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**Generic PBPK-modelling of lipophilic
contaminants in the cow**

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ABSTRACT

European Union (EU) regulatory policy for new and existing substances requires assessments of the risks to man and environment. Against this background the European Union System for the Evaluation of Substances (EUSES) was developed. In EUSES, a Quantitative Structure-Activity Relationship (QSAR) is used to predict residues of lipophilic environmental contaminants in beef and milk. The QSAR is based on octanol-water partition coefficients. It appears that predictions can deviate from experimental data a few orders of magnitude. Therefore the question has been raised whether a mechanistic approach, i.e., Physiologically Based Pharmacokinetic (PBPK) modelling, could contribute to a more reliable assessment methodology. PBPK modelling surely has such potency, but lack of knowledge on model parameters prohibits a modelling approach. Fruitful application of PBPK modelling requires generic relationships between the contaminants molecular structure and properties and its distribution between blood, organs and milkfat, its metabolism in the liver and its absorption from the intestine over the intestinal wall.

SAMENVATTING

Het beleid van de Europese Unie met betrekking tot bestaande en nieuwe stoffen vereist risicoschattingen voor mens en milieu. Daarvoor is een systeem ontwikkeld, EUSES geheten, wat staat voor European Union System for the Evaluation of Substances. Teneinde residuen van lipofiele milieucontaminanten te voorspellen in vlees en melk van koeien, wordt in EUSES gebruik gemaakt van een empirische Quantitatieve Structuur-Activiteit Relatie (QSAR) gebaseerd op de octanol-water partitie van lipofiele stoffen. Het blijkt dat de verschillen tussen schatting en werkelijke, experimenteel gevonden waarden enkele orde-grootten kan bedragen. Daarom is onderzocht of een meer mechanistische benadering, in de vorm van een PBPK (Physiologically Based Pharmacokinetic) model zou kunnen bijdragen tot een betrouwbaarder schatting. Uit dit onderzoek blijkt dat zulks in potentie mogelijk is, maar dat de huidige stand van kennis met betrekking tot modelparameters onvoldoende is om dat te effectueren. Vruchtbaar gebruik van modellen is slechts dan mogelijk, als generieke relaties gelegd kunnen worden tussen molecuulstructuur en -eigenschappen van een lipofiele stof en zijn verdeling over bloed, organen en melkvet, zijn metabolisme in de lever en zijn absorptie over de darmwand.

1. INTRODUCTION

European Union (EU) regulatory policy for new and existing substances requires assessments of the risks to man and the environment. Principles for risk assessment have been laid down in a detailed package of Technical Guidance Documents (TGD). Against this background the European Union System for the Evaluation of Substances (EUSES) was developed. EUSES is the result of a co-ordinated effort of EU Member States, the European Commission and the European Chemical Industry and is available since early 1997 (EC, 1996; Vermeire *et al.*, 1997).

EUSES can be used for tiered risk assessments of increasing complexity on the basis of increasing data requirements. Risk assessments in EUSES is carried out in a step-wise manner encompassing exposure assessment, effects assessments and risk characterisation. Human populations regarded are consumers, workers and men exposed through the environment. The exposure assessment departs from generic model scenarios at the personal level for consumers and workers and at the local, regional and continental (EU) level for man exposed through the environment. Both acute and chronic exposure are considered. The environmental exposure assessment takes account of the complete life cycle of substances as well as their environmental fate in all environmental compartments. At the risk characterisation stage the estimated exposure level is compared to a suitable effect or no-effect parameter at the appropriate time scale and for the relevant route of exposure. The exposure assessment aims at realistic worst case results.

In the EUSES, a Quantitative Structure-Activity Relationship (QSAR) is used, among others, to predict residues of lipophilic environmental contaminants in beef and milk on the basis of Predicted Environmental Concentrations (PECs) in air, grass and soil. The QSAR is based on octanol-water partition coefficients (K_{ow}). This QSAR relates daily ingestion of an environmental contaminant to residues in beef and milk. The QSAR has been proposed by Travis and Arms (1988) and was developed from experimental data reported by several authors. It was obtained by linear regression of the logarithm of the Bio-Transformation Factor (BTF), i.e., the ratio residue concentration/daily intake, against $\log(K_{ow})$. However it appears that the scatter of data is considerable and deviations of observations from the theoretically prediction of a few orders of magnitude in both directions occur (Travis and Arms, 1988). A similar approach was followed by Dowdy *et al.* (1996). They, however, related $\log(BTF)$ to the Molecular Connectivity Index (MCI) and claim a better result. Nevertheless, also their predictions deviate from the experimental data an order of magnitude in both directions.

When simple empirical relationships as in the preceding paragraph contribute a large uncertainty to the risk assessment, one may ask whether there exist more mechanistic based tools that could be used within the EUSES system. Such a tool should combine simplicity and reliability for calculating the BTF in cattle for lipophilic environmental contaminants. Physiologically Based Pharmacokinetic (PBPK) modelling has proved to combine both these properties in relating oral dose and internal concentrations for a large number of pharmacological drugs as well as for some environmental contaminants. From the

concentrations one can estimate residues in beef and milk. So, in this report the theoretical possibility of PBPK modelling to a more reliable assessment methodology will be investigated.

In a PBPK model the disposition of an environmental contaminant is determined by its absorption over the gastrointestinal wall, its partition over the organs, or group of organs, such as the muscle group, and its elimination by way of excretion or metabolism. Note that, in case of metabolism, not the parent compound but (one of) the metabolite(s) may be the toxicological active agent. Generally, the absorption fraction, the partition coefficients and metabolism parameters will depend on the physico-chemical properties of the compound under investigation. Not surprisingly, one tries to find QSARs to relate these properties to the parameters of interest. For example, the distribution over the different compartments as blood, adipose tissue, liver, richly perfused and slowly perfused tissue (muscle group) has been related to the K_{ow} by QSARs (Poulin and Krishnan, 1995; DeJong *et al.*, 1997; Zeilmaker and van Eijkeren, 1997). Poulin and Krishnan (1996^a, 1996^b) relate tissue-blood partition coefficients (via tissue-air and blood-air partition coefficients) to solubility in vegetable oil and saline while Gargas *et al.* (1989) propose a QSAR based on oil-air and water-air partition coefficients. Other authors however suggest connectivity indices and molecule descriptors such as polarity, number of (mono-, trigonal- and tetrahedral-) carbons, number of substituted hydrogens, etc. (Gargas *et al.*, 1988; Parham *et al.*, 1997). For the intrinsic liver clearance, little is known, but a few results have been published, relating molecular structure to metabolism rate (Gargas *et al.*, 1988; Borlakoglu *et al.*, 1991; Tanabe *et al.*, 1981). Also little is known on the absorption fraction that relates the amount of contaminant that is ingested to the amount that is actually taken up over the intestinal wall. This problem does not only concern the environmental compound, but also the physiology of the gastrointestinal tract of the cow and its typical physico-chemical characteristics. For PCBs Tanabe *et al.* (1981) found a relation between molecular weight, i.e. the number of chlorine substitutions, and gastrointestinal absorption in rats.

