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

Transfer model for aflatoxin B1 in dairy cows version 1.1 - model documentation .

2025

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Contents

Summary

The fundamentals of a transfer model for the transfer of aflatoxin B1 and its hydroxylated metabolite aflatoxin M1 from feed to muscle, liver, kidney and milk of dairy cows are presented. The model, which is available as a webtool application (https://feedfoodtransfer.nl/), can be used to compare simulated concentrations with regulatory limits for these food matrices.

1 Introduction

Aflatoxin B1 (AFB) is the most abundant of the aflatoxins, which are secondary metabolites of some Aspergillus fungal species. AFB can be introduced in feed in the field or during storage. When AFB is present in the feed of dairy cows, AFB and its hydroxylated metabolite aflatoxin M1 (AFM) can transfer to meat and milk and humans can be exposed to these contaminants via consumption of these animal products. Both aflatoxins are toxic and carcinogenic. This report describes a transfer model to predict the concentrations of AFB and AFM in meat, liver, kidney and milk from dairy cows after exposure to AFB via feed. The estimated concentrations can be compared to the regulatory limits that have been defined by regulatory agencies in various countries. The model is available as a webtool application (https://feedfoodtransfer.nl/).

2 Model description

2.1 General overview

The transfer of AFB and its metabolite AFM from feed to dairy cows is described using a transfer model based on the steady state model published by van Eijkeren et al. (2006) and the adaptation of that model for a risk assessment (RIVM, 2013), taking the distribution of the chemicals in animal tissues and its pre-steady state characteristics into account (i.e. partition coefficients). This model uses a single compartment where AFB enters the central compartment via absorption across the gut wall (Figure 1). Once AFB is in the central compartment, it is either cleared or metabolized. Clearance or metabolism to any metabolite other than AFM is lumped into clearance while metabolism into AFM is modelled explicitly. Once formed, AFM is also cleared or excreted via milk.

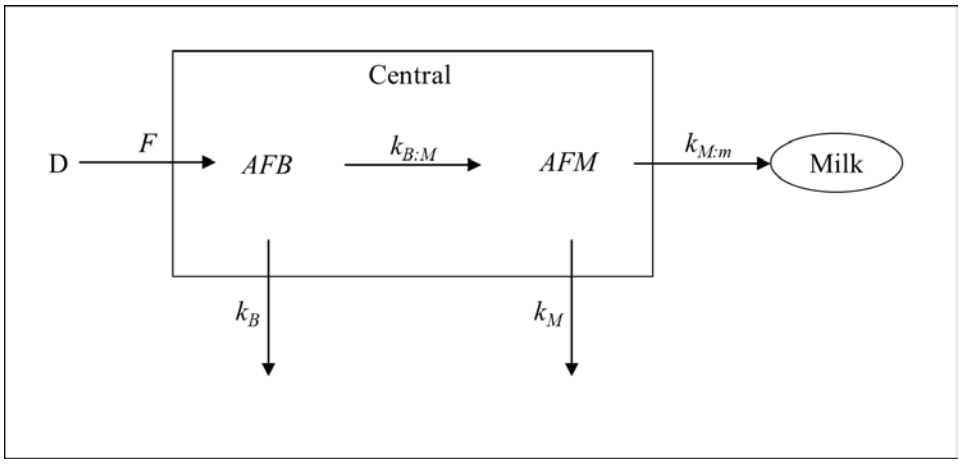

Figure 1. *One compartment model for aflatoxin B1 (AFB) and its metabolite aflatoxin M1 (AFM). Intake occurs via absorption across the gut wall to the central compartment. AFB is converted into AFM and both are cleared from the compartment and AFM is also excreted via milk.*

