National Institute for Public Health
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Ministry of Health, Welfare and Sport

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RIVM letter report 2021-0149
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Synopsis

Transfer models for dioxins and dioxin-like PCBs in wild cattle (case study: Dutch floodplains) – model documentation

In a number of areas between rivers and dykes (floodplains), grazing by wild cattle is used as a form of nature management. Some of these animals are slaughtered to manage the size and composition of the herds. Their meat is then sold as 'wilderness meat'. In 2020, excessive dioxin levels were discovered in the meat of some of the wild cattle as a result of dioxins and dioxin-like PCBs in the grass and soil of floodplains.

In response to this situation, the Dutch National Institute for Public Health and the Environment (*Rijksinstituut voor Volksgezondheid en Milieu* (RIVM)) and Wageningen Food Safety Research developed models to calculate the extent to which these substances end up in the meat of these cattle via grass and soil. This report describes the development of the models and provides the information that researchers will need to be able to use the models. Another report describes how the models were used to predict levels in meat from floodplains.

Three types of wild cattle living in herds were investigated – cattle that do not give milk, cattle that give milk and calves. A separate model was developed for each. The models are based on a previously developed model for dioxins in dairy cows. This model was adapted for the specific characteristics of the type of animal, such as weight.

The model calculations matched closely with a number of levels measured in the meat of cattle. The models are also able to predict how quickly the levels will decrease if the cattle are moved onto cleaner grasslands and soil. This information can be used to estimate when the levels will be below the maximum permitted levels, although these estimates will still need to be verified by means of measurements.

Dioxins and dioxin-like PCBs are chemical substances that are created during the incineration of waste and other substances. Despite the strong decrease in emissions in the last 25 years, dioxins and dioxin-like PCBs are still present in the Netherlands (in grass, the soil and river sediment, for example). Dioxins can be harmful to the immune system, brain development and reproduction.

Keywords: dioxins, PCBs, cattle, floodplains, transfer models, PBK models

Publiekssamenvatting

Overdrachtsmodellen voor dioxinen en dioxineachtige PCB's in wilde runderen (casus: uiterwaarden in Nederland) – modeldocumentatie.

Wilde runderen grazen in enkele gebieden tussen de rivier en dijk (uiterwaarden) als een vorm van natuurbeheer. Sommige dieren worden geslacht om de grootte en samenstelling van de kuddes goed te houden. Het vlees wordt verkocht als 'wildernisvlees'. In 2020 zijn in het vlees van enkele wilde runderen te hoge hoeveelheden dioxinen gemeten. Dit komt door de dioxinen en dioxine-achtige PCB's in het gras en de grond in de uiterwaarden.

Het RIVM en Wageningen Food Safety Research hebben daarom modellen ontwikkeld om te berekenen hoeveel van deze stoffen via gras en grond in het vlees van deze runderen terechtkomen. Dit rapport beschrijft hoe de modellen gemaakt zijn en geeft de informatie die onderzoekers nodig hebben om de modellen te kunnen gebruiken. Een ander rapport beschrijft hoe de modellen zijn gebruikt om hoeveelheden in vlees afkomstig van runderen uit de uiterwaarden te voorspellen.

Er is gekeken naar drie type wilde runderen die in een kudde leven: runderen die geen melk geven, runderen die melk geven, en kalveren. Voor elk type rund is een apart model ontwikkeld. De basis voor deze modellen is een model dat eerder voor dioxinen in de melkkoe is ontwikkeld. Dit model is aangepast aan de kenmerken van het type dier, zoals het gewicht.

De modelberekeningen kwamen goed overeen met enkele metingen in vlees van runderen. Ook kunnen de modellen voorspellen hoe snel de hoeveelheden dalen als de runderen naar gebieden met schoner gras en schonere grond worden gebracht. Met deze informatie kan worden ingeschat wanneer de hoeveelheden onder de maximaal toegestane hoeveelheid zitten. Deze schattingen moeten nog wel met metingen worden gecontroleerd.

Dioxinen en dioxine-achtige PCB's zijn chemische stoffen die bij (vuil)verbranding zijn ontstaan. Ondanks de sterk gedaalde uitstoot in de laatste 25 jaar, komen ze nog steeds voor in Nederland. Bijvoorbeeld in gras, de bodem en rivierslib. Dioxinen kunnen schadelijk zijn voor het immuunsysteem, de ontwikkeling van de hersenen, en de voortplanting.

Kernwoorden: dioxinen, PCB's, runderen, uiterwaarden, overdrachtsmodellen, PBK modellen

Contents

Summary

This report describes the development of three physiologically based kinetic (PBK) models that simulate the transfer of dioxins (polychlorinated dibenzo-p-dioxins and dibenzofurans, PCDD/Fs) and dioxin-like PCBs (DL-PCBs) expressed in toxic equivalents (TEQ) from grass and soil to animal products such as muscle fat of wild cattle grazing in Dutch floodplains. The three models are intended to be used as a typical representation of the three types of wild cattle grazing in the floodplains: beef cattle (bulls and non-lactating cows), lactating cows and their growing calves. The models described in this document have been built based on a PBK model for dairy cattle that was initially developed by Derks et al. (Derks et al., 1993; Derks et al., 1994) and later modified for application in the case of a feed incident with clay (Hoogenboom et al., 2010) and for exposure during grazing in an area previously contaminated by waste incineration (Traag et al., 2006; Zeilmaker et al., 2013). This dairy cattle model was extended to beef cattle and growing calves. For the beef cattle model, milk production was set to zero, and physiological parameters were adjusted. For the growing calf model, body weight increase during the first year was modelled. In addition, seasonal variations in the weight of the fat compartment and, consequently, body weight were added to the adult PBK models.

In order to evaluate the added PBK model functionalities, sensitivity analyses were performed for each of the three models. In addition, results of model simulations were preliminary verified by comparison to measured TEQ levels in body fat and the liver.

All in all, the verification of the PBK model parameters and the first limited evaluation of the simulations with experimental data suggest that the models can accurately simulate the TEQ concentration in tissue fat. The measured TEQ concentrations in muscle fat and liver fat closely resemble those simulated following a worst-case exposure scenario, while being relatively close to the concentrations simulated in the realistic exposure scenario. Nevertheless, additional information on the actual exposure of the animals, and TEQ concentration measurements in tissue fat of the animals are necessary to confirm this finding.

1 Objective and regulatory purpose

In 2020, elevated levels of the dioxins (polychlorinated dibenzo-p-dioxins and dibenzofurans, PCDD/Fs) and dioxin-like PCBs (DL-PCBs) were observed in fat and tissues of wild cattle grazing in floodplains in the Netherlands (NVWA, personal communication). The objective of this project was to predict the accumulation of dioxins and DL-PCBs in wild cattle grazing on river floodplains in the Netherlands, and to simulate the decrease of the dioxin concentration in the cattle after moving to cleaner grounds containing lower exposure to dioxins and DL-PCBs. To this end, physiologically based kinetic (PBK) modelling tools were employed. To account for wild cattle grazing on river floodplains, PBK models were developed for three different types of cattle: 1) beef cattle (representing bulls and non-lactating cows), 2) lactating cows and 3) growing calves.

The PBK models developed in this project were specifically fine-tuned to the characteristics of the Bos taurus breed "Rode Geus" as a representative breed currently grazing in Dutch floodplains. For this breed, analytical data, although still limited, are available. The "Rode Geus" is a crossbreed between "Saler" and "Brandrode" cattle. The PBK models were developed by scaling a PBK model from Friesian dairy cattle (Derks et al., 1993; Derks et al., 1994) to beef cattle. For this purpose, relevant growth and physiological data from Salers were used (as a proxy for absent corresponding data from the "Rode Geus").

This document serves as background document for using the developed PBK models to predict dioxins and DL-PCBs concentrations (expressed in total toxic equivalents [total TEQ]) in cattle. The model simulations performed with these models are described in Notenboom et al. (2021). The document is structured following the Organisation of Economic Co-operation and Development (OECD) guideline for PBK modelling (OECD, 2021) and the remainder of this document will focus on explaining the general structure of the PBK models, the associated model equations, and implementation details. Finally, a preliminary verification of the PBK models is described. Here exposure scenarios based on empirical dioxin and DL-PCB concentrations (expressed in total TEQ) in grass and soil were used to simulate total TEQ concentrations in muscle fat and liver. These concentrations were compared with the empirically measured total TEQ concentrations in kidney fat and liver, under the assumption that TEQ is evenly distributed in the body fat.

2 Background information

Dioxins and DL-PCBs are a group of persistent chemical substances that can be harmful for human health. These chemicals are a mixture consisting of various congeners, of which 29 are generally considered critical. After correction for differences in their toxic potencies (using Toxic Equivalency Factors [TEF-values]) the overall mixture exposure is expressed in toxic equivalents (TEQ). Humans are typically exposed to these contaminants through the consumption of animal products such as fish, eggs, milk, and meat. In turn, dioxins and DL-PCBs in animal products are often the result of the presence of these chemicals in animal feed (such as grass and adhering soil).

In this project we focus on dioxins and DL-PCBs in meat, specifically muscle fat and liver of wild cattle (beef cattle, lactating cows and calves). These compounds accumulate in body fat and in the liver. Cattle that graze on the floodplains are exposed to these compounds via the intake of grass and adhering soil. If the TEQ levels in cattle muscle fat and liver exceed maximum levels established at EU-level, the meat is not allowed for human consumption. A solution to reduce high TEQ levels in cattle is to move cattle to clean(er) grounds (i.e., with lower TEQ levels in grass and soil), and/or to regulate the intake by providing clean(er) feed. This possibly results in TEQ levels below the maximum EU levels. To provide insights into whether these solutions are effective, it is necessary to accurately monitor the TEQ levels in cattle during their life cycle and estimate when the TEQ levels in muscle fat and liver are below the maximum levels. In this context (additional) PBK modelling may help to characterize the accumulation and depletion kinetics and predict the expected TEQ levels in the cattle throughout the years of grazing. In addition, PBK modelling may be used to calculate the time period needed to lower the concentration in muscle fat and liver below the maximum level.

Therefore, this project focused on developing PBK models to simulate the accumulation and depletion kinetics of TEQ in wild cattle, i.e. beef cattle, lactating cows and calves grazing on typical Dutch floodplains along large rivers. It should be stressed that the primary purpose of this cattle is related to nature management of the floodplains and not the production of food of animal origin. However, occasionally, as herds grow too large, animals are slaughtered and their meat is sold as (in Dutch) “wildernisvlees” for human consumption.

3 Qualification

The PBK models described in this document are intended to simulate the TEQ levels in muscle fat and liver of cattle. These models were adapted for the three types of cattle, which should represent a typical herd: lactating cows, adult beef cattle, and growing calves which exclusively depend on milk as feed intake (<6 months). TEQ concentrations in calves were simulated until the age of 1, assuming their diet switches from milk to grass at the age of 6 months. The growing calf model does not cover cattle over the age of 12 months. The quality of the model simulations of the TEQ levels in muscle fat and liver have been verified using measured TEQ values in kidney fat and liver of Rode Geus cows grazing on the floodplains, assuming TEQ is evenly distributed in fat throughout the body.

4 Model development

4.1 General overview

The basic structure of the PBK model developed in this project is given in Figure 1. For adult cattle (i.e., lactating cow and beef cattle), the total intake of TEQ is modelled as the sum of TEQ taken in with grass and from adhering soil. In contrast, for the growing calf model (0-12 months), drinking milk for 6 months from birth was modelled as the sole contributor to the total TEQ intake. However, in practice calves might also have intake of grass and soil, instead of part of their milk consumption leading to lower total TEQ intake, but this is not documented. For the remaining six months, the intake of TEQ by calves was assumed to be solely from grass and soil.

In the PBK model, the TEQ taken in from feed directly enters the liver compartment. This way of modelling mimics the absorption of TEQ orally entering the gastro-intestinal (GI)-tract followed by transport of absorbed TEQ to the liver via the hepatic portal vein as described previously for the models on which this model was built (Derks et al., 1993; Derks et al., 1994; Hoogenboom et al., 2010; Traag et al., 2006; Zeilmaker et al., 2013).

After being taken up by the liver, a fraction of the TEQ is cleared and the remaining TEQ enters the systemic blood flow, from which it can be removed through hepatic clearance and/or excretion to milk. TEQ in the systemic blood flow is distributed over the various parts of the body, which are included in the PBK model as the compartments (blood, fat, liver, slowly perfused organs and richly perfused organs).