The need for thorough knowledge of QSARs relating molecular properties and structure to basic PBPK model parameters is becoming apparent. Indeed, for a fruitful application in risk assessment of PBPK modelling, one has to dispose of generic models in distinction to models which can be applied only to one compound, or to a small group of compounds. Modelling known relationships between a contaminants molecular properties and structure and its compartmental and milkfat partition coefficients, intrinsic liver clearance rate and gastrointestinal absorption fraction will be a major step to genericity in the next decade, as research is going on.

2. PBPK MODEL OF A LACTATING COW

2.1 General description

In Derks *et al.* (1993) a PBPK model for TCDD in the cow has been introduced. A derivate of this original model is augmented with animal growth from birth to maturation and periods of lactation and non-lactation. As such it can be used to simulate the fate of a lipophilic environmental contaminant taken up by the cow during lifetime.

In the PBPK model two functional organ compartments have been defined, i.e. the blood and the liver compartment, and three aggregated compartments, i.e. the slowly perfused (muscle, skin and bone), the richly perfused (intestines, kidney etc.) and the fat compartment (see figure 1; explanation of symbols in the Appendix). In Derks *et al.* (1993) the udder was modelled explicitly. However, the apparent volume of the udder compartment is so small that almost instantaneous equilibration with the blood compartment can be assumed. Therefore, in this report excretion by milk fat is modelled as excretion from the blood compartment.

All these compartments have their own physiological volume. The cardiac blood flow is divided over the different compartments according to their physiological values. Besides the fat compartment, all compartments are assumed to be flow rate limited, i.e. processes like diffusion over membranes and inter-compartmental diffusion are assumed to be fast with respect to compartmental flow. As such, the compartments can be considered as well stirred vessels. The fat compartment on the other hand is supposed to be diffusion limited, which is denoted by the two subcompartments "blood" and "tissue", between which a diffusive transfer flux exists. Nevertheless, these subcompartments are considered to be well stirred vessels too.

In Derks *et al.* (1993) it was found that diffusive flux rate limiting could also be obtained by diminishing the blood flow, which is non-physiological. For the purpose of simplicity, this procedure will be adopted as a first approach in the investigation of appropriateness of PBPK modelling in the EUSES risk assessment procedure (see figure 2).

Keeping track of all the compartmental contaminant mass balances, a system of 5 coupled ordinary differential equations is obtained (see Appendix). Additional features to the model of Derks *et al.* (1993) are animal growth, from birth to maturity, and alternating periods of lactation and non-lactation. Exchange of the contaminant between the cow in calf and the foetus is omitted, but could be added if necessary and if data on foetal growth are available. A listing of the ACSL-code is included in the Appendix.

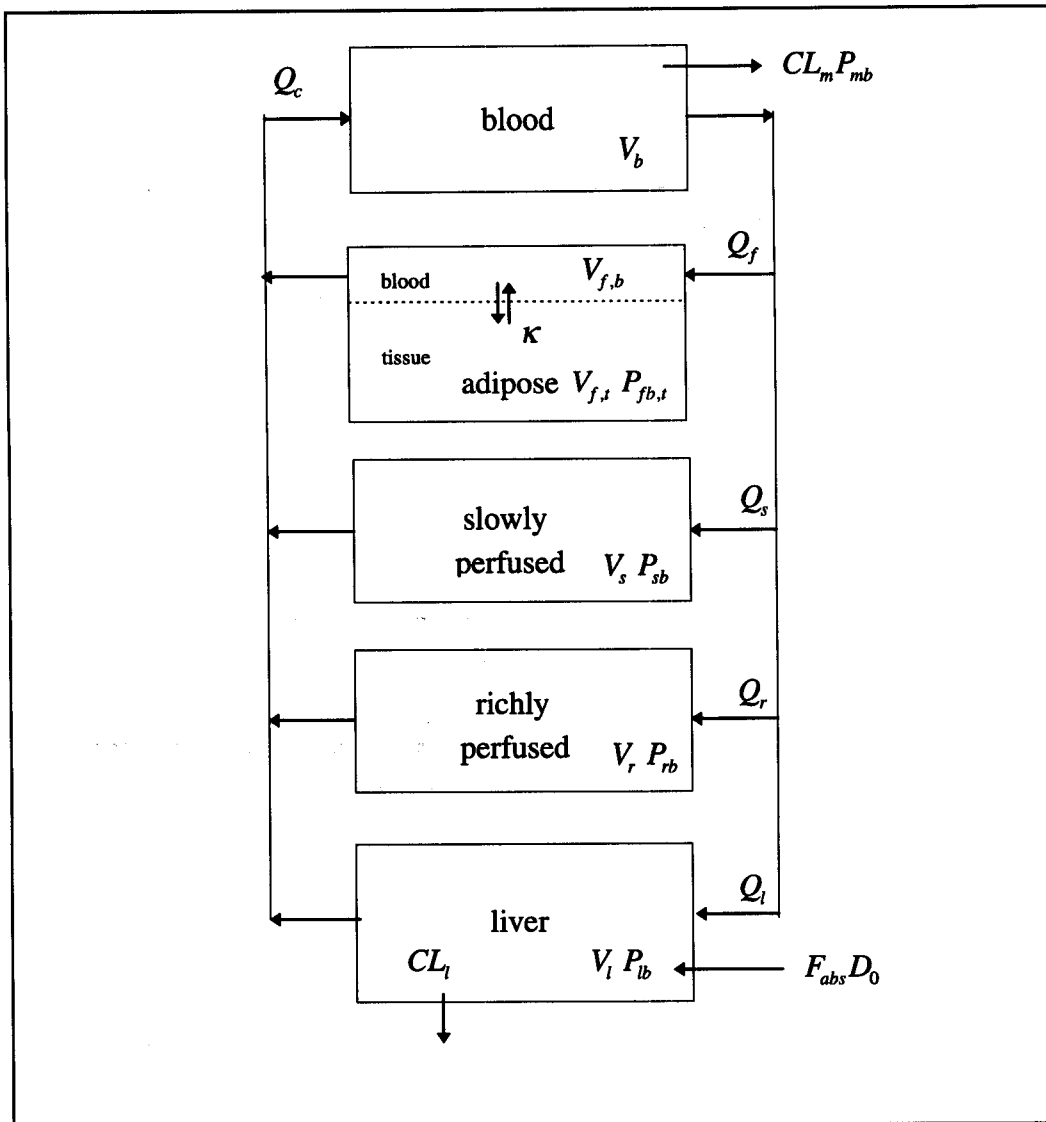


Figure 1. PBPK model scheme (explanation symbols, see Appendix)