2.2 Model equations

The change in the amounts of AFB and AFM at time t ($dB(t)/dt$ and $dM(t)/dt$) in the central compartment are described as:

$$\frac{dB(t)}{dt} = F \cdot D(t) - (k_B + k_{B:M})B(t) \quad (1)$$

$$\frac{dM(t)}{dt} = k_{B:M}\, B(t) - (k_M + k_{M:m})M(t) \quad (2)$$

$B(t)$ [µg]: Amount of AFB in the central compartment at time *t*
$M(t)$ [µg]: Amount of AFM in the central compartment at time *t*
$D(t)$ [µg/day]: Daily intake of AFB
F [-]: Absorption fraction across the gut wall

k_B [day^{-1}]: Elimination rate of AFB through other pathways than to AFM
$k_{B:M}$ [day^{-1}]: Metabolism rate of AFB to AFM
k_M [day^{-1}]: Elimination rate of AFM through other routes than milk
$k_{M:m}$ [day^{-1}]: Milk clearance rate of AFM

2.2.1 *Distribution of aflatoxin in the body*

The concentrations of AFB and AFM in muscle tissues are obtained by dividing the amount of AFB or AFM in the central compartment, X, by the muscle weight (W_{mus}): $C_{X,mus} = \frac{X}{W_{mus}}$. Then the concentration in the liver and kidneys is calculated using the concentration ratios reported by Stubblefield *et al* (1983): $C_{X,organ} = PX_{,organ}C_{X,mus}$. The values of these parameters can be found in Table 1 (paragraph 2.4).

The concentrations of AFB and AFM in muscle, liver and kidney are described in equation 3-8.

$$C_{Bmus} = \frac{B}{W_{mus}} \quad (3)$$
$$C_{Bliv} = PB_{liv} \cdot C_{Bmus} \quad (4)$$
$$C_{Bkid} = PB_{kid} \cdot C_{Bmus} \quad (5)$$
$$C_{Mmus} = \frac{M}{W_{mus}} \quad (6)$$
$$C_{Mliv} = PM_{liv} \cdot C_{Mmus} \quad (7)$$
$$C_{Mkid} = PM_{kid} \cdot C_{Mmus} \quad (8)$$

B [µg]: Amount of AFB in the central compartment
M [µg]: Amount of AFM in the central compartment
C_{Bmus} [µg/kg]: Concentration of AFB in muscle
C_{Bliv} [µg/kg]: Concentration of AFB in liver
C_{Bkid} [µg/kg]: Concentration of AFB in kidney
C_{Mmus} [µg/kg]: Concentration of AFM in muscle
C_{Mliv} [µg/kg]: Concentration of AFM in liver
C_{Mkid} [µg/kg]: Concentration of AFM in kidney
W_{mus} [kg]: Muscle weight in cow
PB_{liv} [-]:i liver-muscle partition coefficient of AFB in cow
PB_{kid} [-]: kidney-muscle partition coefficient of AFB
PM_{liv} [-]:i liver-muscle partition coefficient of AFM in cow
PM_{kid} [kg]: kidney-muscle partition coefficient of AFM

2.2.2 *Aflatoxin excretion to milk*

The concentration of aflatoxin in milk is only computed for AFM, and is described as:
$C_m = k_{M:m} \cdot M/W_{milk}$ (9)
C_m [µg/kg]: Concentration of AFM in milk
W_{milk} [kg/day]: Daily milk production.

2.3 Model assumptions

The following assumptions were made in the presented model:

- The total feed intake is linearly related to milk production. Since the model was fitted using a daily milk production of 35L, the model is assumed to be adequate for a cow with a milk production around 35 L/day.
- Concentrations in the skeletal muscle are similar to those in the non-eliminating tissues. As relative weights of the eliminating tissues are rather small, both AFM and AFB are equally distributed over the body.
- The cow's weight is 640 kg, with a weight of 140 kg for gastrointestinal content + milk + urine, resulting in a clean weight of 500 kg.
- 1 kg milk equals 1 L milk.

2.4 Generic parameters

The distribution of AFB and AFM in muscle, liver and kidney was calculated using values obtained from literature (Table 1).