In the model it is assumed that wild cows only give milk in the spring and summer period (April 1st – October 1st). During the milk production period, cows also have an increased cardiac output (see Table 1). This was also taken into account in the model.

Furthermore, in adult cattle the size of the fat compartment varies during the seasons. Typically cattle net store fat in the spring and summer when grass is abundant, while using the fat in the winter when food is more scarce. To simulate the seasonal variation in the fat compartment, the weight of the fat compartment was modelled as a sinusoidal wave (see Ch 4.2; Eq. 9).

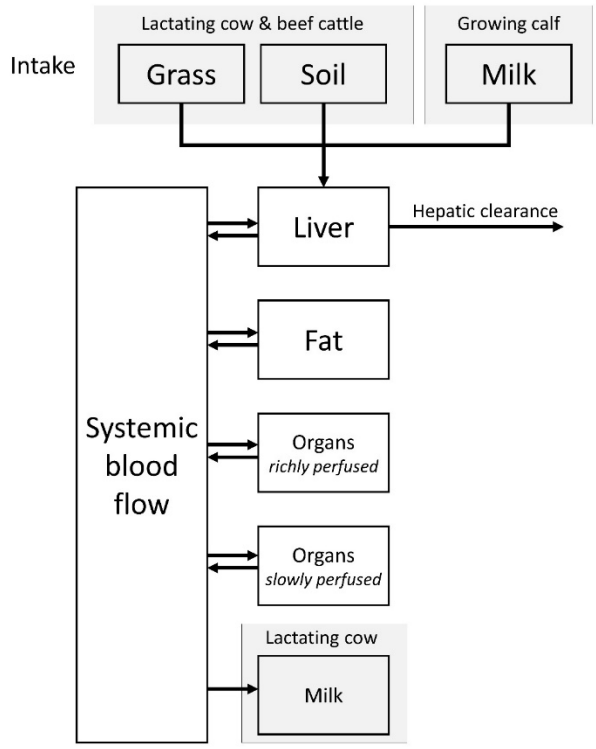

Figure 1 Schematic overview of the PBK model of the dioxin and DL-PCB transfer routes in wild cattle living in a typical herd on Dutch floodplains. Adult cattle (beef cattle and lactating cows) take up dioxins and DL-PCBs through grass and soil, whereas calves take up these compounds through milk. The arrows represent the arterial and venous blood flow between the organ compartments. Note that the sum of these blood flows is equal to the total cardiac output. Excretion is solely performed through hepatic clearance, except for the lactating cows that also excrete dioxins and DL-PCBs through milk.

4.2 Model equations

The PBK models described in this report consist of a set of coupled ordinary differential equations (ODEs). These ODEs describe the movement of a particular substance between liver, fat, slowly perfused organs and richly perfused organs compartments over time. For each (sub)compartment of the models, an ODE was formulated. The ODEs used in the PBK models are presented below. Specifically, equations 1 to 6 describe the change in the amount of TEQ in blood, fat, liver, slowly perfused organs, richly perfused organs, and milk, respectively.

$$\frac{dA_{blood}(t)}{dt} = -\frac{Q_{fat}}{F_q} \times \left(c_{blood}(t) - \frac{c_{fat}(t)}{p_{fat}}\right) - Q_{slowly\ perfused} \times \left(c_{blood}(t) - \frac{c_{slowly\ perfused}(t)}{p_{slowly\ perfused}}\right) - Q_{richly\ perfused} \times \left(c_{blood}(t) - \frac{c_{richly\ perfused}(t)}{p_{richly\ perfused}}\right) - Q_{liver} \times \left(c_{blood}(t) - \frac{c_{liver}(t)}{p_{liver}}\right) - cl_b \times c_{blood}(t) \quad \textbf{1).}$$

$$\frac{dA_{fat}(t)}{dt} = \frac{Q_{fat}}{F_q} \times \left(c_{blood}(t) - \frac{c_{fat}(t)}{p_{fat}}\right) \quad \textbf{2).}$$

$$\frac{dA_{slowly\ perfused}(t)}{dt} = Q_{slowly\ perfused} \times \left(c_{blood}(t) - \frac{c_{slowly\ perfused}(t)}{p_{slowly\ perfused}}\right) \quad \textbf{3).}$$

$$\frac{dA_{richly\ perfused}(t)}{dt} = Q_{richly\ perfused} \times \left(c_{blood}(t) - \frac{c_{richly\ perfused}(t)}{p_{richly\ perfused}}\right) \quad \textbf{4).}$$

$$\frac{dA_{liver}(t)}{dt} = Q_{liver} \times \left(c_{blood}(t) - \frac{c_{liver}(t)}{p_{liver}}\right) - cl_l \times \frac{c_{liver}(t)}{p_{liver}} \quad \textbf{5).}$$

$$\frac{dA_{milk}(t)}{dt} = cl_b \times c_{blood}(t) \quad \textbf{6).}$$

$A_i(t)$ [ng]:	Amount of TEQ in compartment i at time t
$c_i(t)$ [ng/kg]:	Concentration of TEQ in compartment i at time t
cl_b [L/day]:	Blood clearance due to excretion to milk. NB: this is 0 in the beef cattle model and the growing calf model,
cl_l [L/day]:	Hepatic clearance
F_q [-]:	Diffusion limiting flow factor in the adipose tissue
p_i :	Partition coefficient between the blood and compartment i
Q_i [L/day]:	Perfusion to compartment i

The TEQ concentrations in the PBK model compartments c_i are calculated as follows:

$$c_i = \frac{A_i}{V_i} \quad \textbf{7).}$$

$$V_i = rV_i \times (bwLifeMean - WGI) \quad \textbf{8).}$$

where A_i represents the amount of TEQ in compartment i, Vi is the volume of compartment i, rV_i indicates the relative volume of compartment i to the body weight (see Table 1), and WGI denotes the gastro-intestinal tract content weight (see table 2, 3 and 4). bwLifeMean represents the average body weight of the adult cattle including GIT content. However, this body weight is expected to vary over the seasons, since wild cattle typically gain weight in summer when food is abundant and lose weight in winter when food is scarce. As organ weights are not expected to significantly vary over time, the body weight variation was modelled by varying the volume of the fat compartment $V_{fat}(t)$. $V_{fat}(t)$ in adult cattle was calculated separately in Eq. 9 to simulate the seasonal variation in Vfat:

$$V_{fat}(t) = -fatVariation \times \sin\left(\frac{2\pi t}{365}\right) + rV_{fat}(t) \times (bwLifeMean) \quad \textbf{9).}$$

where t denotes the time (days) that has passed in the current calendar year. Note that t=0 indicates January 1st. $fatVariation$ indicates the amplitude of the variation in the fat compartment. The total body weight, and the weight of the fat compartment modelled in the lactating cow, and beef cattle, are visualized in Figure 2.

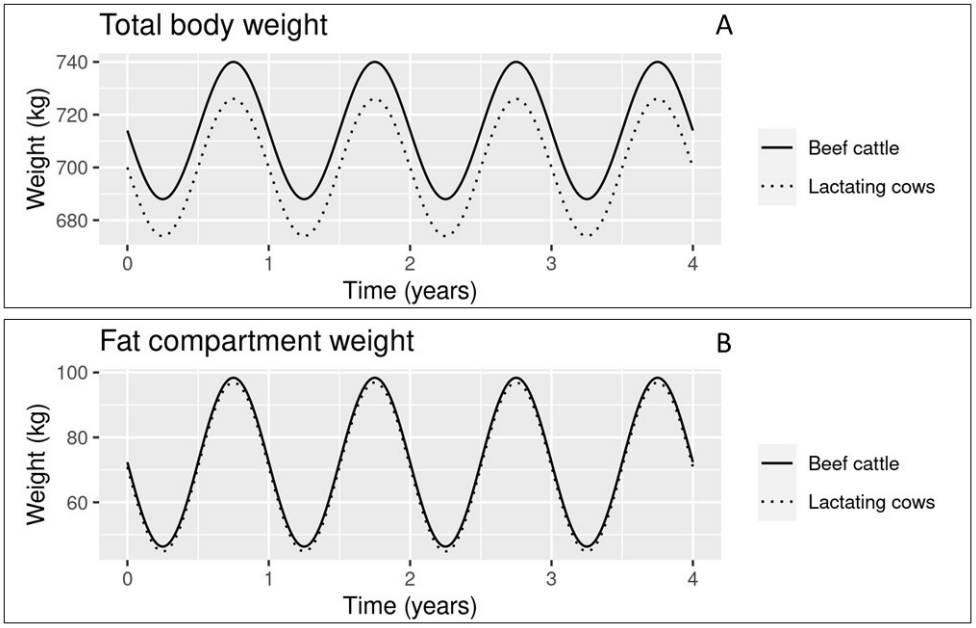

Figure 2 The total body weight variation (A) and the fat compartment weight variation (B) in both beef cattle and lactating cows.

Keeping these variations in the fat compartment in mind, the body weight $BW(t)$ can be calculated as the sum of the separate compartment volumes at time t, assuming the density of all tissues is 1 kg/L:

$$BW(t) = \sum V_i\,(t) \qquad \textbf{10).}$$

$BW(t)$ thus represents the body weight without the GI-tract content weight, which varies over time. Hence, $BW(t)$ shows the weight variations over time, as opposed to bwLifeMean which indicates the yearly average total body weight of adult cattle.

In contrast to the adult cattle models, no fat compartment variations were modelled for the growing calves. The body weight growth of the calves was calculated using the Brody model:

$$BW(t) = bwAdult(1 - b \times e^{(-ct)}) \qquad \textbf{11).}$$

Here, $BW(t)$ denotes the body weight of a calf at time t (days), and bwAdult is the weight that would be reached as an adult, b is a constant that defines the weight at birth, and c is a growth rate. In this model, *bwAdult*=537 (kg), b=0.9255 (-), and c=0.00204 (day^{-1}). Values for this weight growth model were taken from (Domínguez-Viveros et al., 2014). The resulting growth curve is shown in Figure 3.

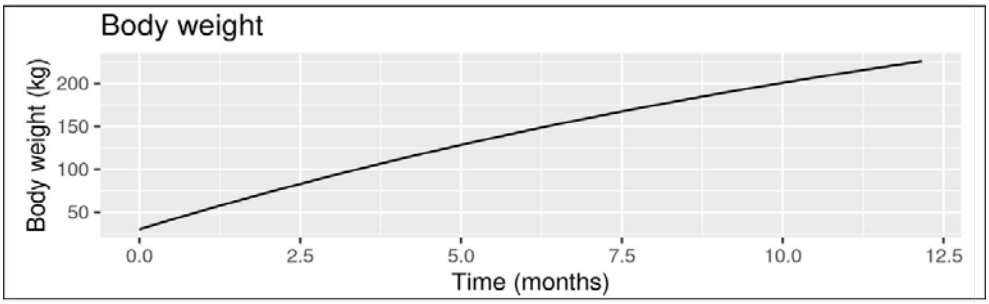

Figure 3 The growth curve for calves used in the simulation for calves aged 0-12 months.

In all three models, the perfusion to compartment *i*, Q_i, is calculated as:

$$Q_i(t) = rQ_i \times q_c(t) \quad \textbf{12).}$$

where rQ_i is the fraction of cardiac output to compartment *i* and $q_c(t)$ is the cardiac output at time t:

$$q_c(t) = q_0 \times \left(\frac{BW(t)}{BW_{ref}}\right)^{0.75} \quad \textbf{13).}$$

Here, q_0 is a reference cardiac output that corresponds to cattle with a body weight BW_{ref} (see Table 1). This equation essentially describes the scaling of a known cardiac output of cattle with a specific body weight to cattle with different body weights. This equation was taken from Dawson (2014).

Clearance of TEQ from the PBK models was modelled in two ways. First, TEQ was cleared through produced milk in the lactating cow model. The clearance rate, cl_b, is calculated as follows:

$$cl_b = pMilkFat \times milkProd \times fatPerc/100 \quad \textbf{14).}$$

Where $pMilkFat$ represents milk-fat partition coefficient, $milkProd$ represents the amount (L/day) of produced milk and $fatPerc$ represents the percentage of fat in the produced milk. Since milk production was only present between April 1st and October 1st, clearance of TEQ through was only possible in this period, and only for the lactating cow model.

The concentration of TEQ in the excreted milk, $c_{milk}(t)$, can be calculated for a given time point between April 1st and October 1st with:

$$c_{milk}(t) = \frac{cl_b \times c_{blood}(t)}{milkProd} \quad \textbf{15).}$$

Here, $c_{blood}(t)$ is the concentration TEQ at time t (days), and milkProd is the volume of produced milk (Table 2).