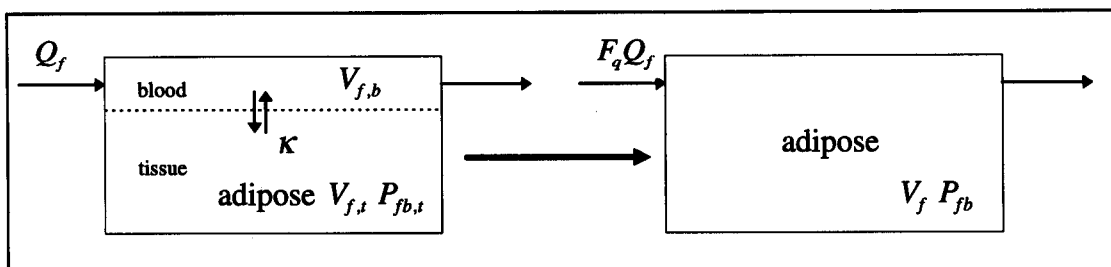


Figure 2. Diffusion limiting by artificial flux limiting

2.2 Distribution

Distribution of the contaminant over the body is described by compartmental partition coefficients that relate the concentration in the compartment to the concentration in its outflowing venous blood. These partition coefficients are dependent on the biochemical properties of the compound. Most often, their values are determined in an *in vitro* experiment, see e.g., Gargas *et al.* (1989) and references therein and Jepson *et al.* (1994). Also, their values can be determined from model calibration of an *in vivo* experiment. Research is starting on combining physico-chemical properties, such as water-octanol partition (K_{ow}) or the molecular structure, with compartmental biochemical properties, such as lipid and water and protein fraction, to obtain reliable estimates (QSARs): Gargas *et al.* (1988), Gargas *et al.* (1989), Poulin and Krishnan (1995, 1996^a, 1996^b), DeJongh *et al.* (1997). One should not only consider physico-chemical properties of the contaminant, but also the composition and structure of the lipid fraction of interest. The lipid fraction in fat cells most presumably differs both in structure and in composition from that in other tissue cells and also from that in milk. For example, the neutral lipid fraction in adipose tissue is almost 100%, while liver contains about 40% phospholipids and muscle and skin 54% (Poulin and Krishnan, 1996^b).

2.3 Elimination

Elimination of the contaminant is supposed to follow two paths: metabolism and excretion. The liver is the site for elimination by way of metabolism. Regarding metabolites, there is a snake in the grass as one of these may be the toxicological active substance, instead of the parent compound. The standard model for metabolism is saturable Michaelis-Menten kinetics, with as parameters the maximum metabolism rate V_{max} and the so called half-velocity concentration, K_M . The latter is the concentration for which metabolism rate is half of its maximum value.

Metabolism parameters can be obtained from *in vitro* experiments, e.g. experiments on microsomes, hepatocytes or liver slices. Also, they can follow from the model calibration of an *in vivo* experiment. Although there is some research ongoing for obtaining QSARs relating physico-chemical properties to values of the Michaelis-Menten parameters (Tanabe *et al.*, (1981); Gargas *et al.* (1988); Borlakoglu *et al.* (1991)), results are far less abundant than for partition coefficients. Also, results for one animal species are difficult to extrapolate to another species. Besides the properties of the compound, one has to know the kind of enzyme(s) capable of metabolising it and liver concentrations of these enzymes in other species. At the moment, this could be a severe flaw in the application of a generic PBPK-model for risk assessment, but future developments could relieve this.

The second path of elimination is excretion by milk. Notably lipophilic contaminants are well excreted from the cow, because of the excretion of about 1 kg of milk fat per day. Besides the milk fat excretion per day, one should know the partition coefficient of milk fat, which can deviate from the partition coefficient of the adipose tissue compartment because of the different composition of fat in adipose tissue and milk fat. In Derks *et al.* (1993) the partition coefficient for the adipose tissue compartment was found to be 280, while the value for milk fat was found to be 430.

Renal clearance and elimination by the lung will not be considered in this first approach.

2.4 Absorption

Another point of concern is the absorption of the orally ingested amount over the gastrointestinal wall. If only a fraction of the intake is effectively taken up, this could lead to an overestimation of risk, when one assumes uptake to be 100%. E.g., for rats the absorbed fraction of B(a)P in a solution of soybean oil that was administered by gavage was found by calibration to be only about 15% (Zeilmaker *et al.*, 1998). Absorption from the amount ingested will not be dependent on the contaminants physico-chemical properties only, but on its vehicle also: grass and soil for cows. The contaminant has to be freed from its matrix first by digestive juices, before it can be absorbed anyway. Perhaps also, enzymatic activity in the gastrointestinal tract could metabolise contaminants. Research for absorption in humans is performed at RIVM and TNO, but the physiology and physico-chemical characteristics of the gastrointestinal tract in humans and ruminants is quite different, which can hamper extrapolation of the results. For PCBs Tanabe *et al.* (1981) found the absorption in the rat to be proportional to the inverse square root of the molecular weight, so this kind of investigation has been performed for one important group of environmental contaminants at least.

2.5 Molecular structure and PBPK model

The only way molecular characteristics enter the current model is by a QSAR that relates the K_{ow} to the partition coefficient. The QSAR is introduced in the Appendix. It appears that in this approach there is hardly any difference in BTF for contaminants with a $\log(K_{ow})$ exceeding 3. For such contaminants, the partition coefficients are almost the same (see Appendix figures A1 through A5, which show partition coefficients of several compartments versus the value of $\log(K_{ow})$). However, in Zeilmaker and van Eijkeren (1998) partition coefficients of B(a)P were found to be much smaller than those of TCDD (Zeilmaker and van Eijkeren, 1998), while both have a comparable K_{ow} : $\log(K_{ow})$ B(a)P about 6, TCDD between 6 and 7 (Mackay *et al.* 1992). This shows the inadequacy of a QSAR based solely on K_{ow} .

