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**PBPK simulated DNA adduct formation: Relevance
for the risk assessment of benzo(a)pyrene**

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CONTENTS

Summary	4
Samenvatting	5
1. Introduction	6
2. PBPK model for B(a)P	7
2.1 General framework	7
2.2 Rat PBPK model	9
2.3 Human PBPK model	9
3. Interspecies scaling of B(a)P DNA adduct formation	11
4. Interspecies extrapolation of B(a)P carcinogenicity: Use of PBPK simulated DNA adduct formation	14
5. Discussion	16
6. Literature	18
Appendix	20

Calculation of the “Risk Specific Dose” and the “Virtual Safe Dose” using the administered amount of B(a)P (external dose concept)

Calculation of the “Risk Specific Dose” and the “Virtual Safe Dose” using the formation of DNA adducts (internal dose concept)

SUMMARY

In a previous report a Physiologically Based Pharmacokinetic (PBPK) model for Benzo(a)Pyrene (B(a)P) is described (Zeilmaker *et al.*, 1998). This model simulates, after chronic exposure, the distribution of B(a)P in the body and the metabolism of B(a)P generating genotoxic metabolites, including the formation of DNA adducts in the liver. This study explores the effect of using PBPK modeling for the interspecies extrapolation of B(a)P DNA adducts. The results of this study indicate that, at equal administered dose, the accumulation of DNA adducts in the human liver is expected to be one order of magnitude higher than in the rat liver.

A method to use PBPK simulated DNA adduct formation, instead of the administered dose of B(a)P, as the starting point for the interspecies extrapolation of B(a)P carcinogenicity is presented.

SAMENVATTING

In een voorgaand rapport is een fysiologisch-kinetisch model ("PBPK" model) voor de orale blootstelling aan B(a)P beschreven (Zeilmaker *et al.*, 1998). Dit model simuleert, na orale blootstelling, de verdeling van B(a)P in het lichaam en de omzetting van B(a)P in genotoxische metabolieten, inclusief de vorming van DNA adducten in de lever. Dit rapport beschrijft een verkennende studie naar de mogelijkheid om PBPK modellering te gebruiken voor de interspecies extrapolatie van de vorming van B(a)P DNA adducten in de lever. De resultaten van deze studie laten zien dat, bij gelijke blootstelling aan B(a)P, de vorming van DNA adducten in de humane lever één orde van grootte hoger ingeschat wordt dan in de ratte lever.

Een methode om PBPK simulaties van de vorming van DNA adducten, in plaats van de toegediende hoeveelheid B(a)P, als uitgangspunt te gebruiken voor de interspecies extrapolatie van de carcinogeniteit van deze verbinding wordt gepresenteerd.

1. INTRODUCTION

In a previous report the development of a PBPK model of B(a)P is described (Zeilmaier *et al.*, 1998). This model incorporates the uptake, the distribution and the metabolism of B(a)P, including the formation of DNA adducts in the liver. Internal dose surrogates simulated by the PBPK model are the amount of B(a)P in the blood and liver, the induction of the B(a)P metabolising P450 proteins Cyp 1A1 and Cyp 1A2 and the accumulation of certain B(a)P DNA adducts in the liver. These endpoints were simulated after intravenous and oral exposure as well as after single and repeated exposure to B(a)P.

The advantage of the PBPK model over a more classical kinetical approach is that with one and the same model a wide variety of experimental studies (*i.v.*, single dose; *p.o.*, single dose and multi-dose oral exposure of B(a)P) and kinetic endpoints (concentration of B(a)P in the blood; P450 induction in the liver, DNA adducts in the liver) can be described. Even more important, the PBPK model gives a framework to extrapolate the mechanism which is thought to dominate B(a)P DNA adduct formation in the liver, i.e. Ah-receptor dependent P450 induction, from the rat to man.

The present study explores the effect of using PBPK simulated DNA adduct formation in a target organ for carcinogenesis, i.e. the liver, instead of the administered dose of B(a)P as the starting point for the calculation of the exposure level which leads to an acceptable B(a)P tumor prevalence in man ("Virtual Safe Dose", VSD). The uncertainty which adheres to the use of PBPK simulated DNA adduct formation instead of the administered dose as the starting point for the calculation of the VSD of B(a)P is discussed.