The second clearance route was through the liver. The hepatic clearance rate, cl_l, is calculated as follows:

$$cl_l = kMet \times V_l \quad \textbf{16).}$$

Where $kMet$ (Tables 2,3 and 4) is the metabolic hepatic rate constant and V_l is the liver volume.

4.3 Model assumptions

To develop the PBK models, various assumptions needed to be made. First, the models are perfusion-limited models, implying that the transport is limited by the blood flow and that partitioning results in an immediate change in the organ concentration. This indirectly implies that perfusion directly leads to a change in concentration in the whole organ. Another assumption was that beef cattle are physiologically the same as lactating cows without any milk production. In addition, the intake of TEQ by growing calves was assumed to be solely caused by drinking milk in the first six months. Although this is not the case in practice, insufficient data on feed intake was available to simulate different exposure routes. After the six months, the intake of TEQ by calves was assumed to be solely from grass and soil. The grass and soil intake of calves was linearly scaled based on their bodyweight relative to that of the lactating cow model:

$$intake_{calf} = intake_{lactating\ cow} \times \frac{BW(t)_{calf}}{bwLifeMean_{lactating\ cow}} \quad \textbf{17).}$$

As energy consumption of cattle varies during the year, a seasonal variation was added to the weight of the fat compartment. In the model, this variation was implemented as a sinusoidal function on the mean weight of the fat compartment (see Eq. 9). The amplitude of the sinus was derived from data reported by Hoch et al. (Hoch et al., 2005). Specifically, they reported that Saler cows on a summer diet were approximately 52 kg heavier than Saler cows with a winter diet. Therefore, the amplitude of the sinusoidal variation implemented in the PBK model was 26 kg. Hence, it was implicitly assumed that the weight variation can be completely attributed to the variation in the fat compartment. Moreover, it must be noted that Hoch et al. performed their experiments on Salers, and not on Rode Geus, so it was assumed that the weight variation is equal for both breeds.

The growing calf model is only valid to predict TEQ levels from birth until the age of 12 months, because the used weight growth model for Saler calves reported by Domínguez-Viveros et al. (2014) is only verified for the first 365 days. Again, the weight growth model was based on an experiment with Salers/Hereford calves and not on Rode Geus calves, but it was assumed the weight growth model was comparable between breeds.

Another assumption is that the weight of the gastro-intestinal content (WGI) is a fixed fraction of the mean total body weight. Derks et al. (1994) implicitly assumed a WGI of 150 kg for a lactating cow with a total average body weight of 600 kg. Since the sum of all compartment weights in Derks et al. (1994) was approximately 450 kg, we derived that the WGI accounted for the remaining 150 kg. In the model described in this report, we therefore assumed that the WGI content was always 25% of the total average body weight (bwLifeMean), in all three cattle models.

Finally, the calculation of the dioxin TEQ concentration in muscle fat was performed as follows:

$$C_{s,f} = \frac{C_s}{0.023} \quad \textbf{18).}$$

This equation shows how to calculate the TEQ concentration in slowly perfused organ fat ($C_{s,f}$) given the simulated TEQ concentration in the slowly perfused organ compartment (C_s). The aforementioned equation is derived based on 2 main assumptions. The first assumption was that muscle fat, like all body fat, is part of the slowly perfused organ compartment. This assumption has been verified for various mammals (Brown et al., 1997). Second, all dioxin TEQ in the extrahepatic tissues was assumed to accumulate in body fat.

From equation 7 and the two aforementioned assumptions it follows that $A_s = C_s \times V_s$. Here, A_s is the amount of TEQ in the slowly perfused organs, C_s represents the TEQ concentration in the slowly perfused tissue compartment, and V_s is the volume of the slowly perfused tissues. Substituting this back into equation 7 gives:

$$C_s = \frac{A_s}{V_s} = \frac{C_{s,f} \times V_{s,f}}{V_s} = C_{s,f} \times v_{s,f} \quad \textbf{19).}$$

Where, $C_{s,f}$ and $V_{s,f}$ represent the TEQ concentration in the slowly perfused tissue fat, and the volume of the slowly perfused tissue fat, respectively. $v_{s,f}$ represents the ratio between the slowly perfused tissue fat volume and the slowly perfused tissue compartment volume.

Assuming an instant equilibrium between blood and the tissue gives

$$C_{Blood} = \frac{C_s}{P_s} \quad \textbf{20).}$$

Where P_s represents the slowly perfused tissue-blood partition coefficient. Substituting equation 19 into 20 and rearranging gives

$$C_{Blood} = \frac{C_{s,f} \times v_{s,f}}{P_s} \quad \textbf{21).}$$

By analogy, the following equations for richly perfused organs and adipose tissue also hold.

$$C_{Blood} = \frac{C_{r,f} \times v_{r,f}}{P_r} \quad \textbf{22).}$$

$$C_{Blood} = \frac{C_{f,f} \times v_{f,f}}{P_f} \quad \textbf{23).}$$

Here, $C_{r,f}$ and $C_{f,f}$ represent the TEQ concentration in the richly perfused organ fat and in the adipose tissue, respectively. $v_{r,f}$ represents the ratio between the richly perfused tissue fat volume and the richly perfused organ compartment volume, whereas $v_{f,f}$ represents the ratio between the adipose tissue fat volume and the adipose tissue volume. Finally, P_r and P_f represent the richly perfused tissue-blood partition coefficient and the adipose tissue-blood partition coefficient, respectively.

Note that:

$$C_{f,f} = C_{s,f} = C_{r,f} \text{ implies } \frac{v_{s,f}}{P_s} = \frac{v_{r,f}}{P_r} = \frac{v_{f,f}}{P_f} \quad \textbf{24).}$$

Rearranging (19):

$$C_{s.f} = \frac{C_s}{v_{s,f}} \quad \textbf{25).}$$

Rearranging (24):

$$v_{s,f} = \frac{P_s}{P_f} \times v_{f,f} \quad \textbf{26).}$$

Substituting (25) in (26):

$$C_{s.f} = \frac{C_s}{\frac{P_s}{P_f} \times v_{f,f}} \quad \textbf{27).}$$

Substituting Ps = 8 (see Table 1), Pf = 280 (see Table 1) and vf,f = 0.8 (Thomas et al, 1962) results in the relation described in equation 18.

4.4 System-dependent parameters

PBK model parameters that are used in all three PBK models are shown in Table 1. The model parameters specific for the lactating cow model, the beef cattle model and the growing calf model are shown in Tables 2, 3 and 4, respectively. For each PBK model parameter, a reference is provided from which the value was obtained. Note that the body weights are given in kilograms, whereas the PBK model compartments represent volumes. In order to relate volume and weight, an assumption was made that density of each PBK model compartment is equal to 1 kg/L.

Table 1 Description of fixed, literature-based input parameters used in all three PBK models.

Parameter	Value [unit]	Description	Reference
bwRef	450 [kg]	Reference body weight (without GI-tract content) used for allometric scaling of cardiac output	(Hoogenboom et al., 2010)
rV_{blood}	0.093 [-]	Weight fraction of the blood compartment with respect to BW(t)	(Derks et al., 1993)
rV_{fat}	0.135 [-]	Average weight fraction of the fat compartment with respect to BW(t)	(Traag et al., 2006)
rV_{liver}	0.019 [-]	Weight fraction of the liver compartment with respect to BW(t)	(Derks et al., 1993)
rV_{rich}	0.069 [-]	Weight fraction of the richly perfused organ compartment with respect to BW(t)	(Derks et al., 1993)
rV_{slow}	0.684 [-]	Weight fraction of the slowly perfused organ compartment with respect to BW(t)	(Derks et al., 1993)
F_q	3 [-]	Diffusion limiting flow factor in the adipose tissue	(Derks et al., 1993)
P_{fat}	280 [-]	Fat-blood partition coefficient	(Derks et al., 1993)
P_{liver}	23 [-]	Liver-blood partition coefficient	(Derks et al., 1993)
$P_{richly\ perfused}$	4 [-]	Richly perfused organ-blood partition coefficient	(Derks et al., 1993)
$P_{slowly\ perfused}$	8 [-]	Slowly perfused organ-blood partition coefficient	(Derks et al., 1993)
rQ_{fat}	0.038 [-]	Fraction of the cardiac output going to the fat compartment	(Derks et al., 1993)
rQ_{liver}	0.458 [-]	Fraction of the cardiac output going to the liver compartment	(Derks et al., 1993)
rQ_{rich}	0.304 [-]	Fraction of the cardiac output going to the richly perfused organ compartment	(Derks et al., 1993)
rQ_{slow}	0.200 [-]	Fraction of the cardiac output going to the slowly perfused organ compartment	(Derks et al., 1993)

Table 2 Description of input parameters used in the lactating cow PBK model.

Parameter	Value [unit]	Description	Reference
bwLifeMean	700 [kg]	The total mean weight of an average "Rode Geus" cow	(Hoch et al., 2005) Observed reference value.
WGI	175 [kg]	Weight of the contents of the GI-tract (=25% of bwLifeMean)	Derived from (Derks et al., 1994)
fatVariation	26 [kg]	Amplitude of the seasonal weight variation of the fat compartment	(Hoch et al., 2005) Observed reference value.
$q0_{milk}$	86500 [L/day]	Cardiac output of the cow during milk production at BW(t) = BWref (April 1st – October). NB: this parameter is only used in the lactating cow model	(Derks et al., 1993) Allometry.
$q0_{dry}$	72600 [L/day]	Cardiac output of the cow outside of the milk production period at BW(t) = BWref. This is the period between April 1st – October	(Derks et al., 1993) Allometry
milkProd	7.5 [L/day]	Milk production	(Zeilmaker et al., 2013) Assumption
fatPerc	4.4 [%]	Fat percentage in milk	(Traag et al., 2006) Observed reference value.
pMilkFat	460 [-]	Milk fat partition coefficient	(Hoogenboom et al., 2010) Model calibration
kMet	36 [1/day]	Metabolic hepatic rate constant	(Hoogenboom et al., 2010) Model calibration for adult non-lactating cattle

Table 3 Description of input parameters used in the non-lactating cattle PBK model.

Parameter	Value [unit]	Description	Reference
bwLifeMean	714 [kg]	The total mean weight of an average Rode Geus bull	(Piedrafita et al., 2003) Observed reference value.
WGI	178 [kg]	Weight of the contents of the GI-tract (=25% of bwLifeMean)	Derived from (Derks et al., 1994)
fatVariation	26 [kg]	Amplitude of the seasonal weight variation in the fat compartment	(Hoch et al., 2005) Observed reference value.
q0	72600 [L/day]	Cardiac output	(Derks et al., 1993) Allometry.
kMet	36 [1/day]	Metabolic hepatic rate constant	(Hoogenboom et al., 2010) Model calibration for adult non-lactating cattle.

Table 4 Description of fixed, literature-based input parameters used in the growing calf PBK model.

Parameter	Value [unit]	Description	Reference
fWGI	0.25 [-]	GI-tract content weight fraction of total body weight	Assumption based on Derks et al. (1993)
q0	72600 [L/day]	Cardiac output	(Derks et al., 1993) Allometry.
kMet	14 [1/day]	Metabolic hepatic rate constant	(Derks et al., 1993) Derived from young beef cattle.

5 Implementation details

The PBK model simulations were developed and run using the R modelling language and using a dedicated PBK development package called mrgSolve. Finally, the package ggplot2 was used to visualize the simulated results. Specifications on the programming packages are listed below:

Name software: R (v. 4.0.5)
Manufacturer: The R Foundation for Statistical Computing
Place of manufacture: online
Year of manufacture: 2021
Description: A programming language for statistical computing

Name software: mrgsolve (0.11.1)
Manufacturer: Kyle T Baron
Place of manufacture: online
Year of manufacture: 2021
Description: An R package that allows solving the ODEs required in the PBK models
url: https://CRAN.R-project.org/package=mrgsolve

Name software: ggplot2
Manufacturer: Hadley Wickham
Place of manufacture: Springer-Verlag New York
Year of manufacture: 2016
Description: An R package that allows visualization of results
url: https://ggplot2.tidyverse.org

6 Simulation of the intended scenario

The scenario described in this document involves a typical herd of cattle (i.e., lactating cows, beef cattle and growing calves) grazing on the floodplains. Adult cattle only take up TEQ (dioxins and DL-PCBs) through grass and adhering soil. The intake of TEQ through eating leaves from other plants and small trees was not taken into account. The only route of TEQ intake for growing calves (< 6 months) was assumed to be from drinking cow's milk. After the six months, the intake of TEQ by calves was assumed to be solely from grass and soil as the adult cattle, but the intake of grass and adhering soil was scaled relatively to their bodyweight.