3. MODEL RESULTS

The model represents a cow with a lifetime of 5 years and 3 lactating periods, starting after parturition at the age of 2, 3 and 4 years. Its initial bodyweight is 75 kg and its weight at maturity is 600 kg. It is assumed that the relative compartmental weights are constant during lifetime and that growth is steady. This assumption is probably violated for the cow in calf. Figure 3 shows the growth curve.

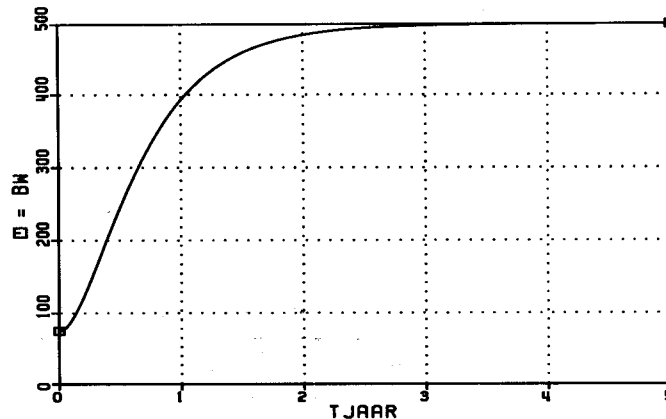


Figure 3. Body weight versus time

Further it is assumed that the cardiac output is scaled to the cardiac output of a 600 kg weighing cow with an allometric power of 0.75. The reference cardiac output during lactation is twice its value during non lactation (Derks *et al.* (1993) and references therein). The relative compartmental blood flows are assumed to be constant during lifetime.

The partition coefficients are calculated as in the Appendix, following the third model. The relative volumes needed for this calculation, i.e., relative compartmental blood, interstitial and cell volumes and relative water and lipid fractions have been estimated from data in Reference Man (1992). The intrinsic liver metabolism clearance is held at 14.5 l/d (Derks *et al.*, 1993) throughout for all values of $\log(K_{ow})$. Also, the gastrointestinal absorption fraction is held at the constant value of 1. During lactation a constant clearance of milk fat of 1 l/d has been assumed, which is in the range given in Derks *et al.* (1993). Figure 4 shows a plot of the concentration of a contaminant with $\log(K_{ow}) = 7$ in milk versus time. In this plot "concentration in milk" should be interpreted as the actual concentration in milk during the lactation period and the concentration as if the cow had been lactating, during the non-lactating period. From this figure one observes that in fact the BTF for a lactating cow is a function of time and changes drastically during the lactation period. Also, one can deduce that the BTF of dairy cattle will be quite smaller than the BTF of beef cattle .

In figure 5. a plot is shown of the logarithm of the BTF versus the logarithm of the K_{ow} . The milk concentration for calculating the BTF is taken to be the concentration at the end of the simulation period of 5 years. The *diamonds* are experimental values reported by Travis and Arms (1988). The upper line, with the *squares*, shows the simulations with the intrinsic liver clearance to be 14.5 l/d. The line parallel with this one, with the *closed circles*,

is calculated with an assumed intrinsic clearance of 10000 l/d. Note that these calculations could never explain the gross dependence of BTF on K_{ow} : because the partition coefficients are almost constant for $\log(K_{ow})$ values greater than 3, also the BTF is almost constant.

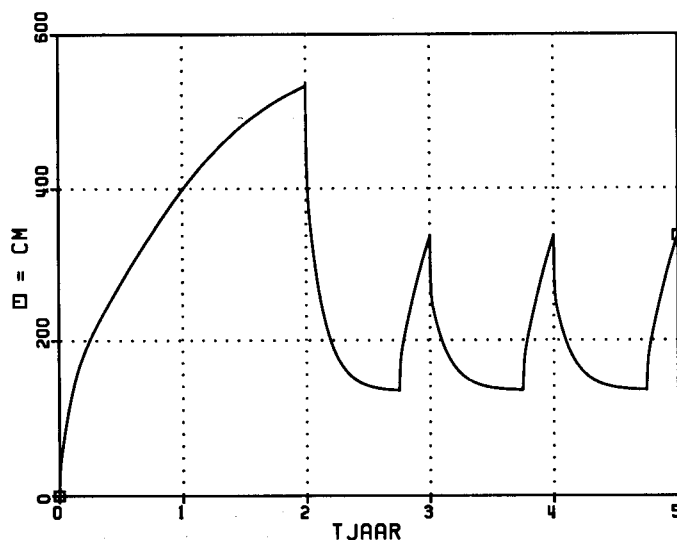


Figure 4. Milk concentration versus time

When the intrinsic liver clearance is scaled to the octanol-water partition coefficient, just by assuming an inverse relation between clearance and $\log(K_{ow})$, the line with *triangles* is obtained. However, this result is, of course, no better than simple linear regression on the data. Still, one may observe that differences in metabolism, based on other molecular structure properties than $\log(K_{ow})$ could explain the variation in the observed experimental observations.

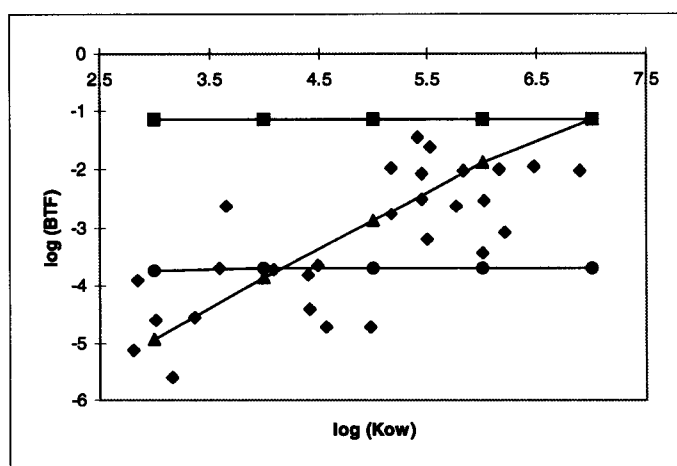


Figure 5. $\log(BTF)$ versus $\log(K_{ow})$ (legend: see text)

4. CONCLUSIONS

The application of PBPK modelling in EUSES requires a more generic approach than the current one. QSARs are needed in order to obtain not only partition coefficients, but also intrinsic liver clearance parameters and gastrointestinal absorption fractions. The current PBPK model makes use of only one molecular property, i.e. its octanol-water partition coefficient, in order to estimate only one of the three parameters that are of interest. From model simulations it appears that this approach is insufficient to explain the reported data, if these are taken for granted.

