The use of physiologically based kinetic (PBK) models in the risk assessment of plant genotoxins

Ivonne M.C.M. Rietjens, Ans Punt*

Division of Toxicology, Tuinlaan 5, 6703 HE Wageningen, The Netherlands *ivonne.rietjens@wur.nl

Abstract

Botanicals and botanical preparations that are part of our modern food chain may contain compounds that are both genotoxic and carcinogenic. The margin of exposure (MOE) approach is considered a useful and pragmatic tool for risk assessment of substances that may be both genotoxic and carcinogenic. A recent inventory of botanical ingredients that are of possible concern for human health because of their genotoxic and carcinogenic properties revealed that the majority of the compounds identified belong to the group of alkenylbenzenes or the group of unsaturated pyrrolizidine alkaloids. For both groups of compounds tumor data that enable definition of a $BMDL_{10}$ for risk assessment by the MOE approach are limited to only a few congeners. In addition, risk assessment of these botanical genotoxins should consider that realistic dietary exposure levels are low while exposure may occur within a complex food matrix and for periods that may be sigificantly shorter than whole life and that effects of genetic polymorphisms or lifestyle factors may have to be taken into account. To facilitate risk assessment of botanical genotoxins we have pioneered the use of physiologically based kinetic (PBK) modelling. The present paper presents examples of how PBK modelling can be used to facilitate risk assessment of plant genotoxins, taking the alkenylbenzenes as model compounds. Together these examples reveal that PBK modelling can facilitate risk assessment and contribute to the development of alternatives for animal testing.

Introduction

Botanicals and botanical preparations that are part of our modern food chain may contain compounds that are both genotoxic and carcinogenic. In 2009, EFSA published an updated guidance on the scientific data needed to carry out a safety assessment of botanicals and botanical preparations (EFSA, 2009). In cases where a botanical preparation contains substances that are both genotoxic and carcinogenic, assessment of the risk for human health is generally complicated. Considering the possible uncertainties and existing disadvantages connected to the use of qualitative and quantitative approaches such as ALARA (as low as reasonably achievable) and low-dose cancer risk extrapolation, the use of a Margin of Exposure (MOE) approach was recommended (EFSA 2005; Barlow et al. 2006; O'Brien J et al. 2006). The MOE is a dimensionless ratio based on a reference point obtained from epidemiologic or experimental data on tumor incidence, such as for example a $BMDL_{10}$ (the

lower confidence limit of the benchmark dose that give 10% extra tumor incidence above background levels) which is divided by the estimated daily intake (EDI) in humans. The MOE approach is considered a useful and pragmatic tool for risk assessment of substances that may be both genotoxic and carcinogenic.

A recent inventory of botanical ingredients that are of possible concern for human health because of their genotoxic and carcinogenic properties revealed that the majority of the compounds identified belong to the group of alkenylbenzenes or the group of unsaturated pyrrolizidine alkaloids (Van den Berg et al. 2011). For both groups of compounds tumor data that enable definition of a $BMDL_{10}$ for risk assessment by the MOE approach are limited to only a few congeners. In addition, risk assessment of these botanical genotoxins should consider that realistic dietary exposure levels are low while exposure may occur within a complex food matrix and for periods that may be sigificantly shorter than whole life and that effects of genetic polymorphisms or lifestyle factors may have to be taken into account. To facilitate risk assessment of botanical genotoxins we have pioneered the use of physiologically based kinetic (PBK) modelling.

A PBK model is a set of mathematical equations that together describe the absorption, distribution, metabolism and excretion (ADME) characteristics of a compound within an organism (Rietjens et al. 2011). PBK models can predict the tissue concentration of a compound or its metabolite(s) in any tissue over time at any dose, allowing analysis of effects at both high but also more realistic low dose levels. Furthermore, PBK models can be developed for different species, which can facilitate interspecies extrapolation. They can also be defined for different congeners in a group facilitating read-across from compounds for which toxicity data are available to analogues for which these data are limited or even absent. In addition, by incorporating equations and kinetic constants for metabolic conversions by individual human samples and/or specific isoenzymes, modeling of interindividual variations and genetic polymorphisms becomes feasible.

