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Model for Estimating Initial Burden and Daily Absorption of Lipophylic Contaminants in Cattle

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Abstract

Many lipophilic organic contaminants in the environment are toxic for humans and animals. The presence of this class of contaminants in animal products (meat and milk) intended for human consumption thus involves health risks to the general population. In order to assess these risks, concentrations of contaminants in domestic animals are monitored by occasional measurements on blood (plasma) or milk of living animals. A computer model to translate such measured concentrations into historical absorption and burden of the compound, and into the future residues expected in meat and milk products, is described. The methodology is based on a Physiologically Based Pharmaco Kinetic model for lipophilic contaminants in domestic animals. Validation of the proposed method was carried out using the results of experiments in which lactating and non-lactating cows were exposed to 2378-TCDD, and lactating and non-lactating goats to lindane and a number of PCB congeners. In the above-mentioned experiments, the animals were internally exposed by injecting known amounts of contaminant into their rumen. Concentration measurements were used to estimate the initial burden and the daily absorption of the PBPK model. Validation was carried out by comparing estimated initial burden and the daily absorption with the doses imposed in the experiment. The results show that the estimated initial body burdens are lower than the doses imposed by the bolus injection in the rumen. This indicates that absorption from the intestine is probably incomplete. The estimates of daily absorption correspond to zero or trace values for the stages in which the animals were fed with food devoid of the contaminant. The model is intended for use by veterinary services/inspections as a screening tool to estimate exposures and residues.

Samenvatting

Veel lipofiele organische milieucontaminanten zijn toxisch voor mens en dier. De aanwezigheid van deze contaminanten in vlees- en/of melkproducten bestemd voor menselijke consumptie kunnen daarom een gezondheidsrisico voor de bevolking vormen. Om de risico's te kunnen beoordelen worden incidenteel concentraties gemeten in bloedplasma of melk van levende dieren. Dit rapport presenteert een computer model om dergelijke metingen te vertalen naar de historische opname van de component en de te verwachten residuen in vlees en melk. De methodologie is gebaseerd op een "Physiologically Based Pharmaco Kinetic" model voor lipofiele contaminanten in landbouwhuisdieren. Validatie van de voorgestelde methode werd uitgevoerd m.b.v. de resultaten van experimenten waarin lacterende en niet-lacterende koeien werden blootgesteld aan 2378-TCDD en lacterende en niet-lacterende geiten aan lindaan en een aantal PCB congeneren. In de bovengenoemde studies werden de dieren blootgesteld door bekende hoeveelheden contaminant in het rumen te spuiten, waarna concentraties in melk, bloedplasma, of vet werden gemeten in de tijd. Deze metingen werden aangewend om de initiële belasting en de dagelijkse absorptie van de contaminant te schatten. Validatie vond plaats door de geschatte waarden te vergelijken met de initiële belasting en de dagelijkse absorptie die waren opgelegd in het experiment. De resultaten laten zien dat na een bolus injectie in het rumen de geschatte initiële belasting van het lichaam over het algemeen lager is dan de opgelegde hoeveelheid. Dit kan duiden op incomplete absorptie uit de darm. De geschatte dagelijkse absorptie was nul of bijna nul voor de perioden dat de dieren schoon voedsel aangeboden kregen. Het model is bedoeld om gebruikt te worden door veterinaire inspecties als een 'screening' instrument om een eerste indruk te krijgen van blootstellingen en residuen en de daarbij behorende gezondheidsrisico's.

CONTENTS

1. Introduction

Many lipophilic organic contaminants in the environment are toxic for humans and animals. A major problem is that these contaminants accumulate in fatty tissues after absorption into the body. Release of lipophilic organic contaminants from the fatty tissues is a very slow process, which involves long retention times. The presence of this class of contaminants in meat and milk may therefore involve health risks for the general population. The Inspectorate for Health Protection, Commodities and Veterinary Public Health has a continuous interest in detecting unacceptable high levels of these contaminants in domestic animals and in identifying the sources of contamination before meat and milk of these animals are sold as consumer-products.

Recognition of contamination of domestic animals requires time consuming research, in which data on exposure, concentrations and body burden are essential. Lipophilic contaminants enter animals by intake of contaminated surface water, meadow grass/soil and supplemental food. It is difficult, if not impossible, to measure all these individual exposure routes into animals and to translate the exposures to the burden of the animals. From a practical point of view occasional measurements of contaminant concentrations in specific tissues and body fluids are therefore often preferred above direct monitoring of exposure. Such concentration measurements include analysis of blood plasma, milk and possibly body fat. Three important questions when interpreting concentrations are: (i) What exposure may lead to high concentrations in the animal, (ii) Is there a way to derive the historical absorption and burden of contaminant from a series of concentration measurements in animal tissues and body fluids, and (iii) If the source of exposure is eliminated, what future residues can be expected in the animal or its excreta. An answer to these questions is required to make a rightful interpretation of the levels detected in an animal. The Inspectorate for Health Protection, Commodities and Veterinary Public Health has requested RIVM to develop a methodology to translate measured concentrations in the body compartments to the historical absorption and burden and to the future residues that can be expected in meat and milk products.

1.1 Aims of this study

In this report a methodology is presented to interpret measurements of concentrations in blood (plasma), fat, and milk. The methodology is based on a Physiologically Based Pharmaco Kinetic model (PBPK model) for organic contaminants in life stock (Olling et al., 1995). The model is used to estimate (i) the average absorption of contaminant from the intestine into the system within a specified period, (ii) the initial amount of contaminant at the beginning of that period, and (iii) to predict the future concentration when the same exposure levels are maintained or terminated. The model is thus partly used in the inverse

way: Instead of predicting concentrations, measured concentrations are used to derive the absorption that is responsible for the presence of a contaminant in the animal. Validation of the proposed methodology was performed with experimental data on 2378-TCDD in lactating and non-lactating cows (Olling et al., 1990; Derks et al., 1991; Olling et al., 1991) and lindane and PCB 169 in lactating and non-lactating goats (Sips et al., 1999). The results are reported in this report. We developed a computer program which contains an implementation of the model.

1.2 Report outline

This report contains 6 chapters and the computer program on a CD-ROM. Chapter 2 discusses the fundamentals of the model. It gives an outline of the concepts of the model, the basic equations and an outline of the proposed methodology for estimating absorption and initial burden of contaminants. Most of the mathematics in this chapter is described in Appendices A and B. Chapter 3 focuses on the application of the model, which includes parameterization and verification of its performance by comparing the results with experimental data. This is done for a limited number of compounds in cows and goats. A user guide is provided in chapters 4 and 5, which leads the reader through the computer program. Chapter 6 presents an executive summary and discusses the perspectives of the model as a tool in estimating the absorption of contaminants from the environment and burden in life stock and. The reader of this report will find the CD-ROM with the computer program in the back of the report. The computer program contains the model connected to a windows user interface (Windows 95/NT).

2. Estimation methodology

This chapter gives an outline of the proposed methodology and the underlying model. Detailed information on mathematical procedures can be found in Appendix B.

2.1 PBPK model

The aim of our study is to develop a method for estimating the initial burden, absorption, and residues of lipophilic contaminants in animals after a period with constant exposure conditions, using a limited number of concentration observations. This requires a conceptual description of the animal and the way the contaminant behaves in the animal. The selected modeling approach follows the basics of the PBPK (Physiologically Based Pharmaco Kinetic) model presented by Olling et al. (1995).
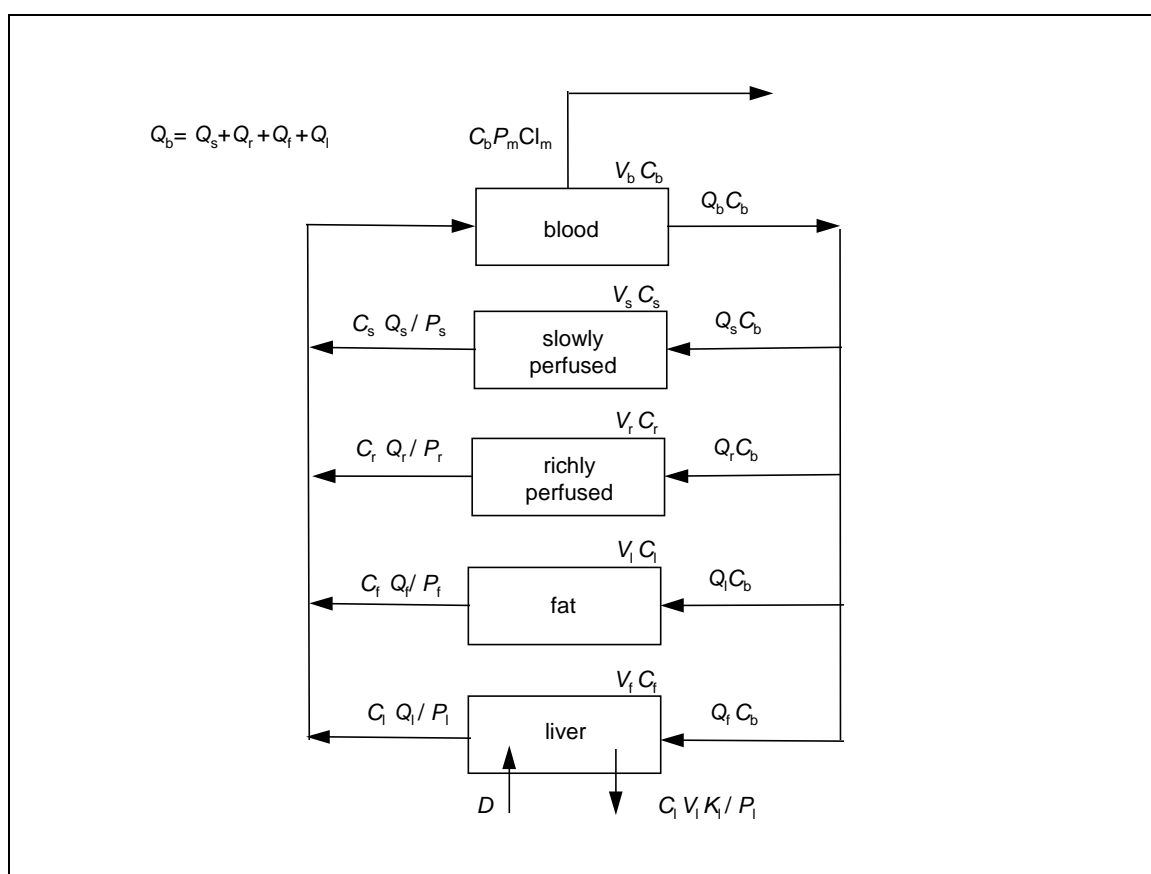
Figure 2.1. PBPK model of organic contaminants in cattle. C indicates concentration, Q effective blood flow rate, and V compartment volume. For a comprehensive explanation of the symbols see Appendix A.

To describe the animal we divided the animal in 5 compartments (Fig. 2.1): a central blood compartment, the liver, fat, richly perfused tissues such as kidney and brain, and slowly perfused tissues such as muscles and bones. This discrete approximation of the animal

physiology is adapted to the expected differences in perfusion, affinity of lipophilic compounds for certain tissues, and metabolic rates in these tissues. It is assumed that blood flows through these compartments at a constant rate, and that the cardic output, Q_b balances the flow through the other compartments. The contaminant is ingested with food. Absorption into the blood stream takes place at the intestine-blood interface, followed by immediate transport via the *Vena Porta* to the liver. Accordingly, in the PBPK model the daily absorption is fully attributed to the liver. The compound may be eliminated from the system by metabolism in the liver, and clearance by excretion into milk fat. The exchange of the compound between blood and the system compartments is driven by concentration differences. Figure 2.1 gives an overview of the contaminant's mass exchange in the modeled animal. A comprehensive list of model assumptions is presented in Appendix B.

The change of mass in the blood compartment can be described by the following linear, ordinary differential equation:

$$\frac{dA_b}{dt} = -\sum_x Q_x \left(C_b - \frac{C_x}{P_x} \right) - Cl_m P_m C_b \tag{1}$$

where A is the amount of contaminant, Q bloodflow, C concentration, P partition coefficient, and Cl_m milk clearance. The subscript b indicates blood, the subscript x all other compartments. For a comprehensive description of the symbols see Appendix A.

The change of mass in the liver is described by the following linear, ordinary differential equation:

$$\frac{dA_l}{dt} = Q_l \left(C_b - \frac{C_l}{P_l} \right) - \frac{K_l}{P_l} A_l + D \tag{2}$$

where K_l is the liver metabolic rate constant, D the daily absorption, and subscript l indicates liver.

For the other compartments (fat, richly perfused tissues, and slowly perfused), i, the following linear, ordinary differential equation describes the change of mass:

$$\frac{dA_i}{dt} = Q_i \left(C_b - \frac{C_i}{P_i} \right) \tag{3}$$

where the subscript i indicates the compartment. Note that the amount of contaminant in a compartment is related to the concentration, and that both may change over time, while all other entities are constants.

2.2 Solution and estimation technique

To solve the above system of differential equations matrix algebra is applied. This solution is fully described in Appendix B. The forcing conditions underlying the solution are the initial burden and the initial distribution over the organs at starting time and a *constant* daily

absorption into the liver. The solution (See Appendix B) provides expressions to calculate the concentrations in the body compartments as a function of time (Eq.(32)). As shown in Eq.(32) the solution comprises a series of exponential terms, each containing a specific time coefficient, an eigen value, λ. The time evolution of these exponential terms determines how long it takes for the system to reach an equilibrium state. The terms that are rapidly dampened are called fast modes, the slowly dampening terms are the so called slow modes. After a relatively long time, in the stage that equilibrium is approximated, only the slow modes contribute to the concentration in the compartments.