For a useful contribution to a more generic risk assessment, it seems appropriate to investigate the relation between molecular properties of the contaminant of interest and its disposition in the cow, i.e. gastrointestinal absorption, intrinsic liver clearance and partitioning over the different compartments. Insufficient current knowledge on these topics prohibits application of PBPK modelling in EUSES for a reliable estimation of the BTF in cattle for lipophilic environmental contaminants yet.

Research with respect to partition coefficients is ongoing. However, from Zeilmaker *et al.* (1998) it appears that the absorption fraction of B(a)P in the rat is only about 15%. If such a value would also be found in the cow, that would reduce the BTF estimation relative to an absorption fraction of 100% with about one decade on the log scale. Also, it appears that the specific liver clearance of B(a)P in the rat is about 3 orders higher than the specific clearance of TCDD. Such a difference in the value of the specific clearance in the cow model would lead to a difference in the simulated BTF of at least two orders. So, it seems that research with respect to absorption and specific clearance is at least as important.

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Appendix

List of symbols

A_b	Amount of contaminant in blood
A_f	Amount in adipose tissue
A_l	Amount in liver
A_r	Amount in richly perfused
A_s	Amount in slowly perfused tissue
C_b	Concentration in blood
C_c	Concentration in cellular fraction of blood
C_{cw}	Concentration in blood cellular water fraction
C_f	Concentration in adipose tissue
C_l	Concentration in liver
C_p	Concentration in plasma
$C_{p,w}$	Concentration in water fraction of plasma
$C_{p,f}$	Concentration in lipid fraction of plasma
C_r	Concentration in richly perfused
C_s	Concentration in slowly perfused tissue
C_t	Concentration in generic compartment ("t = f, l, r, s")
C_{tb}	Concentration in generic blood subcompartment
C_{te}	Concentration in generic extracellular subcompartment
C_{tc}	Concentration in generic cellular subcompartment
C_{tpw}	Concentration in water fraction of generic plasma subcompartment
CL_m	Milkfat clearance
CL_l	Intrinsic liver clearance
D_0	Daily intake
F_{abs}	Fraction absorbed over the gastrointestinal wall
F_q	Factor representing diffusion limitation in adipose tissue
f_p^u	Unbound fraction in plasma
f_c^u	Unbound fraction in blood cells
f_e^u	Unbound fraction in extracellular subcompartments
f_{tc}^u	Unbound fraction in in generic tissue cellular subcompartment ("t = f, l, r, s")
κ	Diffusion parameter (not in model: see F_q)
K_{ow}	K_{ow}
P_{bp}	blood-plasma partition coefficient (PC)
$P_{fb,t}$	fat-blood PC for adipose non-blood ("tissue") subcompartment
P_{fb}	fat-blood PC
P_{lb}	liver-blood PC
P_{mb}	milk-blood PC
P_{rb}	richly perfused tissue-blood PC
P_{sb}	slowly perfused tissue-blood PC

P_{ib}	generic compartmental-blood PC
P_{ip}	generic compartmental-plasma PC ("t = f, l, r, s")
Q_c	cardiac output; total blood flow
Q_f	blood flow through adipose tissue
Q_l	blood flow through liver
Q_r	blood flow through richly perfused tissue
Q_s	blood flow through slowly perfused tissue
v_{cb}	cellular volume fraction of blood
v_{cf}	blood cellular lipid volume fraction of blood
v_{cw}	blood cellular water volume fraction of blood
v_{pb}	plasma volume fraction of blood
v_{pf}	lipid volume fraction of plasma
v_{pw}	water volume fraction of plasma
v_{pf}	lipid volume fraction of plasma
v_{tb}	generic tissue blood volume fraction ("t = f, l, r, s")
v_{te}	generic tissue extracellular volume fraction
v_{tef}	generic tissue extracellular lipid volume fraction
v_{tew}	generic tissue extracellular water volume fraction
v_{tc}	generic tissue cellular volume fraction
v_{tcf}	generic tissue cellular lipid volume fraction
v_{tcw}	generic tissue cellular water volume fraction
V_b	Blood volume
V_f	Fat compartment volume
$V_{f,b}$	Fat blood subcompartment volume
$V_{f,t}$	Fat "tissue" (extracellular and cellular) subcompartment
V_l	Liver volume
V_r	Richly perfused compartment volume
V_s	Slowly perfused compartment volume

QSAR relating K_{ow} to tissue-blood partition coefficients (PC's)

Two different models can be derived and will be presented. The QSAR will be a combination of both. In all models, it will be assumed that the contaminant of interest only binds to the lipid fraction and so does not bind to proteins other than lipoproteins. See also Poulin and Krishnan (1995^a) and DeJong *et al.* (1997). Moreover, it will be assumed that the volumetric fractions of the water and the lipid phase respectively in the extracellular subcompartments will be the same as in plasma.

In both models the blood-plasma PC, P_{bp} will be derived first, and then the tissue-plasma PC, P_{tp} . The tissue-blood PC follows from

$$P_{tb} = \frac{P_{tp}}{P_{bp}}$$

Model 1.

This model is based on the equilibration of concentrations in adjacent water phases.

The blood concentration is a weighted mean of the plasma and cellular fraction of blood respectively:

$$C_b = v_{pb} C_p + v_{cb} C_c$$

where subscript "b" is for blood, "p" for plasma and "c" for cellular fraction. The weights are the volumetric fractions of both the latter in blood.

The plasma concentration is a weighted mean of the concentrations in the water and lipid (fat) phase (neglecting protein binding):

$$C_p = v_{pw} C_{pw} + v_{pf} C_{pf}$$

where subscript "w" is for water phase and "f" is for fat phase. The weights are the volumetric fractions of both in plasma.