2. PBPK MODEL FOR B(a)P

2.1 General framework

In the cell the metabolism of B(a)P is regulated by an intracellular protein, the Ah-receptor. After the binding of B(a)P to the Ah-receptor the formed B(a)P-Ah-receptor complex activates transcription and translation of B(a)P metabolising genes. As a result increased levels of B(a)P metabolising proteins will appear in the cell. In Zeilmaier and Van Eijkeren (1997) a cellular model for the Ah-receptor dependent induction of P450 proteins was developed. This model describes the binding of a ligand to the Ah-receptor and the induction of Ah-receptor dependent *de novo* synthesis of P450 proteins. This cellular model was incorporated in the liver compartment of a PBPK model of B(a)P (Zeilmaier *et al.*, 1998). See Figure 1 for the structure of the B(a)P PBPK model. For further reference and model equations, see *ibid*. Note that in the model the liver is the only organ which contains the Ah-receptor dependent P450 induction mechanism¹. PBPK simulations of B(a)P DNA adduct formation are therefore limited to this organ. The solid lines in Figure 1 represent the flow of B(a)P by the blood circulation through the compartments. The upper dotted arrow represents induction of P450 enzymes mediated by the B(a)P-Ah-receptor complex, delayed by a time lag τ . The lower dotted arrow indicates the instantaneous Ah-receptor dependent P450 storage of B(a)P and, consequently, the metabolic capacity of the liver. This capacity is thought to consist of Ah-receptor dependent P450 activity (see below). The adipose tissue is subdivided in two subcompartments, blood and tissue, with B(a)P being exchanged between these (sub)compartments by diffusion.

¹ Since quantitative information of Ah-receptor dependent P450 induction in the extrahepatic organs is lacking, this cannot be calibrated in these organs. Besides, such a calibration also needs quantitative information on the transport of intermediates of the metabolism of B(a)P from the liver to the extrahepatic organs. Again, such information is not available.

