Inhalation toxicity of cinnamaldehyde:

A physiologically based kinetic modeling approach to study route-to-route extrapolation

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Abstract

Using physiologically based kinetic (PBK) modelling novel, chemical exposure scenarios can be modelled and analysed to form an understanding of the toxicokinetics and possible adverse health effects without having to perform in vivo experiments. In this study, an oral exposure PBK model in rats and humans of cinnamaldehyde (CNMA) was adapted to also model CNMA toxicokinetics after inhalation. The model parameters where derived based on *in vivo* rat exposure data and *in silico* calculated coefficients. The model was then used to predict CNMA tissue concentrations in both rats and humans. CNMA is a reactive aldehyde that is used as a flavouring agent in different food products. More recently CNMA has been adopted as a flavouring agent for electronic cigarettes, i.e., vaping devices. CNMA is a Generally Regarded as Safe (GRAS) flavouring agent. Unfortunately inhalation exposure is not considered for flavouring agents. Worryingly, recent research has linked CNMA to adverse health effects such as oxidative stress en immune suppression in the lungs when inhaled. Simulation results for the human inhalation model show that predicted CNMA concentration are in the range at which immunosuppressive, mitochondrial dysregulation and/or cytotoxicity effects are noted in *in vitro* models using human and mice cells including epithelial, fibroblast and embryonic stem cells. Furthermore, model simulation indicate that CNMA concentration are over 250 times higher in the lung when inhaled compared to oral exposure. The ability to predict tissue concentrations in novel exposure scenarios based on population metrics represents a key feature of PBK modelling which can be used on both novel and known chemicals.

*Keywords:* Inhalation, Physiologically based kinetic modelling, Population, Flavouring, Electronic cigarette

1. Introduction

A decline in cigarette use has been noted by the WHO in almost all regions across the planet (WHO., 2021). This can be ascribed to a number of factors such as increased public awareness of the adverse health effects of smoking cigarettes, the introduction of nicotine patches and by the rise of electronic cigarettes (Hartmann-Boyce et al., 2021). With an electronic cigarette a consumer is no longer exposed to the burning of tabaco and thus to the associated harmful chemicals. Electronic cigarettes work by the vaporization and inhalation of a blend of nicotine, propylene glycol and/or glycerol. Furthermore, electronic cigarette mixtures are often enhanced with the addition of flavouring agents (Omaiye et al., 2019; Page & Goniewicz, 2021). Examples of such flavouring agents are vanillin (vanilla), benzaldehyde (almond) and cinnamaldehyde (cinnamon). A considerable part of the appeal of electronic cigarettes is the absence of a host of toxic and carcinogenic compounds normally found in cigarette smoke. Yet, a mounting body of evidence suggest adverse health effects can be seen after use of electronic cigarettes (Chatham-Stephens et al., 2014; Effah et al., 2022; Hua & Talbot, 2016). The exact cause of these adverse health effects is as of yet unknown.

The reactive aldehyde cinnamaldehyde (CNMA) is an interesting possible candidate that might contribute to adverse health effects. Aldehydes are compounds which possess a carbonyl group with a substituent Hydrogen atom (Lopachin & Gavin, 2014). These carbonyl groups are known for the electrophilic characteristics and associated reactivity. An assortment of aldehydes is associated with adverse health outcomes. These include the known cancer causing agents: Formaldehyde, Acrolein and Crotonaldehyde ( IARC Working Group on the Evaluation of Carcinogenic Risks to Humans., 2021). CNMA is present in multiple vape products at concentrations up to 343mg/ml with possible high retention after exposure (Khachatoorian et al., 2022; Omaiye et al., 2019). CNMA is noted to induce toxicity in in vitro systems including oxidative stress, inflammatory responses and reductions in cell viability (Behar et al., 2014; Bhattacharya et al., 2021; Clapp et al., 2017, 2019; Gerloff et al., 2017; Ka et al., 2003; Muthumalage et al., 2018)

Flavouring agents are regulated in the United States by the Food Additives Amendments to the U.S. Federal Food, Drug, and Cosmetic Act. In Europe they are considered flavouring agents and regulated by de European Food Safety Authority Flavouring and Food Additive panel. Flavouring agents that occur naturally such as CNMA have historically received very little attention as either no adverse effects have been noted in food where its is present naturally or the concentrations used are very low. CNMA is Generally regarded As Safe (GRAS) in both Europe and the United States and therefore allowed respectively either as an flavouring agent or and food additive. Unfortunately, this approach neglects to consider inhalation exposure (Dinu et al., 2020). An infamous example of a flavouring agents that has been noted to cause adverse health effects when inhaled is Diacetyl (Athleen et al., 2002; Hubbs et al., 2008; Morgan et al., 2008) .

Unfortunately no inhalation exposure data is available for CNMA. In cases such as CNMA where no experimental data for the inhalation route is available ECHA recommends using route to route extrapolation with additional testing using PBK modelling (ECHA, 2012). PBK modelling involves mathematically solving the movement of chemicals through the different organs using the blood. This mathematical model is based on physiological parameters such as blood flow and organ weights, chemical specific parameters such as lipophilicity, partition coefficients and metabolic rates. The aim of PBK modelling is to be able to predict the concentration of the target chemical at a specific target site. A PBK modelling approach has multiple advantages in this case. Reduced reliance on ethically troubled animal testing methodologies by employing route-to-route extrapolation based on previously gathered *in vivo* data without the need for additional animal testing. The possibility to employ population based modelling. Population based modelling can reveal sensitive populations otherwise unaccounted for using a “standard” human. And the opportunity to rapidly adapt effective modelling approaches to similar relevant compounds.

For CNMA an PBK model has been developed for oral exposure. The model in question was used to estimate the DNA adduct formation in the liver of rats and humans (Kiwamoto et al., 2016). This model will be used as basis for the creation of inhalation models for both human and rat. This will be done to validate the inhalation approach. When the inhalation model is validated the human model will be adopted into a population model. These models will then be used to asses if exposure to CNMA in a simulated electronic cigarette scenario is predicted to results exposures that could lead to possible adverse effects.

# Abbreviations

PBK Physiologically base kinetic modelling

CNMA Cinnamaldehyde

GRAS Generally regarded as safe

WHO World Health Organisation

AUC Area under the curve

DNA Deoxyribonucleic acid

ODE Ordinary differential equation

LogKow log Octanal water partition coefficient

LogKoa Log octanal air partition coefficient

QSAR

GSA Global sensitivity analysis

1. Materials and methods
   1. Model structure
   2. Kiwamoto

As the basis for an inhalation model of CNMA, a previously published PBK model simulating CNMA tissue levels in rats and humans after oral exposure was used (Kiwamoto et al., 2016). The model consisted of the following compartments; slowly perfused tissue, richly perfused tissue, liver, small intestines, and fat connected to each other by a venous and arterial blood compartment. CNMA is introduced by oral gavage in to the small intestine. From the small intestine it moves to the liver. From the liver CNMA is introduced into the blood and general circulation. An overview of the general structure of the model can be seen in Figure 2. Figure 2 shows the general structure of the Kiwamoto model with the exception of the lung compartment. CNMA partitioning is considered to perfusion limited and driven by partitioning coefficients. These coefficients were estimated based on the logKow values by the method demonstrated by (Dejongh et al., 1997). LogKow were estimated using the Estimation Program Interface (EPI) Suite version 4.10 provided by the US Environmental Protection Agency.