For adult cattle and 6-12 month-old calves, the intake of TEQ through grass and soil was calculated as follows:

$$TEQ_{in} = I_{grass} \times c_{grass} \times f_{grass} + I_{soil} \times c_{soil} \times f_{soil} \quad \textbf{28).}$$

where I_{grass} and I_{soil} denote the daily grass and soil intake (kg 100% dry matter), respectively. c_{grass} and c_{soil} represent the TEQ concentration in grass and soil respectively, and f_{grass} and f_{soil} indicate the absorbed fraction of TEQ from grass and soil, respectively.

The intake of soil (I_{soil}) is calculated as a fraction of the intake of grass (4% of dry matter) (assumption, Traag et al. 2006):

$$I_{soil} = 0.04 \times I_{grass} \quad \textbf{29).}$$

The concentration of TEQ in grass varies over the year. Due to the growth of grass during summer (including spring), the concentration of TEQ is diluted, i.e. it decreases. Hence, the concentration of TEQ in grass was calculated as:

$$c_{grass}(t) = \begin{cases} \left(min\left(c_{grass,min} \times e^{k_{grass} \times (t - t_{winter} + 365)}, c_{grass,max}\right)\right) & t < t_{summer} \\ \left(max\left(c_{grass,max} \times e^{-k_{grass} \times (t - t_{summer})}, c_{grass,min}\right)\right) & t > t_{summer} \;\&\; t < t_{winter} \\ \left(min\left(c_{grass,min} \times e^{k_{grass} \times (t - t_{winter})}, c_{grass,max}\right)\right) & t > t_{winter} \end{cases} \quad \textbf{30).}$$

Here, $c_{grass,max}$ and $c_{grass,min}$ denote the maximum and minimum TEQ concentrations in grass. k_{grass} is the experimentally determined exponential "dilution through growth" factor of TEQ in growing grass (Traag et al., 2006), t_{summer} is the start of the summer season (April 1st), t_{winter} is the start of the winter season (October 1st), and t indicates the number of days that have passed in the calendar year of

interest. Maximal concentrations were assumed on April 1st and minimal on October 1st.

For the growing calf model, TEQ intake was assumed to be solely from drinking milk the first six months. This intake was calculated by:

$$TEQ_{in} = I_{milk} \times c_{milk} \times f_{milk} \quad \textbf{31).}$$

where I_{milk} is the daily milk intake, c_{milk} is the TEQ concentration in the milk, and f_{milk} is the absorbed fraction of TEQ from milk. Note that c_{milk} was derived from the simulated TEQ concentrations in milk of the lactating cow model. However, since the simulated TEQ concentrations in milk vary over time, the average TEQ concentration in milk during the lactating period (April 1st – October 1st) was used as c_{milk}. After the six months, the intake of TEQ by calves was assumed to be solely from grass and soil and was calculated as described above (Eq. 30).

7 Model evaluation

Assessment of the lactating cow model quality was performed using TEQ measurements in kidney fat and liver of cows from a floodplain near the Waal in Beuningen. In order to simulate the intake of TEQ, two different exposure scenarios were defined: a realistic scenario and a worst-case scenario. Defining these scenarios was performed based on TEQ measurements in grass and soil at the floodplain in Beuningen as described by Notenboom et al. (2021). The two scenarios are described below. Note that all TEQ concentrations in grass were converted and reported as 100% dry matter as this is needed as input for the models.

Realistic scenario
The maximum total TEQ concentration in grass was 0.7 ng/kg dry matter (from a sample collected in mid-November), and the minimum concentration applied was 0.3 ng/kg dry matter (from a sample collected in mid-April. The selected TEQ concentration in soil was 16.2 ng/kg (collected in February 2021). The TEQ concentration in soil is assumed to be constant over time.

Worst-case scenario
The worst-case scenario is the same as the realistic scenario, except for the maximum concentration of TEQ in grass ($c_{grass,max}$), for which a value of 9.70 ng/kg dry matter was applied. This concentration was found on the floodplains that had recently been flooded. As a result, the level in grass was strongly increased due to attached sludge, whereas the concentration in soil did not change. The selected TEQ concentration in soil was 16.2 ng/kg (collected in February 2021). The TEQ concentration in soil is assumed to be constant over time.

Finally, for both scenarios, the exponential dilution factor of TEQ in grass, k_{grass}, was 0.0231 day^{-1}, which was obtained from Traag et al. (2006). Furthermore, the applied absorption fraction of TEQ from grass was 0.25 (calibrated value, Zeilmaker et al., 2013), whereas that of soil was 0.43 (calibrated value, Hoogenboom et al., 2010). The intake of grass (I_{grass}) was assumed to be 15 kg dry matter per day, which amounts to 0.6 kg soil per day (Eq. 22) for adult cattle. These values were taken from Traag et al. (2006). For 6-12 months-old calves the same parameter values were used, except the intake of grass and (adhering soil) was scaled relatively to their growing body weight.

To simulate the effect of the transfer of the cattle to cleaner areas with lower concentrations of dioxins and DL-PCBs in grass and soil, the intake of TEQ was changed to a background level at April 1st of the third simulation year. This was done for both the realistic and the worst-case intake scenario. As background level, the maximum total TEQ concentration in grass was 0.49 ng/kg dry matter, and the minimum total TEQ concentration was 0.3 ng/kg dry matter. The total TEQ concentration applied for soil was 1.5 ng/kg dry matter. The total TEQ for grass and soil were both based on control samples collected across the river dyke in mid-November.

Sensitivity analysis

A (local) sensitivity analysis was performed to assess the influence of the model parameters on the TEQ accumulation. In contrast to global sensitivity analyses, a local sensitivity analysis determines the impact of model parameters on the simulation outcome in the vicinity of the chosen parameter values. In addition, the impact of each model parameter is assessed independently from other model parameters. For the present PBK models, local sensitivity analyses were performed based on TEQ concentrations in muscle fat. The sensitivity of a model parameter on the outcome was computed based on the area under the curve (AUC) of the TEQ concentration in muscle fat over time. Specifically, the sensitivity was calculated in the form of an elasticity coefficient S:

$$S = \frac{\Delta AUC/AUC}{\Delta p/p}, \qquad \textbf{25).}$$

where AUC is the area under the curve corresponding with a parameter value p, and ΔAUC is the change in the AUC as a result of a change of the parameter value Δp. Here, the applied change in the parameter values was always 10% of the original value, which means that $\frac{\Delta p}{p} = 1.1$. The higher the sensitivity, the more influential a model parameter is to the model outcome. The results of the sensitivity analyses can be found in Chapter 8.

Verification of the lactating cow model was performed using TEQ measurements of kidney fat and the liver. Specifically, the lactating cow model was used to simulate TEQ concentrations in muscle fat and the liver following the realistic exposure scenario and the worst-case scenario.

8 Results

Figure 4 shows the total simulated TEQ concentration in the muscle fat (A) and liver (B) of lactating cows for the realistic scenario and the worst case scenario based on TEQ measurements in grass and soil at the floodplains in Beuningen. The results shown in this figure show that the measured TEQ concentrations in muscle fat and the liver of three lactating cows that grazed on the floodplains near Beuningen resemble the worst-case scenario simulated with the cow model.

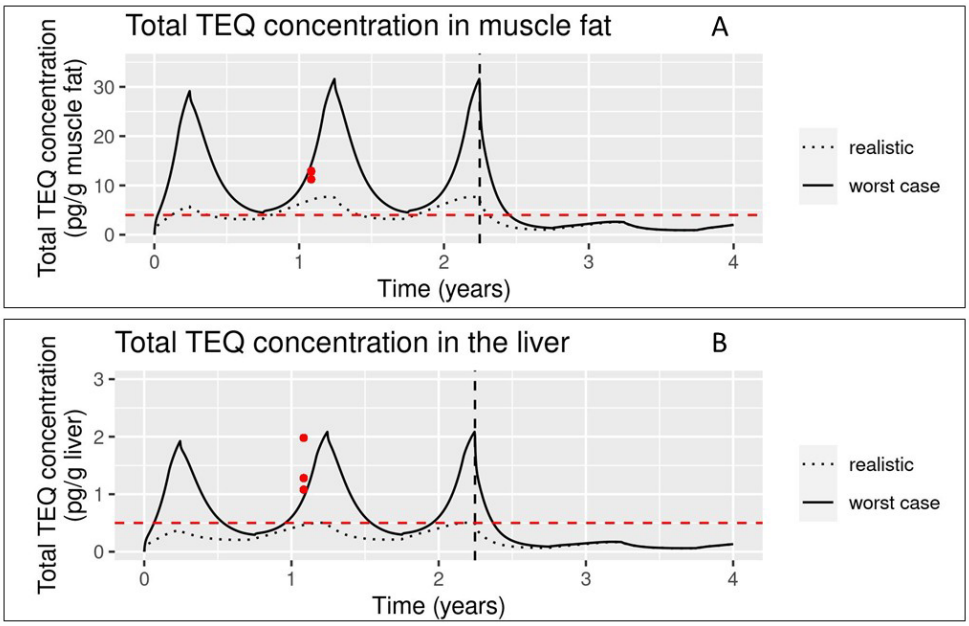

Figure 4 The simulated total TEQ concentration in muscle fat (A) and the liver (B) of lactating cows for the whole simulation duration (=4 years) for the realistic (black dashed line) and worst-case intake scenario (solid black line). Lactation occurred in each year for six months, starting in April. Red dots represent measured values in kidney fat (n=3) (2 measurements overlap) and liver (n=3). At t=820 days a move to cleaner areas was simulated as indicated by the black vertical dashed line). The maximum level for total TEQ in muscle fat and liver is indicated by the red horizontal dashed line.

In addition to the comparison of the model simulation to TEQ concentration measurements, model parameters sensitivity analyses were also performed. The sensitivity analyses were performed separately for each of the three PBK models, and are shown in Figures 5, 6 and 7. A positive elasticity coefficient means that an increase in the parameter value also increases the AUC, and thus increases the total simulated TEQ concentration in muscle fat, over time. In contrast, a negative elasticity coefficient represents that an increase in the parameter value decreases the AUC. Finally, larger values indicate a larger influence of the parameter value on the AUC.

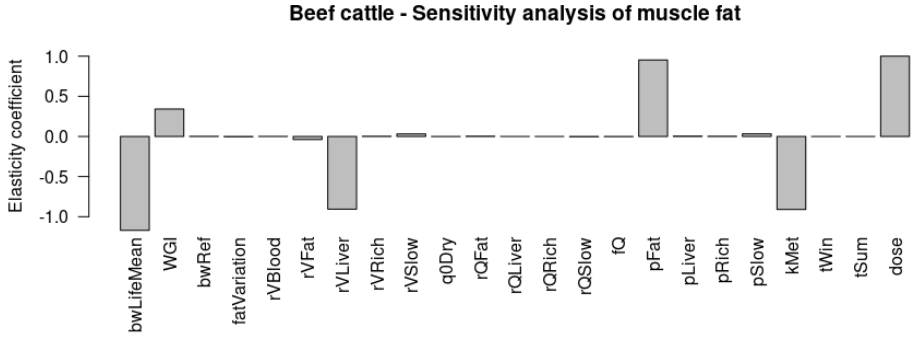

Figure 5 Sensitivity of the beef cattle model parameters on the total TEQ concentration in muscle fat.

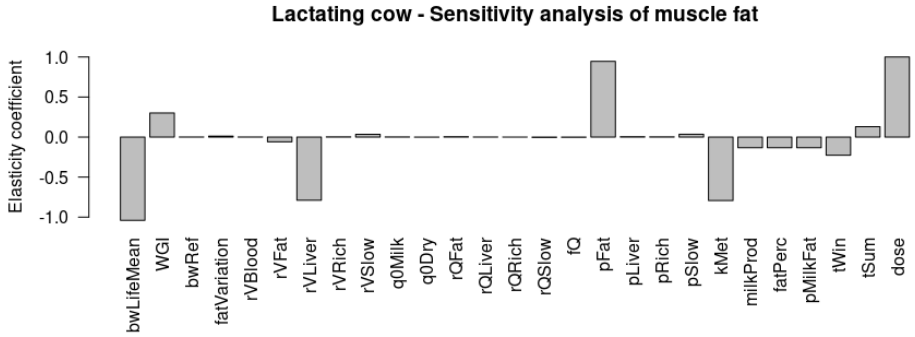

Figure 3 Sensitivity of the lactating cow model parameters on the total TEQ concentration in muscle fat.