Assuming equilibration of the concentrations in both phases following the octanol-water PC, K_{ow} , one obtains

$$C_p = (v_{pw} + v_{pf} K_{ow}) \cdot C_{pw}$$

Likewise,

$$C_c = (v_{cw} + v_{cf} K_{ow}) \cdot C_{cw}$$

The equilibration assumption, $C_{cw} = C_{pw}$, now leads to

$$C_b = [(v_{pw} + v_{pf} K_{ow}) v_{pb} + (v_{cw} + v_{cf} K_{ow}) v_{cb}] \cdot C_{pw}$$

from which

$$P_{bp} = \frac{C_b}{C_p} = v_{pb} + \frac{v_{cw} + v_{cf} K_{ow}}{v_{pw} + v_{pf} K_{ow}} v_{cb}$$

follows.

For tissue, in analogy, the following is obtained.

$$C_t = v_{tb}C_{tb} + v_{te}C_{te} + v_{tc}C_{tc} = \frac{P_{bp}}{v_{pw} + v_{pf}K_{ow}} \cdot v_{tb}C_{tpw} \\ + (v_{tew} + v_{tef}K_{ow}) \cdot v_{te}C_{tpw} \\ + (v_{tcw} + v_{tcf}K_{ow}) \cdot v_{tc}C_{tpw}$$

where subscript "t" is for tissue. Use is made of the relation between the concentrations C_p and C_{pw} above, which hold in all plasma-subcompartments and the equilibration of concentrations in adjacent water phases.

From this relation the tissue-plasma PC is

$$P_{tp} = \frac{C_t}{C_p} = v_{tb}P_{bp} + v_{te} + \frac{v_{tcw} + v_{tcf}K_{ow}}{v_{pw} + v_{pf}K_{ow}} v_{tc}$$

Note that when P_{bp} is about 1 and K_{ow} exceeds 1000, then P_{tp} and also P_{tb} approximately equals the ratio of the relative volumetric lipid fractions in tissue and plasma. See e.g., van der Molen *et al.* (1996), who use this observation as a QSAR for highly lipophilic contaminants.

However, several authors report a much higher fat-blood PC than is possible following this model 1 (e.g., Zeilmaker *et al.*, 1997). Therefore, a more formal approach is given below, leading to a much higher value for the fat-blood PC.

Model 2.

This model is based on the equilibration of non-lipid bound concentrations in adjacent subcompartments.

Again, the blood concentration is a weighted mean of the plasma and cellular fraction of blood respectively:

$$C_b = v_{pb}C_p + v_{cb}C_c$$

Denote with f_p^u the fraction of unbound contaminant in plasma, "u" is for "unbound", then

$$f_p^u = \frac{v_{pw}C_{pw}}{C_p} = \frac{v_{pw}C_{pw}}{v_{pw}C_{pw} + v_{pf}C_{pf}} = \frac{v_{pw}}{v_{pw} + v_{pf}K_{ow}}$$

Likewise, $f_c^u = v_{cw} / (v_{cw} + v_{cf}K_{ow})$. Equilibration of unbound concentrations in the plasma and cellular subcompartments respectively, $f_p^u C_p = f_c^u C_c$, leads to

$$C_b = (v_{pb} + \frac{f_p^u}{f_c^u} v_{cb}) \cdot C_p$$

from which

$$P_{bp} = \frac{C_b}{C_p} = v_{pb} + \frac{v_{pw}}{v_{cw}} \cdot \frac{v_{cw} + v_{cf}K_{ow}}{v_{pw} + v_{pf}K_{ow}} v_{cb}$$

follows. Note that the only difference with respect to model 1 is the factor v_{pw} / v_{cw} before the second term.

For tissue, in analogy, the following is obtained.

$$C_t = v_{ib} C_{ib} + v_{ie} C_{ie} + v_{ic} C_{ic} = (v_{ib} P_{bp} + v_{ic} \frac{f_p^u}{f_e^u} + v_{ic} \frac{f_p^u}{f_{ic}^u}) \cdot C_p$$

Use is made of the equilibration of concentrations in adjacent subcompartments. So, the tissue-plasma PC is

$$P_{ip} = \frac{C_t}{C_p} = v_{ib} P_{bp} + v_{ie} + \frac{v_{pw}}{v_{icw}} \cdot \frac{v_{icw} + v_{icf} K_{ow}}{v_{pw} + v_{pf} K_{ow}} v_{ic}$$

(The assumption in model 1 about the extracellular subcompartment implies $f_p^u / f_e^u = 1$.)

Note again the difference with the PC of model 1, which is the correction factor for relative volumetric water phase fractions. As fat cells contain a relative small water volume, this correction will lead to very high PC's for adipose tissue, while the correction for other tissues will be small. However, this approach leads to a fat-blood PC which on its turn perhaps is far too high.

Model 3.

Therefore, as a compromise between the two models, a third model is used. This model, quite arbitrarily, takes as the correction factor for relative volumetric subcompartmental water content the geometric mean of those factors between model 1 ("correction" factor 1) and model two (correction factor $v_{pw} / v_{(t)cw}$), i.e. correction factor $\sqrt{v_{pw} / v_{(t)cw}}$. As one may see from figure A1,3,4,5 there is not a great absolute deviation in value taking the geometric instead of the arithmetic mean. For adipose tissue, figure A2, the correction factor of model 2, about 11, is reduced to about 3.

Discussion

The three different model outcomes can be seen in the figures A1 through A5, which show PC's versus $\log(K_{ow})$. Note that PC-values do not differ much if $\log(K_{ow})$ is varied above the value 3. Note also the moderate difference for the three models in PC value for all compartments, apart from the adipose tissue compartment. The latter shows great differences, because of its relative small cellular volumetric water fraction.

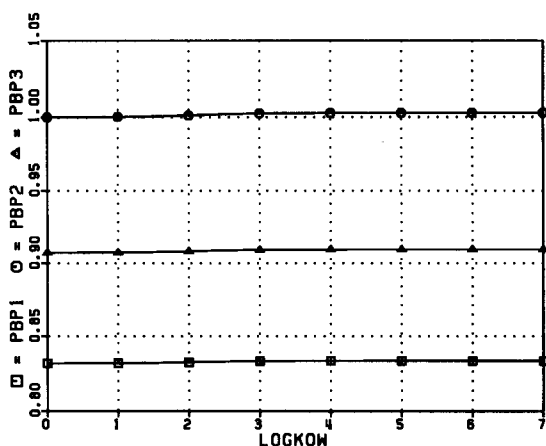


Figure A1. blood-plasma PC, P_{bp} , versus $\log(K_{ow})$; squares: model 1; circles: model 2; triangles: model 3

