Legislation and guidance on human ADME/TK data.

The first three aims enhance the quality and availability of methods and models while the fourth fosters regulatory interest in ADME/TK based human-relevant approaches.

This report presents *in vitro* measurements of blood plasma protein binding (relevant to A and D) and liver hepatocyte clearance (relevant to M and E) of 77 chemicals. Of these, 58 were selected due to their inclusion in a case study being developed by the JRC and others within a consortium of regulatory agencies and regulatory science organisations working together for Accelerating the Pace of Chemical Risk Assessment (APCRA[1]). The remaining chemicals were relevant to in-house JRC projects.

The successful measurements, for 38 chemicals, provided input for TK profiling of the chemicals, in support of qIVIVE.

These ADME/TK data can be informative for several purposes (figure 1.): As such -raw values- data to inform the hazard profile and prioritization of a chemical. Furthermore in a more integrated strategy these data can help filling gaps in: 1. The ab initio framework; 2. In read across approaches and 3. As input parameters into mathematical models.

As a follow-up to the protein binding and hepatocyte stability testing, the chemicals will be subject to further characterisation with the ultimate aim of demonstrating a workflow for chemical profiling based on the concept of *ab initio* assessment (Berggren et al., 2017). This additional objective combines *in vitro* data with intrinsic properties to further predict biological activity by computational modelling. Implicit with the *ab initio* approach is the algorithmic derivation of pertinent chemical properties from first principles, made possible by the availability of advanced software applications with access to comprehensive databases.

Initially, with a preliminary view to demonstrating the feasibility of *ab initio* data compilation, a single physical property, octanol-water partition coefficient (LogKow) was selected as a simple and appropriate parameter for *in silico* derivation, using an available and recognised software application. LogKow indicates relative affinity of a chemical for aqueous (hydrophilic) versus lipid (hydrophobic) phase (solvent or medium).

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[1] https://www.epa.gov/chemical-research/accelerating-pace-chemical-risk-assessment-apcra
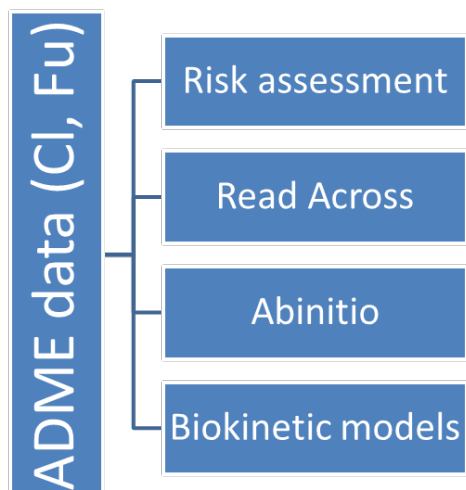
Figure 1. Absorption, Distribution, metabolism and excretion (ADME) data are of relevance and can inform risk assessment strategies, *ab initio* and read across approaches and can be included as input parameters in biokinetic models.

For the biokinetic modelling and qIVIVE extrapolation of toxicity, two approaches were adopted, each able to estimate external dose (exposure) from a predicted adverse outcome:

1. QIVIVE – Estimation of oral equivalents dose (OED) (Wetmore et al., 2012)

2. QIVIVE – Reverse dosimetry (Louisse et al., 2010, Paini et al., 2017)

The measured values and results generated and compiled is this report are available at the JRC data catalogue, EURL ECVAM Collection, including a section on alternatives to animal testing (https://data.jrc.ec.europa.eu/collection/id-0088).

2 Methodology

2.1 Chemicals

The 77 chemicals selected for this study are shown in Table 1. Chemicals #1-58 were selected with APCRA partners, #59-70 were relevant to an in-house JRC study to explore the use of in vitro transcriptomics for grouping and read-across, #71-75 (and #67) are valproic acid analogues used in JRC measurements of hepatocyte clearance (included for comparison) and #76 & #77 were inserted to inform biokinetic model development.

Table 1. List of the 77 Test chemicals with synonyms when available and CAS number.

number	Chemical Name	CAS number	number	Chemical Name	CAS number	number	Chemical Name	CAS number
1	Tetramethylthiuram monosulfide	97-74-5	27	Hexyl salicylate	6259-76-3	53	4,4'-(Oxydiethylene)bis (morpholine) 4,4'-(Oxydi-2,1-ethanediyl)bismorpholine	6425-39-4
2	D-(+)-Xylose	58-86-6	28	4-Hydroxy-2,2,6,6-tetramethylpiperidin1-yl)oxidanyl 4-Hydroxy-TEMPO	2226-96-2	54	Tetrapropyl orthosilicate	682-01-9
3	3,5,5-Trimethylhexyl acetate	58430-94-7	29	Tetrabutylammonium bromide	1643-19-2	55	p-Methylacetophenone 4'-Methylacetophenone	122-00-9
4	Hexanedihydrazide Adipic acid dihydrazide	1071-93-8	30	Benzyl propanoate Benzyl propionate	122-63-4	56	D&C Blue No. 9 7,16-Dichloro-6,15-dihydroanthrazine-5,9,14,18-tetrone	130-20-1
5	2-Phenylethyl phenylacetate	102-20-5	31	Saccharin o-Sulfobenzimide	81-07-2	57	Ethyl 2-cyano-3,3-diphenyl acrylate	5232-99-5
6	Propylsilanetriyl triacetate Propyltriacetoxysilane	17865-07-5	32	Methyl benzoate	93-58-3	58	Vanillin isobutyrate	20665-85-4
7	2-Butyloctan-1-ol 2-Butyl-1-octanol	3913-02-8	33	1,2-Diphenoxy ethane	104-66-5			
8	2,6-Di-tert-butyl-4-[(dimethylamino)methyl] phenol	88-27-7	34	Trimethoxyphenylsilane Phenyltrimethoxysilane	2996-92-1	59	Aflatoxin B1	1162-65-8
9	(-)-Ambroxide	6790-58-5	35	C.I. Direct Red 81 disodium salt	2610-11-9	60	4-(Methylnitroso amino)-1-(3-pyridinyl)-1-butanone	64091-91-4
10	6-Phenyl-1,3,5-triazine-2,4-diamine 2,4-Diamino-6-phenyl-1,3,5-triazine	91-76-9	36	2-Ethylhexyl glycidyl ether	2461-15-6	61	2-Nitrofluorene	607-57-8
11	Bis(2-ethylhexyl) phosphate Bis(2-ethylhexyl) hydrogen phosphate	298-07-7	37	2,6-Dimethyl-2-heptanol	13254-34-7	62	Benzo[a]pyrene	50-32-8
12	Calcium dodecylbenzene sulfonate	26264-06-2	38	Triethoxymethylsilane Methyltriethoxysilane	2031-67-6	63	Cyclosporin A	59865-13-3
13	Neopentyl glycol dibenzoate	4196-89-8	39	(4-Methoxyphenyl) methanol 4-Methoxybenzyl alcohol	105-13-5	64	Piperonyl butoxide	51-03-6
14	Methyl phenylacetate	101-41-7	40	Sodium dodecylbenzene sulfonate Dodecylbenzenesulfonic acid, sodium salt	25155-30-0	65	Rotenone	83-79-4
15	N-Butyldiethanolamine	102-79-4	41	Linalool	78-70-6	66	Diclofenac sodium salt	15307-79-6
16	4,5-Dihydroxy-1,3-dimethylimidazolidin-2-one	3923-79-3	42	N-Dodecanoyl-N-methylglycine N-Lauroylsarcosine N-Dodecanoylsarcosine	97-78-9	67	2-Propylpentanoic acid Valproic acid	99-66-1
17	Ethenylsilanetriyl triacetate Triacetoxy(vinyl)silane	4130-08-9	43	2-Phenoxyethyl isobutyrate	103-60-6	68	Rifampicin	13292-46-1
18	1,4-Butanediol	110-63-4	44	4-Morpholine carboxaldehyde 4-Formylmorpholine	4394-85-8	69	Disulfiram Tetraethylthiuram disulfide	97-77-8
19	Diethylenetriamine	111-40-0	45	2-Phenylethyl 2-methylpropanoate 2-Phenylethyl Isobutyrate	103-48-0	70	Acetaminophen	103-90-2
20	N-Phenyl-1-naphthylamine	90-30-2	46	1-(2-Hydroxyethyl)pyrrolidin-2-one 1-(2-Hydroxyethyl)-2-pyrrolidone	3445-11-2	71	Octanoic acid Caprylic acid	124-07-2
21	Triethoxy-n-octylsilane	2943-75-1	47	Ethyl 2-methylpentanoate	39255-32-8	72	2-Ethylhexanoic acid 2-Ethylcaproic acid	149-57-5
22	N,N'-Disalicylidene- 1,2-diaminopropane N,N'-Bis(salicylidene)-1,2-propanediamine	94-91-7	48	Prop-2-en-1-yl 3-cyclohexylpropanoate Allyl cyclohexanepropionate	2705-87-5	73	Pentanoic acid Valeric acid	109-52-4
23	2,2,2-Trichloro-1-phenylethyl acetate α-(Trichloromethyl)benzyl acetate	90-17-5	49	Bis{2-[2-(propan-2-yl)-1,3-oxazolidin-3-yl]ethyl} hexane-1,6-diylbiscarbamate Bis[2-[2-(1-methylethyl)-3-oxazolidinyl]ethyl] hexan-1,2-diylbiscarbamate	59719-67-4	74	(±)-2-Methylbutyric acid 2-Methylbutanoic acid	116-53-0
24	Nerol (2Z)-3,7-Dimethylocta-2,6-dien-1-ol	106-25-2	50	C.I.Disperse Yellow 42	5124-25-4	75	2-Pentenoic acid	13991-37-2
25	Ethyltriacetoxysilane Triacetoxyethylsilane	17689-77-9	51	Nonanal	124-19-6	76	Amiodarone hydrochloride	19774-82-4
26	Ethenyl(triethoxy)silane Triethoxy(vinyl)silane	78-08-0	52	3-(Dimethylphosphono)-N-methylolpropionamide Dimethyl [3-[(hydroxymethyl)amino] -3-oxopropyl]phosphonate	20120-33-6	77	Caffeine	58-08-2