In the following sections we present examples of how PBK modelling can be used to facilitate risk assessment of plant genotoxins, taking the alkenylbenzenes (Figure 1) as model compounds.
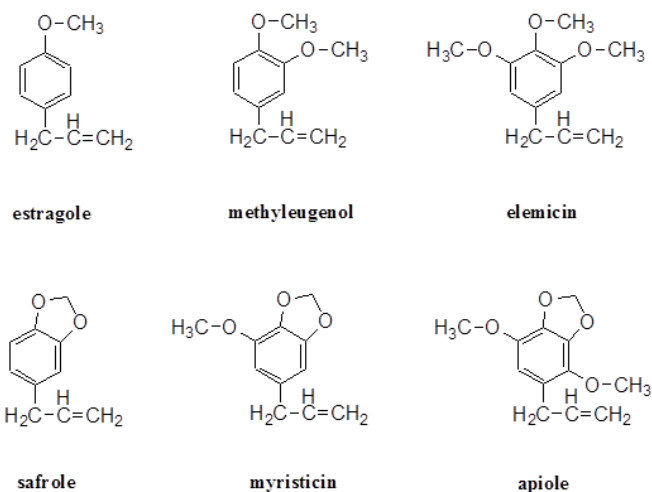
Figure 1. Chemical structures of alkenylbenzenes.

Recent developments that might contribute to an improved and comprehensive risk assessment of food

In risk assessment the aim is to protect human health thus requiring estimation of doses that do not cause adverse effects in humans. Because data from human studies are difficult to obtain, effects are often studied in relevant animal or cellular *in vitro* models. This poses the need for adequate extrapolation from experimental animals to humans, from the *in vitro* to the *in vivo* situation, and/or from high dose levels to realistic low dose levels. Furthermore, effects of genetic polymorphisms or lifestyle factors may have to be taken into account. Thus, several challenges remain within the current risk assessment of plant genotoxins including the following issues.

1) How to account for species differences?
2) How to judge effects at low realistic dietary exposure?
3) How to take interindividual differences into account?
4) What are the consequences of exposure in a complex dietary food matrix?
5) How can we perform a risk assessment in the absence of tumor data?
6) Can we use alternative *in vitro* models?

Physiologically based kinetic (PBK) modelling may facilitate answering these kind of questions in modern risk assessment providing a development that might contribute to an improved and comprehensive risk assessment of genotoxic carcinogens in food. In the following sections this is illustrated based on our work on the use of PBK modelling in the risk assessment of alkenylbenzenes as model compounds. Alkenylbenzenes (Figure 1) are important constituents of herbs and spices like nutmeg, cinnamon, anise star, tarragon, sweet basil, and sweet fennel and are present in the modern food chain mainly as a result of use of these herbs and spices and their essential oils (Rietjens et al. 2014).

1) How to account for species differences?

PBK models provide unique possibilities to account for species differences. They even allow prediction of dose-response curves for the human situation since by replacing all parameters in a PBK model developed and validated for rat, by the corresponding physiological and kinetic human parameters, one can build models for the human situation.

As an example, Figure 2 presents the PBK model based prediction for the formation of the ultimate carcinogenic metabolite 1′-sulfooxyestragole from the alkenylbenzene estragole by rat and human. From these PBK model based predictions it can be concluded that the species differences in bioactivation of estragole to its ultimate carcinogenic metabolite are minimal. Considering that the predicted species differences are lower than 2-fold, the data suggest that the amount of reactive metabolites formed in rat and human is comparable and that rat is an adequate model for human. This indicates that species differences will not play a significant role when extrapolating from rat to human.
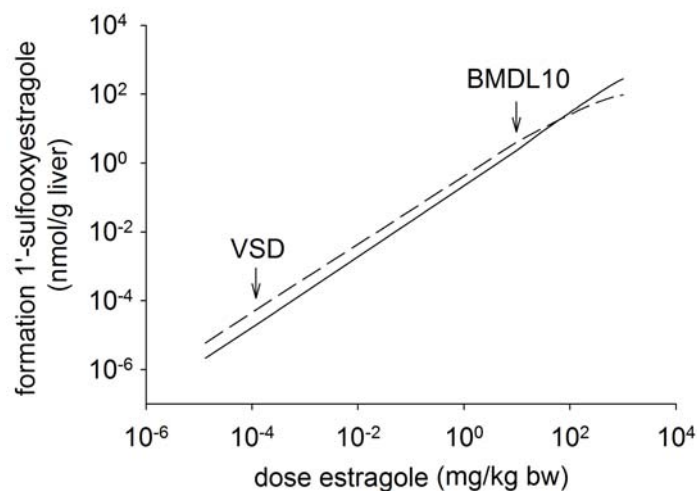

Figure 2. *Prediction of dose-dependent bioactivation of estragole, to its ultimate carcinogenic 1'-sulfooxy metabolite in rat (solid line) and human (dotted line). For further details see Punt et al. (2009) and Rietjens et al. (2010). The BMDL$_{10}$ is the lower confidence limit of the Benchmark Dose (BMD) causing 10% extra tumor incidence, the VSD is the Virtual Safe Dose, the dose level estimated to cause an additional cancer risk of one in a million upon life time exposure.*

2) How to judge effects at low realistic dietary exposure?