CNMA is metabolised into multiple different metabolites. These are a CNMA-Glutathione conjugate, CNMA-protein adducts, Cinnamyl alcohol and Cinnamic acid. An overview of the metabolites can be found in Figure 1. Because Cinnamyl alcohol can be transformed back into CNMA a submodel was added to the model by Kiwamoto et al (2016). This sub model describes the distribution of CNMA alcohol through the body and its metabolism back into CNMA.

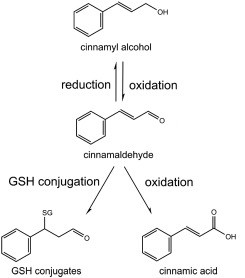


Figure 1: Cinnamaldehyde Metabolism adapted from Kiwamoto et al (2016)

The Kiwamoto model was adapted in a number of different ways. Firstly Kiwamoto wrote the model in the numerical ordinary differential equation(ODE) solver Berkely Madonna (8.3.18 University of California at Berkeley, CA, USA). For this report a different solver was chosen. The model was coded in R (version 4.1.1) and R Studio (version 1.4.1717) using the Rxode, ggplot2 and PKNCA, packages (Fidler et al., 2022). A description of how the code was adapted can be found in supplementary data 1; R code. R was chosen on the basis that R and R studio are open source, have a large assortment of publicly available add on packages, have the ability to perform global sensitivity analysis and lastly ability to perform population based modelling.

The following changes were made to the model code and structure. DNA adduct formation and CNMA-GSH metabolism in the small intestine were removed for the human model as they were shown by (Kiwamoto et al., 2016) to not occur. Inhalation of CNMA into the lungs and subsequent distribution through the body was based on (Jongeneelen & Berge, 2011). This involves the inhalation of CNMA into an alveolar air compartment eq(1) + eq(3) from which CNMA is absorbed into the blood and enters circulation eq(2). CNMA can also re-enter the alveolar air and be exhaled eq(4). The complete model code and equations for both human and rat models can be found in supplementary data 1: R code.

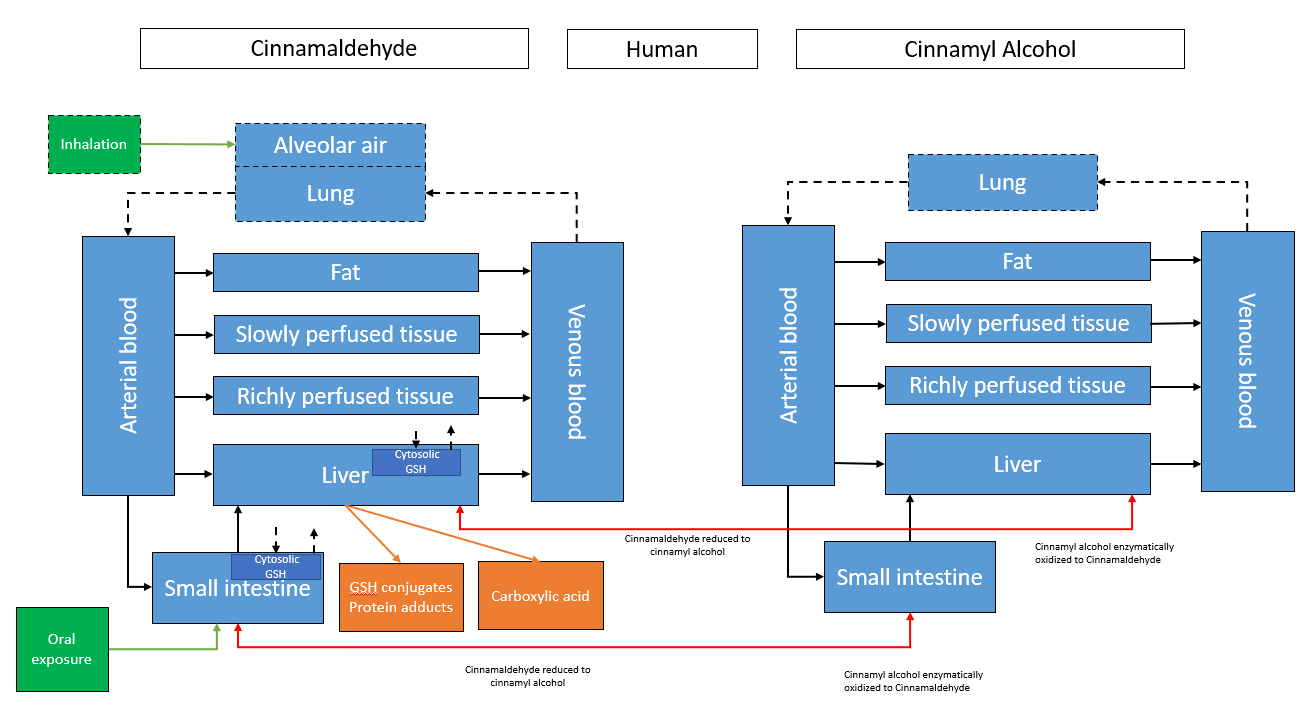


Figure 2: Structure of the inhalation CNMA model in Humans. Dotted lines represent additions to the model compared to Kiwamoto et al. Exposure routes are coloured green. Elimination routes are present in both the liver and small intestine and are coloured orange. In both the small intestine and the liver cytosolic Glutathione concentrations are modelled. Transformations of CNMA to Cinnamyl alcohol and back are represented by red lines.

Rate of Cinnamaldehyde inhalation (μmol/h)

(1)

*P\_V = Pulmonary ventilation (L/h)*

*A\_inhalation\_dose = The dose present in the exposure chamber* *(μmol)*

*Volume\_exposure\_chamber = the volume of the exposure chamber (L)*

Concentration of Cinnamaldehyde in Arterial blood leaving the lungs (μmol/L)

(2)

*Q\_Pu = blood flow to the lungs (L)*

*C\_V = Concentration of CNMA in venous blood (μmol/L)*

*P\_B = blood/air Partition coefficient (unitless)*

Concentration of Cinnamaldehyde exhaled (μmol/L)

(3)

Rate of Cinnamaldehyde exhalation (μmol/h)

(4)

The model equations used in this model was used together with the fit metabolic parameters.

* 1. Inhalation PBK model for CNMA in rats

To validate the inhalation compartment of the model the rat model from Kiwamoto et al (2016) was adapted as noted above. Not only was the model structure adapted all physiological and chemical parameters were checked and changed if necessary. The complete list of physiological parameters can be found in Table 1.