Input data required to perform all calculations are physiological and kinetic parameters and partition coefficients. Suppose that the initial burden and distribution over the organs and the daily absorption into the liver are known. Then the solution is suited to calculate concentrations of contaminants as a function of time. The remaining burden related to this absorption is the burden that is approximated at infinite time, when concentrations are in steady state and fully caused by the imposed dose regimen. However, the above mentioned initial and forcing conditions are not always known. In fact, one of our aims is to estimate the initial burden and the historical daily absorption into the liver from a small set of concentration observations. Note that daily absorption is considered as a constant value within the period. This means that we are looking at the inverse operation compared to what is described above. The first step in this inverse method is to rewrite Eq.(32) for a specific measurement in a compartment at a moment in time (Eq.(34)). The second step is to minimize the number of exponential terms, so that less data points are needed to find the initial burden and the daily absorption (Eq.(35)). The latter can be done by taking measurements that are performed at times that only the slowest or the two slowest modes are still significant. This means that measurements performed at relatively long time after the start e.g. one or two days are of interest. In Appendix B a criterion is given to match the measurements with the significant terms (Eq.(37)). Eq.(35) can be formulated for each measurement. Using yet another matrix solution (Eq.(41)) provides a least square estimate of the parameters of interest: the initial burden and the daily absorption. The minimum number of measurements at different points in time (and thus the minimum number of equations) that is required depends on the number of modes included in the estimation and the number of parameters to be estimated. Table 2.1 gives an overview of the possibilities.

Table 2.1. Minimum number of measurements required at different points in time to estimate daily absorption and/or initial burden.

Estimates	Number of modes	
	1	2
Daily absorption	1	1
Initial burden	1	2
Daily absorption and initial burden	2	3

2.3 Stepwise procedure

Summarizing, the following procedure is followed in practice. We focus on the general case of an animal subjected to a certain exposure scenario involving an organic contaminant. It is important to note that all system parameters (liver metabolic rate constant, milk clearance rate, physiological parameters, and partition coefficients) for the specific animal are to be known. The starting time of this scenario is known, but the initial burden and the daily absorption of contaminant are yet unknown. After a certain time a number of measurements of contaminant concentrations is sequentially carried out in blood plasma, fat, or milk with progressing time. Using the estimation technique (Appendix B) the initial burden and daily absorption of contaminant can be derived from the measurements. The time of measurements should meet the criterion in Eq.(37). The minimum number of measurements needed is displayed in Table 2.1. The next step is to predict residues using the solution of the differential equations (Appendix B) for the change of concentration straightforward. The initial burden and daily absorption found by the estimation technique are employed as input data: Concentrations are now computed as a function of time for any moment after the starting time at which the initial burden was estimated. The steady state burden is defined as the burden that would occur at infinite time.

In the back of this report a CD-ROM is attached with an implementation of the above mathematical procedures for use under windows 95/NT. Documentation to the program is added in Appendix B. Chapter 4 provides a user manual for the computer program.

2.4 Parameter values and validation

When applying the estimation technique the following prerequisite has to be noted: parameterization of the model and some way of validation of the estimation technique have to be completed. Parameterization is the process in which system parameters such as liver metabolic rate constant, milk clearance rate, physiological parameters, and partition coefficients are determined using independent sources of information. The values of these parameters may vary for different compounds and animals. Validation is a controversial term, since this term postulates an absolute truth. In this report we use the term for testing the estimation technique. This is done by using series of concentration measurements obtained in experimental settings as input data in our estimation technique, after which a comparison can be made between the estimated initial burden and daily absorption and those imposed in the experimental setting. The processes of parameterization and validation are discussed in detail in Chapter 3. This report will focus on a limited number of compounds and animals (Table 2.2). The computer program incorporates the parameter values found for those specific combinations of compounds and animals as default values for the compounds in Table 2.2.

Table 2.2. Overview of different combinations of compounds and animals which for which the model was parameterized.

Parameter	Lactating cow	Cow	Lactating goat	Goat
2378-TCDD	+	+		
lindane			+	+
PCB-169			+	+

3. Application of proposed method

In this chapter the estimation technique proposed in chapter two is tested against observations in exposure experiments. Also, parameterization of the model is carried out for a limited number of compounds and animals (See Table 2.2). Two studies were selected in which known amounts of contaminants were fed to animals, after which the concentrations in blood plasma, milk or fat were monitored. The first study includes a series of experiments performed by RIVM and DLO (Derks et al., 1991; Olling et al., 1990; Olling et al., 1991) which focus on the behavior of 2378-TCDD in cows. The second study comprises experiments in which the kinetics of lindane and PCBs in lactating goats governed by repeated imposed exposure events was followed in time (Bode and Hamzink, 1998; Klaassen, 1998; Sips et al., 1999). In both cases average physiological parameters, partition coefficients and liver/milk clearance rates could be considered as *a priori* known values. Accordingly, the concentration measurements can be employed as input data in our estimation technique, after which a comparison can be made between the estimated initial burden and daily absorption and those imposed in the experimental setting. An overview of all experiments is given in Tables 3.1 and 3.2. Table 3.1 explains each case considered, by specifying the animal, the number of animals involved, the type of measurements, and also provides literature references. Table 3.2 focuses on the exposure regimen in each case. In the next sections results are separately discussed for cows and goats.

3.1 Exposure of lactating and non-lactating cows to 2378-TCDD

3.1.1 Exposure regimen

In Tables 3.1 and 3.2 three different studies (cases 1 to 5) are listed which focus on 2378-TCDD in cows. Cases 1 and 2 focus on 4 lactating cows exposed by gavage of a mixture of olive oil and various dioxins and furanes including 2 μg 2378-TCDD in the rumen of the animals. A fraction of the bolus may enter the *Vena Porta* and will directly pass the liver. This amount can be considered as an initial burden in the liver. After this initial bolus the animals were fed with clean food for 93 days. In this period concentrations were monitored in milk and body fat. Case 1 includes the dataset with concentrations in milk, while case 2 focuses on the dataset with concentrations in fat. In cases 3 and 4, lactating and non-lactating cows were studied which had been grazing in a meadow contaminated with 2378-TCDD for several months before starting the experiment. As such, the animals exhibited an unknown initial burden, most likely distributed throughout their body according to steady state partitioning. In the period considered the animals were fed with clean food. The last case, case 5, is a control animal. This animal never resided in a contaminated meadow, and was always fed with clean food.

Table 3.1. Data sets for experimental validation

Case	Animals	n	Measurements			Reference
			plasma	milk	fat	
1	lactating cows	4	-	+	-	Olling et al., 1990
2	lactating cows	4	-	-	+	Olling et al., 1990
3	lactating cows	2	-	+	+	Derks et al., 1991
4	non-lactating cows	3	-	-	+	Olling et al., 1991
5	lactating cow	1	-	+	+	Derks et al., 1991
6	lactating goats	3	+	+	-	Sips et al., 1999
7	lactating goats	3	+	-	-	Sips et al., 1999
8	lactating goats	3	-	+	-	Sips et al., 1999
9	non-lactating goats	3	+	-	-	Sips et al., 1999
10	lactating goats	2	+	+	-	Sips et al., 1999
11	lactating goats	2	+	-	-	Sips et al., 1999
12	lactating goats	2	-	+	-	Sips et al., 1999
13	non-lactating goats	2	+	-	-	Sips et al., 1999

n = number of animals

Table 3.2. Exposure parameters

| Case	Period	Compound	Initial burden		Daily absorption	
			location	mass	agent	mass
1	93 days	2378-TCDD	rumen[*]	2 µg	food	trace
2	93 days	2378-TCDD	rumen[*]	2 µg	food	trace
3	108 days	2378-TCDD	total body	unknown	food	trace
4	220 days	2378-TCDD	total body	unknown	food	trace
5	108 days	2378-TCDD	total body	trace	food	trace
6	24 days	lindane	rumen[*]	50 mg	food	trace
7	24 days	lindane	rumen[*]	50 mg	food	trace
8	24 days	lindane	rumen[*]	50 mg	food	trace
9	24 days	lindane	rumen[*]	50 mg	food	trace
10	22 days	PCB-169	rumen[*]	0.5 mg	food	trace
11	22 days	PCB-169	rumen[*]	0.5 mg	food	trace
12	22 days	PCB-169	rumen[*]	0.5 mg	food	trace
13	22 days	PCB-169	rumen[*]	0.5 mg	food	trace

[*]bolus in rumen.
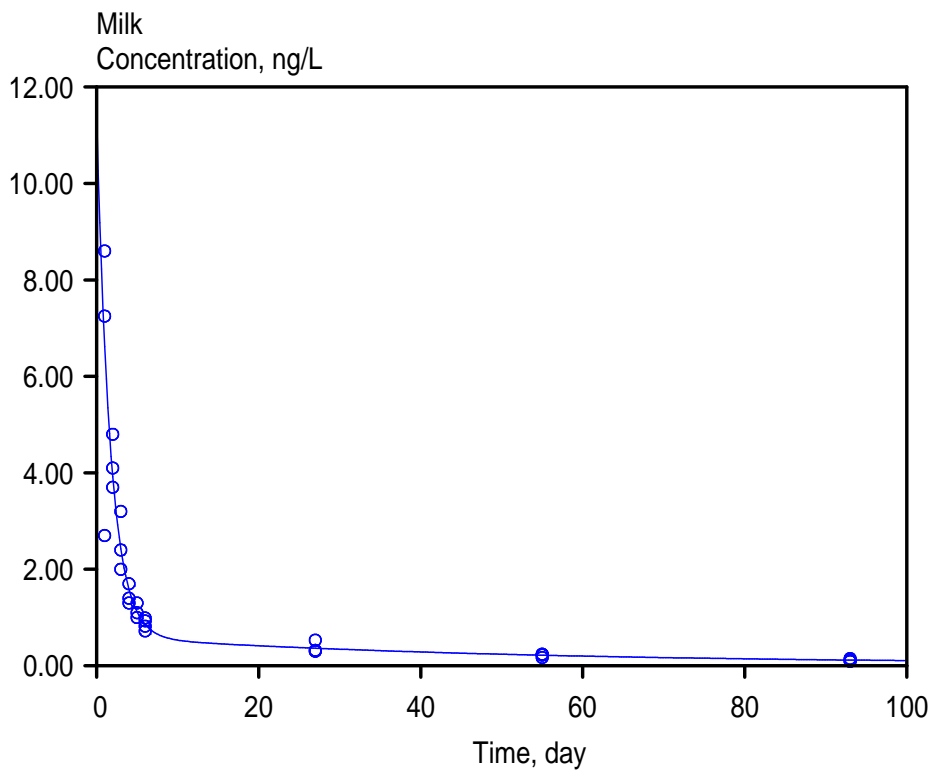
Figure 3.1 Observed (symbols)   and fitted (line) concentration of 2378-TCDD in milk.
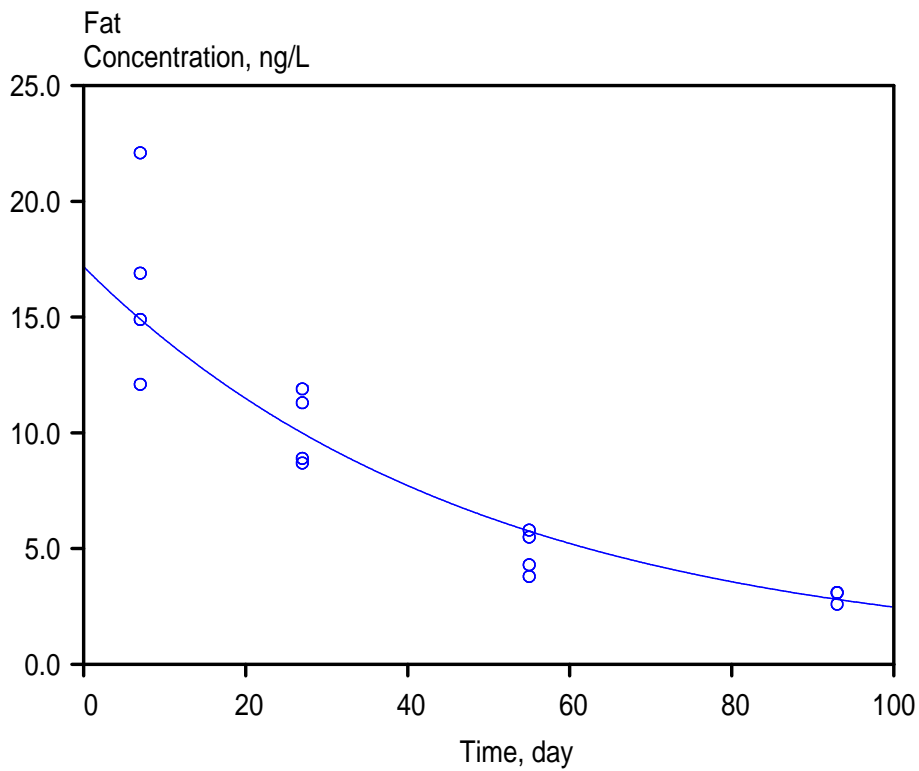Observations represent data from 4 different cows.

Figure 3.2 Observed (symbols) and fitted (line) concentration of 2378-TCDD in body fat.
Observations represent data from 4 different cows.

Table 3.3. Compartment volumes (L) of cows (Derks et al., 1993) and goats (Sips et al., 1999)

Parameter	Lactating cow	Cow	Lactating goat	Goat
Blood	42.0	42.0	4.3	4.30
Liver	8.50	8.50	0.96	0.99
Fat	61.0	135.0	10.5	16.5
Richly perfused tissues	31.0	31.0	10.8	10.8
Slowly perfused tissues	310	385	29.2	29.1

Table 3.4. Total blood flow rates (L/day) through compartments of cows (Derks et al., 1993) and goats (Sips et al., 1999)

Parameter	Lactating cow	Cow	Lactating goat	Goat
Blood	86500	43250	6000	6880
Liver	39600	19800	1500	1720
Fat	3300	1650	300	480
Richly perfused tissues	26300	13150	2100	2340
Slowly perfused tissues	17300	8650	2100	2340

Table 3.5. Milk production in lactating cows (Derks et al., 1993) and goats (Sips et al., 1999).