The example presented in Figure 2 also illustrates that using PBK modelling low dose effects can be predicted. With a PBK model physiologically relevant concentrations of a compound or, when relevant, its active metabolite(s) in any target organ of interest can be

predicted for a certain dose and route of administration. The data presented in Figure 2 reveal that for both rat and human there is a linear relationship between the dose and the level of formation of the ultimate carcinogenic 1'-sulfoxy metabolite. This linear relationship holds from a relatively high daily dose of 50 mg/kg bw which is in the range of the $BMDL_{10}$ (the lower confidence bound of the Benchmark Dose causing 10% effect (BMD_{10})) for tumor formation down to the virtual safe dose (VSD) being the dose estimated to result in one in a million extra cancer incidence (Rietjens et al., 2010). This implies that these PBK data support linear extrapolation from high to low dose levels and exclude a role for saturation of metabolic pathways at dose levels used in experimental animal studies. Thus, PBK modeling provides a unique way to predict responses under realistic low dose regimens which are often experimentally inaccessible.

3) How to take interindividual differences into account?
PBK models can also provide a way to obtain insight in interindividual differences. To this end, kinetic constants for bioactivation and detoxification can be determined using relevant tissue samples from individual human donors thus enabling definition of PBK models for individual human subjects. Based on the values thus obtained for kinetic constants for relevant metabolic pathways a distribution frequency for the whole population can be defined enabling Monte Carlo modelling-based predictions of bioactivation and detoxification for the population as a whole (Punt et al. 2010; Al-Subeihi et al., 2015; Punt et al. 2016).

Figure 3 presents -as an example- the PBK model based prediction of the estragole DNA adduct (N^2-(*trans*-isoestragole-3′-y1)-2′-deoxyguanosine (estragole-3'-N^2-dGuo)) formation in the liver of 19 human individuals. The amount of estragole-3′-N^2-dGuo adducts predicted to be formed at the estimated daily intake of 0.01 mg/kg bw day varied 17.5 -fold between the 19 human individuals.
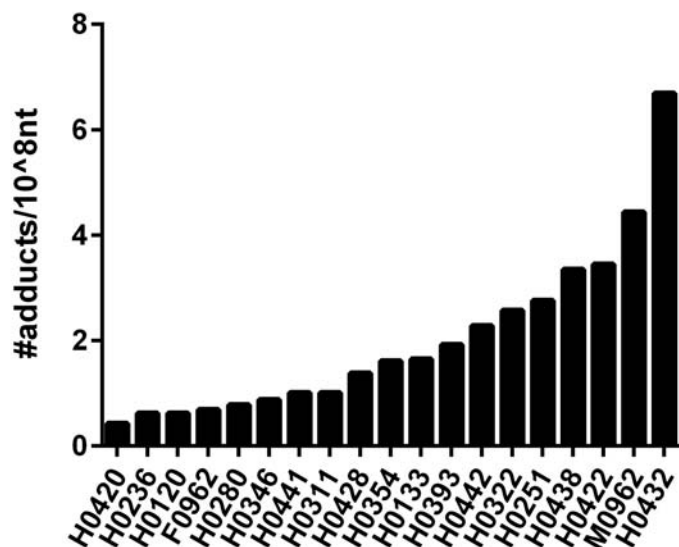
Figure 3. The number of estragole DNA adducts (estragole-3'-N^2-dGuo) at a dose of 0.01 mg/kg bw as predicted by the PBK models for 19 human individuals. (For further details see Punt et al., 2016).

Extending this evaluation to a larger population using Monte Carlo modelling resulted in a median predicted amount of estragole-3'-N^2-dGuo adducts formed for estragole, at the estimated average human daily intake of 0.01 mg/kg bw of 1.6 estragole-3'-N^2-dGuo adducts in 10^8 nts, and of 4.3 and 8.8 adducts in 10^8 nts at the 90th, and the 99th percentiles (Punt et al., 2016). These results can be used to derive a so-called chemical-specific adjustment factor (CSAF) (Gentry et al., 2002; IPCS, 2005, Punt et al., 2010, Al-Subeihi et al., 2015). Following the guidelines of the International Program on Chemical Safety (IPCS, 2005), CSAFs can be calculated as the ratio between given percentiles (such as 90th, 95th, 97.5[th], or 99th) and the median for the whole population. Using the 90th percentile to represent a sensitive individual and the 50th percentile to represent the average individual, the CSAF would amount to 2.6. This predicted CSAF is comparable to the default factor of 3.16 for interindividual kinetic variability and suggests that the default uncertainty factor adequately protects 90% of the population. Using the 99th percentile to represent a sensitive individual the CSAF amounts to 5.4, indicating that protecting 99% of the population would require a higher uncertainty factor than the default value of 3.16. In this way PBK modeling can provide insight in interindividual differences and even contribute to definition of CSAF values to take into account intraspecies differences in risk assessment.