2.2   Plasma protein binding and Hepatocyte metabolic clearance in vitro assays

Human blood plasma protein binding assay using equilibrium dialysis

Plasma protein binding limits the availability of free chemical for cell interaction, with consequences for metabolism and elimination. Equilibrium dialysis provides an accepted method for protein binding assessment, where a semi-permeable membrane separates a protein-containing compartment from a protein-free compartment. Test chemical added to the protein-free compartment is able to pass through the membrane, where a fraction becomes bound to the protein as a ligand. At equilibrium (e.g., 5 hours) with the protein binding at saturation and when the concentration of unbound test chemical is equal on both sides of the membrane, the protein-containing compartment will have a higher overall concentration due to the additional fraction bound to the protein as ligand. This excess concentration then provides a measure of relative binding affinity of the chemical. Of particular significance for kinetic modelling is the fraction unbound (Fu %).

Intrinsic metabolic clearance assay using in vitro human hepatocytes

The liver is the most important site of xenobiotic (chemical) metabolism. Hepatocytes (cells derived from liver tissue) contain the full complement of metabolising enzymes, maintained within the intact cell. Hepatocytes therefore provide an in vitro model for predicting in vivo metabolic clearance. Essentially, the hepatocytes are exposed to the test chemical by incubation in culture medium, preparing replicate samples to enable the interaction to be terminated after appropriate intervals, e.g., from time zero to two hours (e.g., 0, 15, 30, 60, 90, 120 mins). Determination of the supernatant chemical concentration with time allows calculation of any depletion rate occurring due to metabolism and/or other biotransformation processes. Physiologically, the clearance of an eliminating organ (e.g., liver) is the volume of blood cleared of xenobiotic (e.g., drug) by that organ per unit of time. By analogy for in vitro hepatocytes, the principal conventional measurement is intrinsic clearance CLint (or CL) expressed in µL/min/million cells.

Assay implementation

The protein binding and metabolic stability measurements were performed by GVK Biosciences (Hyderabad, India) arranged under a JRC service contract, and concluded with two reports respective of each assay (Srivastava et al., 2019a; 2019b). The reports outline the experimental procedures, summarised in Table 2 (protein binding by equilibrium dialysis) and Table 3 (metabolic stability by hepatocyte clearance).

Table 2. Experimental design for the protein binding measurement

Test System	Human Plasma (K2EDTA)
Concentrations	5µM (with 50µM and 100µM as alternatives)
Incubation Time	5 hours
No of Replicates	Three
Buffer	Phosphate buffer saline, pH 7.4
Final DMSO Conc^n	<0.1%
Bio-analysis	LC-MS/MS (Qtrap5500)
Results Reported	% Bound and Unbound, % Recovery

Table 3. Experimental design for the hepatocyte stability measurement

Test System	Pooled Human Hepatocytes (Gibco lots HUE50-F and HUE121)
Start Concentrations	1µM and 10µM (with 20µM and 100µM as alternatives)
Time Points	0, 15, 30, 60, 90 and 120 minutes
No of Replicates	Three
Final Cell Density	1 million/mL
Incubation Medium	Krebs-Henseleit Buffer
Final DMSO Conc^n	< 0.1%
Bio-analysis	LC-MS/MS (Qtrap5500)
Results Reported	% Remaining, Half-Life, Intrinsic Clearance, Clearance Classification

2.3 Octanol-water partition coefficient

Adhering to the *ab initio* concept, and using ACD/Percepta molecular properties prediction platform, *in silico* LogKow was derived individually for the 77 chemicals based on chemical structure alone, inserted as SMILES (Simplified Molecular Input Line Entry System) code. As a verification exercise of internet sources, the SMILES codes were obtained first from ChemSpider, searched via chemical name, and secondly from ChemIDPlus, searched via CAS number. As an additional check, InChI (International Chemical Identifier) also characteristic of molecular structure was compiled from both sources.

The two sources often have SMILES variants with ChemIDplus providing additional code for molecular conformation (spatial stereochemistry). However, this did not affect LogKow output, which was consistent using either SMILES version, and also the same when InChI was used as input string.

Explanatory notes accompanying the ACD/Percepta software indicate the following for LogKow derivation:

kACD/LogK includes a variety of algorithms for prediction of partition coefficient.. The combination of algorithms delivers coverage for a broad chemical space and provides the most accurate logK values for your compounds.

Classic Algorithm

Based on >12,000 experimental logK values, the Classic algorithm uses the principal of isolating carbons.

GALAS Algorithm (Global, Adjusted Locally According to Similarity)

Based on a training set of >11,000 compounds GALAS provides a value for logK that is adjusted with data from the most similar compounds.

Consensus Model

Using both Classic and GALAS algorithms, the consensus algorithm weights the calculation to the model best suited for each structure.

Thus, the ACD/Percepta software provides three values for LogK, output as pdf reports, including a weighting equation for the Consensus Model. In addition, together with chemical structure, each report also provides a table of available experimental LogK values for similar molecules extracted from built-in databases.

In parallel, for further comparison, LogKow was also taken from Comptox (US EPA chemistry dashboard, https://comptox.epa.gov/dashboard) with preference for experimental values over simulations.