Parameter	Lactating cow	Lactating goat
production rate (L/day)	20.0	0.75
fat in milk (%)	5.0	3.5

Table 3.6. Liver metabolic rate constant (d^{-1}) of 2378-TCDD in cows (Jensen et al., 1981), lindane in goats (Sips et al., 1999), and PCB-169 in goats (Sips et al., 1999).

Parameter	Lactating cow	Cow	Lactating goat	Goat
2378-TCDD	14.5	14.5		
lindane			33	33
PCB-169			40	40

Table 3.7. Partition coefficients relative to blood for 2378-TCDD in (non-)lactating cows (Jones et al., 1987) and lindane and PCB-169 in (non-) lactating goats (Sips et al., 1999).

Parameter	2378-TCDD	Lindane	PCB-169
Liver	23.0	2.1	11.0
Fat	283	44	230
Richly perfused tissues	4.00	2.1	11.0
Slowly perfused tissues	8.00	1.4	7.5
Milk fat*	460	150	800

* lactating animals only

Table 3.8 Imposed initial burden and daily dose and estimated initial burden an daily absorption.

Case	Initial burden		Daily absorption		Steady state burden
	Imposed	Estimated	Imposed	Estimated	
1	2.0 µg	1.20 µg	0 ng	0.81 ng	28.0 ng
2	2.0 µg	1.17 µg	0 ng	0.65 ng	22.4 ng
3	unknown	62.1 ng	0 ng	0.28 ng	9.73 ng
4	unknown	248 ng	0 ng	0.00 ng[*]	0.00 ng
5	0 ng	16.6 ng	0 ng	0.23 ng	7.82 ng
6	50 mg	2.5 mg	0 µg	7.7 µg	115 µg
7	50 mg	2.0 mg	0 µg	23 µg	341 µg
8	50 mg	2.7 mg	0 µg	0.0 µg[*]	0.0 µg
9	50 mg	2.4 mg	0 µg	7.2 µg	174 µg
10	0.50 mg	0.53 mg	0 mg	0.0 mg[*]	0.0 mg
11	0.50 mg	0.31 mg	0 mg	0.0 mg[*]	0.0 mg
12	0.50 mg	0.82 mg	0 mg	0.0 mg[*]	0.0 mg
13	0.50 mg	0.47 mg	0 mg	0.0 mg[*]	0.0 mg

[*] Initially, the estimate of daily absorption was negative. After manually setting the daily absorption value to zero, initial burden was re-estimated (see column 3).

3.1.2 Parameters

System parameters employed in the PBPK model are displayed in Tables 3.3 to 3.7. The physiological parameters and milk production data were derived from Derks et al. (1991), Olling et al., (1990), and Olling et al. (1991). The values in the table reflect averages compiled from all cows included in the above mentioned studies. The liver metabolic rate constant was derived from data presented in Jensen et al. (1981). Partition coefficients were deduced from experimental data in Jones et al. (1987).

3.1.3 Results and discussion

The measured and modeled concentrations of 2378-TCDD in milk for case 1 are displayed in Fig 3.1. In this case both daily absorption and initial burden were estimated. The results are presented in Table 3.8. Fig 3.2 shows the results of case 2 which includes the same cows. However, instead of using milk concentration data in this case fat concentrations were used for estimating daily absorption and initial burden. Comparing the estimated daily absorption and initial burden of cases 1 and 2 shows that estimates based on milk concentrations and fat concentrations are in fair agreement (Table 3.8). The estimated initial burden is about half the dose that was imposed (Table 3.2). The difference between the imposed dose and the estimated initial burden can mainly be attributed incomplete absorption of the compound. An absorption fraction lower than one can be expected, because several processes such as slow desorption from the matrix, incomplete uptake from the *lumen*, and clearance in the *lumen* and the gut wall cells result in partial absorption of the compound in the blood circulation. Part of the contaminant thus leaves the body in faeces, or is transformed before being absorbed over the gut wall. Estimated daily absorption is near zero, which confirms the fact that exposure didn't happen after the bolus injection. The most likely steady state burden in the animals is equal to the amount that remains, forced by the daily absorption. The last column in Table 3.8 displays the estimate of the steady state burden based on Eq.(35), which is very low compared to the initial burden.

Case 3 included cows coming from a contaminated meadow. Here both fat and milk concentrations were used to estimate daily absorption and initial burden. Because the estimation technique is based on minimization of the sum of least squares it is heavily dominated by the measurements in the fat compartment. In this compartment 2378-TCDD concentrations are higher than in milk and thus have a higher weight in the minimization process. The estimated daily absorption from clean food approximates the values estimated in cases 1 and 2, and thus shows that clean food contains only trace of 2378-TCDD. For the estimated initial burden there is no check, because the daily absorption that preceded in the meadow is unknown. To get an idea of the magnitude of exposure we can compare this result with non-lactating cows coming from the same location (case 4) and a clean cow (case 5). In cases 4 and 5, estimated daily absorption is about equal to cases 1 to 3. However, the estimated initial burden for case 4 is higher than for case 3. This may be explained by the lower clearance rate of the non-lactating cows, that lack the loss of 2378-TCDD through milk fat production. For the clean cow (case 5) the estimated initial burden was much lower than in cases 3 and 4. Based on the estimation technique we can conclude that the initial burden in the cows that resided in the contaminated meadows was about 4-15 times higher than the cow from the non-contaminated meadow.

3.2 Exposure of lactating and non-lactating goats to lindane

3.2.1 Exposure regimen

Cases 6 to 9 in Tables 3.1 and 3.2 focus on lindane in goats (Bode and Hamzink, 1998; Sips et al., 1999). In cases 6, 7 and 8 three lactating goats are considered that received an instantaneous dose of lindane at the beginning of the experiment. This was done by gavage of 50 mg of lindane dissolved in olive oil in the rumen. Details are given in Bode and Hamzink (1998). After this initial dose only food devoid of lindane was given to the animals. In this period, concentrations were monitored in blood plasma and in milk. The data recorded a short time after administering the dose were rejected in our current analysis because they don't meet the criteria discussed in Appendix B. For the interpretation of the data in terms of estimating the initial burden and the daily absorption we use the data set of the lactating goats in three different ways: In case 6 concentrations in blood plasma as well as those in milk are used as input, in case 7 only blood plasma data are considered, whereas in case 8 only milk data are used for the analysis. The above mentioned exercises are intended to find out the influence of the data points on the estimates of the initial burden and the daily absorption. In case 9 three non-lactating goats received the same dose regimen as the lactating goats. For these goats the data series consists of blood plasma data only.

3.2.2 Parameters

System parameters in the PBPK model for goats are presented in Tables 3.3 to 3.7. The physiological data and milk production rates reflect the central trend of the values reported in Bode and Hamzink (1998) and Klaassen (1998) for 10 different goats. Sips et al. (1999) determined partition coefficients and liver metabolic rate constants (See Tables 3.6 to 3.7) based on data from the above extensive toxicokinetic study on lindane. (Bode and Hamzink, 1998). In the study by Sips et al. (1999) the above mentioned parameters were determined by parameter optimization. The optimized parameters reflect average values for lindane in goats. In the current exercise we take two series of data from the complete dataset (6 series) to analyze whether the general parameter set can be used to regain the individual daily absorptions and initial burdens specific for that series. This validates the use of the model to estimate initial burden and daily absorption of individual animals using generic parameters.

3.2.3 Results and discussion

Table 3.8 displays the outcomes of the estimation technique together with the imposed dose regimen (cases 6 to 9). A comparison of columns 2 and 3 shows that the estimated initial burden is much lower than the amount that was initially injected into the rumen. The initial burden is about 0.05 times the imposed amount. This trend confirms the results obtained in section 3.1 for 2378-TCDD in cows. The estimated daily absorption is in all cases very low, which is in accordance with the zero dose that was forced after the bolus injection.

The results also give insight in the sensitivity of the estimates on the type of data used. Using milk and blood plasma data individually (cases 7 and 8) or milk and blood plasma concentration data together (case 6) provides three different estimates of the initial burden (Table 3.8) in the same exposure experiment. Table 3.8 shows that the highest estimate is about 1.35 times the lowest estimate, which is fairly good. If we compare the estimates of the initial burden based on blood plasma data in lactating (case 8) and non-lactating goats (case 9) the effect of differences in clearance and physiology becomes clear. Lactating goats have one extra clearance path by the production of milk, which contains contaminant partitioned to the milk fat. Also, lactating goats have less body fat (Table 3.3). The estimated initial burden for the non-lactating goats is about 1.1 times the value for lactating goats (Table 3.8).

3.3 Exposure of lactating and non-lactating goats to PCB-169

3.3.1 Exposure regimen

Cases 10 to 13 in Tables 3.1 and 3.2 comprise experiments where goats were exposed to PCB-169 (Klaassen, 1998). In cases 10, 11 and 12 two lactating goats are considered that received an instantaneous dose of PCB-169 at the beginning of the experiment. This was done by gavage of 0.50 mg of the substance mixed with olive oil in the rumen. After the initial dose only food devoid of the substance was given to the animals. In this period concentrations in blood plasma, and in milk were monitored. For the interpretation of the data in terms of estimating the initial burden and the daily absorption we used the data set in three different ways. In case 10 both concentrations in blood plasma and milk are used as input, in case 11 only blood plasma data are considered, whereas in case 12 only milk data are used the analysis. In case 13 two non-lactating goats received the same dose regimen as the lactating goats. For these goats blood plasma data are available. As such, the above described experimental setups and interpretation are equal to that for lindane in section 3.2.

3.3.2 Parameters

System parameters in the PBPK model for goats are presented in Table 3.3 to 3.7. The physiological data and milk production rates reflect the central trend of the values reported in Sips et al. (1999) for 10 different goats. In order to obtain partition coefficients and the kinetic parameter, basically the same procedure was followed as for the lindane exercise (Section 3.2). Details are given in Sips et al. (1999).

3.3.3 Results and discussion

Table 3.8 (cases 10 to 13) displays the outcomes of the estimation technique together with the imposed dose regimen. A comparison of columns 2 and 3 demonstrates that the estimated

initial burden is 0.6-1.6 times the amount that was initially injected into the rumen. For cases 10 and 13 the estimated initial burden is lower than the imposed amount. This can be expected since several processes prevent complete absorption (see section 3.1). On the other hand, for cases 10 and 12 a higher estimate was found, which is erroneous from a mass balance point of view. This overestimation is probably due to the fact that the measurements of concentration refer to individual cases (see sections 3.3.2 and 3.2.2), whereas central trend parameter values were used for the physiology, partitioning and clearance. Accordingly, in the estimation technique, the mismatch between specific measurements and general parameter values propagate in the estimated daily absorption and initial burden. The estimated daily absorption is zero for all goats in cases 10-13, which is in accordance with the zero dose that was forced after the bolus injection. The sensitivity of the estimates to the type of data used (combination of milk and blood plasma data) can be deduced from data in Table 3.8. Results show that the highest estimate is about 2.6 times the lowest estimate. If we compare the estimates of the initial burden based on blood plasma data in lactating (case 11) and non-lactating goats (case 13) the effect of differences in clearance and physiology becomes clear. The estimated initial burden for the non-lactating goats is about equal to the value for non-lactating goats (Table 3.8).

4. User manual of computer program

4.1 Installing and running the program

The ResAnA (Residue Analysis in Animals) computer program is issued on a CD-ROM. The CD-ROM contains all the files that are necessary to run the program together with a number of sample sessions. Also included are the TCDD, lindane, and PCB-169 data sets discussed in this report. Before installing the program on the hard disk of your computer, please check the following system requirements:

- Intel 80386, 80486 or a pentium processor. Although the program runs on a 80386 system, a 80486 or pentium system is recommended for reasons of speed.
- MS-Windows 95 or NT; installed and working
- 40 Mb of free hard disk space
- 16 Mb RAM free

To install the program insert the CD-ROM in the drive and start the explorer. First, check the presence of the directory C:\RESANA.v10. If this directory is present please rename it, using any other name. Then run the SETUP program from the CD-ROM and follow the directives in the setup procedure.

After installation of the program, it is ready for use. To start the program, simply double click the Resana icon, and the main window will appear. If you are not familiar with the program already, then read section 4.2, which describes the menu structure, and chapter 5, which gives an example for using the program. If you encounter any questions concerning the program, then load the help file from the Resana group by clicking the help icon. Although the numerical parts of the program have been checked thoroughly, numerical errors may occasionally occur. If these errors occur please contact the corresponding author of this report. Before using the computer program, please note the following disclaimer:

> Disclaimer: The National Institute of Public Health and the Environment (RIVM) and/or the authors of this program accept no responsibility for damages that are caused by this program and make no warranty or representation, either expressed or implied, with respect to this software. This software is provided "as is," and you, its user, assume the entire risk when you use it.

4.2 Menu

The main file window of the Resana program contains a menu bar displayed at the top of the window. The menu bar has six main entries for different tasks (Table. 4.1). After activating one of the entries either pop-up menus or dialog boxes will appear to allow a choice from

different options and/or specification of input values. A grayed out entry indicates that the action associated with the entry is currently not available. These entries will become available when sufficient input parameters and options have been specified to allow the action.

Table 4.1 Entries in the main menu of the computer program

Entry	Description
File	Commands on file handling and print, reset, and exit commands
Options	Commands on the specification of input parameters, forcing conditions and program options
Calculate	Commands on calculating daily absorption, initial burden and future concentrations
Report	Commands on the creation and viewing of the detailed written results.
Graph	Commands on the display of the graphical presentation of the results, and a summary of the results
Help	Commands to get information on the program

The *File* entry handles the saving of parameter values and data entries to file, and the retrieving of previously saved sets of parameters and data. It also contains an entry to reset all parameter values and an entry for printing. The *Options* entry covers all operations to specify input parameters, options, measured concentrations, and forcing conditions that are used for the calculations. When the Options menu has been completed, the sub-entries in the *Calculate* entry become available. The Calculate entry initiates calculation with the current set of data and parameters. The *Report* and *Graph* menu entries are used to save and display results of the calculations. The contents of the report and the types of graphs that can be displayed and printed depend on the specifications in the Option pop-up menus. The *Help* entry covers the activation of the box, the status box and provides on-line help. Table 4.1 can be consulted for an overview of the main entries. The main entries are discussed in detail in sections 4.2.1 to 4.2.6.