4) What are the consequences of exposure in a complex dietary matrix?

Current intake of alkenylbenzenes occurs primarily from consumption of foods containing herbs and essential oils containing these compounds. A significant difficulty in evaluating the risks of dietary exposure to alkenylbenzenes is that human exposure results from a complex mixture of food, spice and spice oil constituents which may significantly impact the biochemical fate and toxicological risk. This implies that an important aspect that should be taken into account when assessing the risk of these food-borne genotoxic carcinogens is whether results from long-term animal studies on carcinogenicity with pure compounds dosed by gavage without the natural food matrix being present represent a good starting point for the risk assessment. In this respect, we have previously demonstrated that the natural basil flavone nevadensin (5,7-dihydroxy-6,8,4′- trimethoxyflavone) was able to inhibit estragole DNA adduct (estragole-3'-N^2-dGuo) formation in HepG2 human hepatoma cells and primary rat hepatocytes exposed to the proximate carcinogen, 1′-hydroxyestragole (Jeurisssen et al., 2008; Alhusainy et al., 2010). This inhibition by nevadensin was shown to occur at the level of sulfotransferae (SULT)-mediated bioactivation of 1′-hydroxyestragole into 1′-sulfooxyestragole (Jeurisssen et al., 2008; Alhusainy et al., 2010). Later experiments demonstrated the effect to also occur *in vivo* since co-exposure of rats to estragole and nevadensin at a ratio in which they occur in basil, resulted in a significant reduction in the levels of DNA adducts formed in the liver upon single exposure (Alhusainy et al., 2013). Co-exposure of rats for 8 weeks to the related alkenylbenzene methyleugenol and nevadensin resulted in a significant reduction in not only the level of DNA adducts formed in the liver but also in the early markers of liver carcinogenesis such as the incidence, multiplicity and size of GST-P positive hepatocellular altered foci (HAF) (Alhusainy et al., 2014). This suggests that performing a rodent bioassay with the basil matrix being present will result in higher BMDL$_{10}$ values resulting in higher MOE values in a subsequent risk assessment.

However, it is important to consider whether such an interaction may also occur at realistic lower dose levels. Obviously such a question cannot be studied in a rodent bioassay since at realistic dietary intake levels of estragole tumor formation would not be detectable given the limited number of animals that can be used. However, using PBK modeling such an experiment can be simulated. The mode of action of the nevadensin mediated inhibition of estragole DNA adduct formation and possible tumor formation proceeds by a reversible non-competitive type inhibition with a Ki value of 4 nM (Alhusainy et al., 2010). This inhibition can be incorporated in the equations of the PBK models developed to predict the levels of