2.4 Physiologically based kinetic modelling

TK modelling was implemented using an available software package (https://cran.r-project.org/web/packages/httk/index.html)[2] compatible with r (https://www.r-project.org/) or with r Studio (https://rstudio.com/).A user manual (https://cran.r-project.org/web/packages/httk/httk.pdf) and introduction to httk (Pearce et al., 2017) are also available.
Options for kinetics models in r/httk were:
One compartment model (O'Flaherty, 1981),
Three compartment model (Jamei et al., 2009),
Three compartment steady state model (Wetmore et al., 2012; Wetmore, 2015)
Seven compartment physiologically based (toxico)kinetic model (PB(T)K).
The r-httk package includes a library of several hundred chemicals, searchable by name or CAS number, but new original data may also be introduced (Annex 1).
For a comprehensive output, the Css model and the 7 compartment PBK model was applied, with qIVIVE summarised in Box 1.

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[2] httk refers to high throughput toxico-kinetics, relevant to rapid screening of chemicals

Box 1. Quantitative in vitro in vivo extrapolation (qIVIVE) methods

Two methods were implemented for qIVIVE:

1. QIVIVE – Estimation of oral equivalents dose (OED) (Wetmore et al., 2012)

Step 1. Css (steady state concentration in blood plasma) = k0 / ((GFR*Fu)+(Ql*Fub*Cl/ (Ql+Fu*Cl)))
        LogP = LogKow, Cl = clearance, Fu = unbound fraction in plasma, → Measured
        GFR = glomerular filtration rate, Ql = liver blood flow, ko = input rate → Default from r-httk package   library

Step 2. Execute the Css formula in r-httk package at 24 hours (h) and 7 days (d)

Step 3. Tabulate the Results Css (asa Excel file)

Step 4.   Calculation of OED at 24h (5/38 chemicals) and OED at 7d (38 chemicals)
        OED (mg/kg/d) = IC50/EC50(uM)*(1 mg/kg/d)/Css(uM)

2. QIVIVE – Reverse dosimetry approach (Paini et al., 2017)
        Using the PBK model predicted dose response curves, derive concentration response curves in the   organs; compare this to the nominal concentration experimental values corrected using the Armitage   model.

Step 1. Execute the PBK model at 24h (Cmax) and 7d for area under curve (AUC) over 6 doses.

Step 2. Results (exported to Excel file) used to generate dose response curves.

Step 3. Convert the nominal concentration to free concentration in the well, using the Armitage model (2014).

Step 4. Use the dose response curves (Step 2.) to extrapolate and translate the free concentration tested   to the exposure dose.

Figure 2 provides an overview of the workflow to produce the qIVIVE, including the Excel file names where measured data and numerical results are compiled:

Excel file (1): EURLECVAM_JRC_insilico_HEPCL_ProteinBinding_77_Summary:

All 77 chemicals with LogKow and summary results from the protein binding and hepatocyte clearance measurements.

Excel file (2): EURLECVAM_JRC_insilico_httk_38_analysis:

From the 77 chemicals, summary results for the 38 chemicals where both protein binding and hepatocyte clearance measurement produced a conclusive numerical result.
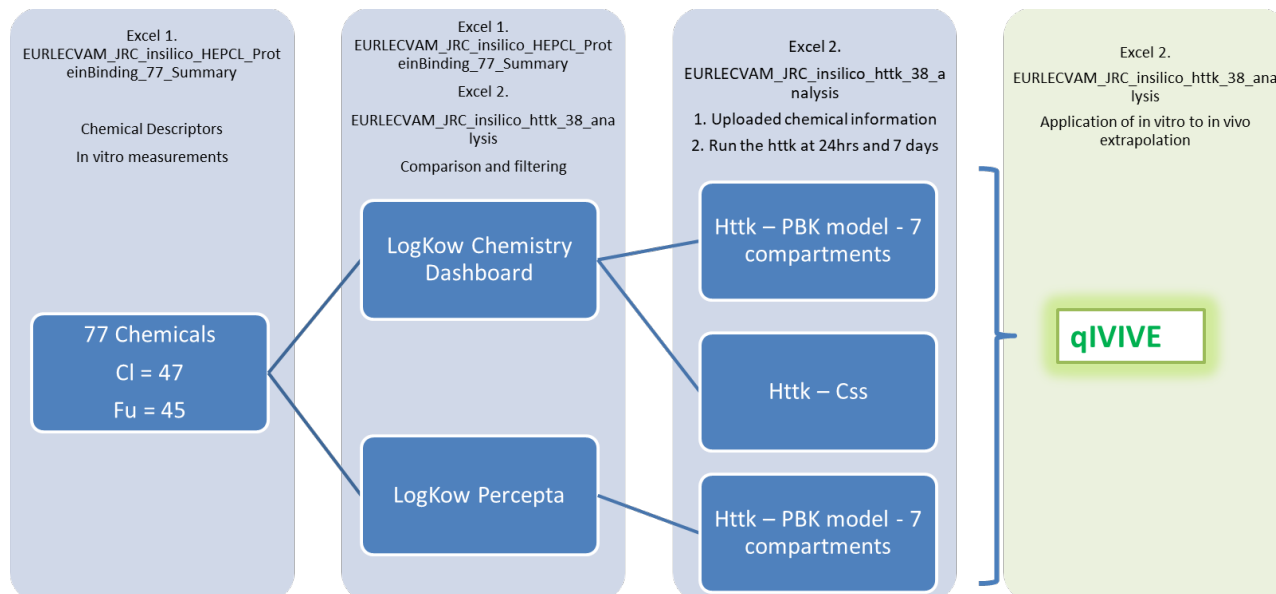

Figure 2. Data compilation and TK modelling overview.

3 Results

3.1 Protein binding and Hepatocyte clearance

Of the 77 chemicals assayed *in vitro*, 45 successful measurements were obtained for protein binding, and 47 for hepatocyte clearance, with coincidence of both in 38 cases. Nevertheless, the testing was exhaustive with various observations of issues relating to solubility, stability and sensitivity, or unsuccessful LC-MS method development (12 chemicals).

Protein binding

For the protein binding, the results were reported as fraction (%) bound and unbound, together with recovery (%).

The following 17 chemicals were found to be highly bound (>90%) in human plasma:

N-Phenyl-1-naphthylamine (CAS# 90-30-2),
Direct Red 81 (CAS# 2610-11-9),
N-Lauroyl sarcosine (CAS# 97-78-9),
Nerol (CAS# 106-25-2),
Hexyl salicylate (CAS# 6259-76-3),
1,2-Diphenoxy ethane (CAS# 104-66-5),
Ethyl 2-cyano-3,3-diphenyl acrylate (CAS# 5232-99-5),
Cyclosporine A (CAS# 59865-13-3),
Rotenone (CAS# 83-79-4),

Diclofenac sodium salt (CAS# 15307-79-6),
Rifampicin (CAS# 13292-46-1),
Amiodarone hydrochloride (CAS# 19774-82-4),
Disperse Yellow 42 (CAS# 5124-25-4),
Tetraethylthiuram disulphide (CAS#97-77-8),
(-)-Ambroxide (CAS# 6790-58-5),
Triethoxy-n-octylsilane (CAS# 2943-75-1),
Octanoic acid (CAS# 124-07-2).

Summary protein binding data are compiled in Excel file, JRC Data Catalogue, EURL ECVAM Collection: Excel file name, EURLECVAM_JRC_insilico_HEPCL_ProteinBinding_77_Summary

Hepatocyte clearance

For the hepatocyte clearance, half-life (mins), percent remaining (at 120 mins), and intrinsic clearance (CLint cells: µL/min/million cells) were calculated together with the following model estimates (refer, for example, to Mehvar, 2018 for explanatory background):

CLint liver: (mL/min/g liver)

CLint invivo: (mL/min/kg BW)

CLinvivo: (mL/min/kg BW) according to the well stirred model

Qh%: (CLinvivo well stirred model*100)/(Qh) taking account of blood flow.