4.2.1   File menu

The File menu consists of five entries. The New entry clears the memory, and resets all previous parameter settings and other options in the program. This entry can be used to start a new session when other parameters are already in operation from previous sessions. The Open entry activates a dialog box to open an existing parameter file. The parameter file contains all paths of the input files and various parameter settings that are used for the calculations. This parameter file is an ASCII text file that must be prepared by entering all input data in the options menu. After this has been completed, the input parameters can be saved as a parameter file using the Save and the Save as entries. In later sessions the saved file can be opened again with the Open entry. The Save as entry activates a dialog box to

specify a file name. The default file extension used in Resana for the parameter file is .PAR. The Save entry overwrites the parameter file that was previously opened. If none was opened before, the Save as dialog box is activated. The Print entry is intended to print the current information displayed in the main window. The entry activates a standard dialog box that contains specifications of print options and printing devices, and asks for reconfirmation of the print job.   The sixth entry of the file menu is the Exit entry, which enables the user to close the program. The program returns one final question by asking the user absolutely and then closes the program in the affirmative.

4.2.2 Options menu

The Options menu is intended to specify input parameters and to select program options. Going through this menu is necessary before calculations can be performed. Alternatively, the user can open a previously saved parameter file which restores all options from that file. The update entry in the calculate menu will become available as soon as the options menu has been completed. The options menu has four entries:

The Animal entry activates a dialog box (Fig. 4.1) in which the exposed animal is selected. Changing the animal in the combobox will result in new corresponding parameter values. However, the user can edit these default values and provide his own suggestions. The default button reinstalls the original values. Animals included in the combo box are listed in Table 2.2.

As soon as the animal has been selected, the Compound entry becomes available. This entry covers specification of the compound to which the animal is exposed. Compounds included in the combo box are listed in Table 2.2. In the dialog box (Fig 4.2) that appears after clicking the menu entry the user specifies the compound. Metabolic parameters and partition coefficients between tissues and blood for the selected compound are displayed in the edit boxes. The user can adapt these parameters for each specific case. Pushing the default button resets the parameters to their original settings.

Control over the Forcing conditions and the time frame of all calculations is provided by the Initial burden and exposure entry. This entry is attached to a dialog box such as displayed in Fig. 4.5. In this dialog box the user specifies whether initial burden and/or daily absorption are to be estimated from concentration measurements or that these are user provided preset values. If the user is providing the initial burden, he or she also has to indicate how the contaminant is distributed: following a steady state distribution, in fat only, or as a bolus in the rumen. Steady state distribution occurs when animals have been under steady state exposure regimen for a long period before the analysis period. Fat only can be used when the fat tissues initially contain the major fraction of contaminant. The "bolus" option is an approximation of a bolus injection of contaminant at starting time into the rumen. Other data

fields which have to be completed are: the starting time, the exposure period, and the number of significant terms (living modes) are to be specified.

The last entry of the option menu "Measurements" is intended to store measurements that refer to the current exercise into an internal database. A dialog box with a list of measurement records is popping up after activating this entry (Fig. 4.3). New records can be added, existing records can be edited or deleted. After pushing Add or Edit a dialog box appears, in which all edit/comboboxes have to be completed to enter/alter one measurement record (Fig.4.4). Mind that the date and time axis do correspond to those of the starting time. One other important item in the Measurement dialog box is the specification of the units of concentration. After the input data and options specification the user should save his settings for later use using Save or Save as.

4.2.3 Calculate menu

The Calculate menu contains only one entry, which becomes available after all necessary input parameters and options have been specified. In all other cases it appears grayed out. Clicking the update entry activates the calculations as described in chapter 2 and Appendix B with the current set of input data. Once these calculations have been performed results can be displayed and reports can be made using the Report and Graph entries.

4.2.4 Report menu

The Report menu covers the creation and display of an ASCII file with a summary of all input data, and the results of the calculations using the selected parameters and options. The entries in the Report menu become available after the user has activated the update entry. The Report menu has two entries. The Create Report entry generates the report file. The file name is selected with a Save As dialog box, and the default file extension is .RPT. Once the report file has been created, it can be viewed in any ASCII file viewer or editor. The standard viewer connected to Resana is notepad. Notepad is activated by clicking the View Report entry, which loads the viewer, after specifying the filename to be viewed in an Open File dialog box.

4.2.5 Graph menu

The Graph menu provides 6 sub-entries to display calculated and measured concentrations in the body compartments and if applicable in milk as a function of time. Printing of these graphs can be done through the File-Print entry. Once the graph is displayed on the screen, the user can modify some of the settings using the Graph settings dialog. This dialog (Fig. 4.6) is activated by selecting the corresponding entry in the graphs menu. Notice that each graph contains an archive box which gives the initial burden and the daily absorption, either estimated from the measurements or specified by the user, together with some other information.

One other entry in the graph menu is the Summary report. Activating this entry provides a dialog box with an overview of the main parameters and gives estimates of the initial burden and the daily absorption.

4.2.6 Help menu

The Help menu accesses the About box and the Help file of the program. The first handle activates the About box, which gives information on the name and authors of the program. The second handle retrieves the Help file. This file contains an index for browsing through the subjects on which help is provided. The search option can be used to find keywords. Some of the help topics on the operation of the program can be directly accessed from the dialog boxes (see, for example Fig. 4.5)
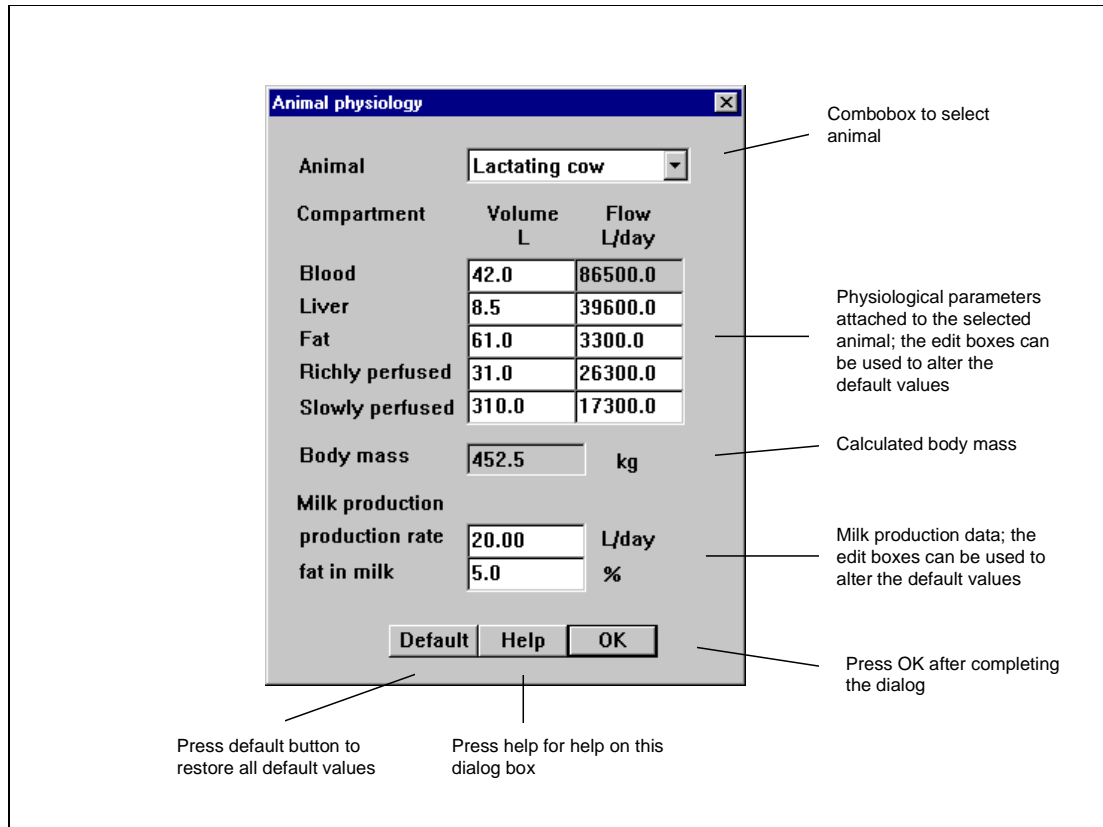
Figure 4.1. Explanation of the Animal physiology dialog box.
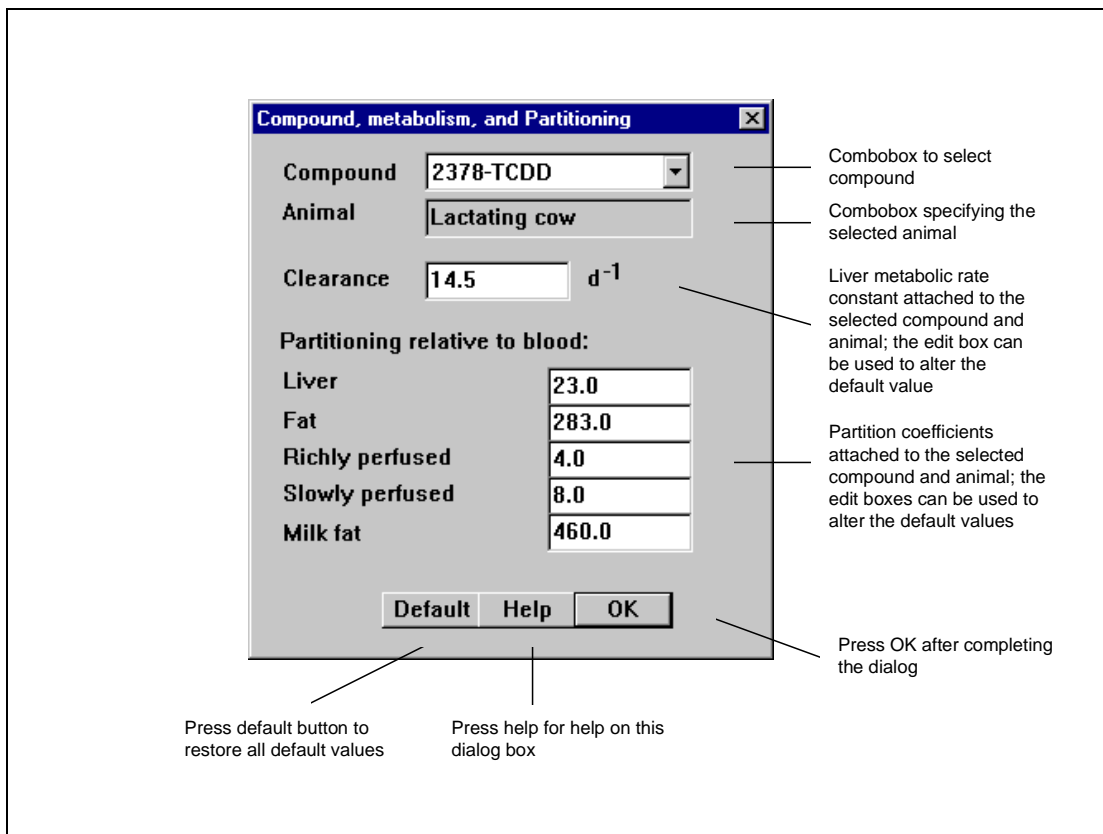

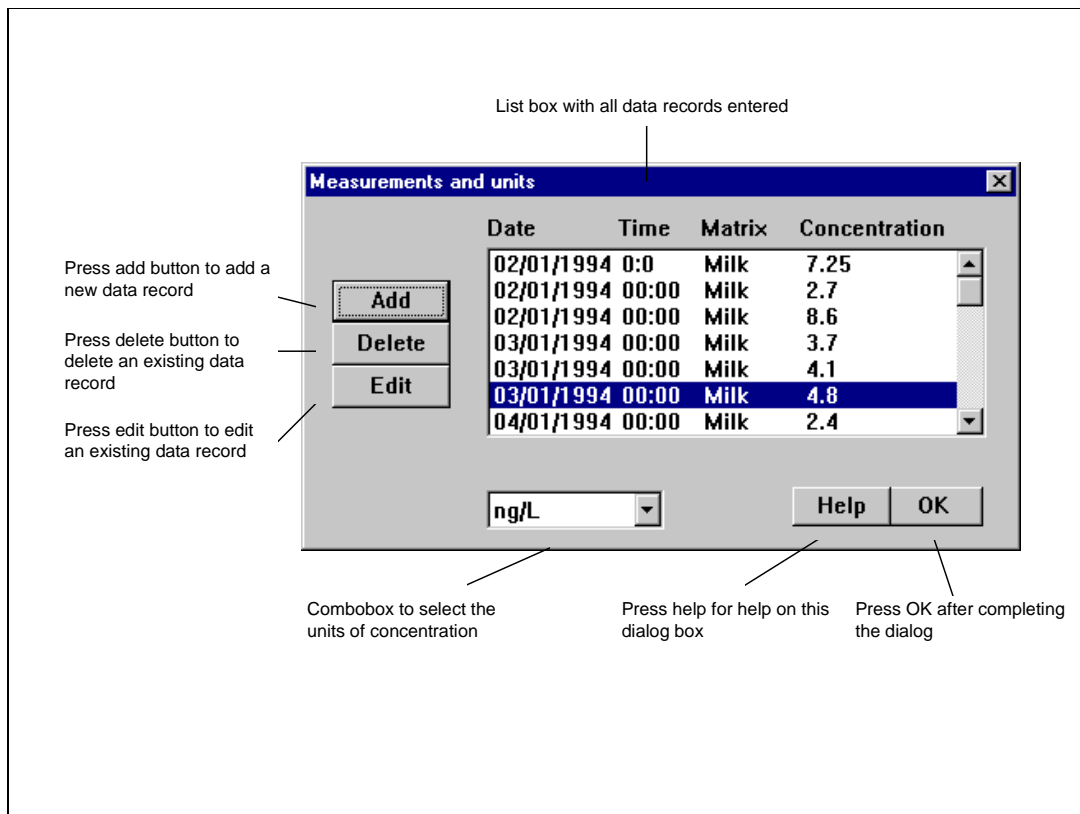Figure 4.2. Explanation of the compound dialog box.