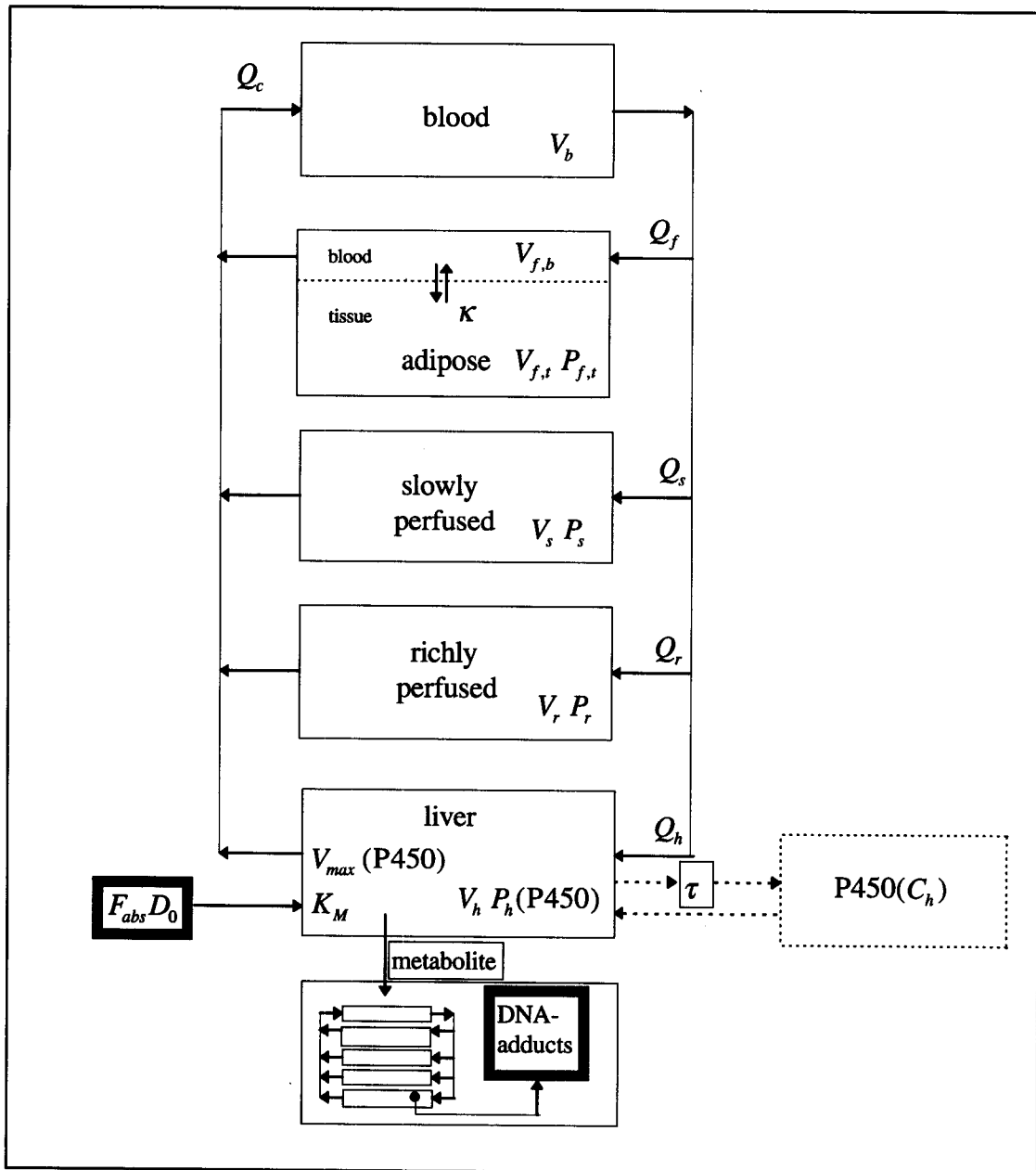


Fig. 1. PBPK model for B(a)P in the rat

V_i : volume of the i^{th} organ, $i = \text{blood, adipose, slowly perfused organs, richly perfused organs, liver}$;
 P_i : partition coefficient of the i^{th} extrahepatic organ; $P_h(P450)$: partition coefficient of the liver which depends on the P450 content of this organ; Q_i : blood flow to the i^{th} organ; K_M : Michaelis-Menten constant for hepatic B(a)P metabolism; $V_{max}(P450)$: metabolic capacity of the liver which depends on the P450 content of this organ; $V_{f,i}$: volume of the lipid fraction of the adipose tissue; $V_{f,b}$: volume of blood in the adipose tissue; D_0 : amount administered; F_{abs} : fraction of D_0 which is absorbed from the gastrointestinal tract.

2.2 Rat PBPK model

For the calibration of the model the following endpoints were available: the concentration of B(a)P in the blood; the induction of Ah-receptor dependent P450 activity, i.e. Ethoxyresorufin-o-dealkylase activity (EROD), in the liver and the amount of DNA adducts in the liver. These endpoints were measured after *i.v.*, single dose; *p.o.*, single dose and multi-dose oral exposure (concentration of B(a)P in the blood) and after repeated exposure (EROD induction; DNA adduct formation). Regarding the accumulation of DNA adducts in the liver ³²P labeling studies revealed the formation of four B(a)P DNA adducts (referred to as adducts 1, 2, 3 and 4). Of these adducts adduct 2 was identified as benzo(a)pyrene-diol-epoxide-N2-deoxyguanine (BPDE-N2-dG). This adduct, which is also found in humans (Bartsch, 1996), is known to be associated with the induction of point mutations and carcinogenicity (Thakker *et al.*, 1985; Denissenko *et al.*, 1996). Adducts 1, 3 and 4 were only identified by their P³² label. In order to facilitate the calibration of the PBPK model the major adduct, i.e. adduct 4, was chosen for this purpose. Although this adduct accounts for about 90% of all adducts identified by ³²P-postlabeling in the liver its role in carcinogenesis is still unresolved. However, as all four adducts showed almost similar formation and removal characteristics (Kroese, in preparation) conclusions which are based on this calibration procedure also apply to adducts 1, 2 and 3.

In calibrating the model data on the disappearance of B(a)P from the blood after single and repeated exposure was used as a marker for basal and inducible B(a)P metabolism. Induced EROD activity after repeated exposure was used as a marker for Ah-receptor dependent P450 induction and DNA adduct formation as a marker for the formation of genotoxic B(a)P metabolites. The availability of these data only allows the calibration of a relative simple metabolic pathway of B(a)P. Furthermore, only quantitative information (basal and inducible amounts) of the B(a)P metabolising P450 enzymes 1A1 and 1A2 is available (Zeilmaker and van Eijkeren, 1997). The concomitant simulation of the above mentioned endpoints is only possible with a model which contains (at least) two independent B(a)P metabolic pathways: one pathway (relatively high basal capacity, relatively low inducible capacity) which is responsible for the bulk of the metabolism of B(a)P and one pathway which is mainly associated with EROD activity and DNA adduct formation (see accompanying report). In concordance with the role of 1A1 in EROD induction and B(a)P DNA adduct formation (Eberhart *et al.*, 1992) we assumed the latter pathway to be associated with 1A1. In contrast to 1A1 1A2 has the kinetic properties which are needed to describe the bulk of the B(a)P metabolism, i.e. relatively high basal, but low inducible, B(a)P metabolism. For this reason the kinetic properties of 1A2 (basal and maximally inducible amount of the protein in the liver) were used to characterise this pathway.