Table 1: Physiological parameters for the rat inhalation model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Description | Value | Unit | Reference |
| BW  Tissue volumes | Body Weight | 0.25 | Kg | (Brown et al., 1997) |
| V\_F | Fat | 0.0175 | L | *“”* |
| V\_L  V\_SI  V\_A  V\_V  V\_RP  V\_SP  V\_Pu  Cardiac parameters  Q\_C  Q\_F  Q\_L  Q\_SI  Q\_RP  Q\_SP  Q\_Pu  Uptake  P\_V  Ka | Liver  Small intestine  Arterial Blood  Venous Blood  Richly perfused  Slowly perfused  Lung  Cardiac output  Fat  Liver  Small intestine  Richly perfused  Slowly perfused  Lungs  Pulmonary ventilation  Uptake rate constant | 0.0085  0.0035  0.00475  0.01475  0.00925  0.169  0.00125  5.4  0.07  0.13  0.12  0.64  0.17  Q\_C  0.75  1.97 | L  L  L  L  L  L  L  L/h  L/h  L/h  L/h  L/h  L/h  L/h  L/h  Per hour | *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  (Brown et al., 1997) based on a mean of 50ml/min per 100g  Calculation based on (Ans et al, 2022) |

As noted in Table 1 the uptake rate constant was calculated based on Punt et al,(2022). The calculation that were used are displayed below.

Apparent permeability

(5)

*TPSA = Topological polar surface area of CNMA = 17.1 Computed by Cactvs 3.4.8.18 (PubChem release 2021.05.07)*

Effective permeability

(6)

Uptake rate constant

(7)

*R = intestinal radius in cm = 0.126* (Kothari & Rajagopalan, 2020)

The physiological parameters are based on (Brown et al., 1997). The movement of CNMA through the body is governed by partition coefficient that describe the partitioning of CNMA between different compartments. A central partition coefficient is the octanol water partition coefficient, logKow this was calculated using EPIsuite (Version 4.5 SP1). The organ blood partition coefficients where calculated based on the LogKow using (Dejongh et al., 1997). The blood air partition coefficient was not present in the oral uptake model and thusly had to be defined. This was calculated using the method provided by (Jongeneelen & Berge, 2011). This method estimates the blood/air partition coefficient based on a dimensionless Henry coefficient that was calculated as is shown in eq(8). Eq(9) uses the Henry coefficient in combination with the octanal/air partition coefficient to estimate a blood/air partition coefficient. A complete list of partition coefficients used for the rat inhalation model can be found in Table 2.

Dimensionless Henry coefficient

(8)

*Vapour pressure = 0.0337 (mmHg)*

*Molecular weight = 132.16*

*Water solubility = 2150 (mg/l)*

*Gas constant = 3.45\*10^-6 (atm-m3/mole)*

Blood : air partition coefficient

(9)

*Koa = octanal/air partition coefficient = 13.18 (EPIsuite Version 4.5 SP1)*

Kiwamoto *et al* also used Episuite and Dejongh *et al* to calculate the partition coefficients yet some values do not agree as can be seen in Table 2. Sadly, Kiwomato *et al* provides no specifics as to how they were derived and thusly the reason for the difference remains unclear. Partition coefficient calculates used for this report can be found at [*https://github.com/jjLugt/Cinnamaldehyde-pbk/blob/main/QSAR%20calculations.R*](https://github.com/jjLugt/Cinnamaldehyde-pbk/blob/main/QSAR%20calculations.R).

Table 2: Partition coefficients for CNMA and Cinnamyl alcohol used in the rat inhalation model. Values as used in Kiwamoto et al (2016) added for comparison.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Partition coefficient | Description | Value | Kiwamoto | Reference |
| P\_F  P\_L | Fat:Blood  Liver:Blood | 17.42  1.18 | 14.2  1.21 | (Dejongh et al., 1997) |
| P\_SI  P\_RP  P\_SP  P\_PB  P\_Pu  Cinnamyl alcohol  P\_OH\_F  P\_OH\_L  P\_OH\_SI  P\_OH\_RP  P\_OH\_SP  P\_OH\_Pu | Small intestine:Blood  Richly perfused:Blood  Slowly perfused:Blood  Blood:Air  Lung:Blood  Fat:Blood  Liver:Blood  Small intestine:Blood  Richly perfused:Blood  Slowly perfused:Blood  Lung:Blood | 1.18  0.81  0.39  1.81  1.18  1.71  0.81  0.81  0.81  0.39  1.81 | 1.21  1.21  0.57  NA  NA  14.6  1.22  1.22  1.22  0.57  NA | “”  “”  “”  “”  (Jongeneelen & Berge, 2011)  (Dejongh et al., 1997)  “”  “”  “”  “”  “” |

The last group of parameters that has yet to be defined are the parameters that are associated with metabolism of CNMA, Cinnamyl alcohol and Glutathione. As Kiwamoto et al (2016) experimentally derived these parameters they were used as is. A Complete overview of these parameters can be found in supplementary data 2: parameters.

* 1. Single human male inhalation model

The same approach was used with the the single human model as with the rat model. This means adopting the parameters as used by Kiwamoto *et al* and supplementing these parameters with additional parameters as needed. Physiological parameters were derived from IRCP values (Alexaklrin Obninsk et al., 2003).

Table 3 Physiological parameters for the single human male inhalation model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Description | Value | Unit | Reference |
| BW  Tissue volumes | Body Weight | 70 | Kg | (Alexaklrin Obninsk et al., 2003) |
| V\_F | Fat | 15 | L | *“”* |
| V\_L  V\_SI  V\_A  V\_V  V\_RP  V\_SP  V\_Pu  Cardiac parameters  Q\_C  Q\_F  Q\_L  Q\_SI  Q\_RP  Q\_SP  Q\_Pu  Uptake  P\_V  Ka | Liver  Small intestine  Arterial Blood  Venous Blood  Richly perfused  Slowly perfused  Lung  Cardiac output  Fat  Liver  Small intestine  Richly perfused  Slowly perfused  Lungs  Pulmonary ventilation  Uptake rate constant | 1.8  0.6  1.4  4.1  3.4  43.1  0.5  390  5.2  14.1  8.6  47.3  24.8  Q\_C  540  0.62 | L  L  L  L  L  L  L  L/h  L/h  L/h  L/h  L/h  L/h  L/h  L/h  Per hour | *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  *“”*  (Alexaklrin Obninsk et al., 2003)  (Punt, 2022)  R = 1.26 cm (Helander & Fändriks, 2014) |

The human partition coefficients were calculated in a similar manner as the rat partition coefficients. The LogKow values were calculated using EPIsuite (Version 4.5 SP1). Based on the LogKow the partition coefficients where calculated using both (Dejongh et al., 1997; Jongeneelen & Berge, 2011). Similar unexplained differences in partition coefficients could be found as with rats. The uptake rate Ka was calculated using Punt *et al* (2022) with an intestinal radius of 1.26 cm (Helander & Fändriks, 2014) . The complete list of sources/calculations can be found in the supplementary data 2: parameter