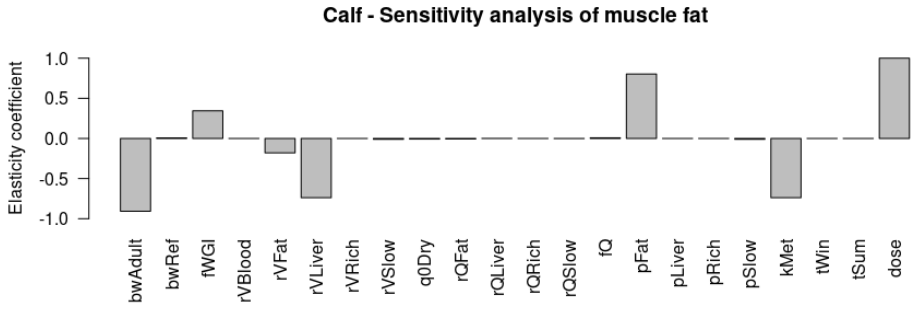

Figure 7 Sensitivity of the growing calf model parameters on the total TEQ concentration in muscle fat.

Unsurprisingly, the TEQ dose taken in from feed is an influential parameter, as the accumulated TEQ in muscle fat almost linearly scales with the dose (i.e., elasticity coefficient of approximately 1). Another parameter that is expected to heavily influence the simulated TEQ levels is the body weight. The higher the body weight, the lower the TEQ concentration in muscle fat. The reason for this is that the volume of the muscle fat compartment linearly increases with body weight, thus explaining the negative correlation (i.e., elasticity coefficient of approximately -1).

In all three models, the rVLiver, pFat and kMet strongly affect the simulated TEQ levels. The rVLiver determines the size of the liver compartment, and pFat the fat-blood partition coefficient. The rVLiver value was previously used by Derks et al. (1993) and is considered to be accurate since similar liver weights have been reported in literature (Berende, P.L.M., 1998).

kMet is the hepatic clearance rate. A higher hepatic clearance rate reduces the accumulation of TEQ in the liver, and subsequently reduces the TEQ levels in muscle fat. Because of this strong influence on the model outcome, kMet should be determined accurately. In the model described in this document, a kMet of 36 day^{-1} was used. This value was taken from (Hoogenboom et al., 2010). Since this value was calibrated for lactating cows, it is unclear whether it is also accurate for the beef cattle model. For the kMet used for the growing calve model it is also unclear, because this was based on an experiment with young beef cattle (Derks et al., 1993). This uncertainty should be considered when interpreting the PBK model simulations.

Interestingly, q0Dry and q0Milk only have a minor effect on the TEQ concentration in muscle fat. As a result, also the bwRef, which was used to scale the q0's, has only a minor influence on the TEQ concentration. Uncertainties and assumptions on these parameter values are therefore expected to not affect the validity of the three models.

In contrast, bwLifeMean and WGI have a larger influence on the TEQ concentrations in muscle fat. This can be explained by the fact that an

increase in the bwLifeMean would increase the sizes of the model compartments, thus decreasing the concentration of TEQ by dilution. Hence, an increase in bwLifeMean reduces the TEQ concentration in muscle fat. In contrast, an increase in the WGI would result in an increased concentration of TEQ in muscle fat, since an increased WGI directly reduces the sizes of the remaining model compartments.

The fatVariation parameter (i.e., the amplitude of the fat compartment weight variation) also has a limited influence on the TEQ concentration in muscle fat. However, here it is important to recall that the sensitivity was measured based on the AUC. Although the AUC was hardly affected by the fatVariation parameter since it is based on a sinusoidal wave, the TEQ concentration over time was certainly influenced by this parameter. This finding shows that the interpretation of this sensitivity analyses must be performed with care.

9 Discussion of the regulatory application

The PBK models presented in this report are built upon previously developed and optimized PBK models (Derks et al., 1993; Derks et al., 1994; Hoogenboom et al., 2010). The validity and reliability of these PBK models have been verified in several case studies (Hoogenboom et al., 2010; Traag et al., 2006; Zeilmaker et al., 2013). In addition, the simulation of the TEQ concentration in muscle fat and liver of lactating cows was in good agreement with the TEQ concentration measurements in kidney fat and liver of three lactating cows, based on the worst-case scenario. Additional features described in this report, namely, the growing calf model, and the seasonal variation of the fat compartment, have not yet been experimentally evaluated. The growth curve of the calves is fitted based on data of over 20,000 Saler calves, while the variations of the fat compartment are based on experimental body weight variations observed in cattle.

One of the assumptions used to create the beef cattle model was that beef cattle are physiologically the same as lactating cows without milk production. As previously described in section 8, this assumption might lead to underestimations of the TEQ concentration in beef cattle, since beef cattle typically have less body fat. Therefore, experimental measurements of the body composition of Rode Geus beef cattle could be performed to further refine the beef cattle model.

Another assumption described in this report was that the WGI is 25% of the bwLifeMean, and that the WGI is constant over time. The value of the WGI was derived from Derks et al. (1994). Their PBK model consisted of multiple compartment that added up to 450 kg, which was used to describe a lactating cow of 600 kg. The WGI of 25% is also similar to that used by Zeilmaker et al. (2013), who used a WGI of 140kg for lactating cows of 650 kg (i.e., 21.5%). It is largely unknown to which extent the value of 25% is accurate, and whether this value also holds for beef cattle or growing calves. In addition, variations in the WGI over time are not included in the models described in this report. These uncertainties should be considered when interpreting model simulations.

The sensitivity analyses (Fig. 5, 6 and 7) identified parameters that heavily influence the model predictions. One of these parameters is the hepatic clearance rate, *kMet*. In the adult PBK models described in this report, the *kMet* value (36 day^{-1}) was taken from (Hoogenboom et al. (2010). This value is significantly higher than that initially found by (Derks et al., 1994) (14 day^{-1}). The reason for this difference is that the *kMet* of 14 day^{-1} was based on young (200 kg) beef cattle. Hoogenboom et al. (2010) recalibrated the *kMet* for adult lactating cattle. The validity of this choice was experimentally confirmed. Nevertheless, it is important to stress the importance of finding an adequate estimate for kMet, as an overestimation of this parameter can lead to underestimations of the TEQ concentration in muscle fat.

It is extremely important to define the exposure scenario as accurately as possible. The daily total TEQ intake from grass and soil or milk is a major contributor to the simulated TEQ levels in muscle fat. In addition, the absorption fractions (i.e., fGrass, fSoil and fMilk), heavily affect the dose accumulated in muscle fat. Therefore, the cattle's grazing behaviour, absorption fractions, feed intake, and TEQ concentrations in feed should be estimated as precisely as possible.

All in all, the verification of the PBK model parameters and the first limited evaluation of the simulations with experimental data suggest that the lactating cow model can accurately simulate the TEQ concentration in muscle fat. The measured TEQ concentrations in muscle fat and liver fat closely resemble those simulated following a worst-case exposure scenario, while being relatively close to the concentrations simulated in the realistic exposure scenario. Nevertheless, additional information on the exposure scenario, and experimental TEQ concentration measurements in body fat of wild cattle are necessary to confirm this finding.

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Annex I R-Code meat cattle model simulation

Model code:

```
meatCattleSeasonalVariations<- '
$PARAM
@annotated
bwLifeMean  : 714     : life weight [kg];
WGI         : 178     : GI-tract content [kg]
bwRef       : 450     : referentie gewicht [kg];
fatVariation: 26      : Amplitude of seasonal variation in fat compartment
[kg];
rVBlood     : 0.093   : Blood volume [-];
rVFat   : 0.135   : relative fat volume [-];
rVLiver : 0.019   : relative liver volume [-];
rVRich  : 0.069   : relative richly perfused organs volume [-];
rVSlow  : 0.684   : relative slowly perfused organs volume [-];
q0Dry   : 72600   : cardiac  output when no milk production [L/day];
rQFat   : 0.038   : relative blood flow fat compartment [-];
rQLiver : 0.458   : relative blood flow liver compartment [-];
rQRich  : 0.304   : relative blood flow richly perfused organs [-];
rQSlow  : 0.200   : relative blood flow slowly perfused organs [-];
fQ      : 3.      : diffusion limiting flow factor [-];
pFat    : 280.    : Fat partition coefficient [-];
pLiver  : 23.     : Liver partition coefficient [-];
pRich   : 4.      : Richly perfused organ partition coefficient [-];
pSlow   : 8.      : Slowly perfused organs partition coefficient [-];
kMet    : 36.     : Liver clearance [per day];
tWin    : 275.    : Start of winter [day]
tSum    : 90.     : Start of summer [day]

$GLOBAL
#define masBal (aBlood+aFat+aSlow+aRich+aLiver+aMet)

$MAIN
double vFat = -
fatVariation*sin(2*M_PI*(TIME)/365)+rVFat*(bwLifeMean-WGI);
double vBlood = (bwLifeMean-WGI)*rVBlood;
double vLiver = (bwLifeMean-WGI)*rVLiver;
double vRich = (bwLifeMean-WGI)*rVRich;
double vSlow = (bwLifeMean-WGI)*rVSlow;
double bw = vFat+vBlood+vLiver+vRich+vSlow;

double clL = (vLiver*kMet);

double qF      = rQFat*qC;
double qffq    = qF/fQ;
double qS      = rQSlow*qC;
double qR      = rQRich*qC;
double qL      = rQLiver*qC;
double qC      = q0Dry*pow((bw/bwRef),0.75);
```

```
$INIT @annotated
aBlood  : 0 : blood;
aFat    : 0 : fat;
aLiver  : 0 : liver;
aRich   : 0 : richly perfused organs;
aSlow   : 0 : slowly perfused organs;
aMet    : 0 : metabolised particles;

$ODE
double cBlood   = aBlood/vBlood;
double cFat     = aFat/vFat;
double cSlow    = aSlow/vSlow;
double cRich    = aRich/vRich;
double cLiver   = aLiver/vLiver;
double cMeatFat = cSlow/(pSlow*0.8/pFat);

dxdt_aBlood = -qffq*(cBlood-cFat/pFat)-qS*(cBlood-cSlow/pSlow)-
qR*(cBlood-cRich/pRich)-qL*(cBlood-cLiver/pLiver);
dxdt_aFat = qffq*(cBlood-cFat/pFat);
dxdt_aSlow = qS*(cBlood-cSlow/pSlow);
dxdt_aRich = qR*(cBlood-cRich/pRich);
dxdt_aLiver = qL*(cBlood-cLiver/pLiver)-clL*cLiver/pLiver;
dxdt_aMet  = clL*cLiver/pLiver;

$CAPTURE
cFat
cLiver
cBlood
cSlow
cRich
cMeatFat
masBal
vBlood
vFat
vRich
vSlow
vLiver
bw
'
```