2.3 Human PBPK model

The human PBPK model was obtained by scaling the rat model to man. In this scaling rat model parameters were replaced by their human equivalents. The latter parameters were as follows. B(a)P partition coefficients in the rat and in man were assumed to be equal. As the lipid content of rat and human organs do not differ much we feel that this assumption is justified. Similarly, the concentration of the Ah-receptor in the rat and the human liver is almost equal (Zeilmaker and van Eijkeren, 1998). In the non-induced liver the P450 associated enzyme activity EROD is thought to be associated with both 1A1 and 1A2 (Zeilmaker and Van Eijkeren, 1997). In primary human hepatocytes the basal level of this activity is low (Schrenk *et al.*, 1995), indicating fairly low 1A1 and 1A2 levels. This conclusion is supported by the finding that also the basal levels of 1A1 and 1A2 mRNAs are low in uninduced primary human hepatocytes (Abdel-Razzak *et al.*, 1994). On the other hand the enzyme activity POD, which is thought to be associated with 1A2, seems to be almost equal in rat liver and human liver microsomes (Jansen, personal communication). In the PBPK model the uncertainty in the basal levels of 1A1 and 1A2 in the human liver is taken care of by assuming these levels to range, in comparison with the basal levels of these proteins in the rat liver, from virtually absent to equal to levels in the rat liver. In the induced liver EROD activity is thought to be mainly associated with 1A1 (Zeilmaker and Van Eijkeren, 1997). In primary human hepatocytes the inducibility of EROD activity is twenty-fold lower than in the rat liver (Schrenk *et al.*, 1991, 1995), indicating that the inducible level of 1A1 in the human liver is about twenty times lower than in the rat liver. As 1A1 and 1A2 are almost equally induced in human liver cells and rat liver cells (Zeilmaker and Van Eijkeren, 1997; Abdel-Razzak *et al.*, 1994) this conclusion was assumed to hold for 1A2 as well. Compelled by necessity the following parameters were assumed to be equal in the rat and in man: the rate constant for the decay of the B(a)P-P450 complex, the half-life of P450 proteins in the human liver, the fraction of B(a)P which is absorbed in the gastro-intestinal tract, the rate constant for the absorption of B(a)P from the gastro-intestinal tract and the rate of the formation and the repair of DNA adducts in the liver.

3. INTERSPECIES SCALING OF B(a)P DNA ADDUCT FORMATION

At first glance one expects the accumulation of DNA adducts in the liver to be determined by the metabolism of B(a)P to chemically reactive electrophiles, the reaction of these intermediates with DNA to DNA adducts and the repair rate of the formed DNA adducts. Consequently, interspecies differences in DNA adduct formation are expected to be caused by interspecies differences in these processes. Unfortunately the information on these processes in the human liver is limited. Though the basal levels of 1A1 and 1A2 associated enzyme activity are low in human liver cells (see foregoing paragraph) the absolute amounts of these proteins are not known in this cell type. Furthermore actually no information is available on the efficacy of DNA adduct formation in the human liver.

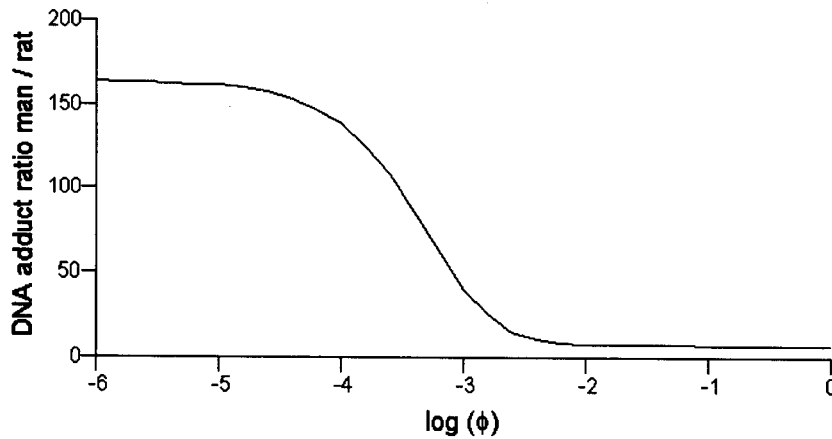
How does the interspecies scaling of DNA adduct formation works out when the above mentioned uncertainties are taken into account? This question was addressed with the PBPK model as follows. With regard to the uncertainty of the basal amounts of 1A1 and 1A2 in the human liver simulations of DNA adduct formation in the human liver were started with the assumption that the amounts of 1A1 and 1A2 in this organ are equal to those in the rat liver. Then the amounts of both 1A1 and 1A2 were simply scaled down to a situation in which only traces of these proteins are present in the human liver. The effect of this down scaling on the amount of B(a)P DNA adducts which, at relative low doses, is expected to accumulate in the human liver relative to the rat liver is shown in Fig. 2a.