Table 4:Human partition coefficients for CNMA and Cinnamyl alcohol used in the single human inhalation model. Values as used in Kiwamoto et al (2016) added for comparison.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Partition coefficient | Description | Value | Kiwamoto | Reference |
| P\_F  P\_L | Fat:Blood  Liver:Blood | 47.75  1.83 | 39.3  2.04 | (Dejongh et al., 1997) |
| P\_SI  P\_RP  P\_SP  P\_PB  P\_Pu  Cinnamyl alcohol  P\_OH\_F  P\_OH\_L  P\_OH\_SI  P\_OH\_RP  P\_OH\_SP  P\_OH\_Pu | Small intestine:Blood  Richly perfused:Blood  Slowly perfused:Blood  Blood:Air  Lung:Blood  Fat:Blood  Liver:Blood  Small intestine:Blood  Richly perfused:Blood  Slowly perfused:Blood  Lung:Blood | 1.81  1.81  1.5  0.29  1.81  49.26  1.18  1.18  1.18  1.53  1.18 | 2.04  2.04  1.57  NA  NA  40.5  2.09  2.09  2.09  1.60  NA | “”  “”  “”  “”  (Jongeneelen & Berge, 2011)  (Dejongh et al., 1997)  “”  “”  “”  “”  “” |

And lastly for the metabolic parameters they were derived by Kiwamoto et al (2016) and thusly they were used as is. A Complete overview of these parameters can be found in supplementary data 2: parameters.

* 1. Human population based PBK model

To more accurately model possible variation between individuals, it was decided to create a population based model. Instead of modelling a single individual population based modelling runs the same model many different times each time with a different parameter set to represent different individuals from a population. The base model used is the same as for the single human and can be seen in Figure 2. As the basis for the population parameters the Popgen webservice was used. Popgen is a web based application which generates a population of individuals with necessary parameters for pbk modeling based on several initial parameters (Willmann et al., 2007). Using Popgen two data sets consisting of 1000 individuals were generated. One female data set and one male data set.

Popgen does not include alle necessary parameters and was missing pulmonary ventilation and a blood compartment. Pulmonary ventilation was derived again using the ICRP values (Alexaklrin Obninsk et al., 2003). The male value for the data set is based on a normal distribution and 3 standard deviations (99.7%) of variance based on the male mean of 540 L/h. the female value for the data set is based on a normal distribution and 3 standard deviations (99.7%) of variance based on the female mean of 390 L/h. Blood compartment volumes were based on calculations shown in (Price et al., 2003). The calculation used is eq(10) based on this total blood volume 2/3 was defined as arterial blood and 1/3 as venous blood.

Blood volume (L)

(10)

A complete overview of initial parameters and calculations can be found in the supplementary data 2: parameters.

* 1. Model validation

A PBK model attempts to predict tissue concentrations based on a host of different parameters. To ascertain if such a model accurately predicts this tissue concentration the preferred method is to compare model output to relevant *in vivo* data. Such an approach was used by Kiwamoto *et al* (2016) as there is oral exposure data available for rats and limited data for humans. As this report is interested in inhalation exposure *in vivo* exposure data is preferred to validate the model. Unfortunately, exceedingly little inhalation exposure data that is coupled with PBK relevant parameters is available in either human or rats (plasma concentration, tissue concentrations etc). In order to bridge this gap a read across approach using the similar chemical Benzaldehyde was considered. Unfortunately, this chemical is similarly data poor and thus could not be used. As no inhalation data is available for either rats or humans and a chemical read across wasn’t possible either validation of this model will be purely based on oral absorption performance.

* 1. Route to route extrapolation

A plausible exposure scenario for use with the human models was constructed based on limited data from Electronic cigarette liquids (Khachatoorian et al., 2022). Exposure was modeled as being a three minute ‘smoke’ break using an Electronic cigarette every 30 minutes for 6 hours. A total of 12 exposures. As Khachatorian et al (2022) has shown that an Electronic cigarette user consumes on average 567 mg of Electronic cigarette liquid per session. If we assume a worst-case scenario with a CNMA concentration of 343 mg/ml (Omaiye et al., 2019) this would yield an exposure of 197.6 mg of CNMA per session. For an average person weighing this would yield approximately 2.8 mg/kg-BW per smoking session.

1. Global sensitivity analysis

PBK models use a large set of parameters not all of which have a large impact on model outcome. Knowing which parameters have an significant impact on model outcome allow a model user to ensure those parameters are defined as accurately as possible (Hsieh et al., 2018). Furthermore, the important parameters can be analysed for abnormalities which can help in the quest for discovering hidden errors in the code.To evaluate the sensitive parameters of the single human and rat models global sensitivity analyses where performed. This was done using a method demonstrated by Kasteel *et al* (2021) (Kasteel et al., 2021). This involves the `soboljansen` function from the Package `sensitivity´ (Bertrand Iooss et al., 2022). This method requires the following input.

Two parameters set which serve as upper and lower bounds. These two parameter sets were generated based on the single human parameter set. As an upper bound the parameter values +1% and as a lower bound the parameter values -1% where used. Between these bounds the parameter values assumed to be normally distributed. The soboljansen function will then generate a large data set of possible parameter combinations and will run the model using these parameter combinations. The output of these runs will then be compared to evaluate the impact on the out come of the model when parameters are changed. The output of this analysis is a ranking of influential parameter using two indexes. The total effect and main effect. The total effects have cumulative value of 1 representing the contribution of a parameter to the overall change in output of interest. The main effect similarly has a cumulative value of 1 representing the contribution of this parameter independent of its effects on other parameters. To illustrate lets take Body Weight. Body weight is expected to have a large total effects as a large number of parameters such as organ volumes, cardiac output and so on are influenced by body weight. Yet, it is expected that body weight does not necessarily directly have a large impact on CNMA concentration and thusly a smaller main effect is expected. The total and main effects for alle parameters were investigated at multiple time points for both oral and inhalation exposures. The corresponding code can be found in the supplementary data section 1: R code. Or alternatively on https://github.com/jjLugt/Cinnamaldehyde-pbk .

1. Results
   1. Global Sensitivity analysis

As noted above global sensitivity analyse (GSA) can be a valuable tool in both checking the model for errors as ensuring correct parameters are used. As the out come of a model is both dependent on exposure dose and exposure route multiple GSA’s have been performed for both rat and human models.

In rats the sensitive parameters determining the CNMA concentration in blood with oral and inhalation exposure were investigated. The results are presented in Figure 3 and Figure 4. During oral exposure the two most important parameters excluding T= 30 min where the uptake rate constant (Ka) and cardiac output (QC). At T=30 min the initial concentration of Gluthation in the liver (init\_GSH\_L) is the second most important parameters followed by the first order rate constant for the conjugation GSH with CNMA(k\_L\_GST). It can be noted that after 90 min the partition coefficient between air and blood for CNMA (PB) and pulmonary ventilation (PV) steadily increase in relevance. Body weight(BW) has a moderate impact at all time points. The sensitive parameters for inhalation exposure are noted to be considerably different. The two most important parameters are PB followed by BW. Both blood flow to Richly perfused tissue (Q\_RP) and volume of slowly perfused tissue (V\_SP) are moderately important at all time points.