Simulation code:

```
############################
# Dose events for meat cow
############################
library(ggplot2)
library(mrgsolve)
library(stringr)

source('models/meatCattleModelSeasonalVariation.R')

simMeatCattle <- function(grass, soil, scenario, tclean) {
  # intake default assumptions
  iGrass    <- 15           # Average grass intake [kg/day];
```

```
  fGrass    <- 0.25          # Fraction of dioxin absorbed from grass [-]
  tHGrass   <- 30            # half time decay of dioxin concentration in
grass [day];
  kGrass    <- log(2)/tHGrass # exponential growth factor [per day]
  cGrassMax <- grass         # Maximum dioxin concentration in grass
[ng/kg dry matter]
  cGrassMin <- 0.3           # Minimum dioxin concentration in grass
[ng/kg dry matter]

  iSoilFraction <- 0.04      # soil intake (as a fraction of grass intake) [-
]
  fSoil <- 0.43              #  fraction of dioxin in soil absorbed [-]
  iSoil <- iSoilFraction * iGrass # soil intake [kg/day]
  cSoil0 <- soil           # contamination of dioxin in soil [ng/kg]

  simulationTime <- 365*4    # duration of simulation [day]
  tSum    <- 90              # start of summer [day];
  tWin    <- 275             # start of winter [day];
  tClean  <- tclean            # start of cows on clean food, hence no
dioxin intake [day];

  # Create dose event
  doseEvent <- ev(amt=0, cmt='aLiver', addl=simulationTime, ii=1)
%>% realize_addl()
  doseTiming <- seq(0,simulationTime)%%365

  # Calculate dioxin intake per day through grass in the specified
scenario
  cGrass <- pmin(cGrassMin*exp(kGrass*(doseTiming+(365-tWin))),
cGrassMax)
  cGrass[doseTiming>tSum & doseTiming<=tWin] <-
pmax(cGrassMax*exp(-kGrass*(doseTiming-
tSum)),cGrassMin)[doseTiming>tSum & doseTiming<=tWin]
  cGrass[doseTiming>=tWin] <-
pmin(cGrassMin*exp(kGrass*(doseTiming-tWin)),
cGrassMax)[doseTiming>=tWin]
  # Calculate dioxin intake per day through grass in a reference scenario
(used when t>tClean)
  cGrassRef <- pmin(cGrassMin*exp(kGrass*(doseTiming+(365-tWin))),
0.492)
  cGrassRef[doseTiming>tSum & doseTiming<=tWin] <-
pmax(0.492*exp(-kGrass*(doseTiming-
tSum)),cGrassMin)[doseTiming>tSum & doseTiming<=tWin]
  cGrassRef[doseTiming>=tWin] <-
pmin(cGrassMin*exp(kGrass*(doseTiming-tWin)),
0.492)[doseTiming>=tWin]

  # After tClean, cGrass becomes cGrassRef
  cGrass[seq(0,simulationTime)>=tClean]<-
cGrassRef[seq(0,simulationTime)>=tClean]
```

```
  # dioxin intake per day through soil
  cSoil <- rep(cSoil0, length(cGrass))

  # After tClean, cSoil becomes cGrassRef
  cSoilRef <- rep(1.54, length(cGrass))
  cSoil[seq(0,simulationTime)>=tClean] <-
cSoilRef[seq(0,simulationTime)>=tClean]

  # Create dose event: Once a day, the cattle take up the specified dose
  inGrass <- fGrass*iGrass*cGrass
  inSoil <- fSoil*iSoil*cSoil
    dose <- inGrass+inSoil

  doseEvent@data$amt <- dose
  doseEvent@data$rate <- dose

  # Load meat cow model
  vleeskoeModel <- mcode("meatCow", meatCattleSeasonalVariations)
  sim <- vleeskoeModel %>% ev(doseEvent) %>% mrgsim()

    # Save results
  saveRDS(inGrass,
paste(paste("results/updated_results/simResults/inGrass_", scenario,
sep=""), ".rds",sep=""))
  saveRDS(inSoil,
paste(paste("results/updated_results/simResults/inSoil_", scenario,
sep=""), ".rds",sep=""))
  saveRDS(sim,
paste(paste("results/updated_results/simResults/meatCattle_simulation
_", scenario, sep=""), ".rds",sep=""))
}

tClean <- 365*2+90
simMeatCattle(grass=0.492, soil=1.54, scenario="reference",
tclean=tClean)
simMeatCattle(grass=0.7, soil=16.20, scenario="realistic",
tclean=tClean)
simMeatCattle(grass=9.70, soil=16.20, scenario="worstcase",
tclean=tClean)

####### Visualize data ################
sim_reference <-
readRDS("results/updated_results/simResults/meatCattle_simulation_re
ference.rds")
sim_realistic <-
readRDS("results/updated_results/simResults/meatCattle_simulation_re
alistic.rds")
sim_worstcase <-
readRDS("results/updated_results/simResults/meatCattle_simulation_w
orstcase.rds")
```

```
inGrass_realistic <-
readRDS("results/updated_results/simResults/inGrass_reference.rds")
inSoil_realistic <-
readRDS("results/updated_results/simResults/inSoil_reference.rds")
inGrass_realistic <-
readRDS("results/updated_results/simResults/inGrass_realistic.rds")
inSoil_realistic <-
readRDS("results/updated_results/simResults/inSoil_realistic.rds")
inGrass_worstcase <-
readRDS("results/updated_results/simResults/inGrass_worstcase.rds")
inSoil_worstcase <-
readRDS("results/updated_results/simResults/inSoil_worstcase.rds")

# Calculate how long it takes until concentrations drop below the dioxin
TEQ norm of 4 ng/kg
t_realistic <-
which.max((as.data.frame(sim_realistic)[,"cMeatFat"]<4)[- (1:tClean)])
t_worstcase <-
which.max((as.data.frame(sim_worstcase)[,"cMeatFat"]<4)[-
(1:tClean)])

# plot concentration of dioxin in meat fat
simMeatLoevesteinPlot <- ggplot(as.data.frame(sim_realistic),
aes(x=time/(365), y=cMeatFat)) +
  geom_line(linetype="dotted") +
  geom_line(data = as.data.frame(sim_worstcase), aes(x=time/(365),
y=cMeatFat, linetype="solid")) +
  scale_linetype_manual(name = "",
                        values = rep(c("dotted"="dotted", "solid"="solid")),
                        labels=c("realistic", "worst case")) +
  ggtitle("Total TEQ concentration in meat fat") +
  xlab("Time (years)") +
  ylab(str_wrap("Total TEQ concentration (pg/g meat fat)", 23)) +
  geom_vline(xintercept=tClean/365, linetype="dashed", color =
"black") +
  geom_hline(yintercept=4, linetype="dashed", color = "red")

# plot concentration of TEQ in liver
simLiverPlot <- ggplot(as.data.frame(sim_realistic), aes(x=time/(365),
y=cLiver)) +
  geom_line(linetype="dotted") +
  geom_line(data = as.data.frame(sim_worstcase), aes(x=time/(365),
y=cLiver, linetype="solid")) +
  scale_linetype_manual(name = "",
                        values = rep(c("dotted"="dotted", "solid"="solid")),
                        labels=c("realistic", "worst case")) +
  ggtitle("Total TEQ concentration in the liver") +
  xlab("Time (years)") +
  ylab(str_wrap("Total TEQ concentration (pg/g liver)", 23)) +
  ylim(0,3) +
```

```
  geom_vline(xintercept=tClean/365, linetype="dashed", color =
"black") +
  geom_hline(yintercept=0.5, linetype="dashed", color = "red")

# plot intake of dioxins through grass and soil
intake <- data.frame(time=rep(seq(1:1461), 4),
                     val=c(inGrass_realistic,inGrass_worstcase,
inSoil_realistic, inSoil_worstcase),
                     intake=c(rep("grass", length(inGrass_realistic)*2),
rep("soil", length(inGrass_realistic)*2)),
                     type=c(rep("realistic",length(inGrass_realistic)),
rep("worst case",length(inGrass_realistic)),
rep("realistic",length(inGrass_realistic)), rep("worst
case",length(inGrass_realistic))))

intakePlot <- intake %>% ggplot() +
  geom_line(aes(x=time/(365), y=val, color=interaction(intake, type),
linetype=interaction(intake, type))) +
  scale_color_manual(name = "Intake scenario", values = rep(c("blue",
"blue", "red", "red"), times = 2)) +
  scale_linetype_manual(name = "Intake scenario", values =
rep(c(1,2,1,4))) +
  xlab("Time (years)") +
  ylab(str_wrap("Daily total TEQ intake (ng/day)", 26)) +
  ggtitle("Total TEQ intake through grass and soil")

# plot body weight
bodyWeightPlot <- as.data.frame(sim_realistic) %>% ggplot() +
  geom_line(aes(x=time/(365), y=bw)) +
  geom_line(aes(x=time/(365), y=vFat, linetype="dotted")) +
  scale_linetype_manual(name = "",
                        values = rep(c("solid"="solid", "dotted"="dotted")),
                        labels=c("Total body weight", "Fat compartment")) +
  ggtitle("Body weight variation") +
  xlab("Time (years)") +
  ylab("Weight (kg)")

### Save images ###
ggsave("results/updated_results/reportFigs/meatCattle/simMeat.jpg",
       plot=simMeatPlot,
       unit="mm",
       width=150,
       height=50)

ggsave("results/updated_results/reportFigs/meatCattle/simLoevesteinM
eat.jpg",
       plot=simMeatLoevesteinPlot,
       unit="mm",
       width=150,
       height=50)
```

```
ggsave("results/updated_results/reportFigs/meatCattle/simMeatZoom.jp
g",
      plot=simZoomMeatPlot,
      unit="mm",
      width=150,
      height=50)

ggsave("results/updated_results/reportFigs/meatCattle/simLiver.jpg",
      plot=simLiverPlot,
      unit="mm",
      width=150,
      height=50)

ggsave("results/updated_results/reportFigs/meatCattle/simLiverLoevest
ein.jpg",
      plot=simLiverLoevesteinPlot,
      unit="mm",
      width=150,
      height=50)
ggsave("results/updated_results/reportFigs/meatCattle/Intake.jpg",
      plot=intakePlot,
      unit="mm",
      width=150,
      height=50)

ggsave("results/updated_results/reportFigs/meatCattle/Body
weight.jpg",
      plot=bodyWeightPlot,
      unit="mm",
      width=150,
      height=50)
```

Annex II R-Code lactating cow model simulation

Model code:

```
dairyCattleSeasonalVariations<- '
$PARAM
@annotated
bwLifeMean  : 700     : life weight [kg];
WGI         : 175     : GI-tract content [kg]
bwRef       : 450     : referentie gewicht(?) [kg];
fatVariation: 26      : Amplitude of seasonal variation in fat compartment
[kg];
rVBlood     : 0.093   : Blood volume [-];
rVFat   : 0.135   : relative fat volume [-];
rVLiver : 0.019   : relative liver volume [-];
rVRich  : 0.069   : relative richly perfused organs volume [-];
rVSlow  : 0.684   : relative slowly perfused organs volume [-];
q0Milk  : 86500   : cardiac output during milk production [L/day];
q0Dry   : 72600   : cardiac  output when no milk production [L/day];
rQFat   : 0.038   : relative blood flow fat compartment [-];
rQLiver : 0.458   : relative blood flow liver compartment [-];
rQRich  : 0.304   : relative blood flow richly perfused organs [-];
rQSlow  : 0.200   : relative blood flow slowly perfused organs [-];
fQ      : 3.      : diffusion limiting flow factor [-];
pFat    : 280.    : Fat partition coefficient [-];
pLiver  : 23.     : Liver partition coefficient [-];
pRich   : 4.      : Richly perfused organ partition coefficient [-];
pSlow   : 8.      : Slowly perfused organs partition coefficient [-];
kMet    : 36.     : Liver clearance [per day];
milkProd: 7.5       : Milk production [L/day];
fatPerc : 4.4     : Fat percentage in milk [%];
pMilkFat: 460.    : Milk-fat partition coefficient [-];
tWin    : 275.    : Start of winter [day]
tSum    : 90.     : Start of summer [day]

$GLOBAL
#define masBal (aBlood+aFat+aSlow+aRich+aLiver+aMet)

$MAIN
double vFat = -
fatVariation*sin(2*M_PI*(TIME)/365)+rVFat*(bwLifeMean-WGI);
double vBlood = (bwLifeMean-WGI)*rVBlood;
double vLiver = (bwLifeMean-WGI)*rVLiver;
double vRich = (bwLifeMean-WGI)*rVRich;
double vSlow = (bwLifeMean-WGI)*rVSlow;
double bw = vFat+vBlood+vLiver+vRich+vSlow;
double clM = milkProd*fatPerc/100;
```

```
double qC      = 0;
double clB     = 0;
double milkOn   = 0;

if((int(TIME)%(365)<tWin) && (int(TIME)%(365)>=tSum)) {
  qC      = q0Milk*pow((bw/bwRef),0.75);
  clB     = pMilkFat*clM;;
  milkOn   = 1;
}
else {
  qC      = q0Dry*pow((bw/bwRef),0.75);
  clB     = 0;
  milkOn   = 0;
}

double qF      = rQFat*qC;
double qffq    = qF/fQ;
double qS      = rQSlow*qC;
double qR      = rQRich*qC;
double qL      = rQLiver*qC;
double clL     = (vLiver*kMet);

$INIT @annotated
aBlood  : 0 : blood;
aFat    : 0 : fat;
aLiver  : 0 : liver;
aRich   : 0 : richly perfused organs;
aSlow   : 0 : slowly perfused organs;
aMet    : 0 : metabolised particles;
aMilk   : 0 : milk;

$ODE
double cBlood   = aBlood/vBlood;
double cFat     = aFat/vFat;
double cSlow    = aSlow/vSlow;
double cRich    = aRich/vRich;
double cLiver   = aLiver/vLiver;
double cMilk    = clB*cBlood/milkProd;
double cMeatFat = cSlow/(pSlow*0.8/pFat);

dxdt_aBlood = -qffq*(cBlood-cFat/pFat)-qS*(cBlood-cSlow/pSlow)-
qR*(cBlood-cRich/pRich)-qL*(cBlood-cLiver/pLiver)-clB*cBlood;
dxdt_aFat = qffq*(cBlood-cFat/pFat);
dxdt_aSlow = qS*(cBlood-cSlow/pSlow);
dxdt_aRich = qR*(cBlood-cRich/pRich);
dxdt_aLiver = qL*(cBlood-cLiver/pLiver)-clL*cLiver/pLiver;
dxdt_aMet  = clL*cLiver/pLiver;
dxdt_aMilk = clB*cBlood;
```

```
$CAPTURE
cFat
cLiver
cBlood
cSlow
cRich
cMilk
cMeatFat
masBal
vBlood
vFat
vRich
vSlow
vLiver
bw
```