formation of the reactive 1′-sulfooxy metabolite and subsequent DNA adduct formation in the liver. Upon incorporating this reversible mode of SULT inhibition by nevadensin into the PBK model, the dose dependency of this matrix derived combination effect can be quantified (Rietjens et al., 2015). Figure 4 presents an overview of the outcomes obtained. This reveals that the matrix-derived combination effect will be significant at dose levels used in rodent bioassays (50 mg/kg bw), but that the effect is predicted to become less significant at lower dose levels being even absent at realistic human exposure levels (0.05 mg/kg bw). This is due to the fact that nevadensin is a reversible non-competitive inhibitor and that upon realistic low dose exposure the concentrations reached in the liver will be lower than the Ki of 4 nM for SULT inhibition by nevadensin, thus not resulting in effective inhibition. This result implies that when real food preparations would be tested in rodent bioassays at high dose levels the results obtained may not be representative for the human situation with low dose exposure and not necessarily provide a better starting point for risk assessment than testing the compound of concern in isolation. The results obtained even indicate that the experiments with the pure compound may provide a better starting point for the risk assessment of low dose exposure than testing of basil itself. As illustrated by this example PBK model based analyses is an excellent way to study the consequences of matrix derived combination effects at realistic low dose exposure levels.
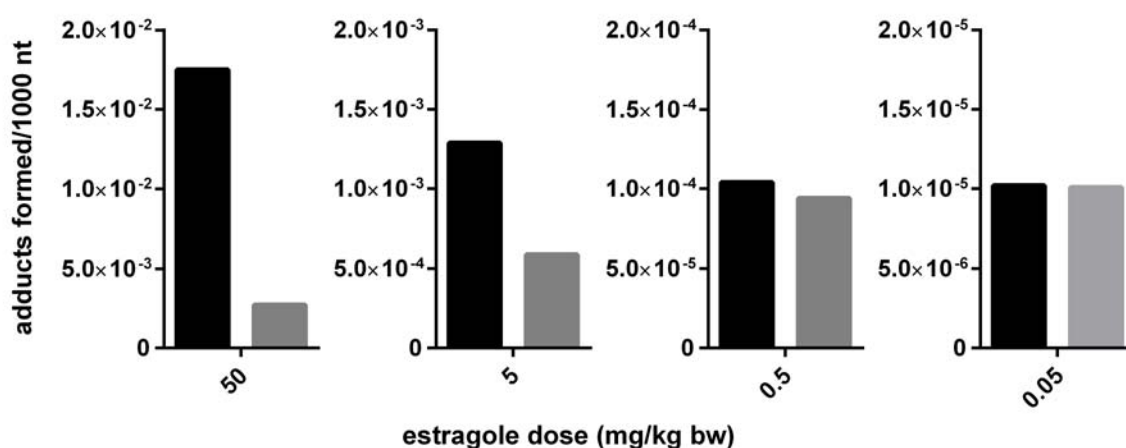

Figure 4. PBK-model based prediction of the dose dependent effect of the basil ingredient nevadensin on the DNA adduct formation in rats by the genotoxic carcinogen estragole. Black (grey) bars represent the predicted DNA adduct formation in absence (or presence) of nevadensin. The ratio of nevadensin to estragole was kept constant at the possible ratio in basil. The PBK model used was the model described by Alhusainy et al. (2013). For further details see Rietjens et al. (2015).

5) How can we perform a risk assessment in the absence of tumor data?

For risk assessment of genotoxic carcinogens data from long-term rodent carcinogenicity studies are essential. Such studies may provide a $BMDL_{10}$ required to apply the MOE approach. However such long-term carcinogenicity studies are not always available for compounds of concern and this raises the question on how we can perform a risk assessment for genotoxic carcinogens in the absence of adequate tumor data. Also here the group of alkenylbenzenes can provide an example since for several congeners of the group, including elemicin, myristicin and apiole tumor data that allow definition of a $BMDL_{10}$ value are not available. In such a situation PBK modelling may facilitate read-across to congeners within the group for which tumor data are available. For example a first estimate of the risk of exposure to elemicin, for which tumor data for definition of a $BMDL_{10}$ are absent, was made using PBK modelling based read-across to methyleugenol and estragole for which *in vivo* animal tumor data are available (Van den Berg et al., 2012).

PBK modelling based predictions of bioativation of elemicin, estragole and methyleugenol to their ultimate carcinogenic 1'-sulfoxymetabolites revealed that compound differences in formation of the 1'-sulfoxymetabolites are limited with bioactivation of elemicin being 11- and 2-fold lower than that of estragole and methyleugenol respectively. Extrapolation of the $BMDL_{10}$ values for estragole and methyleugenol to a possible $BMDL_{10}$ for elemicin, based on the differences in formation of the related ultimate carcinogenic 1'-sulfoxymetabolites, results in a $BMDL_{10}$ value for elemicin of 30.6 mg/kg bw per day (Van den Berg et al., 2012). This $BMDL_{10}$ value was used to perform a risk assessment for elemicin using the MOE approach indicating that elemicin poses a lower priority for risk management compared to its structurally related analogs estragole and methyleugenol. This example illustrates that PBK modelling may facilitate a read-across in risk assessment from compounds for which *in vivo* toxicity studies are available to a compound for which only limited toxicity data have been described, thus contributing to risk assessment for a genotoxic carcinogen in the absence of tumor data and the development of alternatives for animal testing.

6) Can we use alternative *in vitro* models?