In figure 2a. the amount of DNA adduct 4 in the human liver relative to the rat liver, i.e. the ratio between DNA adducts in the human liver in comparison with the rat liver, is shown in dependence of a scaling factor, ϕ . This scaling factor characterises the basal amount of 1A1 and 1A2 in the human liver relative to the rat liver. A value of $\phi \approx 0$ corresponds with a negligible basal level of both 1A1 and 1A2 in the human liver. In this situation the PBPK model calculates that the accumulation of DNA adduct 4 in the human liver will exceed that in the rat liver by a factor 160. At the other extreme $\phi = 1$ corresponds with both basal levels of 1A1 and 1A2 which are equal in the human liver and the rat liver. The PBPK model calculates that in this situation the accumulation of DNA adduct 4 in the human liver still will exceed that in the rat liver by a factor 7. This factor illustrates that next to differences in B(a)P metabolising enzymes and efficacy of DNA adduct repair interspecies differences in physiology (cardiac output, organ blood flows and organ weights) considerably contribute to the observed difference in B(a)P DNA adduct formation. Note the clear non-linear dependency of the accumulation of DNA adduct 4 on ϕ . Also note that, given the model as it is, the phenomenon depicted in Fig. 2a applies to DNA adducts 1,2 and 3 as well.

The explanation of the phenomenon depicted in Fig. 2a is as follows. In the model the amount of DNA adducts formed depends on two factors: the amount of 1A1 in the liver and the amount of B(a)P which is available for metabolism to genotoxic intermediates, the latter amount being determined by 1A2. Starting with the rat, this organism possesses a relative high basal amount of 1A2 in comparison with 1A1 in the liver. Furthermore, at low doses the basal amounts of 1A1 and 1A2 are relative high in comparison with the induced amounts of these proteins. As a result the metabolism of B(a)P is expected to be mainly determined by the basal amounts of 1A1 and 1A2, with the 1A2 pathway being dominant over the 1A1 pathway. Consequently, relative few B(a)P will be available for 1A1 metabolism, i.e. will be converted into DNA adducts. A gradual downscaling of the basal amounts of 1A1 and 1A2 then will shift the metabolism of B(a)P more and more from basal to induced metabolism. As 1A1 and 1A2 are induced with equal efficacy this results in a shift in the metabolism of B(a)P towards the 1A1 pathway. As a results more DNA adducts will be formed. So, in general, a down scaling of the basal amounts of 1A1 and 1A2 is expected to lead to an increased formation of DNA adducts.

Fig. 2. PBPK simulation of the accumulation of DNA adduct 4 in the human liver relative to the rat liver (external dose rate in man and in the rat: 207 ng B(a)P/kg/day)

2A. Down scaling of both IA1 and IA2 from the rat liver to the human liver



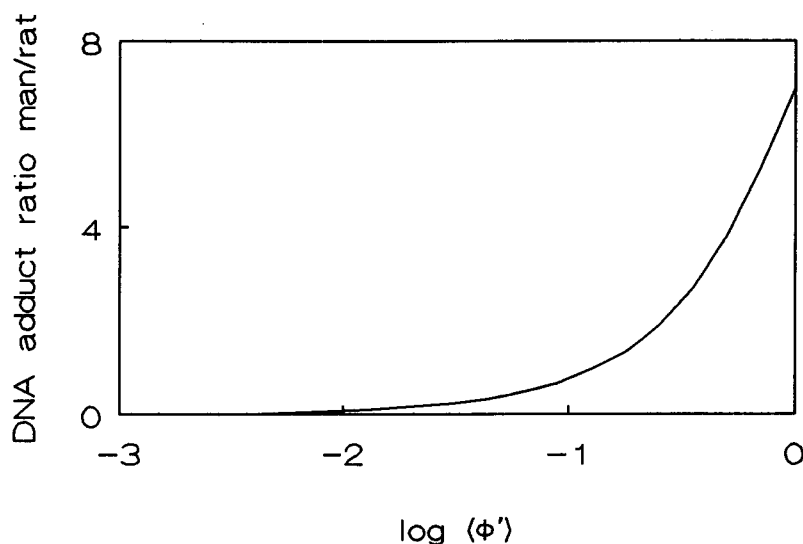
Legend to Fig. 2A.

In the rat the dose level which, after life-long exposure, is associated with a tumor prevalence of 10^{-6} in the liver, i.e. the so-called Risk Specific Dose, was calculated to be 207 ng B(a)P/kg/day (see Appendix for details of this calculation). At this dose level the PBPK model was also used to calculate the amount of adduct 4 in the human liver. In this calculation the uncertainty in the (basal) amount of IA1 and IA2 was explicitly taken into account, i.e. the calculation was performed as a function of the scaling factor ϕ . This factor characterises the basal amount of IA1 and IA2 in the human liver relative to the rat liver. A value of $\phi \approx 0$ corresponds with negligible low basal levels of IA1 and IA2 in the human liver. At the other extreme $\phi = 1$ corresponds with basal levels of IA1 and IA2 which are equal in the human liver and the rat liver. A scaling of ϕ from 1 to (approximately) 0 just means a down scaling of both the amounts of IA1 and IA2 in the human liver relative to the rat liver. The calculated amount of DNA adduct 4 in the human liver was divided by the amount of this adduct in the rat liver (= DNA adduct ratio man/rat).