Simulation code:

```
################################
# Dose events for dairy cattle #
################################
library(ggplot2)
library(mrgsolve)
library(stringr)

source('models/dairyCattleModelSeasonalVariation.R')

simDairyCattle <- function(grass, soil, scenario,  tclean) {
  # intake default assumptions
  iGrass    <- 15            # Average grass intake [kg/day];
  fGrass    <- 0.25          # Fraction of dioxin absorbed from grass [-]
  tHGrass   <- 30            # half time decay of dioxin concentration in
grass [day];
  kGrass    <- log(2)/tHGrass # exponential growth factor [per day]
  cGrassMax <- grass         # Maximum dioxin concentration in grass
[ng/kg dry matter];
    cGrassMin <- 0.3         # Minimum dioxin concentration in grass
[ng/kg dry matter]

  iSoilFraction <- 0.04      # soil intake (as a fraction of grass intake) [-
]
  fSoil <- 0.43              #  fraction of dioxin in soil absorbed [-]
  iSoil <- iSoilFraction * iGrass # soil intake [kg/day]
  cSoil0<- soil              # contamination of dioxin in soil [ng/kg];

   simulationTime <- 365*4     # duration of simulation [day]
  tSum    <- 90              # start of summer [day];
  tWin    <- 275             # start of winter [day];
  tClean  <- tclean          # start of cows on clean food, hence no dioxin
intake [day];
```

```
  # Create dose event
  doseEvent <- ev(amt=0, rate=0, cmt='aLiver', addl=simulationTime,
ii=1) %>% realize_addl()
  doseTiming <- seq(0,simulationTime)%%365

  # Calculate dioxin intake per day through grass in the specified
scenario
  cGrass <- pmin(cGrassMin*exp(kGrass*(doseTiming+(365-tWin))),
cGrassMax)
  cGrass[doseTiming>tSum & doseTiming<=tWin] <-
pmax(cGrassMax*exp(-kGrass*(doseTiming-
tSum)),cGrassMin)[doseTiming>tSum & doseTiming<=tWin]
  cGrass[doseTiming>=tWin] <-
pmin(cGrassMin*exp(kGrass*(doseTiming-tWin)),
cGrassMax)[doseTiming>=tWin]
    # Calculate dioxin intake per day through grass in a reference
scenario (used when t>tClean)
  cGrassRef <- pmin(cGrassMin*exp(kGrass*(doseTiming+(365-tWin))),
0.492)
  cGrassRef[doseTiming>tSum & doseTiming<=tWin] <-
pmax(0.492*exp(-kGrass*(doseTiming-
tSum)),cGrassMin)[doseTiming>tSum & doseTiming<=tWin]
  cGrassRef[doseTiming>=tWin] <-
pmin(cGrassMin*exp(kGrass*(doseTiming-tWin)),
0.492)[doseTiming>=tWin]

  # After tClean, cGrass becomes cGrassRef
  cGrass[seq(0,simulationTime)>=tClean]<-
cGrassRef[seq(0,simulationTime)>=tClean]

  # dioxin intake per day through soil
  cSoil <- rep(cSoil0, length(cGrass))

  # After tClean, cSoil becomes cGrassRef
  cSoilRef <- rep(1.54, length(cGrass))
  cSoil[seq(0,simulationTime)>=tClean] <-
cSoilRef[seq(0,simulationTime)>=tClean]

  # Create dose event: Once a day, the cattle take up the specified dose
  inGrass <- fGrass*iGrass*cGrass
  inSoil <- fSoil*iSoil*cSoil

  dose <- inGrass+inSoil

  doseEvent@data$amt <- dose
  doseEvent@data$rate <- dose

  # Load milk cow model
  melkkoeModel <- mcode("milkCow", dairyCattleSeasonalVariations)
  sim <- melkkoeModel %>% ev(doseEvent) %>% mrgsim()
```

```
  # Save results
  saveRDS(inGrass,
paste(paste("results/updated_results/simResults/inGrass_", scenario,
sep=""), ".rds",sep=""))
  saveRDS(inSoil,
paste(paste("results/updated_results/simResults/inSoil_", scenario,
sep=""), ".rds",sep=""))
  saveRDS(sim,
paste(paste("results/updated_results/simResults/dairyCattle_simulation
_", scenario, sep=""), ".rds",sep=""))

}

tClean <- 365*2+90
simDairyCattle(grass=0.492, soil=1.54, scenario="reference",
tclean=tClean)
simDairyCattle(grass=0.7, soil=16.20, scenario="realistic",
tclean=tClean)
simDairyCattle(grass=9.70, soil=16.20, scenario="worstcase",
tclean=tClean)


####### Visualize data ################
sim_reference <-
as.data.frame(readRDS("results/updated_results/simResults/dairyCattle
_simulation_reference.rds"))
sim_realistic <-
as.data.frame(readRDS("results/updated_results/simResults/dairyCattle
_simulation_realistic.rds"))
sim_worstcase <-
as.data.frame(readRDS("results/updated_results/simResults/dairyCattle
_simulation_worstcase.rds"))
inGrass_realistic <-
readRDS("results/updated_results/simResults/inGrass_reference.rds")
inSoil_realistic <-
readRDS("results/updated_results/simResults/inSoil_reference.rds")
inGrass_realistic <-
readRDS("results/updated_results/simResults/inGrass_realistic.rds")
inSoil_realistic <-
readRDS("results/updated_results/simResults/inSoil_realistic.rds")
inGrass_worstcase <-
readRDS("results/updated_results/simResults/inGrass_worstcase.rds")
inSoil_worstcase <-
readRDS("results/updated_results/simResults/inSoil_worstcase.rds")

# plot concentration of dioxin in meat fat (regular plot)
simMeatPlot <- ggplot(as.data.frame(sim_realistic), aes(x=time/(365),
y=cMeatFat)) +
  geom_line(linetype="dotted") +
```

```
  geom_line(data = as.data.frame(sim_worstcase), aes(x=time/(365),
y=cMeatFat, linetype="solid")) +
  geom_point(aes(x=1.083, y=12.80), color="red", size=1) +
  geom_point(aes(x=1.083, y=11.23), color="red", size=1) +
  geom_point(aes(x=1.083, y=12.93), color="red", size=1) +
  scale_linetype_manual(name = "",
                        values = rep(c("dotted"="dotted", "solid"="solid")),
                        labels=c("realistic", "worst case"),
  ) +
  ggtitle("Total TEQ concentration in meat fat") +
  xlab("Time (years)") +
  ylab(str_wrap("Total TEQ concentration (pg/g meat fat)", 23)) +
  ylim(0,35) +
  geom_vline(xintercept=tClean/365, linetype="dashed", color =
"black") +
  geom_hline(yintercept=4, linetype="dashed", color = "red")

# plot concentration of TEQ in liver (regular plot)
simLiverPlot <- ggplot(as.data.frame(sim_realistic), aes(x=time/(365),
y=cLiver)) +
  geom_line(linetype="dotted") +
  geom_line(data = as.data.frame(sim_worstcase), aes(x=time/(365),
y=cLiver, linetype="solid")) +
  geom_point(aes(x=1.083, y=1.08), color="red", size=1) +
  geom_point(aes(x=1.083, y=1.98), color="red", size=1) +
  geom_point(aes(x=1.083, y=1.28), color="red", size=1) +
  scale_linetype_manual(name = "",
                        values = rep(c("dotted"="dotted", "solid"="solid")),
                        labels=c("realistic", "worst case"),
  ) +
  ggtitle("Total TEQ concentration in the liver") +
  xlab("Time (years)") +
  ylab(str_wrap("Total TEQ concentration (pg/g liver)", 23)) +
  ylim(0,3) +
  geom_vline(xintercept=tClean/365, linetype="dashed", color =
"black") +
  geom_hline(yintercept=0.5, linetype="dashed", color = "red")

# plot concentration of dioxin in milk
milkPlot <- ggplot(as.data.frame(sim_realistic), aes(x=time/(365),
y=cMilk)) +
  geom_line(linetype="dotted")+
  geom_line(data = as.data.frame(sim_worstcase), aes(x=time/365,
y=cMilk, linetype="solid")) +
  scale_linetype_manual(name = "",
                        values = rep(c("dotted"="dotted", "solid"="solid")),
                        labels=c("realistic", "worst case"),
                        ) +
  ggtitle("Total TEQ concentration in milk") +
  xlab("Time (years)") +
```

```
  ylab(str_wrap("Daily total TEQ intake (ng/day)", 26)) +
  geom_vline(xintercept=tClean/365, linetype="dashed", color =
"black")

# plot intake of dioxins through grass and soil
intake <- data.frame(time=rep(seq(1:1461), 4),
                     val=c(inGrass_realistic,inGrass_worstcase,
inSoil_realistic, inSoil_worstcase),
                     intake=c(rep("grass", length(inGrass_realistic)*2),
rep("soil", length(inGrass_realistic)*2)),
                     type=c(rep("realistic",length(inGrass_realistic)),
rep("worst case",length(inGrass_realistic)),
rep("realistic",length(inGrass_realistic)), rep("worst
case",length(inGrass_realistic))))

intakePlot <- intake %>% ggplot() +
  geom_line(aes(x=time/(365), y=val, color=interaction(intake, type),
linetype=interaction(intake, type))) +
  scale_color_manual(name = "Intake scenario", values = rep(c("blue",
"blue", "red", "red"), times = 2)) +
  scale_linetype_manual(name = "Intake scenario", values =
rep(c(1,2,1,4))) +
  xlab("Time (years)") +
  ylab(str_wrap("Daily total TEQ intake (ng/day)", 26)) +
  ggtitle("Total TEQ intake through grass and soil")

# plot body weight
bodyWeightPlot <- as.data.frame(sim_realistic) %>% ggplot() +
  geom_line(aes(x=time/(365), y=bw)) +
  geom_line(aes(x=time/(365), y=vFat, linetype="dotted")) +
  scale_linetype_manual(name = "",
                        values = rep(c("solid"="solid", "dotted"="dotted")),
                        labels=c("Total body weight", "Fat compartment")) +
  #ggtitle("Body weight variation") +
  #scale_y_continuous(trans = squash_axis(150, 450, 6), breaks =
c(0,50,100,150,450,500,550,600)) +
  xlab("Time (years)") +
  ylab("Weight (kg)")

### Save images ###
ggsave("results/updated_results/reportFigs/dairyCattle/simMeat.jpg",
       plot=simMeatPlot,
       unit="mm",
       width=150,
       height=50)

ggsave("results/updated_results/reportFigs/dairyCattle/simLiver.jpg",
       plot=simLiverPlot,
       unit="mm",
       width=150,
```

```
      height=50)

ggsave("results/updated_results/reportFigs/dairyCattle/simMeatZoom.jp
g",
      plot=simZoomMeatPlot,
      unit="mm",
      width=150,
      height=50)


ggsave("results/updated_results/reportFigs/dairyCattle/Milk.jpg",
      plot=milkPlot,
      unit="mm",
      width=150,
      height=50)

ggsave("results/updated_results/reportFigs/dairyCattle/Intake.jpg",
      plot=intakePlot,
      unit="mm",
      width=150,
      height=50)

ggsave("results/updated_results/reportFigs/dairyCattle/Body
weight.jpg",
      plot=bodyWeightPlot,
      unit="mm",
      width=150,
      height=50)
```