In the absence of *in vivo* toxicity data one could also consider the use of alternative non-animal based testing models. The development of non-animal based testing strategies of chemicals is important in current human safety testing. Many efforts in this area focus on the development and use of especially *in vitro* testing strategies using cells in culture that

provide concentration-response curves on adverse effects. However, concentration-response curves from *in vitro* models are inadequate for human risk and safety assessment because risk assessment requires *in vivo* dose-response curves to obtain points of departure (PODs). PBK modeling can overcome this limitation because PBK models can facilitate translation of *in vitro* concentration-response curves into predicted *in vivo* dose-response curves using so-called reverse dosimetry (Figure 5). In reverse dosimetry *in vitro* concentrations are set equal to plasma or tissue levels of the respective compound in the PBK model, following which the PBK model can calculate the corresponding *in vivo* dose levels, thereby facilitating definition of the *in vivo* dose-response curve. Proofs of principle for this *in vitro-in silico* PBK model based reverse dosimetry approach have been provided in our previous work predicting for example developmental toxicity of glycol ethers, phenol and retinoic acid (Louisse et al., 2010; Strikwold et al., 2013; Louisse et al., 2015) and kidney toxicity of aristolochic acid I (Abdullah et al., 2016). The *in vivo* predictions made by these models based on mainly *in vitro* data were generally within one order of magnitude or even similar to the *in vivo* data available in literature.
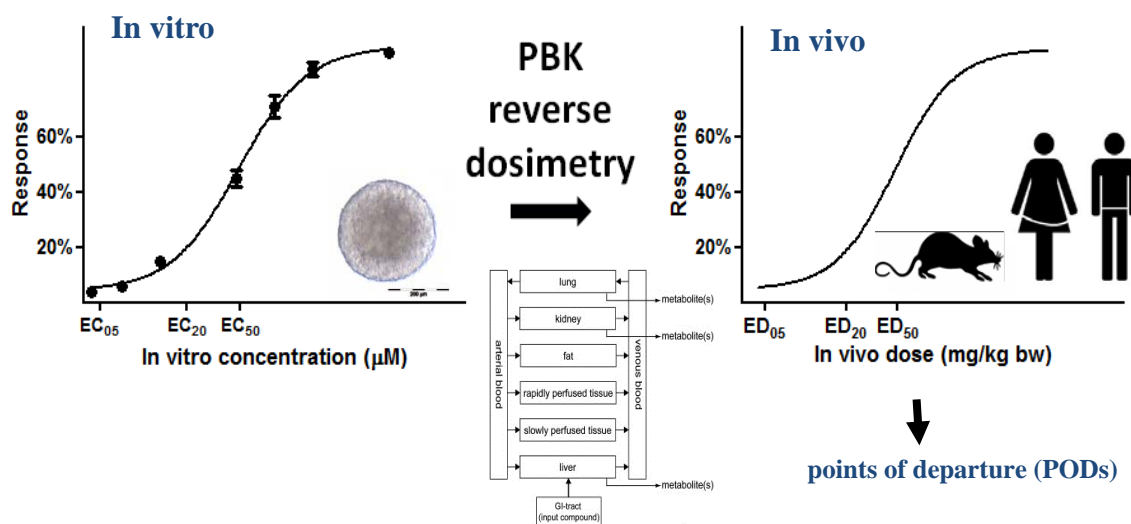

Figure 5. *Schematic presentation of PBK modeling-based reverse dosimetry enabling definition of in vivo dose-response curves for all species including human based on concentration-response curves from in vitro assays. These in vivo dose-response curves are required by risk assessors to derive points of departure (PODs) to set safe exposure limits.*

Application of this approach to the field of genotoxic carcinogens clearly awaits the development of an *in vitro* assay to predict carcinogenicity. Perhaps novel developments in the field of array technologies including arrays for quantification of kinase activities may proof useful for this in the near future. A new bioassay for the *in vitro* detection of

carcinogenicity may be based on arrays detecting effects on kinase activity because at the present state-of-the-art it is accepted that the modes of actions underlying the hallmarks of cancer often relate to an effect on kinase activity influencing subsequent downstream cellular signaling pathways (Hanahan and Weinberg, 2011).

Emerging challenges that may arise in the near future, knowledge gaps and research needs

The rate at which *in vitro* toxicity data are currently generated is high. For example the EPA ToxCast project recently completed the evaluation of over 2,000 chemicals from a broad range of sources, including industrial and consumer products, food additives, and potentially "green" chemicals that could be safer alternatives to existing chemicals. These chemicals were tested in over 700 different high-throughput *in vitro* screening assays covering a range of endpoints and signaling pathways. In order to use these data for risk assessment purposes, the *in vitro* concentration-response data should be translated to *in vivo* dose-response data applying PBK modelling-facilitated reverse dosimetry.