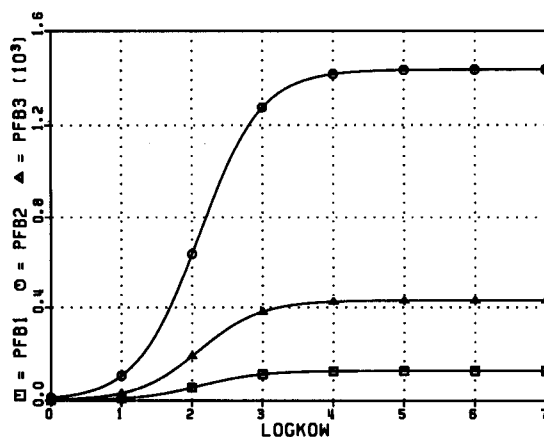


Figure A2. Adipose tissue-blood PC, P_{fb} , versus $\log(K_{ow})$; legend: see figure A1

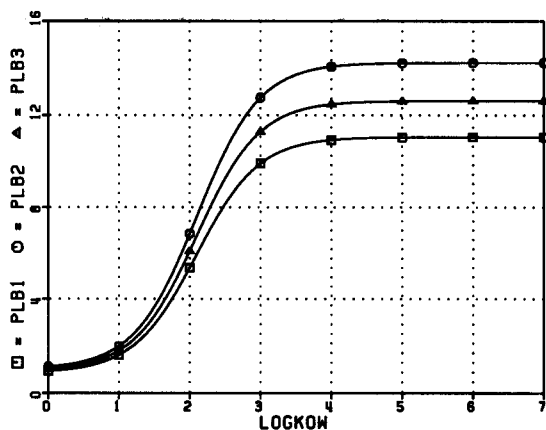


Figure A3. Liver-blood PC, P_{lb} , versus $\log(K_{ow})$; legend: see figure A1

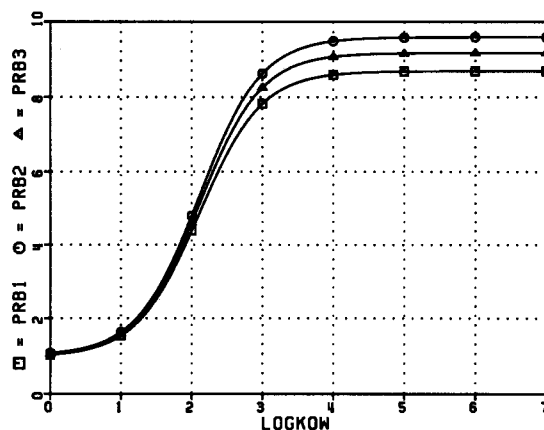


Figure A4. Richly perfused tissue PC, P_{rb} , versus $\log(K_{ow})$; legend: see figure A1

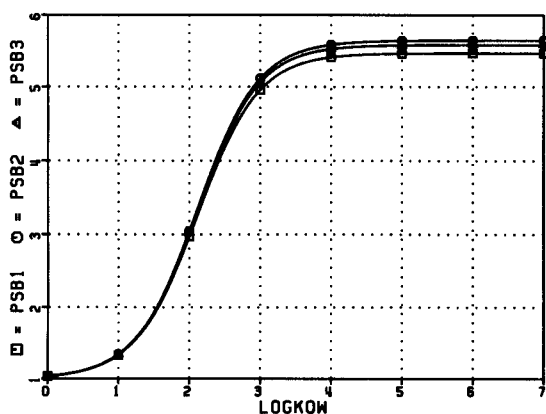


Figure A5. Slowly perfused tissue PC, P_{sb} , versus $\log(K_{ow})$; legend: see figure A1

Mathematical model

Mathematically, the PBPK model consists of a system of 5 differential equations, that can be derived from mass balance considerations, i.e. the amount accumulated in a compartment is the difference of the amounts entering the compartment by transport and leaving it by transport, excretion or metabolism. Milk clearance is modelled as excretion from the blood compartment, liver clearance as metabolism in the liver. Transport is by blood flow, denoted by Q . This way, the following system is obtained:

$$\begin{aligned}\frac{dA_b}{dt} &= -F_q Q_f (C_b - C_f / P_{fb}) - Q_l (C_b - C_l / P_{lb}) - Q_r (C_b - C_r / P_{rb}) \\ &\quad - Q_s (C_b - C_s / P_{sb}) - CL_m P_{mb} C_b \\ \frac{dA_f}{dt} &= F_q Q_f (C_b - C_f / P_{fb}) \\ \frac{dA_l}{dt} &= Q_l (C_b - C_l / P_{lb}) - CL_l C_l / P_{lb} + F_{abs} D_0 \\ \frac{dA_r}{dt} &= Q_r (C_b - C_r / P_{rb}) \\ \frac{dA_s}{dt} &= Q_s (C_b - C_s / P_{sb})\end{aligned}$$

Here, A 's denote compartmental amounts of the contaminant, C 's concentrations, Q 's blood flows and P 's partition coefficients. The subscripts denote "b" for the blood compartment, "f" for adipose tissue, "l" for liver, "r" for richly perfused tissue and "s" for slowly perfused tissue. F_q represents diffusion limiting in adipose tissue, CL_m is the milk clearance, CL_l the intrinsic liver clearance and F_{abs} is the fraction of the oral intake that is taken up over the gastrointestinal wall.

The first line describes the rate of change of the amount of contaminant in the blood compartment, the second line the same in the adipose compartment, the third in the liver compartment, the fourth in the richly perfused compartment and the fifth in the slowly perfused compartment. Note the flow factor for the adipose tissue compartment representing diffusion limited transport. The "arterial" concentration entering the peripheral compartments and leaving the blood compartment is C_b . The "venous" concentrations leaving the peripheral compartments and entering the blood compartment are the total compartment concentrations divided by the tissue-blood PC, i.e., C/P subscripted appropriately. CL_m is the milk clearance parameter from blood, CL_l is the intrinsic liver clearance.

ACSL model code

PROGRAM koe

INTEGER Ion

ARRAY Ton(4)

' Bodyweight parameters; Bw0 initial, Bws steady'

' tHalf growth rate, Bwref reference weight'

CONSTANT Bw0=75.,Bws=600.,tHalf=0.5,Bwref=600.

' Relative compartmental volumes'

' b blood, f fat, l liver, r richly perfused, s slowly perfused'

CONSTANT Vrelb=0.093,Vrelf=0.135,Vrell=0.019,Vrelr=0.069,Vrels=0.684

' total cardiac reference output'

CONSTANT Qlac=86500.,Qnolac=43250

' Relative compartmental blood flows'

CONSTANT Qrelf=.038,Qrell=0.458,Qrelr=0.304,Qrels=0.200

' Diffusion limiting flow factor'

CONSTANT Fq=3.