With reference to Qh%, 49 chemicals were then conclusively assigned by the contractor to a clearance classification (low: <30%, moderate: 30-70%, High: >70%):

Low clearance (metabolism) at two tested concentrations (18 chemicals):

Adipic acid dihydrazide (CAS# 1071-93-8),

2,4-diamino-6-phenyl-1,3,5-triazine (CAS# 91-76-9),

Tetrabutyl ammonium bromide (CAS# 1643-19-2),

O-sulfobenzimide (CAS# 81-07-2),

N-Butyl diethanolamine (CAS# 102-79-4),

Direct Red 81 (CAS# 2610-11-9),

4-Formyl morpholine (CAS# 4394-85-8),

1-(2-Hydroxyethyl)-2-pyrrolidone (CAS# 3445-11-2),

Nerol (CAS# 106-25-2),

Rifampicin (CAS# 13292-46-1),

Acetaminophen (CAS# 103-90-2),

Caffeine (CAS# 58-08-2),

Diethylene triamine (CAS# 111-40-0),

2-Butyl-1-octanol (CAS# 3913-02-8),

Triethoxy methylsilane (CAS# 2031-67-6),

2-Propyl pentanoic acid (CAS# 99-66-1),

2-Ethylhexanoic acid (CAS 149-57-5),

2-Pentenoic acid (CAS# 13991-37-2).

Moderate clearance (metabolism) at two tested concentrations (10 chemicals):

4-Hydroxy-TEMPO (CAS# 2226-96-2),

Bis (2-ethylhexyl) phosphate (CAS# 298-07-7),

Cyclosporin A (CAS# 59865-13-3),

Amiodarone hydrochloride (CAS# 19774-82-4),

Aflatoxin B1 (CAS# 1162-65-8),

4-(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (CAS# 64091-91-4),

Triethoxy-n-octylsilane (CAS# 2943-75-1),

2-Nitrofluorene (CAS# 607-57-8),

Benzo(a)pyrene (CAS# 50-32-8),

Octanoic acid (CAS# 124-07-2).

High clearance at two tested concentrations (12 chemicals):

Neopentyl glycol dibenzoate (CAS# 4196-89-8),

N-phenyl-1-naphthylamine (CAS# 90-30-2),

N-Lauroyl sarcosine (CAS# 97-78-9),

N,N'-Bis (salicylidene)-1,2-propane diamine (CAS# 94-91-7),

2-Phenylethyl phenylacetate (CAS# 102-20-5),

Hexyl salicylate (CAS# 6259-76-3),

4-Methoxyl benzyl alcohol (CAS# 105-13-5),

Ethyl 2-cyano-3,3-diphenyl acrylate (CAS# 5232-99-5),

Vanillin isobutyrate (CAS# 20665-85-4),

Diclofenac sodium salt (CAS#15307-79-6),

Disperse Yellow 42 (CAS# 5124-25-4),

2-Phenylethyl isobutyrate (CAS# 103-48-0).

Concentration dependent clearance at two tested concentrations (6 chemicals):

2,6-di-tert-butyl-4-dimethylaminomethylphenol (CAS# 88-27-7),

4,4'-(Oxydi-2,1-ethanediyl) bismorpholine (CAS# 6425-39-4),

Tetramethyl thiuram monosulfide (CAS# 97-74-5),

1,2-Diphenoxy ethane (CAS# 104-66-5),

Rotenone (CAS# 83-79-4),

Tetraethylthiuram disulphide (CAS# 97-77-8).

Low clearance at higher tested concentration (2 chemicals):

Bis[2-[2-(1-methylethyl)-3-oxazolidinyl] ethyl] hexan-1,2-diyl biscarbamate (CAS# 59719-67-4),

Dimethyl [3-[(hydroxymethyl)amino]-3-oxopropyl] phosphonate (CAS# 20120-33-6).

Summary clearance data are compiled in Excel file (Can be found in the JRC Data Catalogue, EURL ECVAM collection).

EURLECVAM_JRC_insilico_HEPCL_ProteinBinding_77_Summary

Inconclusive results

A number of technical issues were observed during the experimental assay and analytical programme, precluding conclusion of a discrete numerical result in many cases. For the protein binding assay, insolubility or instability in plasma were variously noted for about 20 chemicals. Similar issues were reported for about 10 chemicals in the hepatocyte clearance assay, including analytical limitations relating to sensitivity. For 12 chemicals, LC-MS method development was not achieved. Nevertheless, all reported data provide qualitative insight into the respective properties and behaviour of the individual chemicals investigated, where details are available in the summary Excel files (JRC Data Catalogue, EURL ECVAM Collection).

3.2 Chemical LogKow profiling

First, the 38 chemicals with discrete CL and Fu data were assigned a colour classification based on LogKow variation between the four respective values. This enabled immediate indication of any significant variability, which occurred for four chemicals:
Aflatoxin B1, LogKow : 2,03 ; 0,45; 1,08; 2,01
Cyclosporin A, LogKow : 2,92; 3,35; 0,41;
Tetrabutyl ammonium bromide, LogKow : 0,641; -1,72; -0,12; -0,64
Diclofenac sodium salt, LogKow : 0,7; 4,06; 4,61; 4,48
Secondly, using the four LogKow values per chemical, ADME was evaluated using the seven compartment PBK model [24h (Cmax) and 7d (AUC)] to produce four corresponding predictions for concentration, Cmax, in liver and blood.
For the 24h simulations Cmax was consistent for 25 chemicals, while the remaining 13 showed significant variation. For the 7d prediction of AUC, 24 chemicals were consistent and 14 had significant variation in model output simulation of AUC.

3.3 Quantitative In vitro to In vivo Extrapolation (QIVIVE)

Biokinetic models were used to extrapolate from in vitro concentration to in vivo external dose. Using the numerical data generated by measuring protein binding (Fu) and hepatocyte clearance (CL) together with MW and LogKow, were introduced into the biokinetic models to simulate Css and Cmax and AUC, allowing qIVIVE, as outlined in Figure 3.
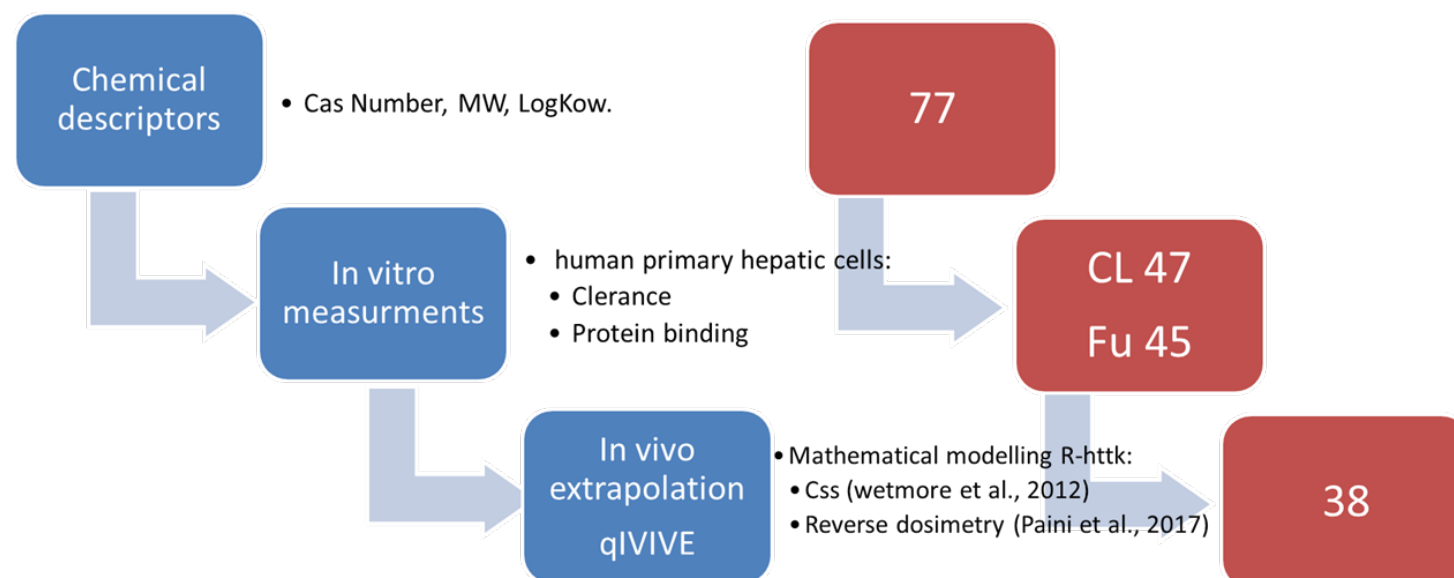