Remark

In the simulation shown in Fig. 2a both the basal amounts of 1A1 and 1A2 were straightforwardly scaled down to the same extent, i.e. both amounts were scaled down with the same factor. As shown this leads to a higher formation of DNA adducts in the human liver than in the rat liver. A quite different outcome of the simulation is expected when the basal amounts of 1A1 and 1A2 are scaled down at a different extent. Two extreme situations may then be discriminated: down scaling of 1A2 occurring faster than the down scaling of 1A1 or the reverse situation. In comparison with Fig. 2a, a faster down scaling of 1A2 than 1A1 will result in even higher DNA adduct formation in the human liver (data not shown). In contrast, as shown in Fig. 2b, when 1A1 is scaled faster than 1A2 just the rat liver is expected to display the highest DNA adduct levels (note that in this simulation the 1A2 level in the human liver is held at the level of this protein in the rat liver). In this report this issue will not be addressed further.

2B. Down scaling of only 1A1 from the rat liver to the human liver



Legend to Fig. 2B.

In the rat a dose rate of 207 ng B(a)P/kg/day was calculated to be the Risk Specific Dose, i.e. the rate associated with a tumor prevalence of 10^{-6} in the liver. At this dose level the PBPK model was used to calculate the amount of adduct 4 in the human liver. In this calculation the uncertainty in the (basal) amount of 1A1 was explicitly taken into account, i.e. the calculation was performed as a function of the scaling factor ϕ' . This factor characterises the basal amount of 1A1 in the human liver relative to the rat liver. A value of $\phi' \approx 0$ corresponds with negligible low basal level of 1A1 in the human liver. At the other extreme $\phi' = 1$ corresponds with a basal level of 1A1 which is equal in the human liver and in the rat liver. A scaling of ϕ' from 1 to (approximately) 0 just means a down scaling of the basal amount of 1A1 in the human liver relative to the rat liver. The calculated amount of DNA adduct 4 in the human liver was divided by the amount of this adduct in the rat liver (= DNA adduct ratio man/rat).

4. INTERSPECIES EXTRAPOLATION OF B(a)P CARCINOGENICITY: USE OF PBPK SIMULATED DNA ADDUCT FORMATION

Relevance of DNA adducts for B(a)P tumour formation

Studies with chemical carcinogens have repeatedly shown that, in general, there is no clear-cut relationship between the accumulation of DNA adducts and the organotrophic action of chemical carcinogens (see for example De Vries *et al.* 1997 for a discussion of the relationship between B(a)P DNA adducts, B(a)P mutagenicity and B(a)P tumorigenicity). With this we mean that DNA adducts may be found all over the body, whereas in only a few organs tumours will become manifest. On the other hand, when carcinogenesis is considered as a process which consists of a discrete number of succeeding events ultimate tumour growth will only occur when all events have taken place in (at least) one cell. When the formation of pre-mutagenic DNA adduct is one such event DNA adduct formation is a *conditio sine qua non* for tumour growth, though adduct formation alone does not suffice for tumour formation. In this way of reasoning pre-mutagenic DNA adducts are merely considered as lesions which potentially predispose a cell for malignant transformation. For example, in the case of B(a)P DNA adduct 2 has been found to induce point mutations in the P53 gene, a mutation found in association with lung tumor formation in man (Denissenko *et al.*, 1996). For this reason we consider the accumulation of pre-mutagenic B(a)P DNA adducts as a critical factor in target organs for carcinogenicity.

The calculation of an acceptable human exposure level to B(a)P using DNA adduct formation: An example.

In the Netherlands the acceptable human exposure to B(a)P ("Virtual Safe Dose") is currently calculated on the basis of the administered amount of this chemical, i.e. on the amount which is daily administered per unit body weight. In this method the carcinogenicity of B(a)P is straightforwardly extrapolated from the rat to man using the assumption that equal administered amounts of B(a)P, after life-long exposure, lead to an equal cancer incidence in man and in the rat (see Appendix). This calculation results in a VSD of 57 ng B(a)P/kg/day.

When the accumulation of DNA adducts instead of the administered amount, is taken as indicator for B(a)P's carcinogenic potency and DNA adduct formation is extrapolated from the rat liver to the human liver in accordance with the conditions as described in Fig. 2a the PBPK model calculates the life-long dose rate which is expected to lead to a tumour occurrence of 10^{-6} in the human liver to lie between 1.3 ($\varphi \approx 0$) and 27 ($\varphi = 1$) ng B(a)P/kg/day (see Fig. 3). This dose rate is much lower than the rate which is expected to lead to a 10^{-6} tumour occurrence in the rat, i.e. 207 ng B(a)P/kg/day.