The sensitive parameters in the Human model where investigated using two different exposures . Firstly, a high dose of 250 mg/kg-BW to compare to the Rat model and secondly a 2.8mg/kg-BW dose comparable to a high Electronic cigarette exposure dose. Secondly, we considered both the concentration of CNMA in blood and the concentration in lung If we consider the two different exposures. It can be noted that in both generally the same set of parameters is important. From these PB and PV are the most important parameters. Following in slightly different order are the following parameters; V\_SP, Q\_SP, Q\_RP and V\_Pu. Absent are parameters responsible for metabolic processes. This suggest that CNMA concentrations are dictated by perfusion dynamics.The sensitive parameters for oral exposure in Humans can be found in the supplementary data 3: supplementary results.

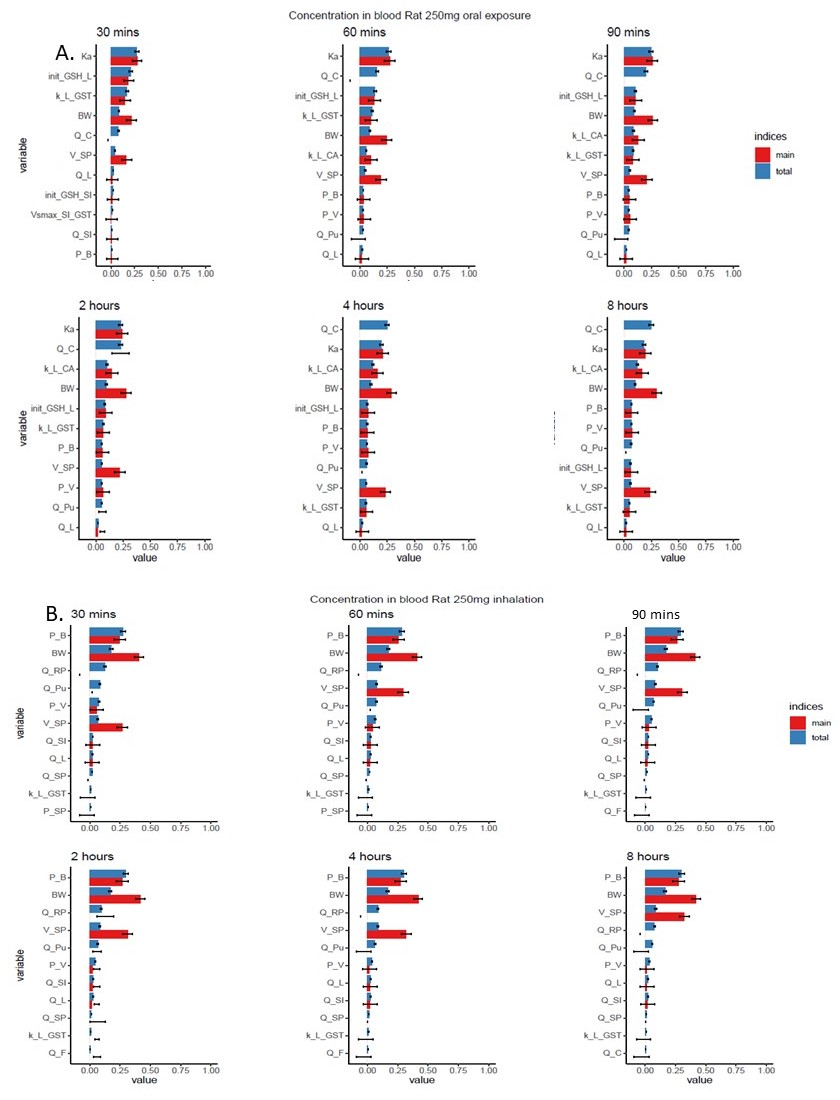


Figure 3: Global sensitivity analyses results for CINMA exposure both inhalation and oral in Rat 250 mg/kg-BW. (A) top ten sensitive parameters influencing the concentration of CNMA in the liver.(B) top ten sensitive parameters influencing the concentration of CNMA in blood. Error bars indicate the 95% confidence interval.

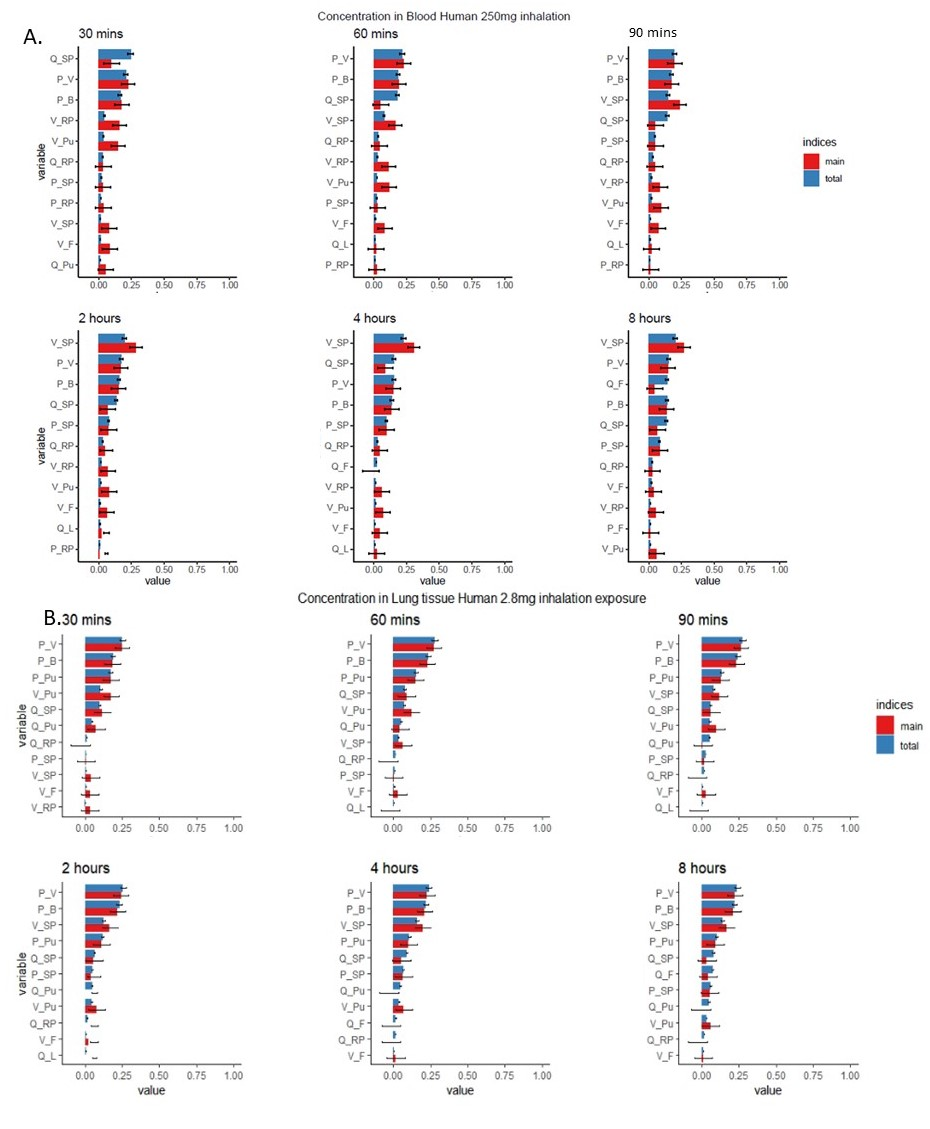


Figure 4: Global sensitivity analyses results for inhalation exposure with the human model. A; top ten sensitive parameters for inhalation exposure to a 250 mg/kg-BW dose. B; top ten sensitive parameters for inhalation exposure to a 2.8 mg/kg-BW dose. Error bars indicate the 95% confidence interval.