Figure 3. QIVIVE summary workflow, application of the CL and Fu in models to extrapolate oral equivalent dose

Estimation of oral equivalent dose (OED) (Wetmore et al., 2012)

Following the methodology, Css (steady state concentration in blood plasma) was derived for the 38 chemicals by simulation using r-httk package, function Calc_css_ analytics (see Annex for code). Oral equivalent dose (OED) in human was then calculated using the following formula:
OED (mg/kg/d) = IC50/EC50(µM)*(1 mg/kg/d)/Css(µM)
Css was calculated for 24h (hours) and 7d (days). However for only 5 chemicals out of the 38 was steady state reached at 24 hours. Therefore, for the remainder OED calculations were based on 7 days. Table 4 provides some example results for OED. By using default values for AC50 (set as 1 µM) and Dose (set as 1 mg/kg BW) and using the Concentration at steady state calculated using the r-httk package. For Acetaminophen an inhouse value measuring cytotoxicity was available and was used to extrapolate to the OED.

Table 4. Example OED results following Wetmore et al. (2012)

Compound	CAS	Plasma concentration returned in uM units.	Dose mg/kg bw	AC50 uM	OED (mg/kg*d)
(-)-Ambroxide	6790-58-5	32,7	1	1	0,0306
Acetaminophen	103-90-2	0,07465	1	1	13,396
Adipic acid dihydrazide	1071-93-8	0,293	1	1	3,413
Aflatoxin B1	1162-65-8	0,1097	1	1	9,116
Amiodarone hydrochloride	19774-82-4	1,178	1	1	0,849
Benzo(a)pyrene	50-32-8	22,61	1	1	0,044
C.I.Disperse Yellow 42	5124-25-4	26,25	1	1	0,039
Caffeine	58-08-2	0,06479	1	1	15,435
Cyclosporin A	59865-13-3	386,1	1	1	0,003
Acetaminophen*	103-90-2	0,07465	1	19,17	256,799

*Acetaminophen EC50 results from in-house lab measurements.

Reverse dosimetry Approach

Using the r-httk 7 compartment PBK model, dose (concentration) response curves were derived for 24h respective of liver and blood. The nominal concentrations were corrected according to the model of Armitage (2014). The PBK model was then run for 6 doses considering distribution in several compartments (e.g., liver, blood, lungs, kidney, etc.) for 11 of the 38 chemicals. For the remaining (27 chemicals) concentration was simulated in the blood and liver compartments only.
Figure 4 shows the dose response concentration at 24h for caffeine (other curves can be found in the Excel file 2, EURLECVAM_JRC_insilico_httk_38_analysis Tab dose response curves).

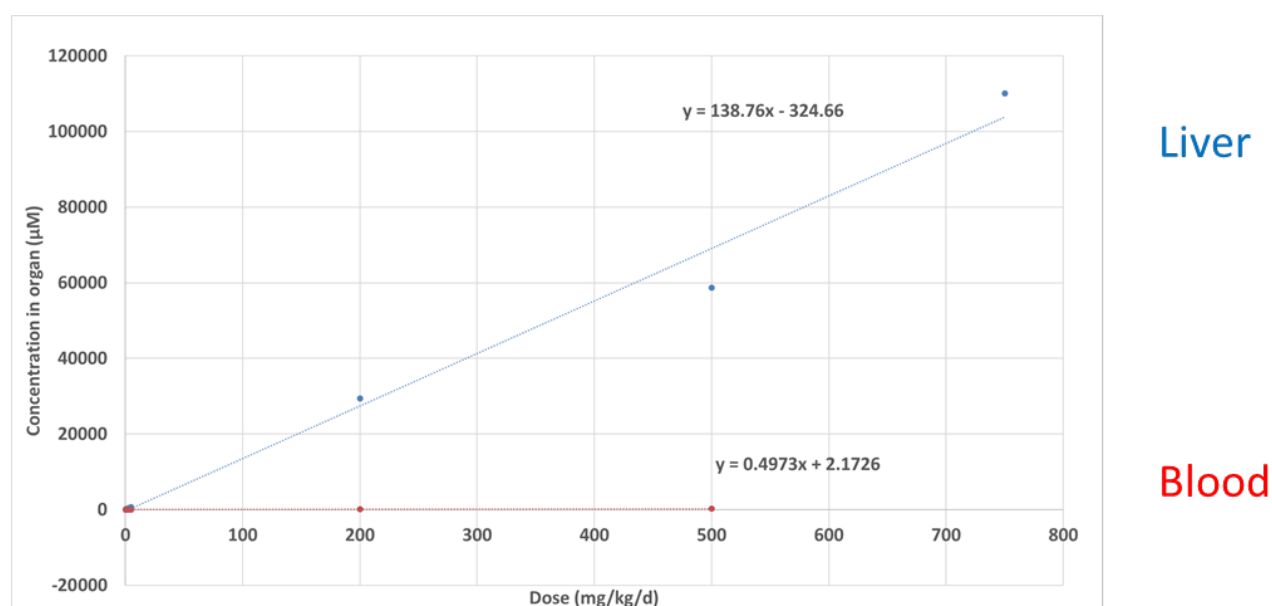Figure 4. Dose (concentration) response curve at 24h for caffeine in liver and blood compartments.

The nominal concentration (Cnom) applied to the test system were converted to the free concentration (Cwat) in the well by applying the Armitage model 2014, results are in the excel sheet under the Armitage tab; example of Cnom versus Cwat are reported in table 5.

The Armitage model, published in 2014, is a mathematical model describing the dispersion of a chemical in the well; is an excel tool, that helps to predict the free available chemical concentration in an in vitro experiment, such other models are: Fischer et al., 2017; Fisher et al., 2019; Kramer, 2010; Zaldivar Comenges et al., 2017.

Table 5. Selected results from the Armitage model CNom versus CWat in uM.

Name	CAS	$C_{NOM,initial}$	C_{WAT}
(-)-Ambroxide	6790-58-5	50	0.33093259
2-Propyl pentanoic acid	99-66-1	50	28.88916582
Acetaminophen	103-90-2	50	49.83472498
Amiodarone hydrochloride	19774-82-4	50	0.004794881
Caffeine	58-08-2	50	49.93611056
Diclofenac sodium salt	15307-79-6	50	6.608700739
Hexyl salicylate	6259-76-3	50	2.899934916
Aflatoxin B1	1162-65-8	50	44.39820034
Benzo(a)pyrene	50-32-8	50	0.060055204
Cyclosporin A	59865-13-3	50	23.793035
Rotenone	83-79-4	50	2.408435086

By using the dose response curves achieved in Step 2 by applying the 7 compartment PBK model and by plotting the external dose simulated versus concentration we can use the equation representing the curve for extrapolation. The extrapolation can be made between the dose and the testedk free concentration, for instance Acetaminophen was tested in house at the nominal concentration of 19,17 µM after filtering via the Armitage model the concentration available to be uptake in the cell was refined to 19,136 µM. This concentration was used in the equation for acetaminophen obtained in liver and blood, and the external equivalent dose was estimated for both organs (Table 6). For the current experimental setting, the acetaminophen value was obtained by in house HepaRG experiments, the liver curve would be the best organ to make the extrapolation.