Annex III R-Code growing calf model simulation

Model code:

```
calfModel<- '
$PARAM
@annotated
bwAdult : 537     : Adult weight [kg];
bwRef   : 450     : Reference weight for cardiac output calculation [kg];
fWGI    : 0.25    : GI-tract content weight as a fraction of the body
weight [-];
rVBlood : 0.093   : Blood volume [-];
rVFat   : 0.135   : relative fat volume [-];
rVLiver : 0.019   : relative liver volume [-];
rVRich  : 0.069   : relative richly perfused organs volume [-];
rVSlow  : 0.684   : relative slowly perfused organs volume [-];
q0Dry   : 72600   : cardiac  output when no milk production [L/day];
rQFat   : 0.038   : relative blood flow fat compartment [-];
rQLiver : 0.458   : relative blood flow liver compartment [-];
rQRich  : 0.304   : relative blood flow richly perfused organs [-];
rQSlow  : 0.200   : relative blood flow slowly perfused organs [-];
fQ      : 3.      : diffusion limiting flow factor [-];
pFat    : 280.    : Fat partition coefficient [-];
pLiver  : 23.     : Liver partition coefficient [-];
pRich   : 4.      : Richly perfused organ partition coefficient [-];
pSlow   : 8.      : Slowly perfused organs partition coefficient [-];
kMet    : 14.     : Liver clearance [per day];
tWin    : 275.    : Start of winter [day];
tSum    : 90.     : Start of summer [day];

$GLOBAL

#define masBal (aBlood+aFat+aSlow+aRich+aLiver+aMet);

$MAIN

double bwLife   = bwAdult*(1-0.9255*exp(-0.00204*TIME));
double bw       = bwLife*(1-fWGI);
double vBlood   = rVBlood*bw;
double vFat     = rVFat*bw;
double vLiver   = rVLiver*bw;
double vRich    = rVRich*bw;
double vSlow    = rVSlow*bw;

double clL = (vLiver*kMet);

double qF       = rQFat*qC;
double qffq     = qF/fQ;
double qS       = rQSlow*qC;
double qR       = rQRich*qC;
double qL       = rQLiver*qC;
```

```
double qC      = q0Dry*pow((bw/bwRef),0.75);

$INIT @annotated
aBlood  : 0 : blood;
aFat    : 0 : fat;
aLiver  : 0 : liver;
aRich   : 0 : richly perfused organs;
aSlow   : 0 : slowly perfused organs;
aMet    : 0 : metabolised particles;

$ODE
double cBlood   = aBlood/vBlood;
double cFat     = aFat/vFat;
double cSlow    = aSlow/vSlow;
double cRich    = aRich/vRich;
double cLiver   = aLiver/vLiver;
double cMeatFat = cSlow/(pSlow*0.8/pFat);

dxdt_aBlood = -qffq*(cBlood-cFat/pFat)-qS*(cBlood-cSlow/pSlow)-
qR*(cBlood-cRich/pRich)-qL*(cBlood-cLiver/pLiver);
dxdt_aFat = qffq*(cBlood-cFat/pFat);
dxdt_aSlow = qS*(cBlood-cSlow/pSlow);
dxdt_aRich = qR*(cBlood-cRich/pRich);
dxdt_aLiver = qL*(cBlood-cLiver/pLiver)-clL*cLiver/pLiver;
dxdt_aMet  = clL*cLiver/pLiver;

$CAPTURE
cFat
cLiver
cBlood
cSlow
cRich
cMeatFat
bwLife
masBal
'
```

Simulation code:

```
############################
# Dose events for milk cow
############################
library(ggplot2)
library(mrgsolve)
library(stringr)

source('models/calfModel.R')


simCalf <- function(scenario, tclean) {

  # intake default assumptions
  tClean  <- tclean                # start of clean period, i.e., dioxin intake
stops [day];
  simulationTime <- 365            # duration of simulation [day]
```

```
  time <- seq(0,simulationTime)
  iMilk    <- seq(7.5,7.5,length.out=simulationTime+1)  # Average milk
intake [L/day];
  fMilk    <- 1               # Absorption of TEQ through milk [-] :

  bwCalf <- 537*(1-0.9255*exp(-0.00204*time));
  bwAdult <- 700
  fraction <- bwCalf/bwAdult


  # Background grass and soil
  iGrass    <- 15*fraction    # Average daily grass intake [kg/day];
  fGrass    <- 0.25          # Fraction of dioxin absorbed from grass [-]
  tHGrass   <- 30             # half time decay of dioxin concentration in
grass [day];
  kGrass    <- log(2)/tHGrass # exponential growth factor [per day]
  cGrassMax <- 0.433          # Maximum dioxin concentration in grass
[ng/kg dry matter];

  cGrassMin <- 0.3            # Minimum dioxin concentration in grass
[ng/kg dry matter]

  iSoilFraction <- 0.04       # soil intake (as a fraction of grass intake) [-
]
  fSoil <- 0.43              #  fraction of dioxin in soil absorbed [-]
  iSoil <- iSoilFraction * iGrass # soil intake [kg/day]
  cSoil<- 1.54               # contamination of dioxin in soil [ng/kg];

  tSum    <- 0               # start of summer [day];
  tWin    <- 185              # start of winter [day];

  # Take milk concentration from literature or from previous model
simulation
  getMilk <- "model"

  if (getMilk=="literature") {
    cMilk <- 2  # concentration of TEQ in milk [ng/L]
  }
  if (getMilk =="model") {
    cMilk <-
readRDS(paste(paste("results/updated_results/simResults/dairyCattle_s
imulation_", scenario, sep=""), ".rds", sep=""))$cMilk[1:(365*3)]  #
concentration of TEQ in milk [ng/L] for the first three years
    cMilk <- mean(cMilk[cMilk>0])
  }

  # Create dose event
  doseEvent <- ev(amt=0, rate=0, cmt='aLiver', addl=simulationTime,
ii=1) %>% realize_addl()
  doseTiming <- seq(0,simulationTime)%%365

  # Create dose event: Once a day, the calf take up the specified dose
through milk
  dose <- fMilk*iMilk*cMilk
```

```
  # Calculate dioxin intake per day through grass in a reference scenario
(used when t>tClean)
  cGrassRef <- pmin(cGrassMin*exp(kGrass*(doseTiming+(365-tWin))),
0.492)
  cGrassRef[doseTiming>tSum & doseTiming<=tWin] <- 0
  cGrassRef[doseTiming>=tWin] <-
pmin(cGrassMin*exp(kGrass*(doseTiming-tWin)),
0.492)[doseTiming>=tWin]

  # From moment t == tClean, the cattle will be fed with clean hay, and
no dioxin intake will take place
  inGrass <- fGrass*iGrass*cGrassRef
  inGrass[doseTiming<tClean]<-NA
  inSoil <- fSoil*iSoil*cSoil
  inSoil[doseTiming<tClean]<-NA
  dose[doseTiming>=tClean] <-
inGrass[doseTiming>=tClean]+inSoil[doseTiming>=tClean]

  doseEvent@data$amt <- dose
  doseEvent@data$rate <- dose

  # Load growing calf model
  calfModel <- mcode("calfModel", calfModel)

  sim <- calfModel %>% ev(doseEvent) %>% mrgsim()

  saveRDS(inGrass,
paste(paste("results/updated_results/simResults/calf_inGrass_",
scenario, sep=""), ".rds",sep=""))
  saveRDS(inSoil,
paste(paste("results/updated_results/simResults/calf_inSoil_", scenario,
sep=""), ".rds",sep=""))
  saveRDS(sim,
paste(paste("results/updated_results/simResults/calf_result_", scenario,
sep=""), ".rds", sep=""))
  saveRDS(dose,
paste(paste("results/updated_results/simResults/calf_intake_",
scenario, sep=""), ".rds", sep=""))

}
tClean <- 185
simCalf(scenario="reference", tclean=tClean)
simCalf(scenario="realistic", tclean=tClean)
simCalf(scenario="worstcase", tclean=tClean)

####### Visualize data ###############
sim_realistic <-
as.data.frame(readRDS("results/updated_results/simResults/calf_result
_realistic.rds"))
sim_worstcase <-
as.data.frame(readRDS("results/updated_results/simResults/calf_result
_worstcase.rds"))
intake_realistic <-
readRDS("results/updated_results/simResults/calf_intake_realistic.rds")
```

```
intake_worstcase <-
readRDS("results/updated_results/simResults/calf_intake_worstcase.rds")
inGrass_reference <-
readRDS("results/updated_results/simResults/calf_inGrass_reference.rds")
inSoil_reference <-
readRDS("results/updated_results/simResults/calf_inSoil_reference.rds")

# plot concentration of dioxin in meat fat
simMeatPlot <- ggplot(as.data.frame(sim_realistic), aes(x=time/(30),
y=cMeatFat)) +
  geom_line(linetype="dotted") +
  geom_line(data = as.data.frame(sim_worstcase), aes(x=time/30,
y=cMeatFat, linetype="solid")) +
  scale_linetype_manual(name = "",
                        values = c("dotted"="dotted", "solid"="solid"),
                        labels=c("realistic", "worst case")) +
  ggtitle("Total TEQ concentration in meat fat") +
  xlab("Time (months)") +
  ylab(str_wrap("Total TEQ concentration (pg/g meat fat)", 23)) +
  geom_vline(xintercept=6, linetype="dashed", color = "black")   +
  geom_hline(yintercept=4, linetype="dashed", color = "red")


# plot concentration of dioxin in the liver
simLiverPlot <- ggplot(as.data.frame(sim_realistic), aes(x=time/(30),
y=cLiver)) +
  geom_line(linetype="dotted") +
  geom_line(data = as.data.frame(sim_worstcase), aes(x=time/30,
y=cLiver, linetype="solid")) +
  scale_linetype_manual(name = "",
                        values = c("dotted"="dotted", "solid"="solid"),
                        labels=c("realistic", "worst case")) +

  ggtitle("Total TEQ concentration in the liver") +
  xlab("Time (months)") +
  ylab(str_wrap("Total TEQ concentration (pg/g liver)", 23)) +
  geom_vline(xintercept=6, linetype="dashed", color = "black") +
  geom_hline(yintercept=0.5, linetype="dashed", color = "red")


# plot intake of total TEQ
in_realistic<-c(intake_realistic[0:tClean], rep(NA,366-(tClean)))
in_worstcase<-c(intake_worstcase[0:tClean], rep(NA,(366-tClean)))
inGr<-c(rep(NA,tClean),inGrass_reference[(tClean+1):366])
inS<-c(rep(NA,tClean),inSoil_reference[(tClean+1):366])

intake <- data.frame(time=rep(seq(1:length(intake_realistic)),2),
                     val=c(in_realistic,in_worstcase, inGr, inS),
                     type=c(rep("milk: realistic",length(intake_realistic)),
                            rep("milk: worst case",length(intake_worstcase)),
                            rep("grass: background",length(inGr)),
                            rep("soil: background",length(inS))))
newIntake <- intake
```

```
newIntake$type<- factor(newIntake$type, levels=c("milk: realistic",
"milk: worst case", "grass: background", "soil: background"))

intakePlot <- newIntake %>% ggplot() +
  geom_line(aes(x=time/(30), y=val, linetype=type, color=type)) +
  scale_color_manual(name = "Intake scenario", values = c("blue",
"red", "purple", "purple")) +
  scale_linetype_manual(name = "Intake scenario", values = c(1,1,1,2))
+
  ggtitle("Total TEQ intake through milk") +
  xlab("Time (months)") +
  ylab(str_wrap("Daily total TEQ intake (ng/day)", 26))

# Plot body weight
bodyWeightPlot <- as.data.frame(sim_realistic) %>% ggplot() +
  geom_line(aes(x=time/(30), y=bwLife*0.75)) +
  ggtitle("Body weight") +
  xlab("Time (months)") +
  ylab("Body weight (kg)")

### Save images ###
ggsave("results/updated_results/reportFigs/calf/simMeat.jpg",
       plot=simMeatPlot,
       unit="mm",
       width=150,
       height=50)

ggsave("results/updated_results/reportFigs/calf/simLiver.jpg",
       plot=simLiverPlot,
       unit="mm",
       width=150,
       height=50)

ggsave("results/updated_results/reportFigs/calf/Intake.jpg",
       plot=intakePlot,
       unit="mm",
       width=150,
       height=50)

ggsave("results/updated_results/reportFigs/calf/Body weight.jpg",
       plot=bodyWeightPlot,
       unit="mm",
       width=150,
       height=50)
```

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