Afbeelding met diagram

Automatisch gegenereerde beschrijving

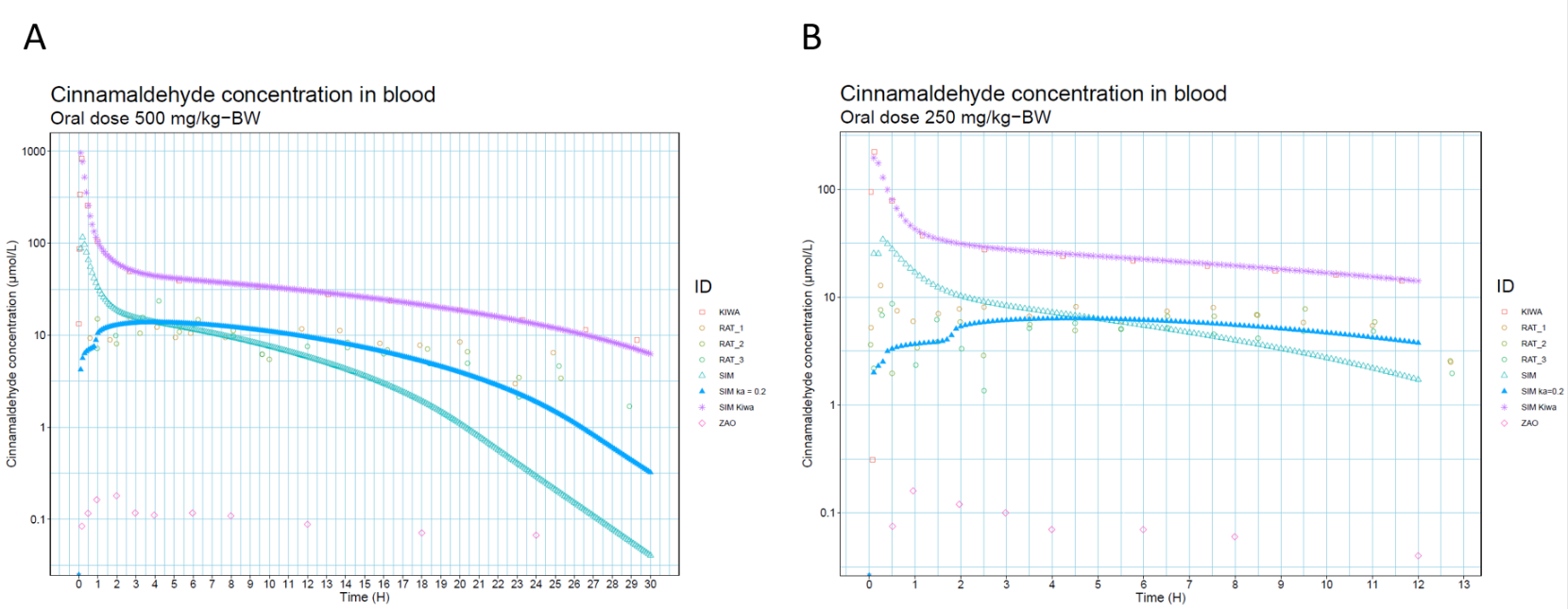


Figure 5: CNMA blood concentration comparison between simulated data and Kiwamoto. (🞏)The purple squares are the simulation data form Kiwamoto et al. (○) Yuan et al data is represented by the Rat 1-3 points. (△) R model simulation data is represented by the SIM line.(▲) R model simulation data with fitted Ka of 0.2 is represented by the SIM ka = 0.2 line.(◊) Zao et al data is represented by Zao data points. (🞻)R model with Kiwamoto parameters is represented by SIM kiwa.

* 1. Evaluation Rat model

As the inhalation model is based on an existing model the first objective was seeing if it was possible to recreate the results of this model using the translated model in R code. In order to do this oral and IV exposure data was used from the literature. Different exposure doses are available from literature these include 500 mg/kg-BW, 250 mg/kg-BW oral exposures (Yuan et al., 1992; Zhao et al., 2014) already considered in the Kiwamoto report and more recent pharmacokinetic study’s concerning 375 mg/kg-BW, 15 mg/kg-BW and 50 mg/kg-BW exposures(Dong et al., 2022; Ji et al., 2015; Yong et al., 2020). Lastly two iv exposures will be considered 10 and 20 mg/kg-BW (Shetty et al., 2020; Zhao et al., 2014). The plasma concentration of CNMA as measured in (Zhao et al., 2014) (Zao) and (Yuan et al., 1992)(RAT\_1,2,3) are presented in Figure 4. Also presented in Figure 4 are the simulation results as presented in Kiwamoto et al (Kiwa) and the simulation results found when running the inhalation model (SIM). As expected the results of the Kiwamoto model and the inhalation model can be seen to differ substantially with a Cmax value of 829 μmol/L and 116 μmol/L respectively. A 7.15 fold higher Cmax value with the Kiwamoto model. To evaluate whether this difference was due to errors in the R code a new simulation was run this time using all of the parameters as specified in Kiwamoto et al (SIM kiwa). This resulted in a Cmax value of 769 μmol/L or a 0.93 fold difference compared to Kiwamoto. The inhalation model performance compared to the experimental data found in Yao et al. A 6.56 fold difference can be found between the inhalation model 116 μmol/L)and the Yao data 17.69 μmol/L. This represents a improvement compared to model performance found in Kiwamoto et al from 56 fold compared to 6.56 fold. As the model still overpredicts in the time points shortly after exposure. Another model run was done with a reduced absorption rate constant to simulate a slower uptake rate in the small intestine. This resulted in a inhalation model Cmax 13.81 μmol/L that was 0.78 fold that of the Yao data 17.60 μmol/L. The remaining data is presented in Figure 5, in this figure predicted vs outcome plots are presented for the different exposures. If relevant the results of the inhalation model with adapted Ka are also shown. To compare the results between the inhalation model and adapted model the Root mean square deviations are shown. This is a measure of the size of the deviation from the observed values.