Given that the definition of a PBK model for each individual compound can be resource and time consuming, it is obvious that in order to be able to judge the impact of the *in vitro* toxicity data for the *in vivo* situation, efforts have to be directed at the development of efficient and generic PBK models for large groups of compounds. In an optimal situation the PBK models should be able, with a minimum amount of efforts to generate the required parameter values, to predict whether data obtained in an *in vitro* assay translate to an *in vivo* relevant dose or that dose levels required to obtain the relevant plasma or tissue concentrations turn out to be unrealistically high.

Development of such generic PBK models is an important challenge for the near future. Within the American EPA (Environmental Protection Agency) ToxCast programme an initial strategy to define such generic PBK models has been described. The PBK models in the EPA strategy only include a few kinetic processes and contain only the following experimental parameters (Wetmore et al., 2012):
- Plasma protein binding.
- Metabolic clearance measured at two concentrations using hepatocytes.
- Bi-direction permeability of an intestinal barrier using Caco-2 cells in a transwell model.
- Red blood cell partitioning.

To improve the model and make it generic for a larger chemical domain it is likely that additional parameters will have to be included.

Another emerging challenge and research need which should be addressed to advance the risk assessment of food includes especially the risk assessment of carcinogens. As outlined above an *in vitro* assay that enables definition of concentration-response curves for tumor development would be a real challenge for future research. Also better understanding of the translation of DNA adduct formation into mutagenicity and eventually carcinogenicity (Paini et al., 2011) would close important knowlegde gaps in the current risk assessment of carcinogens in our food.

Conclusions/Concluding remarks

Together the examples presented reveal that PBK modelling can facilitate extrapolation in risk assessment from compounds, exposure regimens or species for which *in vivo* toxicity studies are available to compounds, exposure regimens, species or even individuals for which no or only limited toxicity data have been described, thus contributing to better risk assessment as well as to the development of alternatives for animal testing.

Acknowledgement

References

Abdullah R, Alhusainy W, Woutersen J, Rietjens IMCM, Punt A (2016) Combining *in vitro* cytotoxicity data with physiologically based kinetic (PBK) modeling to define *in vivo* dose-response curves for kidney toxicity of aristolochic acid I in rat, mouse and human. (submitted)

Alhusainy W, Paini A, Punt A, Louisse J, Spenkelink B, Vervoort J, Delatour, T, Scholz G, Schilter B, Adams T, van Bladeren PJ, Rietjens IMCM (2010) Identification of nevadensin as an important herb-based constituent inhibiting estragole bioactivation and physiology-based biokinetic modeling of its possible *in vivo* effect. Toxicology and Applied Pharmacology 245 (2010) 179-190.

Alhusainy W, Paini A, van den Berg JHJ, Punt A, Scholz G, Schilter B, van Bladeren PJ, Taylor SV, Adams TB, Rietjens IMCM (2013) *In vivo* validation and physiologically based modelling of the inhibition of SULT-mediated estragole DNA adduct formation in the liver of male Sprague-Dawley rats by the basil flavonoid nevadensin. Molecular Nutrition and Food Research 57, 1969-1978.

Alhusainy W, Williams, Jeffrey AM, Iatropoulos MJ, Taylor S, Timothy B. Adams TB, Rietjens.IMCM (2014) The natural basil flavonoid nevadensin protects against induction of markers of hepatocarcinogenicity by methyleugenol in male F344 rat. Food Chemical Toxicology 74, 28-34.

Al-Subeihi AAA, Alhusainy W, Kiwamoto R, Spenkelink B, van Bladeren PJ, Rietjens IMCM, Punt A (2015) Evaluation of the interindividual human variation in bioactivation of methyleugenol using physiologically based biokinetic modeling and Monte Carlo simulations
Toxicology and Applied Pharmacology 283, 117-126.

Barlow S, Renwick AG, Kleiner J et al. (2006) Risk assessment of substances that are both genotoxic and carcinogenic. Food and Chemical Toxicology 44, 1636-1650.

EFSA (2005) Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. The EFSA Journal, 282, 1-31.

EFSA (2009) Guidance on safety assessment of botanicals and botanical preparations intended for use as food supplements. EFSA Journal 7, 1249

Gentry PR, Hack CE, Haber L, Maier A, Clewell III HJ (2002) An approach for the quantitative consideration of genetic polymorphism data in chemical risk assessment: Examples with warfarin and parathion. Toxicological Sciences 70, 120-139.

Hanahan D, Weinberg, RA (2011) Hallmarks of cancer: The next generation. Cell, 144, 646-674.