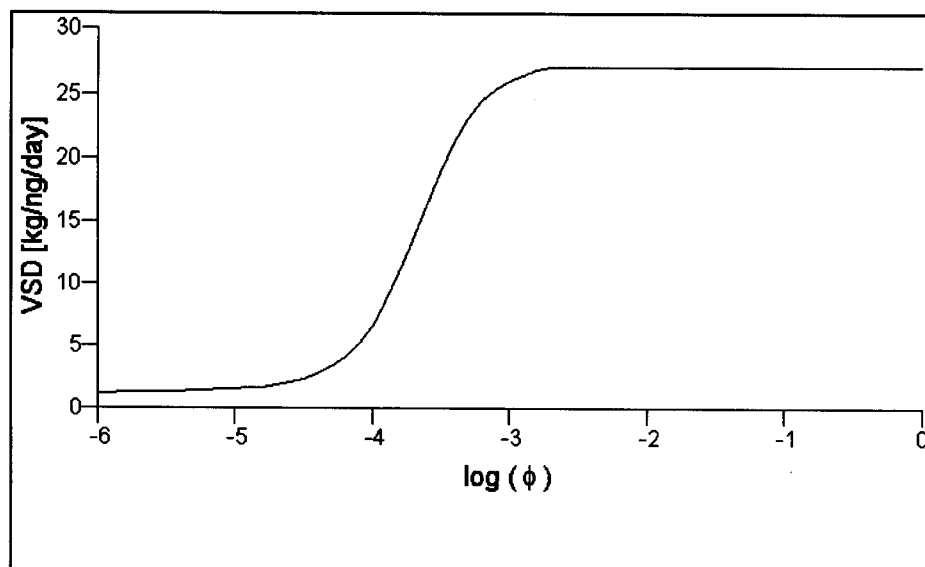


Fig. 3 PBPK calculation of the acceptable human exposure to B(a)P

The acceptable human exposure to B(a)P was defined as the life-long, dose rate of which, in humans lead to the occurrence of liver tumors at a frequency of 10^{-6} . In calculating this exposure the basal amounts of 1A1 and 1A2 were scaled down from the rat liver to the human liver as depicted in Fig. 2a.

5. DISCUSSION

The toxicological risk of chemicals is usually evaluated on the basis of the administered amount of the chemical. The genotoxic carcinogen B(a)P does not constitute an exception to this rule. In the Netherlands the acceptable human exposure for oral B(a)P exposure is calculated by a straightforward extrapolation of the experimentally observed dose-response of B(a)P carcinogenicity in the rat to man. In this extrapolation man and rat are, after life-time exposure, assumed to be equally sensitive for B(a)P. Consequently, the dose response curve of B(a)P carcinogenicity as observed in the rat is assumed to hold for man as well. Furthermore, the sequence of events which lead from the entrance of B(a)P in the body to the occurrence of tumors is not explicitly taken into account. Alternatively the extrapolation of B(a)P carcinogenicity may be based on the mechanism by which B(a)P induces tumors, i.e. the resultant of B(a)P genotoxicity, cytotoxicity and tumorigenicity. As the (exact) mechanism of B(a)P induced carcinogenicity is not known the latter approach is not (yet) feasible. This, however, does not exclude the possibility to incorporate at least some of the events which are thought to play a role in B(a)P tumour formation into the interspecies extrapolation of the carcinogenicity of this compound.

This report explores the possibility to extrapolate the hepatic carcinogenicity of B(a)P from the rat to man on the basis of the accumulation of DNA adducts. This extrapolation was performed with the aid of a PBPK model. This model describes, in the rat and in man, the metabolism of B(a)P to genotoxic intermediates and its immediate result, the formation of B(a)P DNA adducts. When the PBPK model was used to scale the formation of B(a)P DNA adducts from the rat liver to the human liver it was found that, at equal administered dose levels, the human liver accumulates substantially more DNA adducts than the rat liver.

In interpreting this finding a number of uncertainties which underlie the PBPK calculations should be kept in mind. These uncertainties relate to the validity of the PBPK model in simulating B(a)P metabolism and B(a)P DNA adduct formation in the rat and the procedure for the interspecies scaling of B(a)P metabolism.

Firstly, the toxicokinetics of B(a)P in the rat could only be simulated with a PBPK model in which DNA adduct formation and the bulk of B(a)P metabolism are mediated by two different metabolic pathways (Zeilmaker and Van Eijkeren, 1998). In concordance with experimental findings in primary rat hepatocytes 1A1, and not 1A2, was modeled as the enzyme which is responsible for the conversion of B(a)P to its carcinogenic and mutagenic metabolites, in particular (+)-*anti*-benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide (Eberhart *et al.*, 1992). The bulk of B(a)P metabolism could well be described by an enzymatic pathway which mimics 1A2. To our knowledge no suitable experimental data set yet exists which makes the evaluation of this assumption possible.