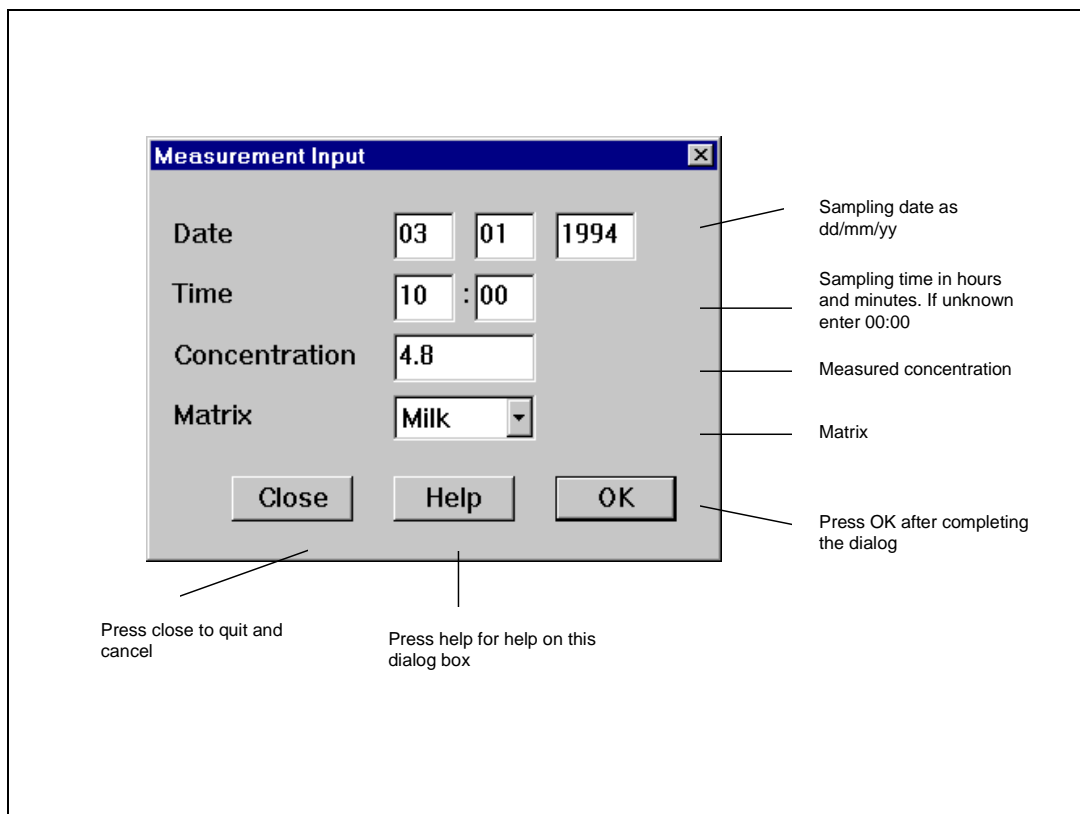Figure 4.3 Explanation of the measurements and units dialog box.

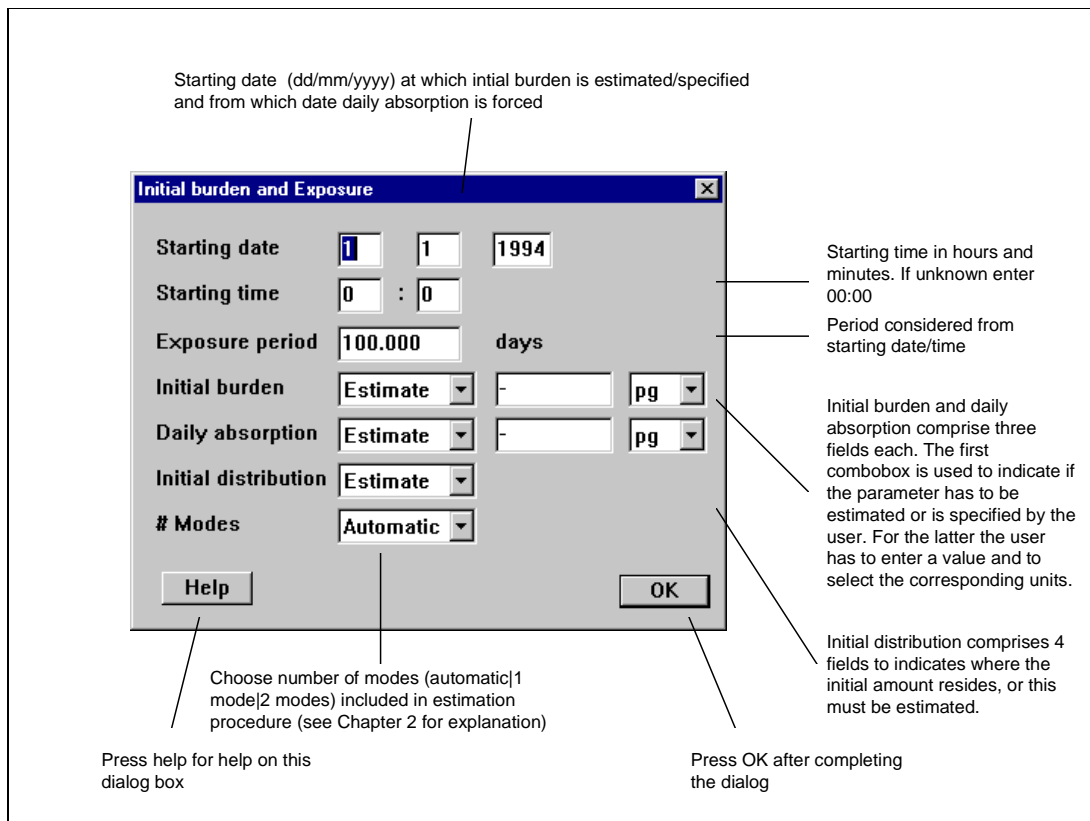Figure 4.4 Explanation of the measurement input and units dialog box.

Starting date (dd/mm/yyyy) at which intial burden is estimated/specified
and from which date daily absorption is forced

Initial burden and Exposure

Starting date	1	1	1994
Starting time	0	:	0
Exposure period	100.000	days
Initial burden	Estimate	-	pg
Daily absorption	Estimate	-	pg
Initial distribution	Estimate
# Modes	Automatic

Help   OK

Starting time in hours and minutes. If unknown enter 00:00

Period considered from starting date/time

Initial burden and daily absorption comprise three fields each. The first combobox is used to indicate if the parameter has to be estimated or is specified by the user. For the latter the user has to enter a value and to select the corresponding units.

Initial distribution comprises 4 fields to indicates where the initial amount resides, or this must be estimated.

Choose number of modes (automatic|1 mode|2 modes) included in estimation procedure (see Chapter 2 for explanation)

Press help for help on this dialog box

Press OK after completing the dialog

Figure 4.5. Initial burden and Exposure dialog box.

Graph settings

Minimum x-value	0.00
Maximum x-value	100.00
Minimum y-value	0.00
Maximum y-value	12.00
Number of tics x-axis	5
Number of tics y-axis	6
Archive	yes
Line color	Blue

OK

Controls the range of the x axis and the y axis

Controls the number of divisions of the x-axis and the y-axis

Controls presence of an archive box with background information

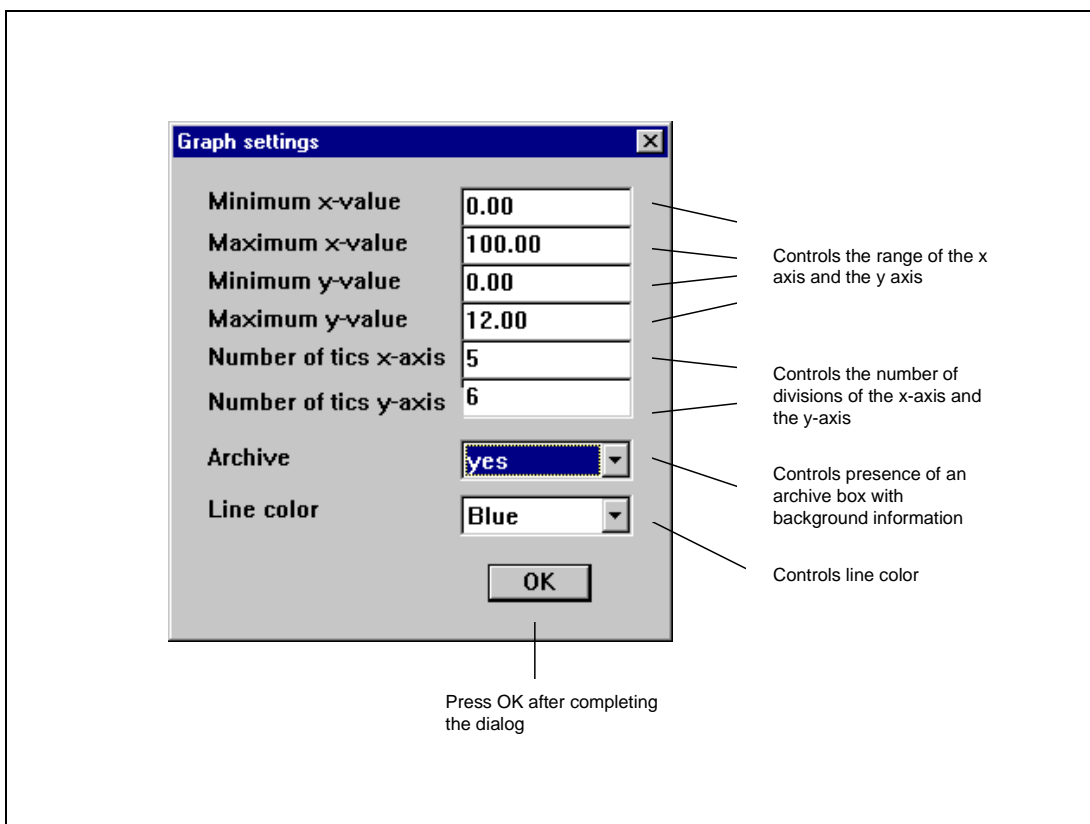Controls line color

Press OK after completing the dialog

Figure 4.6. Graph settings dialog box.

5. Model exercises

This chapter contains a sample session, which demonstrates the use of the model, starting from a practical problem. An outline of all steps is given in Tables 5.1 and 5.2. In Table 5.1 steps are listed to perform calculations of concentrations in different tissues and organs when initial burden and/or daily absorption are known. In Table 5.2 steps are listed to estimate initial burden and/or daily absorption from measured concentrations. The following experiment is the opening:

> Four lactating cows received a bolus of olive oil containing 2 μg of 2378-TCDD, whcih was injected into the rumen. The cows were then kept in the stable and they were fed with products which were assumed to be devoid of 2378-TCDD. An overview of events and measurements is given in Appendix C. The experimentalist wants to know what is the initial amount directly taken up by the animals at the beginning of the experiment and what residues can be expected after 100 days. Also an estimate is desired of the absorption of 2378-TCDD from the food, which is expected to be clean.

5.1 Input of data

First start the program and activate the options menu. Next click the Animal entry, after which a dialog box pops up. Select "lactating cow" from the combobox, after which default parameters are loaded. Because you don't know any specific data on the cows that are studied leave the values as they are. Then leave this dialog by clicking OK and continue with the compound dialog. Select the compound that is studied from the combobox, in this case 2378-TCDD, after which default values appear for all parameters. If the user can provide accurate data for those parameters, he or she can change any of the default value. A simple click on the default button will restore the default values for a specific combination of a compound and an animal. Leave the compound dialog by clicking OK

Then enter the "Forcing conditions" entry. This activates the dialog box displayed in Fig. 4.5. The starting date is the beginning of experiment: the date at which you want to know the initial, direct uptake of 2378-TCDD from the rumen. The starting time must also be entered. Because this time is not available in Appendix C, a practically dummy value will do, e.g. 00:00 hrs. The exposure period is set to 100 days because we are interested in residues at that point in time. The comboboxes that follow are all focusing on the estimation procedure. Because you are interested in estimates of both initial burden and daily absorption, select for both "Estimate" from the comboboxes. In the editboxes to the right of the comboboxes automatically a hyphen appears, because the user does not have to specify an (initial) value. Also the user may skip the specification of units. The initial distribution is automatically set to "Estimate". The next entry is a combobox intended for specifying the number of modes to

be included in the estimation. For normal use the number of modes can best be kept to the default "Automatic". If the user wants to select manually one or two modes, he or she can find an explanation in Chapter 2 and Appendix B. Leave the "Forcing conditions" dialog by clicking OK.

The fourth entry in the options menu is the Measurements entry, which activates the dialogbox displayed in Fig. 4.3. First select the units of concentration that are used in the combobox at the bottom. Then a list of measurements can be entered by adding records (Appendix C). Mistakes can be corrected using the delete and edit buttons on a highlighted record in the list. For explanation of the dialog box used for adding/editing a new record see Fig.4.4. . Leave the "Measurements" dialog by clicking OK.