Results for all chemicals can be found in Excel file 2, EURLECVAM_JRC_insilico_httk_38_analysis. For all the other chemicals, in-house measured concentration were not available the Cnom was left as default to 50 µM.

Table 6. Extraction of the qIVIVE results, results area available in Excel file 2, EURLECVAM_JRC_insilico_httk_38_analysis

Compound name	CAS number	$C_{NOM,initial}$	C_{WAT}	Dose response curves		external dose based on Cven			external dose based on Cliver		
Units	*-			Curve Cblood	Curve Cliver	a Cblood	b Cblood	mg/kg BW	a Cliver	b Cliver	mg/kg BW
(-)-Ambroxide	6790-58-5	50,00	0,33	y = 0,4407x + 0,0305	y = 82,949x + 0,0322	0,4407	0,0305	0,690	82,949	0,0322	0,004
1,2-Diphenoxy ethane	104-66-5	50,00	4,71	y = 9,5901x - 71,975	y = 17,705x - 2,461	9,5901	71,975	7,996	17,705	2,461	0,405
2-Butyl-1-Octanol	3913-02-08	50,00	0,52	y = 0,5737x + 0,0376	y = 105,55x - 1,1043	0,5737	0,0376	0,842	105,55	1,1043	0,015
2-Ethylhexanoic acid	149-57-5	50,00	32,20	y = 1,5457x + 0,0737	y = 97,583x + 4,6535	1,5457	0,0737	20,782	97,583	4,6535	0,282
2-Nitrofluorene	607-57-8	50,00	11,70	y = 0,6176x + 0,0233	y = 76,159x + 3,1614	0,6176	0,0233	18,905	76,159	3,1614	0,112
2-Propyl pentanoic acid	99-66-1	50,00	29,03	y = 0,0123x - 0,01	y = 45,204x - 27,223	0,0123	0,01	2361,063	45,204	27,223	1,244
4-Formyl morpholine	4394-85-8	50,00	37,21	y = 8,0436x + 0,4067	y = 13,033x + 0,7016	8,0436	0,4067	4,576	13,033	0,7016	2,801
4-hydroxy-tempo	2226-96-2	50,00	49,75	y = 0,9133x + 0,0412	y = 3,3743x + 0,1272	0,9133	0,0412	54,422	3,3743	0,1272	14,705
4-Methoxyl Benzyl alcohol	105-13-5	50,00	49,28	y = 0,2472x + 0,0115	y = 1,6475x + 0,0852	0,2472	0,0115	199,291	1,6475	0,0852	29,858
Acetaminophen	103-90-2	50,00	49,84	y = 0,1269x + 0,0054	y = 30,419x + 1,2819	0,1269	0,0054	392,686	30,419	1,2819	1,596
Acetaminophen	103-90-2	19,17	19,12	y = 0,1269x + 0,0054	y = 30,419x + 1,2819	0,1269	0,0054	150,627	30,419	1,2819	0,586

4 Conclusions

Following the aims of the EURL ECVAM TK strategy, data for clearance and protein binding were generated and are now available via the EURL ECVAM collection in the JRC data catalogue. These data will inform several projects (APCRA among others).
The ADME data measured and generate are of high relevance and have multiple applications. They can inform risk assessment for prioritization, can be used in *ab initio* or read-across approaches, and can be input to mathematical modelling as done in the current report. To illustrate the importance of ADME data we applied the CL and Fu into two different biokinetic models to calculate in a reverse dosimetry fashion by qIVIVE the human "potential" exposure doses. This was done by applying the models available in the r-httk package from US EPA. Showing that both approaches presented can be informative in risk assessment context.

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Annexes

Annex 1. r-httk in house manual

Modeling using httk of the CL and Fu measured data. The present manual illustrated how to use new in vitro data in r/httk from US EPA. R and R studio were used and recommended packages where downloaded.

Analysis will be done using calc_analytic_css, and Calc_css and PBTK

Preparation of your virtual working bench

Step 1: Install R or Rstudio, install package httk, automatically all the packages needed are imported and installed when httk is installing. In addition some r packages are suggested to be installed in https://cran.r-project.org/web/packages/httk/index.html

Imports: deSolve, msm, data.table, survey, mvtnorm, truncnorm, stats, graphics, utils, magrittr
Suggests: ggplot2, knitr, rmarkdown, R.rsp, GGally, gplots, scales, EnvStats, MASS, RColorBrewer, TeachingDemos, classInt, ks, stringr, reshape, reshape2, gdata, viridis, CensRegMod, gmodels, colorspace

Import new data into httk libraries

Step 2. Convey all the information needed to run (input parameters, name the column: Compound, CAS, logP, MW, Clint, Fubound.plasma) the model in one excel table (see table Input Table Analysis 05/04/2020)). Model parametrization that was introduced in the httk (###########Input Table Analysis 05/04/2020 ####)

1. Add_chemtable
This function adds chemical-specific information to the table chem.physical_and_invitro.data. This table is queried by the model parameterization functions when attempting to parameterize a model, so adding sufficient data to this table allows additional chemicals to be modeled.

Usage add_chemtable(new.table, data.list, current.table=NULL, reference=NULL, species=NULL, overwrite = F)
        chem.physical_and_invitro.data   <-   add_chemtable(my.new.data,   current.table=chem.physical_and_invitro.data,   data.list=list(Compound="Name", CAS="CAS", DTXSID="NULL", MW="MW", logP="LogP", Funbound.plasma="Fu", Clint="CLint"), species="Human")

2. chem.physical_and_invitro.data Physico-chemical properties and in vitro measurements for toxicokinetics
This data set contains the necessary information to make basic, high-throughput toxicokinetic (HTTK) predictions for compounds, including Funbound.plasma, molecular weight (g/mol), logP, logMA (membrane affinity), intrinsic clearance(uL/min/10^6 cells), and pKa. These data have been compiled from multiple sources, and can be used to parameterize a variety of toxicokinetic models. See variable EPA.ref for information on the reference EPA

```
####Libraries needed to run the httk
rm(list = ls())
library(httk)
library(ggplot2)
library(openxlsx)
a<-chem.physical_and_invitro.data

####new database
K <-read.csv("C:/Users/meinema/chemicals.csv", sep = ";")
my.new.data <- as.data.frame(K)

chem.physical_and_invitro.data <- add_chemtable(my.new.data,
                current.table=chem.physical_and_invitro.data,
                data.list=list(
                Compound="Compound",
                CAS="CAS",
```

```
                    MW="MW",
                    logP="LogP",
                    Funbound.plasma="Fu",
                    Clint="CLint"),
                    species="Human",
                    reference="MyPaper 2015",
                    overwrite=TRUE)
```

3. Parameterize_PBTK (see table below)

parameterize_pbtk(chem.cas = NULL, chem.name = NULL, dtxsid = NULL, species = "Human", default.to.human = F, tissuelist = list(liver = c("liver"), kidney = c("kidney"), lung = c("lung"), gut = c("gut")), force.human.clint.fup = F, clint.pvalue.threshold = 0.05, adjusted.Funbound.plasma = T, regression = T, suppress.messages = F, restrictive.clearance = T, minimum.Funbound.plasma = 1e-04

4. A. For the steady state the formula is: calc_css(), again you have to specify chemical, time, type of model (in this case pbtk). calc_analytic_css Calculate the analytic steady state concentration.