IE the higher the value the worse a model corresponds to the observed data. Adapting the model with a reduced Ka value improved model performance in 3 of the 5 cases with oral absorption and had a negligible or slight negative impact on the remaining two cases. With 500, 250, 20 and 15 mg/kg-BW doses most data points where within a 2 fold difference of the observed values. 50 mg/kg-BW values remained within a 5 fold difference and 15 mg/kg-BW doses within a 10 fold difference. 375 and 10 mg/kg-BW doses differed greatly from predicted values

Table 5: Table showing the results of a 250 mg/kg-BW dose in the single rat inhalation model. Exposure route either oral or inhalation.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Rat | Cmax ( | Tmax (h) | AUC->last  (µmol/l-h) | AUC->inf  (µmol/l-h) |
| **Oral**  Small intestine | 1384.1 | 0.1 | 796.65 | 796.7 |
| Liver  Blood  Lung  Fat  Richly perfused tissue  Slowly perfused tissue  **Inhalation**  Blood  Lung  Fat  Richly perfused tissue  Small intestine  Slowly perfused tissue  Liver | 92.2  60.84  30.37  30.37  26.94  8.3  14.75  10.48  10.48  9.28  5.54  3.06  1.32 | 0.3  0.3  0.3  0.3  0.3  0.4  0.8  0.5  0.5  0.6  0.6  0.7  0.39 | 227.01  163.32  81.52  81.52  72.23  24.05  188.45  130.4  130.4  115.42  73.67  38.19  21.8 | 227.26  165.51  81.62  81.62  72.31  24.08  227.22  157.1  157.1  139.06  86.63  46.05  26.59 |

Table 6: Table showing the results of a 250 mg/kg-BW dose in the single human inhalation model. Exposure route either oral or inhalation.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Human | Cmax ( | Tmax (h) | AUC->last  (µmol/l-h) | AUC->inf  (µmol/l-h) |
| **Oral**  Small intestine | 2982.11 | 0.1 | 3521.35 | 3521.7 |
| Fat  Liver  Blood  Lung  Richly perfused tissue  Slowly perfused tissue  **Inhalation**  Fat  Richly perfused tissue  Lung  Blood  Slowly perfused tissue  Small intestine  Liver | 0.44  6.33  1.8  0.49  0.47  0.22  81.89  57.55  57.59  48.69  30.74  7.48  0.14 | 4.5  0.1  0.2  0.2  0.3  0.9  5.8  0.2  0.1  0.2  1.1  0.2  0.2 | 8.43  7.83  2.4  0.65  0.65  0.54  1566.02  121.88  120.64  117.19  101.27  15.51  0.29 | 17.34  7.88  2.4  0.66  0.66  0.54  3261.41  122.44  121.2  119.49  101.72  15.59  0.29 |

* 1. Interspecies comparison.

The Human model has no major changes to model structure that would lead to differences in simulation outcome compared to the rat model. The differences between the models is mainly in the specific input parameters concerning partition and physiological parameters. Table 1 shows the results of 4 simulations consisting of an oral and an inhalation exposure to both the single human and single rat model. When oral exposure is considered between human and rat simulation results a similar picture emerges. A very high AUC value for the primary exposed organ Small intestine followed by the Liver. The liver is directly linked to the small intestine in this model so this was expected. Followed by AUC values in the rest of the organs that are at least an order of magnitude lower. If we compare inhalation exposure between human and rat simulation results differ more compared to oral exposure. The organ with the highest Cmax and AUC value in rat is blood. In Humans this is Fat.

* 1. Human model evaluation

In order evaluate the performance of the single and population based inhalation model only very limited metabolic data is available. In the study two male participants were given 0.7 mg/kg-BW CNMA orally. This resulted in 96.2% and 96.5% of the administered dose being excreted in the urine as hippuric acid, benzoyl glucuronide, HPPA or benzoyl acid al of which are downstream products of the Cinnamic acid metabolite(Maria & Peters, 1993) . In Figure 6 the results of 0.7 mg/kg-BW oral exposure simulation using the human population model can be seen. The median percentage metabolized for both females and males 97.6% after 24h. with 99.72% of results laying between 98.42% and 96.3%.

As no read across data of in vivo data was available no further validation steps were performed. In order to evaluate whether inhalation exposure leads to differences in sensitive organs two simulation where performed. In Figure 7 the results of these simulation can be seen. Populations where simulated as being exposed once to either an oral or inhalation dose of 250 mg/kg-BW. The concentrations of the various organs were then collected between 0 and 24 hours. Firstly, as the simulations where ran with both a female and a male data set the results of these simulations were compared. Specifically, the mean values of these results were tested using Tukey HSD and a Welch T test. In all cases the male and female values differed significantly (P>0.05). With exception of the small intestine during oral exposures AUC values for females were higher then for males. If we consider the differences between exposure methods it can be observed that there are considerable differences. Mean CNMA AUC values for both males and females during inhalation exposure where higher in the following organs. Lung (252.6 fold), Blood(76.6 fold), Fat(126.6 fold), Slowly perfused tissue(318.8 fold) and Richly perfused tissue(284.5). Mean CNMA AUC values for both males and females were lower in the following organs; Liver(30.1 fold) and small intestine(75 fold). As a singular 250 mg/kg-BW inhalation dose is not representative of normal exposures during electronic cigarette usage, repeat dosing of 2,8 mg/kg-BW as described in Exposure modeling was simulated. The results of this simulation are presented in Figure 8.

Similar steps were performed as before. Inter sex comparisons shown significant differences in the mean CNMA AUC values between females and males with higher values observed in female simulations. If compared to 250 mg/kg-BW inhalation exposure multiple inhalation exposures to 2.8 mg/kg-BW yielded the following results. CNMA AUC values were lower in all organs. Lung (4.5 fold), Blood(4.7 fold), Fat(2.2 fold), Slowly perfused tissue(5.12 fold), Richly perfused tissue(4.8), Liver(6.2) and Small Intestine(10.4 fold). The general distribution over organs was similar to 250 mg/kg-BW inhalation with the highest concentrations noted in fat. Cmax results can be found in Supplementary data 3: supplementary graphs.

In the context of exposure to electronic cigarette smoke the first organ to be exposed and the organ most associated with adverse effects of cigarette smoke is the lungs. Using the human population based inhalation model, the CNMA concentration in lung tissue was simulated after multiple exposures to 2.8 mg/kg-BW. The results of this simulation can be seen in Figure 9. After the start of exposure the CNMA concentration in the lung rapidly increases to a maximum of around 32 uM after which it rapidly decreases in about 30 min to zero. Inter individual variation in results are modest for the exposure peak the lower bound is 29.3 μmol/l, the mean is 32.8 μmol/l and the upper bound is 39.0 μmol/l. This variation is not evenly distributed between genders as can be seen in Figure 8 with female individuals CNMA concentrations being significantly higher than male individuals

4. Discussion

This report presents an human population inhalation model for exposure to CNMA. Population models allow a researcher to more accurately predict chemical concentrations in the general public. This is especially pertinent in the case CNMA as its use in Electronic cigarette devices has not been comprehensively studied and thus in vivo data is still missing. This population model was based on real population data using the online tool Popgen. The pharmacokinetic parameters such as logKow and the partition coefficients were derived using QSAR’s.