Secondly, in calibrating the human PBPK model quite a few parameters were found unknown. In simulating the accumulation of DNA adducts in the human liver these parameters were assumed to be equal to their rat equivalents. For example, the efficacy of the formation of B(a)P DNA adducts and their rate of repair were assumed to be equal in the rat liver and in the human liver. Furthermore, the extrapolation of the basal amounts of the B(a)P metabolising proteins 1A1 and 1A2 was performed by straightforwardly scaling the absolute amounts of these proteins down from the rat liver to the human liver (see Fig 2a). In this extrapolation the ratio of the amounts of these proteins was held constant in the human liver, i.e. at the value as observed for this ratio between basal 1A1 and 1A2 in the rat liver. As is shown in Fig. 2b the accumulation of DNA adducts in the human liver over the rat liver is very sensitive for this assumption. The accuracy of the calculation of the accumulation of B(a)P DNA adducts in the human liver over the rat liver as depicted in Fig. 2a can therefore only be improved by measuring the basal amounts of 1A1 and 1A2 and DNA adduct levels in the human liver.

6. LITERATURE

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Appendix

Calculation of the “Risk Specific Dose” and the “Virtual Safe Dose” using the administered amount of B(a)P (external dose concept)

In the Netherlands the high to low dose extrapolation of carcinogenicity in animals is based on linear extrapolation of the observed tumor frequency. As an example we present here this extrapolation for B(a)P induced liver carcinogenesis in the female rat.

In a carcinogenicity study of B(a)P (Kroese, in preparation) a dose level of 2.1 mg B(a)P/kg/day was found to induce tumors in the liver of female rats at a frequency of 3.8%, i.e. the Risk Specific Dose (RSD)_{0.038} equals 2.1 mg/kg/day². Up to this dose level the dose-response relationship for B(a)P induced liver cancer in the rat is assumed to be linear, thus:

$$\text{Observed tumor frequency} = \frac{0.038}{2.1} \times \text{dose rate}$$

with dose rate in mg/kg bw/day. A tumor frequency of 10⁻⁶ then corresponds with a dose rate of about 57 ng B(a)P/kg/day (= RSD_{10⁻⁶}).

Now, assume a tumor frequency of 10⁻⁶ to be acceptable in man. Furthermore, let the dose rate which corresponds with this frequency be called the “Virtual Safe Dose” (VSD) and, at low doses, the dose response relationship for B(a)P carcinogenesis to be equal in rat and in man. The RSD_{10⁻⁶} in the rat then equals the VSD.

Calculation of the “Risk Specific Dose” and the “Virtual Safe Dose” using the formation of DNA adducts (internal dose concept)

Alternatively, the formation of DNA adducts instead of the administered dose may be used as the starting point for the calculation of the RSD. In this extrapolation the high to low dose extrapolation of carcinogenicity is not based on the administered amount of B(a)P but on the amount of DNA adducts which results from this administration. The extrapolation procedure then is as follows. A dose level of 2.1 mg B(a)P/kg/day was found to induce a tumor frequency of 3.8 % in the liver of female rats. At this dose level the “steady state” level of DNA adduct 4 was found to be around 2850 adducts per 10¹⁰ nucleotides. Up to this dose level the (assumed) relationship between B(a)P induced liver cancer and levels of DNA adduct 4 then is assumed to be linear³, or:

$$\text{Observed tumor frequency} = \frac{0.038}{2850} \times N$$

² In practice this dose level is corrected for a “less than life-time exposure period” and a “less than life-time observation period”, which for the rat is 1000 days (HCN, 1995). In this report these corrections were not taken into account because survival of females after 2 years of exposure was about 50%.

³ note that this calculation implies that the accumulation of DNA adduct 4, or any other adduct whose accumulation closely correlates with this adduct, is directly or indirectly involved in the genesis of the observed tumors

with N the number of DNA adducts 4 in the liver. The adduct level which corresponds with a tumor frequency of 10^{-6} then is 0.075 adducts per 10^{10} nucleotides. The rat PBPK model calculates that this adduct level will be reached after life-long exposure to 207 ng B(a)P/kg/day. So in this calculation the $RSD_{10^{-6}}$ is equal to 207 ng B(a)P/kg/day. When, at low doses, the relationship between DNA adduct 4 and tumor prevalence is the same in the rat and in man and a tumor frequency of 10^{-6} is acceptable in man (note that, by assumption, the level of DNA adduct 4 in the human liver which corresponds with this frequency is, as in the rat liver, 0.075 adducts per 10^{10} nucleotides) the PBPK model calculates the VSD to lie between 1.3 ($\phi \approx 0$) and 27 ($\phi = 1$) ng B(aP)/kg/day in man (see Fig. 3).