5. A. Made a loop to calculate all at once (Below the R code used, ##########steady state ####)

4. B. To run the PBK model the function is: solve_pbtk(), you then have to specify the chemical, the dose, the time etc.

solve_pbtk : This function solves for the amounts or concentrations in uM of a chemical in different tissues as functions of time based on the dose and dosing frequency. Use the function solve_pbtk to run the PBTK seven compartment model. You have to set adjusted.Funbound.plasma=FALSE (as shown in the code) to take into account your data and not the one from the paper.

5. B. Calculation where done one by one 38 code were generate. The loop did not produce the max conc in organ but total time curve run. (below the Code ##########PBTK ####)

6. Example results from the run, could be written in xlsx. The results are displayed in a table that can be used to generate figures (to make plot see below, ###plot.data###) and for downstream analysis.

CODE

##########steady state, in loop ####

chemical_CAS<-c('6790-58-5', '3445--11--2', '104-66-5','91-76-9', '88-27-7', '3913--02--08', '149-57-5', '607-57-8', '99-66-1', '64091-91-4', '6425-39-4', '4394-85-8', '2226-96-2', '105-13-5', '103-90-2', '1071-93-8', '1162-65-8', '19774-82-4', '50-32-8', '5124-25-4', '58-08-2', '59865-13-3', '15307-79-6', '111-40-0', '2610--11--9', '5232-99-5', '6259-76-3', '102-79-4', '106-25-2', '97-78-9', '90-30-2', '124-07-2', '81-07-2', '83-79-4', '1643-19-2', '97-77-8', '97-74-5', '2943-75-1')

for (CAS in chemical_CAS) {

print(CAS)

m2<-calc_css(chem.cas = CAS, adjusted.Funbound.plasma = FALSE, days= 1, species="Human", model='pbtk')

print(m2)

}

chemical_CAS<-c('6790-58-5', '3445--11--2', '104-66-5', '91-76-9', '88-27-7', '3913--02--08', '149-57-5','607-57-8', '99-66-1', '64091-91-4','6425-39-4','4394-85-8','2226-96-2','105-13-5', '103-90-2', '1071-93-8','1162-65-8', '19774-82-4', '50-32-8', '5124-25-4','58-08-2', '59865-13-3', '15307-79-6','111-40-0','2610--11--9', '5232-99-5', '6259-76-3', '102-79-4', '106-25-2', '97-78-9','90-30-2', '124-07-2', '81-07-2', '83-79-4', '1643-19-2', '97-77-8', '97-74-5', '2943-75-1')

for (CAS in chemical_CAS) {

```
print(CAS)

m1<-calc_css(chem.cas= CAS, adjusted.Funbound.plasma = FALSE, days= 7, species="Human", model='pbtk')  print(m1)

}

chemical_CAS<-c('6790-58-5', '3445--11--2', '104-66-5', '91-76-9', '88-27-7', '3913--02--08', '149-57-5','607-57-8', '99-66-1', '64091-
91-4','6425-39-4','4394-85-8','2226-96-2','105-13-5', '103-90-2', '1071-93-8','1162-65-8', '19774-82-4', '50-32-8', '5124-25-4','58-08-
2', '59865-13-3', '15307-79-6','111-40-0','2610--11--9', '5232-99-5', '6259-76-3', '102-79-4', '106-25-2', '97-78-9','90-30-2', '124-07-2',
'81-07-2', '83-79-4', '1643-19-2', '97-77-8', '97-74-5', '2943-75-1')

for (CAS in chemical_CAS)  {

print(CAS)

a<-calc_analytic_css(chem.cas=CAS, adjusted.Funbound.plasma=FALSE, model='pbtk')

print(a)

 }

###########PBTK ####

s1<- solution<-solve_pbtk(chem.cas='6790-58-5', adjusted.Funbound.plasma = FALSE, days=1, dose = 1,iv.dose=F, species="Human")

kwrite.xlsx(s1, "C:/Rstudio/chemicallistPBTK")

s1<-as.data.frame(s1)

max(s1$Cart)

max(s1$Cven)

max(s1$Cliver)

max(s1$Crest)

max(s1$Clung)

max(s1$Ckidney)


###plot.data###

a1 <- ggplot(s1,aes(time, Cliver)) + geom_line() +
  ylab("Liver Concentration (uM)") +
  xlab("Day") + theme(axis.text = element_text(size = 16),
            axis.title = element_text(size = 16),
            plot.title = element_text(size = 17, hjust = 0.5)) +
  ggtitle("(-)-Ambroxide")
a1

a2 <- ggplot(s1,aes(time,Cart)) + geom_line()+
  ylab("Blood Concentration (uM)") +
  xlab("Day") + theme(axis.text = element_text(size = 16),
            axis.title = element_text(size = 16),
            plot.title = element_text(size = 17, hjust = 0.5)) +
  ggtitle("(-)-Ambroxide")
a2
```
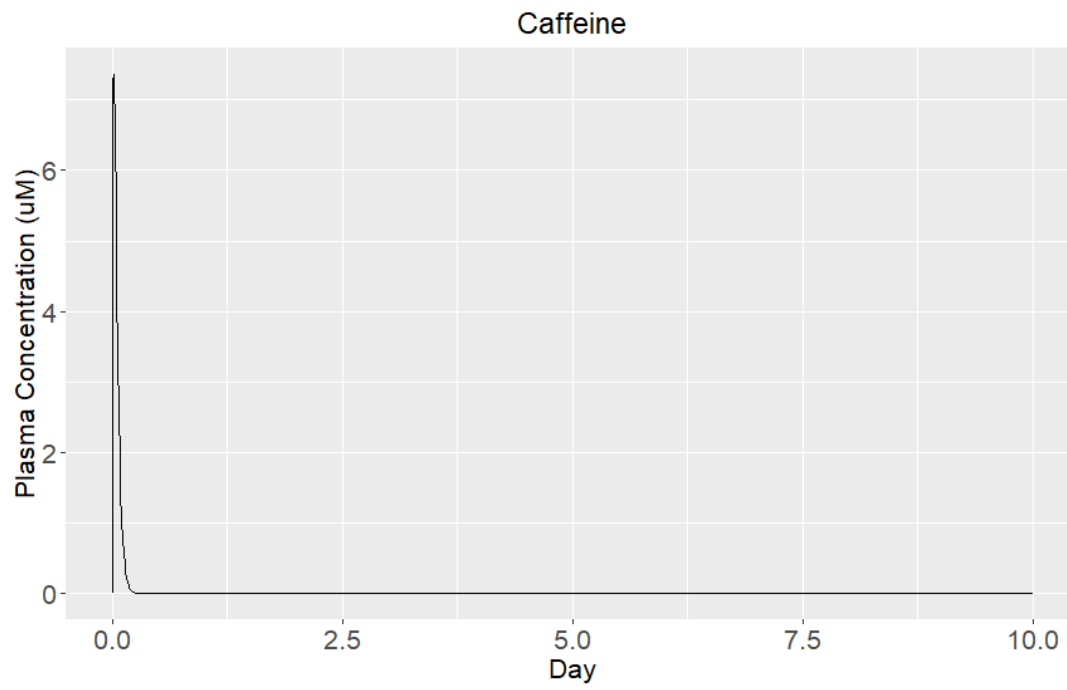
Figure 2. Graphical representation of the caffeine plasma concentration

###########Input Table Analysis 05/04/2020 ####

Table. Chemical list, CAS, MW, LogP, Fu, Clint, uploaded into httk library to run different biokinetic models