Previously, a oral exposure model for CNMA was developed for rats and humans by Kiwamoto et al. This model was recapitulated in this report using the coding language R. The chemical specific parameters where calculated using relevant QSARs. Using the R code model and the newly calculated parameters this report was able generate oral absorption results to within 6 .5 fold of in vivo data See Figure 4. This represents a improvement compared to Kiwamoto et al as the general oral absorption model yielded results withing 56 fold of in vivo data. Kiwamoto et al generated results within a 6 fold difference only after the addition of a separate oral absorption model. For 500, 250, 20 and 15 mg/kg-BW doses most predicted data points where within a 2 fold difference compared to in vivo data. If a model predicts within 2 fold of in vivo data it is considered adequate for risk assessment (WHO, 2010). As noted by Kiwamoto et al significant differences between in vivo data source complicate overall characterization of model performance. The proposed mechanism of differences in sample preparation is unlikely to be the explanation for the 375 and 10 mg/kg-BW dosing differences as the model underestimates the CNMA concentration. Additionally (Shetty et al., 2020) found no appreciable CNMA degradation in samples at room temperature. As oral absorption predictions were not the focus of this report no further analysis was performed to explain these differences apart from the observation that heterogeneity in in vivo data is a complicated problem to solve. Furthermore, it was noted that reduction of the absorption rate constant further to 0.2 increased model fit in most cases. Fitting the absorption rate constant might work to improve model prediction yet it might not accurately represent CNMA dynamics in vivo as it is noted to be absorbed rapidly (Wu et al., 2022; Yingrong et al., 2009; Zhao et al., 2014). A possible explanation for high uptake rate is active transport into the gut. A Scopus search for CNMA AND transporter revealed no literature and CNMA was found not to be transported in Caco 2 cells (Wu et al., 2022). Lastly, in the model bioavailability is assumed to be 100% yet there is data suggesting this might be as low as 20% (Zhao et al., 2014). Therefore, more accurate simulation of bioavailability is a promising avenue for further improvement of the model.

One of the factors of rewriting the model in the R code is the possibility to perform a global sensitivity analysis (GSA). Is a preferable alternative to a Local sensitivity analysis as it is able to work with parameter sets which contain correlated parameters(Li et al., 2010; Liu et al., 2020). Yet, great difficulties were experienced in trying to implement a Sobol GSA approach during the writing of this report. The difficulties were of two general categories. Firstly, great computational demands for running GSA models with many input parameters. With 59 parameter inputs in the model running a GSA analysis demanded dozens of Gigabytes(GB) of Random access memory (RAM) and consumed up to 100 GB of storage per run. Secondly, GSA approaches are as of yet quite novel approach and therefor trouble shooting specific programming implementations was difficult. The GSA approach used in this report was as noted Sobol. This approach in its naïve form assumes non correlated inputs parameters. Another Sobol method has been developed to specifically deal with correlated parameters sets this method is called extended Sobol (Kucherenko et al., 2012). It is noted that this approach has been characterized as being so complicated that the usefulness is limited(Liu et al., 2020). Because of these considerations only a normal Sobol analysis was performed.

With relatively good performance in the rat oral model and agreement of the human model with the limited human metabolism data. The rat and human inhalation model was compared to results form the oral human and oral rat model. In the results it is clear that cinnamaldehyde appears to exhibits first pas metabolism. First pas metabolism is the phenomenon in which an orally taken chemical exhibits low systemic availability due to either poor absorption or fast metabolism(Doherty & Pang, 1997). As noted earlier absorption/bio availability is not modeled in these models so this effect is due to fast metabolism of CNMA in both rats and humans. In contrast to this the inhalation models do not exhibit this first pas effect and consequently have considerably higher AUC concentrations. The above mentioned effect is visible in both rats and human simulations. An difference between the simulations is the amount of CNMA that can be found in fat in comparison to other organs in human simulation results with inhalation AUC values being at least 10 fold higher that other organs. These differences are likely the results of a combination of two factors. Humans have considerably more adipose tissue in comparison to rats with 7% for rats and 21% for humans(Alexaklrin Obninsk et al., 2003). Added to this is that the predicted Fat:Blood Partition coefficient in Humans is 47.75 and 17.42 for Rats.

The difference between oral exposure and inhalation exposure were analyzed based on the simulation results shown in Figure 7 and Figure 8. As noted earlier the model predicts a clear first pas effect which ensures that CNMA concentration in the oral results are at least 75 fold lower than those seen in inhalation exposure simulation results. With the exception of liver and small intestine results as these are where higher in oral exposure scenario’s. This large difference in CNMA AUC values demonstrates the need to consider exposure pathways and the effects these can have on the target organ concentration. The most sensitive organ in inhalation exposure is predicted to be fat where a significant amount of CNMA is predicted to accumulate. CNMA is predicted to remain here for a significant time after 24h as can be seen in Table 1 specifically in the difference between AUC->end and AUC->inf. There were no gender differences in sensitive organs. A significant difference can be seen in all AUC values comparing males and females with inhalation exposure leading consistently to higher female AUC values. These differences are expected to be cause by differences in body composition such as higher fat percentage and smaller mean liver values see supplementary data for associated graphs. Multiple exposures to concentration relevant for Electronic cigarette use did not change the overall distribution of CNMA but AUC values were between 2.2 and 10.4 fold lower. As lung are the main organ of interest for electronic cigarette exposures the concentration of CNMA was plotted to be able to compare it to in vitro data. This was done as CNMA has been noted to induce toxicity in different lung cells in vitro(Behar et al., 2014; Clapp et al., 2019; Ka et al., 2003). Furthermore, there is data suggesting immune suppressing effects of CNMA(Chapman et al., 2019; Clapp et al., 2017). The effective concentration in these studies differ from 49 uM to 10.000 uM. Ka et al found the 49 uM IC50 value for LL3 mouse lung carcinoma cells. And Clapp et al 2017 and 2019 found IC50 values for Neutrophils, Macrophages and Natural killers cells ranging from 30 uM and 243 uM. If we compare this to predicted AUC->24h values using the population model which is a mean of around 30 μmol/l-hr. This would mean that worst case exposure Electronic cigarette exposure can lead to adverse immune effects due to the exposure of CNMA in Electronic cigarettes. A limitation of this study is that Glutathione (GHS) is not modeled in the lungs. And, it is therefore possible that the GHS available in the lungs would reduce the available CNMA below levels predicted in this model.

In conclusion in this report an human inhalation and population based CNMA model is presented. This population based model was then used to predict CNMA concentration after a worst case CNMA exposure as part of electronic cigarette emission. This model predicts significant difference in CNMA concentrations in the body when comparing inhalation to oral exposure and predicts that these concentration differ significantly between males and females. When a worst case CNMA exposure is modelled the model predicts CNMA concentration in the lung which are associated with adverse effects in *in vitro* systems. A major drawback of the model is lack of in vivo data available to validate model prediction for inhalation exposures. Promising future steps are either generating in vivo data for CNMA or finding a suitable read across chemical to validate inhalation predictions. Adoption of the model for other aldehydes and the consideration of other chemicals associated with electronic cigarette emissions.

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1. An example appendix

Supplementary data to this report is separated in three files

Supplementary data 1: R code

Supplementary data 2: parameters

Supplementary data 3: Supplementary results

Additionally all code used in this project can be found at the the following Github page.

https://github.com/jjLugt/Cinnamaldehyde-pbk

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