After completing all dialogs first save your data by clicking the "Save" or "Save as" entries in the File menu. A dialog box is employed to save the data in a file with the standard extension .PAR. Upon saving all parameter settings and data points are stored and can be retrieved for later sessions using the "Open" entry in the File menu.
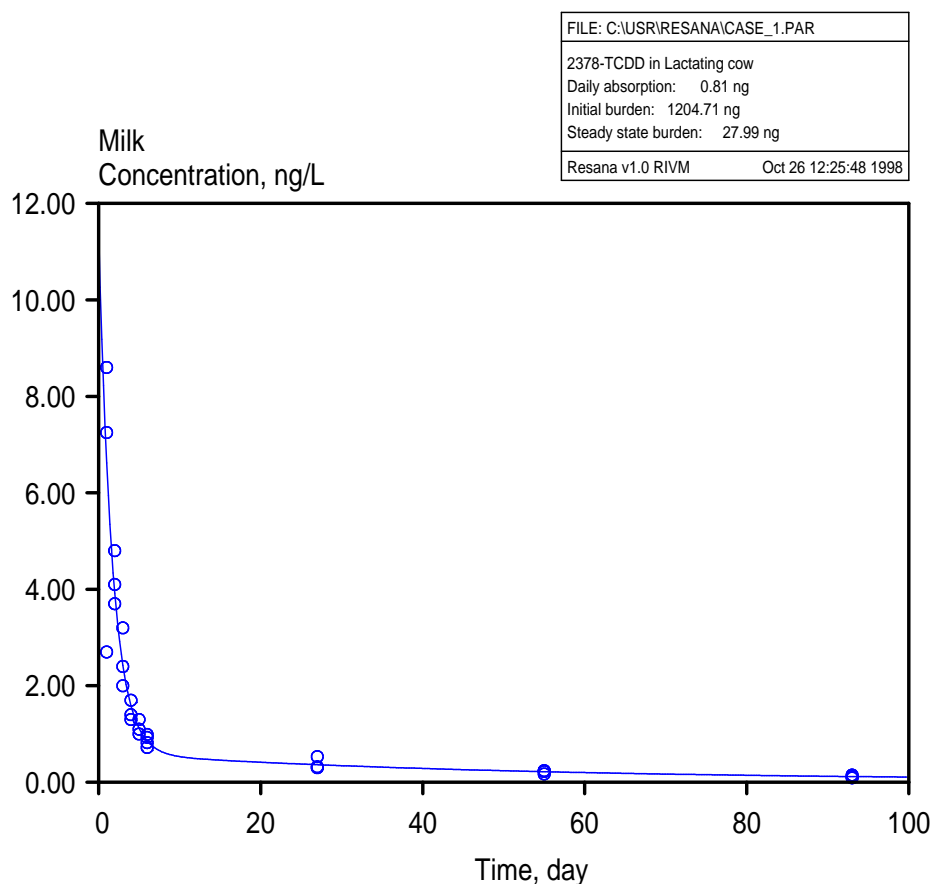

Figure 5.1. Estimated initial burden and daily absorption and fitted (line) and observed (symbols) concentration of 2378-TCDD in milk. The results refer to the example described in chapter 5. The number of tics and ranges of the axes have been adapted using the "Graph Settings" dialog.

5.2 Graphs and reports

With all required input data specified, you can now proceed with the calculations. The "Update" entry from the Calculate menu no longer appears grayed out. A simple click on this button runs the program and loads the results into the memory. To see the estimated initial burden and daily absorption together with the measured and fitted concentrations in milk click the graph-Concentration vs. time-Milk entry. This figure (Fig 5.1) displays the concentration in milk calculated by the model using the estimated parameters and all data points. In the upper right corner there is an archive box which displays the estimated parameter values. The archive box can be switched off using the "Graph-Graph Settings" entry (see section 4.6). The parameter values can also be retrieved in a separate box by clicking the "Graph-Summary" report entry. Printing the graph is established by using the "File-Print" entry. The user can generate an extensive report with details on input data and the mathematics by clicking the "Report-Create" entry. This results in the activation of a "Save file" box, where the user can specify the name of the report. The default extension is .RPT. The report can be viewed by clicking the "View report" entry, which activates a "File open" box to select the report file.

Coming back to the original questions the following results are obtained: The initial burden is 1205 ng , which is about 50 % of the amount that was injected into the rumen. The daily absorption from the clean food is estimated at a level of 0.81 ng, which can be neglected compared to the initial burden. So the food may be considered to be clean. Looking in the report file shows that after 100 days there is still 120 ng of 2378-TCDD in the body of the cows.
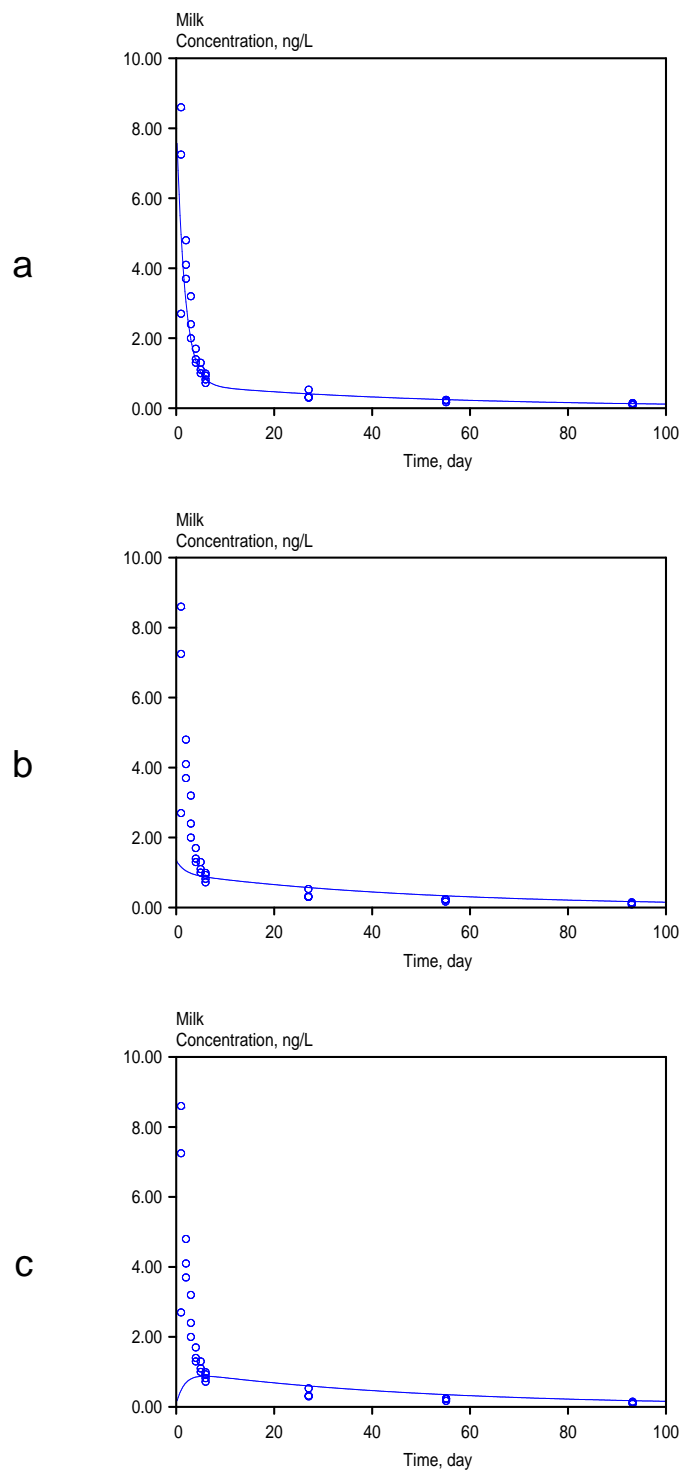
Figure 5.2 Calculated and observed concentration of 2378-TCDD in milk based on estimated initial burden and daily absorption, but with different assumption on the initial distribution: (a) Contaminant initially present in rumen, (b) contaminant initially present following a steady state distribution, and (c) contaminant initially present in fat.

5.3 Further analyses

After the estimation of the initial burden and daily absorption you can perform several analyses with the program to gain insight into the process of displacement and clearance of 2378-TCDD in the body of the cows and to explore the sensitivity to changing the input parameters. For example the estimated initial burden and daily absorption can be entered as specified values in the "Forcing conditions" dialog box. To do so, the user has to choose "Specified" in the combobox, to enter values in the text edit boxes to the right, and to select the corresponding units of mass. Then in the dialog box that specifies the initial distribution choose between "Steady", "Fat", and "Bolus".

Steady assumes an initial distribution over the organs according to steady state partitioning at the starting time. Fat attributes all mass to the fat compartment, while bolus assumes all mass to be initially present in the rumen. Results of new calculations can be obtained by reactivating the Update entry. Fig 5.2 shows the outcomes for milk concentration for the three different assumptions on the initial distribution. The assumption that all initial mass was present in the rumen gives identical result to those of the case where parameters and distribution were estimated (Compare Figs 5.1 and 5.2a). A high initial blood concentration is caused by initial uptake, which has to pass the blood circulation before it is redistributed over all other organs (mainly the fat compartment). The subsequent lowering has two reasons. The first is transfer to other tissues and organs, and the second reason is clearance due to loss by milk production and decay in the liver. In case of steady state, the partition coefficients dictate that at the start of the experiment 2378-TCDD concentrations in milk are much less than in the experimental case (Compare Figs 5.1 and 5.2b). The initial steady state distribution attributes most of the contaminant to the fat compartment. The lowering of the concentration is caused by liver and milk clearance. Redistribution of contaminant over the compartments is solely forced by the former two processes. Notice that the concentrations calculated using an initial bolus and an initial steady state distribution converge with progressing time. In the case that the initial burden is believed to be present in fat, redistribution over all organs occurs from the fat. However, because this process has to occur through blood flow, and is thus perfusion-limited, first a rise of blood and thus milk concentration occurs, followed by continuous lowering due to clearance (Fig 5.2c).

Another interesting option is to change some of the physiological parameters and to evaluate the effect on the parameter estimates. For example if you change the default milk production of 20 L/day to 30 L/day and run the program again the following results are obtained: initial burden: 1598 ng, daily absorption 2.03 ng, and residual burden after 100 days 120.6 ng (Fig 5.3). Obviously, there is a strong sensitivity of the parameters to the milk production rate.
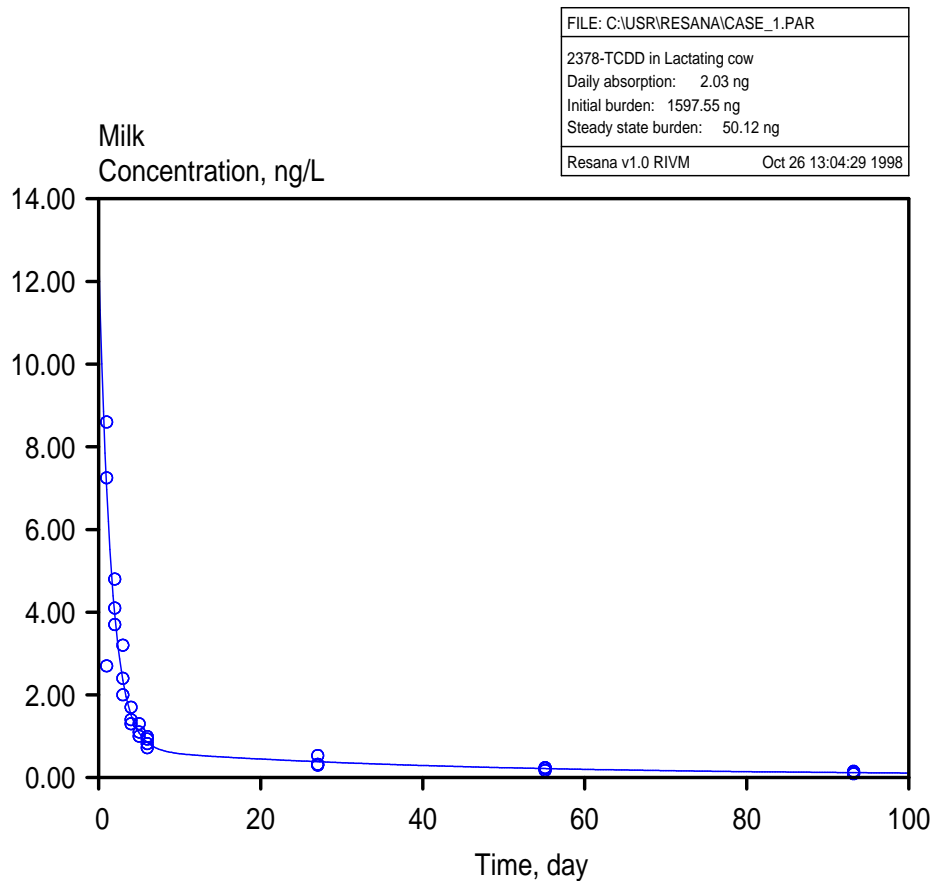
Figure 5.3 Estimated initial burden and daily absorption and fitted and observed concentration of 2378-TCDD in milk. The results refer to the example described in chapter 5, but with a higher milk production rate (30 L/day instead of 20 L/day).

Table 5.1 Summary of actions in Resana for calculating concentrations at known initial burden and/or daily absorption of organic contaminants.