	Compound	CAS	MW	LogP	Fu	CLint
1	(-)-Ambroxide	6790-58-5	236.40	5.110	1.5400	2.25
2	1-(2-Hydroxyethyl)-2-pyrrolidone	3445--11--2	129.16	-0.819	264.1200	0.66
3	1,2-Diphenoxy ethane	104-66-5	214.26	3.810	0.0001	5.48
4	2,4-diamino-6-phenyl-1,3,5-triazine	91-76-9	187.20	1.360	34.3000	0.20
5	2,6-di-tert-butyl-4-dimethylamino methylphenol	88-27-7	263.42	4.180	26.4900	3.44
6	2-Butyl-1-Octanol	3913--02--08	186.33	4.760	210.7500	0.83
7	2-Ethylhexanoic acid	149-57-5	144.21	2.640	13.1600	0.60
8	2-Nitrofluorene	607-57-8	211.22	3.370	177.1700	3.58
9	2-Propyl pentanoic acid	99-66-1	144.21	2.750	21.4000	1.36
10	4-(Methylnitroso amino)-1-(3-pyridinyl)-1-butanone	64091-91-4	207.23	0.282	96.4700	4.37
11	4,4?-(Oxydi-2,1-ethanediyl) bismorpholine	6425-39-4	244.33	-0.554	111.4400	3.67
12	4-Formyl morpholine	4394-85-8	115.13	-1.120	95.2800	0.45
13	4-hydroxy-tempo	2226-96-2	172.24	0.670	108.6500	9.83
14	4-Methoxyl Benzyl alcohol	105-13-5	138.16	1.100	93.9200	50.95
15	Acetaminophen	103-90-2	151.16	0.460	93.9800	0.31
16	Adipic acid dihydrazide	1071-93-8	174.20	-1.950	96.0100	0.92
17	Aflatoxin B1	1162-65-8	312.27	2.030	10.4500	7.12
18	Amiodarone hydrochloride	19774-82-4	681.80	7.380	0.0001	6.71
19	Benzo(a)pyrene	50-32-8	252.30	6.130	38.2600	4.64
20	C.I.Disperse Yellow 42	5124-25-4	368.00	4.600	0.0001	34.16
21	Caffeine	58-08-2	194.19	-0.070	141.4200	0.11
22	Cyclosporin A	59865-13-3	1202.61	2.920	0.0001	4.71
23	Diclofenac sodium salt	15307-79-6	318.13	0.700	0.4100	34.79
24	Diethylene triamine	111-40-0	103.13	-1.510	96.7800	0.33

###########Example of Results calc_css Analysis 05/04/2020 ####
```
 "6790-58-5"
Plasma concentration returned in uM units.
 32.7
 "3445--11--2"
Plasma concentration returned in uM units.
 0.5343
 "104-66-5"
Plasma concentration returned in uM units.
 571.9
 "91-76-9"
Plasma concentration returned in uM units.
 1.13
 "88-27-7"
Plasma concentration returned in uM units.
 7.468
 "3913--02--08" […results for the 38 chemicals]
```
###########Example of Results solve_PBTK Analysis 06/04/2020 ####

```
> s7<- solution<-solve_pbtk(chem.cas= '149-57-5', adjusted.Funbound.plasma = FALSE, days=1, dose =
1,iv.dose=F, species="Human")
Human amounts returned in umol and concentration returned in uM units.
AUC is area under plasma concentration in uM * days units with
Rblood2plasma = 17.2.
Warning messages:
1: In predict_partitioning_schmitt(parameters = schmitt.params, species = species,  :
  Membrane affintity (MA) predicted with method of Yun and Edginton (2013)
2: In available_rblood2plasma(chem.cas = chem.cas, species = species,  :
  Human Rblood2plasma calculated with calc_rblood2plasma.
> write.xlsx(s7, "C:/Rstudio/2-Ethylhexanoic acid.xlsx")
> s7<-as.data.frame(s7)
> max(s7$Cart)
[1] 1.628
> max(s7$Cven)
[1] 1.628
> max(s7$Cliver)
[1] 97.6
> max(s1$Crest)
[1] 4.506
> max(s1$Clung)
[1] 2.806
> max(s1$Ckidney)
[1] 26.03
```
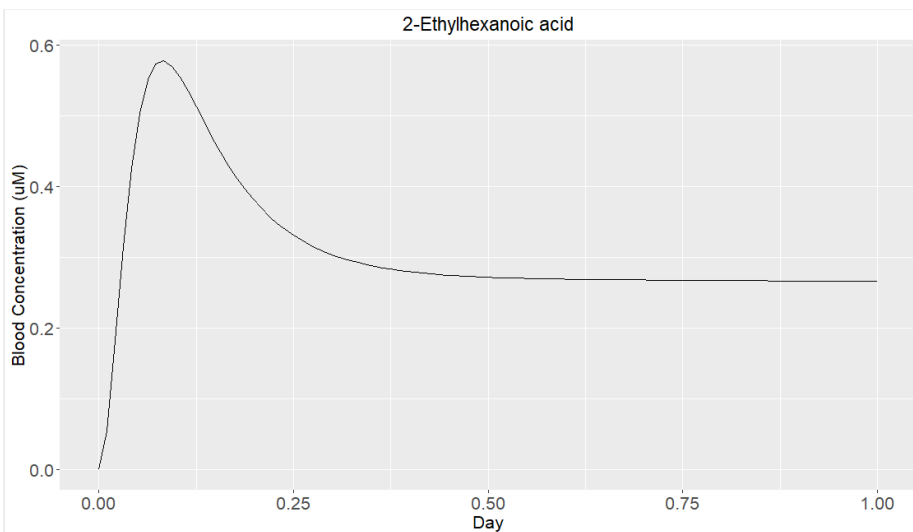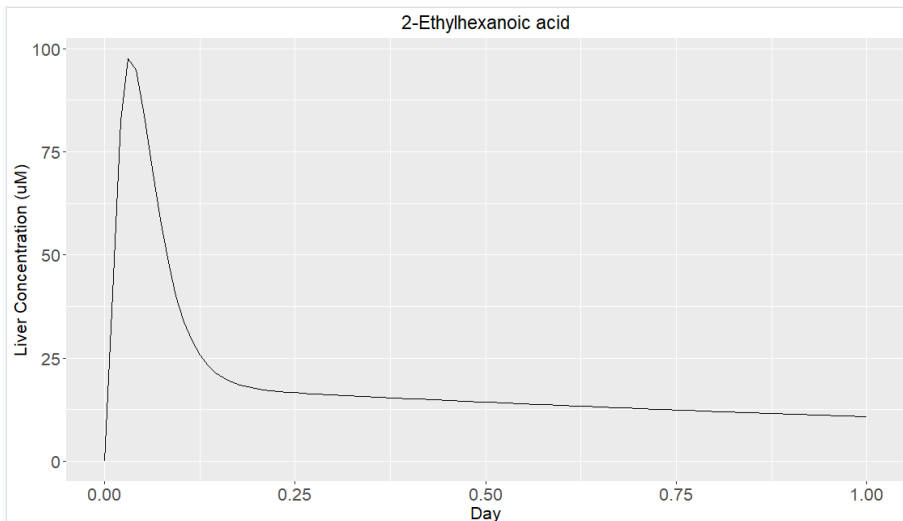

####Write to excel####

The table obtained with httk can be exported as an excel file or other formats

To export the table as an excel file, first install and upload the package openxlsx and then use the function write.xlsx. You define the table that you want to export and you set the destination and the name of the new file.

write.xlsx(s1, "C:/Rstudio/chemicallistPBTK")

Log Kow analysis (results not shown in the excel)

You can use just one logP per time. So when you upload the table you specify the logP you want to use. For example in the picture you seek that logP="logP" because we are using the one we collected on the dashboard. If you want to use another one you should put logP="logP_m" etc. before doing this is better to clean the environment with the command: rm(list = ls()).

So, **step1:** upload of the database with one logP specified. You run all the models you want.

Step2: clean the environment with the command rm(list = ls())

Step3: upload again the database specifying a different logP. You run all the models you want