IPCS (2005). International Programme on Chemical Safety. Chemical-specific adjustment factors for interspecies difference and human variability: Guidance document for use of data in dose/concentration-response assessment. Harmonization Project Document No. 2. World Health Organization, Geneva (Switzerland). http://whqlibdoc.who.int/publications/2005 /9241546786_eng. pdf.

Jeurissen SMF, Punt A, Delatour T, Rietjens IMCM (2008) Basil extract inhibits the sulfotransferase mediated formation of DNA adducts of the procarcinogen 1'-hydroxyestragole by rat and human liver S9 homogenates and in HepG2 human hepatoma cells. Food and Chemical Toxicology 46, 2296-2302.

Louisse J, de Jong E, van de Sandt JJM, Blaauboer BJ, Woutersen RA, Piersma AH, Rietjens IMCM, Verwei M (2010) The use of *in vitro* toxicity data and physiologically based kinetic modeling to predict dose-response curves for *in vivo* developmental toxicity of glycol ethers in rat and man. Toxicological Sciences 118, 470-484.

Louisse J, Bosgra S, Blaauboer BJ, Rietjens IMCM, Verwei M (2015) Prediction of *in vivo* developmental toxicity of all-*trans*-retinoic acid based on *in vitro* toxicity data and in silico physiologically based kinetic modeling. Archives of Toxicology 89, 1135-1148.

O'Brien J Renwick AG et al. (2006) Approaches to the risk assessment of genotoxic carcinogens in food: A critical appraisal. Food and Chemical Toxicology 44, 1613-1635.

Paini A, Scholz G, Marin-Kuan M, Schilter B, O'Brien J, Van Bladeren PJ, Rietjens IMCM (2011) Quantitative comparison between *in vivo* DNA adduct formation from exposure to selected DNA-reactive carcinogens, natural background levels of DNA adduct formation and tumor incidence in animal bioassays. Mutagenesis 26, 605-618.

Punt A, Paini AA, Freidig AP, Delatour T, Scholz G, Schilter B, van Bladeren PJ, Rietjens IMCM (2009) Use of physiologically based biokinetic (PBBK) modeling to study estragole bioactivation and detoxification in human as compared with male rat. Toxicological Sciences 110, 255-269.

Punt A, Jeurissen SMF, Boersma MG, Delatour T, Scholz G, Schilter B, van Bladeren PJ, Rietjens IMCM (2010) Evaluation of human interindividual variation in bioactivation of estragole using physiologically based biokinetic modeling. Toxicological Sciences 113, 337-348.

Punt A, Paini A, Spenkelink B, Scholz G, Schilter B, van Bladeren PJ, Rietjens IMCM (2016) Evaluation of interindividual human variation in bioactivation and DNA binding of estragole in liver predicted by physiologically based biodynamic (PBBD) and Monte Carlo modelling (submitted).

Rietjens IMCM, Punt A, Schilter B, Scholz G, Delatour T, van Bladeren PJ (2010) *In-silico* methods for physiologically based biokinetic models describing bioactivation and detoxification of coumarin and estragole: Implications for risk assessment.
Molecular Nutrition and Food Research 54, 195-207.

Rietjens IMCM, Louisse J, Punt A (2011) Tutorial on physiologically based kinetic (PBK) modelling in molecular nutrition and food research. Molecular Nutrition and Food Research 55, 941-956.

Rietjens IMCM, Adams TB, Bastaki M, Cohen SM, Fukushima S, Harman C, Gooderham NJ, Hecht S, Marnett LJ, R. Smith RL, Taylor SV (2014) The impact of structural and metabolic variation on the toxicity and carcinogenicity of hydroxy- and alkoxy- substituted allyl- and propenylbenzenes. Chem Res Toxicol 27, 1092-1103.

Rietjens IMCM, Tyrakowska B, van den Berg SJPL, Soffers AEMF, Punt A (2015) Matrix and combination effects in absorption, distribution, metabolism and excretion (ADME) of food-borne toxic compounds; implications for risk assessment. Toxicology Research 4, 23-35.
Van den Berg SJPL, Restani P, Boersma MG, Delmulle L, Rietjens IMCM (2011) Levels of genotoxic and carcinogenic ingredients in plant food supplements and associated risk assessment. Food and Nutrition Sciences 2, 989-1010.

Van den Berg SJPL, Punt A, Soffers AEMF, Vervoort J, Ngeleja S, Spenkelink B, Rietjens IMCM (2012) Physiologically based kinetic models for the alkenylbenzene elemicin in rat and human and possible implications for risk assessment. Chemical Research in Toxicology 25, 2352-2367.