' relative volumes'

' plasma in blood, cells in blood, water in plasma'

' fat in plasma, water in blood cells, fat in cells'

CONSTANT vpb=.5,vcb=.5,vpw=.95,vpf=.0075,vcw=.63,vcf=.005

' blood in fat, extracellular in fat, cellular in fat'

' water in fat cells, lipid in fat cells'

CONSTANT vfb=.02,vfe=.065,vfc=.915,vfcw=.07,vfcf=.87

' idem as for fat in liver'

CONSTANT vlb=.16,vle=.09,vlc=.75,vlcw=.61,vlcf=.09

' idem as for fat in richly perfused'

CONSTANT vrb=.05,vre=.2,vrc=.75,vrcw=.71,vrcf=.07

' idem as for fat in slowly perfused'

CONSTANT vsb=.03,vse=.15,vsc=.82,vscw=.76,vscf=.04

CONSTANT logKow=7.

' Clearance parameters; Kl specific volumetric liver clearance'

' Pvet relative fat content for milk'

CONSTANT Kl=14.5,CLm0=1.,Pvet=.035

' Initial values'

CONSTANT Ab0=0.,Af0=0.,Al0=0.,Ar0=0.,As0=0.

' Daily dose rate, intestinal absorption coefficient'

CONSTANT D0=10.,Fabs=1.

CONSTANT tSTOP=1825.,Cint=1.,Jaar=365.

INITIAL

Kow=10**logKow

```

' Partition coefficient QSAR'
' blood-plasma'
Pbp=vpb+vcb*SQRT(vpww/vcw)*(vcw+Kow*vfc)/(vpw+Kow*vpf)
' fat-plasma'
Pfp=vfb*Pbp+vfe+vfc*SQRT(vpww/vfcw)*(vfcw+Kow*vfcf)/(vpw+Kow*vpf)
' liver-plasma'
Plp=vlb*Pbp+vle+vlc*SQRT(vpww/vlcw)*(vlcw+Kow*vfcf)/(vpw+Kow*vpf)
' richly perfused-plasma'
Prp=vrb*Pbp+vrc+vrc*SQRT(vpww/vrcw)*(vrcw+Kow*vrcf)/(vpw+Kow*vpf)
' slowly perfused-plasma'
Psp=vsb*Pbp+vse+vsc*SQRT(vpww/vscw)*(vscw+Kow*vscf)/(vpw+Kow*vpf)
' partition coefficients with respect to blood'
Pfb=Pfp/Pbp $ Plb=Plp/Pbp $ Prb=Prp/Pbp $ Psb=Psp/Pbp
' partitioncoefficient for milkfat'
Pm=1.5*Pfb
Ton(1)=2.*Jaar $ Ton(2)=3.*Jaar $ Ton(3)=4.*Jaar $ Ton(4)=tSTOP
- CLm=0. $ Q0=Qnolac $ Ion=1 $ SCHEDULE milkon.AT.(Ton(Ion))
END

```

DYNAMIC

DISCRETE milkon

' lactating status'

CLm=CLm0

Q0=Qlac

SCHEDULE milkof.AT.(Ton(Ion)+0.75*Jaar)

END

DISCRETE milkof

' non-lactating status'

CLm=0.

Q0=Qnolac

Ion=Ion+1

SCHEDULE milkon.AT.(Ton(Ion))

END

DERIVATIVE

' Growth of bodyweight (Von Bertalanffy-like)'

Bw=Bw0+(Bws-Bw0)*(1.-EXP(-t/(tHalf*Jaar)))**2

' resulting compartmental volumes'

Vb=Vrelb*Bw \$ Vf=Vrelf*Bw \$ Vl=Vrell*Bw \$ Vr=Vrelr*Bw

Vs=Vrels*Bw

' amount per day absorbed, liver clearance'

Dabs=Fabs*Bw*D0 \$ CLI=V1*KI

' Cardiac output related to bodyweight'

$Qc=Q0*(Bw/Bwref)**(3./4.)$

' resulting compartmental blood flows'

$Qf=Qrelf*Qc$ \$ $Ql=Qrell*Qc$ \$ $Qr=Qrelr*Qc$

$Qs=Qrels*Qc$

' diffusion limiter for fat compartment'

$QfFq=Qf/Fq$

' Compartmental concentrations'

$Cb=Ab/Vb$ \$ $Cf=Af/Vf$ \$ $Cl=Al/Vl$ \$ $Cr=Ar/Vr$ \$ $Cs=As/Vs$

' Rates of change of amounts'

' blood'

$dAbdt=-QfFq*(Cb-Cf/Pfb)-Ql*(Cb-Cl/Plb)-Qr*(Cb-Cr/Prb)...$

$-Qs*(Cb-Cs/Psb)-CLm*Pm*Cb$

' fat'

$dAfdt=QfFq*(Cb-Cf/Pfb)$

' liver'

$dAldt=Ql*(Cb-Cl/Plb)-CLI*Cl/Plb+Dabs$

' richly perfused'

$dArdt=Qr*(Cb-Cr/Prb)$

' slowly perfused'

$dAsdt=Qs*(Cb-Cs/Psb)$

' Integration of the resulting dsystem of differential equations'

$Ab=INTEG(dAbdt,Ab0)$

$Af=INTEG(dAfdt,Af0)$ \$ $Al=INTEG(dAldt,Al0)$

$Ar=INTEG(dArdt,Ar0)$ \$ $As=INTEG(dAsdt,As0)$

END

' concentration in milk, instead of milkfat'

$Cm=((1.-Pvet)/Kow+Pvet)*Pm*Cb$ \$ $tJaar=t/Jaar$

TERMT(t.EQ.tSTOP)

END

END

The CONSTANT statements declare the model parameters.

The INITIAL block calculates parameter values from the preceding basic ones and sets constants necessary for the calculation.

The DYNAMIC block performs the model calculations. It consists of two DISCRETE blocks for putting on/off laction, and a DERIVATIVE block for integratinf the set of differential equations from the preceding section.

Note that only the PC's are calculated depending on only the octanol-water partition K_{ow} . It is desirable to not only calculate these parameters, but also the intrinsic liver clearance Cl_{met} and not only depending on K_{ow} , but also on other molecular structure properties.