Step	Action	Entry
1	Select animal	Options-Animal
2	Select compound	Options-Compound
3	Enter forcing conditions by specifying the time frame, values for initial burden and daily absorption, and specify the initial distribution.	Options-Forcing conditions
4	Select the units of concentration which are used	Options-Measurements
5	Save input settings	File-Save as
6	Run the calculation module	Calculate-Update
7	Display the concentration in different compartments, tissues, or milk	Graph-Concentration vs. time menu
8	Adapt graph settings if necessary	Graph-Graph Settings
9	Display the forcing conditions	Graph-Summary report
10	Print graphs displayed in window	File-Print graph
11	Write results to ASCII file	Report-Create report
12	View ASCII file with results	Report-View report
13	Exit	File-Exit

Table 5.2 Summary of actions in Resana for estimating the initial burden and/or daily absorption of organic contaminants from concentration measurements

Step	Action	Entry
1	Select animal	Options-Animal
2	Select compound	Options-Compound
3	Enter forcing conditions by specifying the time frame and what to estimate. Select "estimate" for the initial burden, daily absorption and the initial distribution if both initial burden and daily absorption are to be estimated . If only one of the parameters is to be estimated select "specified" for the know parameter and enter a value	Options-Forcing conditions
4	Select the units of concentration which are used	Options-Measurements
5	Enter measured values of concentration by clicking the add button. See for the number of values which is necessary to estimate 1 or 2 parameters Table 2.1	Options-Measurements
6	Save input settings	File-Save as
7	Run the calculation module	Calculate-Update
8	Display the concentration in different compartments, tissues, or milk	Graph-Concentration vs. time menu
9	Adapt graph settings if necessary	Graph-Graph Settings
10	Display the estimated parameters	Graph-Summary report
11	Print graphs displayed in window	File-Print graph
12	Write results to ASCII file	Report-Create report
13	View ASCII file with results	Report-View report
14	Exit	File-Exit

6. Executive summary and perspectives

The presence of lipophilic organic contaminants in animal products (e.g. meat and milk) intended for human consumption involves possible health risks to the general population. The Inspectorate for Health Protection, Commodities and Veterinary Public Health has a continuous interest in detecting the presence of unacceptable high levels of these contaminants in domestic animals before their meat and milk are sold as consumer-products. For this purpose concentrations of contaminants in domestic animals are monitored by performing occasional measurements in blood (plasma) or milk of living animals. Three important questions when interpreting the contaminant levels are: (i) What exposure to these compounds can lead to high concentrations in the animal, (ii) Is there a way to derive the historical daily absorption and burden of contaminant from a series of simple concentration measurements in blood plasma, milk or body fat and (iii) If the source of exposure is eliminated, what residues can be expected in the animal.

6.1 Model development and validation

The Inspectorate for Health Protection, Commodities and Veterinary Public Health has requested RIVM to develop a methodology to translate measured concentrations in blood plasma, fat, or milk to the historical daily absorption and burden and to the future residues that can be expected in meat and milk products. The current study presents such methodology. The methodology is based on a Physiologically Based Pharmaco Kinetic model (PBPK model) for lipophilic organic contaminants in cattle (Olling et al., 1995). The model is used to estimate (i) the absorption of contaminant from the intestine in a specified period, (ii) the initial amount of contaminant in the body at the beginning of that period, and (iii) to predict the future concentrations when the same exposure levels are maintained. The model is thus partly used in the inverse way: Instead of predicting concentrations, measured concentrations are used to derive the absorption that is responsible for the presence of a contaminant in the animal. A computer program was developed which contains an implementation of the model.

Validation of the proposed methodology was carried out for 2378-TCDD in lactating and non-lactating cows (Olling et al., 1990; Derks et al., 1991; Olling et al., 1991) and lindane and PCB in lactating and non-lactating goats (Sips et al., 1999). In the above mentioned experimental studies the animals were exposed to known amounts of contaminant, and concentrations in blood plasma, milk, or fat were monitored with progressing time. These concentration measurements were used to estimate the initial burden and the daily absorption of the contaminants.   Validation was carried out by comparing estimated initial burden and the daily absorption with those imposed in the experiment. System parameters in the PBPK model could be considered as *a priori* known constants. The results show that for a bolus

injection in the rumen the estimated initial burdens are lower than the dose imposed by the bolus injection. This is to be expected because passage through the intestine is too fast for complete desorption from the matrix and subsequent absorption of the contaminant into the blood stream. Part of the contaminant will thus leave the body in faeces, and does not contribute to initial burden. The model estimates daily absorptions which correspond to zero or trace values for stages in which the animals were fed with food devoid of the contaminant. Accordingly, the model seems to be a suitable screening tool to estimate historical daily absorption and burden of lipophilic contaminants in domestic animals using simple concentration measurements.

6.2 Limitations

The proposed methodology has a number of limitations. Firstly, the underlying model itself imposes a limitation. Every model is a simplification of the real world. The art of modeling is to focus on the level of simplification of reality that is necessary to interpret the research questions. The limitations related to simplification in the current model are presented explicitly in Appendix B and concern the processes described by the model, the imposed forcing conditions and the initial condition. The model is a dynamic model which is capable of describing changes of concentration with time in the compartments of the modeled animal. The model uses an initial condition which is the initial burden due to exposure during the time before the period considered in the analysis. The forcing condition dictates that during the period considered, the absorption rate expressed as 'daily absorption' is about constant. If not so, e.g. due to alternating stages in which the animals are stabled or in the field, those periods should be taken into account separately.

The second limitation is related to the fact that the estimates are based on measurements. The quality of those measurements as well as the level to which they represent the organ or fluid from which samples were taken is not always clear. If poor data are used as input this will propagate in the estimates of daily absorption and initial burden.

A third limitation, and probably the most important one, is formed by the parameterization. Using the model requires physiological data of animals and compound properties (partition coefficients and clearance rates). In the current study we looked at lindane and PCBs in non-lactating and lactating goats and 2378-TCDD in non-lactating cows and lactating cows. The parameters found for these combinations of compounds and animals cannot be used for other compounds and animals. Parameterization must be looked upon as a returning issue when using the model for multiple animals and compounds. Data presented in literature and establishing QSARs (Quantitative Structure Activity Relations) can be of help in this respect.

6.3 Perspectives

The current model has been implemented in a computer program with a graphical user interface (GUI) for use under windows 95/NT. It is a versatile tool that can be used to evaluate different compounds in different animals. The model can be used as a predictive tool, in order to analyze the effect of different initial burdens and daily absorptions on the progress of concentrations in body compartments and milk with time. This type of employment can be done without any measurements of concentrations. When estimates of initial burden and daily absorption are aimed at, just 1 to 3 measurements of concentrations in milk, blood plasma, or fat are required for such analysis.

The GUI and the link with practical problems makes the computer model very suitable for the Inspectorate for Health Protection, Commodities and Veterinary Public Health to apply it in the field for screening purposes. The current version of the model should be considered as an evaluation version. Further development is to be guided by critical comments from (potential) users. Possibilities include the development of a comprehensive database with parameter values for different animals and compounds. Also, the model itself can be extended by allowing different stages of exposure, including a module to translate intake to absorption, and to make the model suitable to evaluate non-lipophilic compounds. A third extension that has to be added is an option that allows for estimating the uncertainty in the estimated parameters and predictions for data series with multiple data points, that amply exceed the minimum number of point required .

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Acknowledgement

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Appendix A List of symbols and abbreviations

a   vector containing contaminant amounts (masses) in compartments i, A_i

b   known vector in **Ax**=**b** matrix operation

d   vector containing scaled daily absorption (as a fraction of the total daily absorption) to compartments i, d_i

$\mathbf{s}_i$ eigen vector i

t time [T]

t_0 starting time [T]

$t_{1/2,i}$ half-life corresponding to the ith eigenvalue [T]

x   unknown vector in **Ax**=**b** matrix operation

A_i amount (mass) of contaminant in compartment i [M]

A   matrix in **Ax**=**b** matrix operation

D total daily absorption of contaminant from intestine into the animal [M T^{-1}]

d_i fraction of total daily absorption received by compartment i

f_i correction factor for intracompartmental diffusion in compartment i

F_m fat fraction of milk

K_i first order metabolic rate constant in compartment i referenced to blood [T^{-1}]

M   system matrix

P_i partition coefficient between blood and compartment i

R subset of eigen values

S   matrix of n eigen vectors

Q_i^{*} physiological flow rate of blood through a compartment i [L^3 T^{-1}]

Q_i effective flow rate of blood through a compartment i [L^3 T^{-1}]

Cl_m milk clearance [L^3 T^{-1}]

X set of periferal compartments f, l, r, and s

Y set of all compartments b, f, l, r, and s

δ_i ith absorption coefficient

λ_i ith eigenvalue

ξ_i ith mass coefficient

ξ_{i0} ith mass coefficient at $t=t_0$

Λ diagonal matrix with eigenvalues

b   blood

f   fat

l   liver

m   milk

mf   milk fat

r   richly perfused tissues

s   slowly perfused tissues

Abbreviations are printed in normal letters, scalars in italics, vectors in small bold letters, and matrices in capital bold letters. Dimensions are printed within rectangular brackets.

Appendix B Mathematical model

Model description

The fate of organic contaminants in animals is modeled by a Physiologically Based Pharmacokinetic model (Fig 2.1). In the proposed model the animal is divided into five compartments: blood, liver, fat, slowly perfused tissues, and richly perfused tissues. The following assumptions are made concerning the physiology of the animal:

- Blood is flowing through all compartments at constant rates.
- Milk production occurs at a constant rate, and is zero for non-lactating animals.
- The effective flow in fat is reduced compared to the real flow in order to compensate for intracompartmental diffusion (Derks et al., 1993).

The following assumptions apply to the contaminant and the distribution through the animal:

- The contaminant is taken in with food. Absorption into the blood stream takes place at the intestine-blood interface, where the freshly absorbed contaminant first passes the liver through the *Vena Porta*. The uptake from the intestine can therefore be modeled as administration into the liver.
- Partitioning of contaminants follows linear equilibrium between blood and organs.
- The blood-to-plasma concentration ratio is approximately one.
- Partitioning between blood and milk is derived from milk fat/blood partitioning and fat content of the milk.
- Metabolism of the contaminant is restricted to the liver according to first order kinetics.

Noticing the above assumptions the mass balance for a contaminant in blood is

$$\frac{dA_b}{dt} = -\sum_x Q_x \left(C_b - \frac{C_x}{P_x} \right) - \text{Cl}_m P_m C_b, \quad x \in X = \{l, s, r, f\} \tag{4}$$

The mass balances for a contaminant in organs and tissues are perceived as

$$\frac{dA_x}{dt} = Q_x \left(C_b - \frac{C_x}{P_x} \right) - \frac{K_x}{P_x} C_x V_x + Dd_x, \quad x \in X = \{l, s, r, f\} \tag{5}$$

C_y is the concentration in compartment volume y:

$$C_y = A_y / V_y, \ y \in Y = \{b, l, s, r, f\} \tag{6}$$

The effective bloodflow, Q_x, through a compartment is related to the physiological blood flow by:

$$Q_x = f_x Q_x^*, \quad x \in X = \{l, s, r, f\} \tag{7}$$

where the values for the factors for the effective blood flow rate correcting for diffusion (Derks et al., 1993) are:

$$f_x = 1, \ x \in \{s, r, l\}$$
$$f_x = 0.33, \ x \in \{f\} \tag{8}$$

The blood-milk partition coefficient is equal to

$$P_m = P_{mf} F_m \tag{9}$$

For a complete list of symbols and their meaning see Appendix A. In the above notation *l* indicates the liver, *s* indicates slowly perfused tissues, *r* indicates richly perfused tissues, *f* indicates fat, *mf* indicates milk fat, and *b* indicates blood.

Note that the effective cardic output is equal to the sum of effective flow rates through the remaining compartments:

$$Q_b = \sum_x Q_x, \ x \in X = \{l, s, r, f\} \tag{10}$$

The assumptions dictate the following values to be attributed to the metabolic rate constants:

$$K_x = 0, \ x \in \{b, s, r, f\}$$
$$K_x \neq 0, \ x \in \{l\} \tag{11}$$

and the following values to the normalized absorption factors d_x:

$$d_x = 0, \ x \in \{b, s, r, f\}$$
$$d_x = 1, \ x \in \{l\} \tag{12}$$

Matrix relationships

The method to solve the above described system of 5 coupled linear ordinary differential equation uses matrix algebra. In Matrix notation the system of differential equations given by (4) and (5) is written as:

$$\frac{d\mathbf{a}}{dt} = \mathbf{M}\mathbf{a} + D\mathbf{d} \tag{13}$$

where the vector $\mathbf{a}$ containing the amount of contaminant in the compartments is:

$$\mathbf{a} = [A_b, A_f, A_l, A_r, A_s]^T \tag{14}$$

$\mathbf{M}$ is the system matrix, given as:

$$\mathbf{M} = \begin{bmatrix} -\dfrac{Q_b + \mathrm{Cl}_m P_m}{V_b} & \dfrac{Q_f}{P_f V_f} & \dfrac{Q_l}{P_l V_l} & \dfrac{Q_r}{P_r V_r} & \dfrac{Q_s}{P_s V_s} \\[2mm] \dfrac{Q_f}{V_b} & -\dfrac{Q_f}{P_f V_f} & 0 & 0 & 0 \\[2mm] \dfrac{Q_l}{V_b} & 0 & -\dfrac{Q_l + V_l K_l}{P_l V_l} & 0 & 0 \\[2mm] \dfrac{Q_r}{V_b} & 0 & 0 & -\dfrac{Q_r}{P_r V_r} & 0 \\[2mm] \dfrac{Q_s}{V_b} & 0 & 0 & 0 & -\dfrac{Q_s}{P_s V_s} \end{bmatrix} \tag{15}$$

The vector with the partitioning of the daily absorption over the compartments reads:

$$\mathbf{d} = [0, 0, 1, 0, 0]^T \tag{16}$$

The following relationships apply to Eq.(13) by the rules of matrix algebra:

$$\mathbf{M}\mathbf{s}_i = \lambda_i \mathbf{s}_i \tag{17}$$

where $\mathbf{s}_i$ is the ith eigen vector and λ_i the ith eigen value. Properties of the matrix in Eq. (15) dictate that the eigen values are real and negative. Let $\mathbf{S}$ be the matrix consisting of the 5 eigen vectors:

$$\mathbf{S} = [\mathbf{s}_1, \mathbf{s}_2, \mathbf{s}_3, \mathbf{s}_4, \mathbf{s}_5] \tag{18}$$

and Λ the matrix with the eigen values on the diagonal and zero elements outside the diagonal:

$$\Lambda_{ii} = \lambda_i \tag{19}$$

then
$$\mathbf{MS} = \mathbf{S}\Lambda \tag{20}$$
and for the transposed system matrix $\mathbf{M}$ (Olling et al., 1995)
$$\mathbf{M}^T \mathbf{S}^{-T} = \mathbf{S}^{-T}\Lambda \tag{21}$$
Eqs. (17) to (21) provide the relationships to determine the eigen vectors, eigen values, and transposed eigen vectors present in the columns of $\mathbf{S}^{-T}$. These are necessary in the development of the solution in the next section.

Analytical solution
The mass vector and the normalized absorption vector are composed of linear combinations of the eigen vectors:
$$\mathbf{a} = \sum_j \xi_j \mathbf{s}_j \tag{22}$$

$$\mathbf{d} = \sum_j \delta_j \mathbf{s}_j \tag{23}$$

The coefficients ξ and δ can be isolated by decomposition of the above mentioned vectors. For the coefficients ξ decomposition gives:

$$\mathbf{s}_i^{-T} \cdot \mathbf{a} = \mathbf{s}_i^{-T} \cdot \sum_j \xi_j \mathbf{s}_j = \sum_j \xi_j \mathbf{s}_i^{-T} \cdot \mathbf{s}_j = \xi_i \tag{24}$$

For the vector, ξ , with the coefficients ξ_i we thus obtain

$$\mathbf{S}\xi = \mathbf{a} \tag{25}$$

For δ the same procedure leads to:
$$\mathbf{s}_i^{-T} \cdot \mathbf{d} = \delta_i \tag{26}$$
and

$$\mathbf{S}\delta = \mathbf{d} \tag{27}$$

In order solve Eq.(13) we have to find an expression for the change of the coefficients ξ_i with time. Reformulation of vector $\mathbf{a}$ in terms of ξ provides such an expression:

$$\mathbf{s}_i^{-T} \cdot \frac{d\mathbf{a}}{dt} = \mathbf{s}_i^{-T} \cdot \mathbf{M} \sum_j \xi_j \mathbf{s}_j + \mathbf{s}_i^{-T} \cdot D \sum_j \delta_j \mathbf{s}_j \tag{28}$$

$$\frac{d\xi_i}{dt} = \lambda_i \xi_i + D\delta_i \tag{29}$$

When the daily absorption is constant, integration yields:

$$\xi_i(t) = -D\frac{\delta_i}{\lambda_i} + (\xi_{i0} + D\frac{\delta_i}{\lambda_i})\exp(\lambda_i t) \tag{30}$$

where

$$\mathbf{s}_i^{-T} \cdot \mathbf{a}_0 = \xi_{i0} \tag{31}$$

Combination of (22) and (30) yields the expression to calculate the mass distribution in the animal as a function of time

$$\mathbf{a}(t) = \sum_j \left[-D\frac{\delta_j}{\lambda_j} + (\xi_{j0} + D\frac{\delta_j}{\lambda_j})\exp(\lambda_j t) \right] \mathbf{s}_j \tag{32}$$

The amounts of contaminant in the compartments to which the solution converges when steady state is accomplished is found by taking:

$$\lim_{t\to\infty} \mathbf{a}(t) = \sum_j \left[-D\frac{\delta_j}{\lambda_j} \right] \mathbf{s}_j \tag{33}$$

In Eq.(33) the exponential terms become zero at infinite time, because the eigenvalues are negative.

Estimation procedure for the initial burden and the daily absorption

When Q observations have been made, then for a specific observation in compartment p at time t_q Eq.(32) is written as:

$$V_p C_p(t_q) = a_p(t_q) = D\left[-\sum_j \frac{\delta_j}{\lambda_j} s_{pj} + \sum_j \frac{\delta_j}{\lambda_j} s_{pj} \exp(\lambda_j t_q) \right] + \sum_j \xi_{j0} \exp(\lambda_j t_q) s_{pj} \tag{34}$$

with $q \in \{1,...,Q\}$. The daily absorption and the initial mass distribution of a contaminant in the animal at $t=0$ can now be calculated from Q observations of concentration at different points in time. For each observation one equation of the form of Eq.(34) can be formulated. The number of equations (and observations) necessary to estimate the initial condition and the daily absorption depend strongly on the number of the exponential terms in Eq.(34) that have numerical significance at the times of observation. Let that number of significant terms be R and $r \in \{1,...,R\}$, then Eq.(34) becomes:

$$V_p C_p(t_q) = a_p(t_q) = D\left[-\sum_j \frac{\delta_j}{\lambda_j} s_{pj} + \sum_r \frac{\delta_r}{\lambda_r} s_{pr} \exp(\lambda_r t_q) \right] + \sum_r \xi_{r0} \exp(\lambda_r t_q) s_{pr} \tag{35}$$

When $t_{1/2,i}$ is defined as the half-life corresponding to the ith eigenvalue:

$$t_{1/2,i} = -\ln(2)/\lambda_i \tag{36}$$

then a practical rule of thumb to select the significant terms is to include all those eigen values where

$$3t_{1/2,i} > \min(t_1,...,t_Q) \tag{37}$$

Eq.(35) can now be rearranged in different ways to compute:

1. The daily absorption, D, when this is the only unknown entity:

$$D\left[-\sum_j \frac{\delta_j}{\lambda_j} s_{pj} + \sum_r \frac{\delta_r}{\lambda_r} s_{pr} \exp(\lambda_r t_q) \right] = a_p(t_q) - \sum_r \xi_{r0} \exp(\lambda_r t_q) s_{pr} \tag{38}$$

This requires at least 1 equation of the above form and a know initial distribution $\mathbf{a}_0$. However, it is strongly recommended to use a few more observations.

2. The initial distribution of the contaminant over the compartments, when the distribution is unknown:

$$\sum_r \xi_{r0} \exp(\lambda_r t_q) s_{pr} = a_p(t_q) - D\left[-\sum_j \frac{\delta_j}{\lambda_j} s_{pj} + \sum_r \frac{\delta_r}{\lambda_r} s_{pr} \exp(\lambda_r t_q) \right] \tag{39}$$

This requires at least R equations of the above form and a known daily absorption D.

3. The initial distribution of the contaminant over the compartments and the daily absorption, when both are unknown:

$$D\left[-\sum_j \frac{\delta_j}{\lambda_j} s_{pj} + \sum_r \frac{\delta_r}{\lambda_r} s_{pr} \exp(\lambda_r t_q)\right] + \sum_r \xi_{r0} \exp(\lambda_r t_q) s_{pr} = a_p(t_q) \qquad (40)$$

This requires at least $R+1$ equations of the above form.

The system of equations that has to be solved in all 3 cases takes the following form

$$\mathbf{Ax} = \mathbf{b} \qquad (41)$$

where $\mathbf{b}$ is the vector containing the known right side terms, $\mathbf{A}$ the matrix containing the coefficients dictated by the physical model, and $\mathbf{x}$ is the unknown left hand side vector, solution of which yields the values of interest. Note that in case 1 the matrix consists of 1 vector, in case 2 of R vectors and case 3 of $R+1$ vectors. The $\mathbf{x}$ vector is found by a least squares solution.

Algorithms to calculate $\mathbf{a}(t)$, $\mathbf{a}_0$, *or* D
Using the mathematical model in a computer program requires algorithms to handle the matrix operations discussed in the above section. In our approach we used LAPACK driver routine (version 2.0) DGEEV developed by the Univ. of Tennessee, Univ. of California Berkeley, NAG Ltd., Courant Institute, Argonne National Lab, and Rice University , September 30, 1994. The purpose of this routine is to compute for an N-by-N real nonsymmetric matrix $\mathbf{M}$, the eigenvalues and, optionally, the left and/or right eigenvectors. Another routine from the same package applied in our computer program is DGELS. This routine solves overdetermined or underdetermined real linear systems involving an M-by-N matrix $\mathbf{A}$, or its transpose, using a QR or LQ factorization of $\mathbf{A}$. It is assumed that $\mathbf{A}$ has full rank.
The computer code in the C programming language can be found on the world wide web at the following address:

<center>http://gams.nist.gov/,</center>

and more specifically:

<center>http://math.nist.gov/cgi-bin/gams-serve/list-package-components/CLAPACK.html.</center>

Calculating $\mathbf{a}(t)$
For a known initial mass distribution over the compartments, specified parameter values, and a known daily absorption, the concentration in each compartment can be calculated by the following steps:
1. Fill the system matrix, the normalized absorption vector and the vector containing the initial amount of contaminant in the compartments.
2. Determine the eigen values, the eigen vectors, and the transposed eigen vectors using LAPACK routine DGEEV
3. Decompose the scaled absorption vector using (23) and decompose the vector with the initial amounts using Eq.(31) with LAPACK routine DGELS.
4. Use Eq.(32) to compute the mass distribution over the compartments. Concentrations follow from Eq.(6).

Assessment of the daily absorption

For a known initial mass distribution over the compartments, specified parameter values, and a set of observations (at least one) at different moments in time, the daily absorption is estimated by the next 4 steps:

1. Fill the system matrix, the normalized absorption vector and the vector containing the initial amount of contaminant in the compartments.
2. Determine the eigen values, the eigen vectors, and the transposed eigen vectors using LAPACK routine DGEEV
3. Decompose the scaled absorption vector using (23) and decompose the vector with the initial amounts using Eq.(31) with LAPACK routine DGELS.
4. Use Eqs.(38) and (41) to estimate D. The solution requires filling of the **A** matrix (1 column) and the **b** vector. The LAPACK routine DGELS is used to make a least squares estimate of D, which is returned in the 1-element result vector **x**.

Assessment of the initial burden

For a known daily absorption, specified parameter values, and a set of at least R observations at different moments in time, the initial burden is estimated by the next 6 steps:

1. Fill the system matrix and the normalized absorption vector.
2. Determine the eigen values, the eigen vectors, and the transposed eigen vectors using LAPACK routine DGEEV.
3. Determine the order of eigen values, and check which exponential terms in Eq. (34) are significant at the time that the observations have been performed. The criterion for testing the significance is provided by Eq.(37).
4. Decompose the scaled absorption vector using (23) with LAPACK routine DGELS.
5. Use Eqs.(39) and (41) to estimate the coefficients ξ_{r0}. The solution requires filling of the **A** matrix (R columns) and the **b** vector. The LAPACK routine DGELS is used to make least squares estimates of ξ_{r0}, which are returned in the result vector **x**. The result vector contains R elements.
6. Use Eq.(22) to calculate the initial burden in each compartment from the estimated coefficients ξ_{r0}.

Assessment of the initial burden and daily absorption

For specified parameter values, and a set of at least $R+1$ observations at different moments in time, the initial burden and the daily absorption are estimated by the next 6 steps:

1. Fill the system matrix and the normalized absorption vector.
2. Determine the eigen values, the eigen vectors, and the transposed eigen vectors using LAPACK routine DGEEV.
3. Determine the order of eigen values, and check which exponential terms in Eq. (34) are significant at the time that the observations have been performed. The criterion for testing the significance is provided by Eq.(37).
4. Decompose the scaled absorption vector using (23) with LAPACK routine DGELS.
5. Use Eqs.(40) and (41) to estimate the coefficients ξ_{r0}. The solution requires filling of the **A** matrix ($R+1$ columns) and the **b** vector. The LAPACK routine DGELS is used to make least squares estimates of ξ_{r0} and D , which are returned in the result vector **x**. The result vector contains $R+1$ elements.
6. Use Eq.(22) to calculate the initial burden in each compartment from the estimated coefficients ξ_{r0}.

Computer program
The PBPK model and the solution methods for assessing daily absorption and initial burden from measurements of concentrations in the animal have been implemented in a computer program. This program is written in the C++ programming language and consists of a object oriented windows interface which handles input and output of data and results, and C code for all calculations. All matrix operations are handled by the DGEEV and DGELS routines from the LAPACK package. The development environments for the computer program consisted of Borland C++ v4.5 and v5.0, both connected to Borland code guard. Code guard is a tool for tracing runtime errors. Compiled computer code includes the 32 bits executable for windows 95 and windows NT 4.0.

Appendix C Input data in the example exercise

Measurements of 2378-TCDD in milk used in the example exercise of chapter 5. At the
starting date (01/01/1994) 2 µg 2378-TCDD was injected in the rumen of the cows.

Date	Concentration in milk (ng L^{-1})			
	1	2	3	4
02/01/1994	8.6	7.25	2.7	-
03/01/1994	4.8	4.1	3.7	-
04/01/1994	3.2	2.4	2.0	-
05/01/1994	1.7	1.4	1.3	-
06/01/1994	1.3	1.1	1.0	-
07/01/1994	0.99	0.93	0.82	0.72
28/01/1994	0.53	0.32	0.31	0.30
25/02/1994	0.24	0.23	0.22	0.17
04/04/1994	0.15	0.13	0.10	0.09

Appendix D Mailing list

1-5   Inspectie Gezondheidsbescherming, Waren en Veterinaire zaken
6   Directeur Generaal van de Volksgezondheid
7   Voorzitter Gezondheidsraad
8   Hoofdinspecteur Inspectie W&V
9   Inspectie W&V Regionale Dienst Noord
10   Inspectie W&V Regionale Dienst Oost
11   Inspectie W&V Regionale Dienst Zuid
12   Inspectie W&V Regionale Dienst Noord-West
13   Inspectie W&V Regionale Dienst Zuid-West
14   DGM, directie Stoffen, Veiligheid en Straling
15   dr J.H.M. Nieuwenhuijs, Inspectie W&V
16   dr ir G. Kleter, Inspectie W&V
17   dr ir P.C. Bragt, Inspectie W&V
18   ing P.C. Beijen, Inspectie W&V
19   drs R.A.A. Oosterom, Inspectie W&V Regionale Dienst Noord-West
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32   C.J. Berkhoff, LOC-RIVM
33   dr R. Hoogerbrugge, LOC-RIVM
34   dr ir H.J.G.M. Derks, LGO-RIVM
35   drs A. van der Giessen, CIM-RIVM
36   dr ir E. Lebret, LBM-RIVM
37   dr M.P. van Veen, LBM-RIVM
38   dr ir M.J. Zeilmaker, LBM-RIVM
39   ing H.J. Bremmer, LBM-RIVM
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