Table 1. Generic parameter values for dairy cows

Parameter	Value	Unit	Source
$\boldsymbol{F}$	0.4	day^{-1}	Hoogenboom et al. (2001)
$\boldsymbol{W_{mus}}$	200	kg	(Stubblefield et al., 1983)
$\boldsymbol{PB_{liv}}$	13.4	-	(Stubblefield et al., 1983)
$\boldsymbol{PB_{kid}}$	125	-	(Stubblefield et al., 1983)
$\boldsymbol{PM_{liv}}$	15	-	(Stubblefield et al., 1983)
$\boldsymbol{PM_{kid}}$	2.9	-	(Stubblefield et al., 1983)

2.5 System-dependent parameters

As described by the RIVM (2013) the model parameters were fitted using data from Frobish *et al.* (1986) and were verified using data from Britzi et al. (2013). This fitting procedure used lumped parameters to substitute k_B, $k_{B:M}$, k_M and $k_{M:m}$, i.e.. :

$\lambda_B = k_B + k_{B:M}$ (10)

$\lambda_M = k_M + k_{M:m}$ (11)

λ_B [day^{-1}]: Total elimination rate of AFB
λ_M [day^{-1}]: Total elimination rate of AFM

Stubblefield *et al.* (1983) describes that the ratio between AFB and AFM in the body of a lactating cow is 0.4. This can be applied to the steady state solution:

$$0 = k_{B:M}\,B - \lambda_M M \Longrightarrow \frac{B}{M} = \frac{\lambda_M}{k_{B:M}} = 0.4 \Longrightarrow k_{B:M} = \frac{\lambda_M}{0.4}. \quad (12)$$

So to summarize, this system was fitted for λ_B and λ_M using data from Frobish et al. (1986),:

$\frac{dB(t)}{dt} = F \cdot D(t) - \lambda_B B(t)$ (13)

$$\frac{dM(t)}{dt} = \frac{\lambda_M}{0.4} B(t) - \lambda_M M(t) \quad (14)$$

resulting in $\lambda_B = 17$ and $\lambda_M = 1.7$. Since $k_{B:M} = \frac{\lambda_M}{0.4}$ was already derived, k_B can be derived from equation (10) $\lambda_B = k_B + k_{B:M}$.

Using the transfer rate, we can derive $k_{M:m}$.

$$TR = k_{M:m} \frac{M}{D} \quad (15)$$

During steady state we have:

$$M = \frac{k_{B:M}}{\lambda_M} \; and \; B = \frac{k_{B:M}}{\lambda_M} \frac{F \cdot D}{\lambda_B} \quad (16)$$

Substituting equation 16 in 15 results in:

$$TR = \frac{k_{B:M}}{\lambda_M} \frac{F \cdot k_{M:m}}{\lambda_B} \quad (17)$$

According to van Eijkeren et al. (2006), the TR for AFB from feed to milk depends on the daily milk production, according to

$$TR = \frac{0.032 \cdot W_{milk}}{17 + W_{milk}} \quad (18)$$

Using a W_{milk} of 35 L/day then gives a TR of 0.022. The $k_{M:m}$ can be now be derived from equation 17, given that $TR = 0.022$ (based on (van Eijkeren et al., 2006)) and $F = 0.4$ (Hoogenboom et al., 2001), $k_{M:m} = 0.375$

See Table 2 for a full overview of the parameter values.

Table 2. Calibrated parameter values used in the transfer model for milk production around 35 liters per day

Parameter	Description	Value	Unit
k_B	AFB elimination rate	12.75	day^{-1}
$k_{B:M}$	AFB to AFM rate	4.25	day^{-1}
k_M	AFM elimination rate	1.325	day^{-1}
$k_{M:m}$	AFM to milk rate	0.375	day^{-1}

Note that the TR and thus the estimated levels in milk, meat, liver and kidney in this model are fitted for dairy cows with a daily milk production of 35L. Several studies reported that the TR depends on the daily milk production (see also Masoero et al., 2007; Signorini et al., 2011; Britzi et al., 2013). Since the AFB and AFM milk and tissue concentrations scale inversely with the milk production because of the fixed TR value of 0.022 that is fitted for a milk production 35 kg/day, the model underestimates the concentrations in milk and the body for animals with a high milk production. Conversely, the model overestimates the concentrations in milk and the body for animals with a low milk production (RIVM, 2013). Due to the uncertainties related to the TR with varying milk productions, we decided to keep the daily milk production parameter constant at 35L, as this was the value used for model calibration.

3 Software details

The transfer model simulations were developed and run using the R modelling language and using the deSolve package. Specifications on the programming packages are listed below:

Name software: R (tested with v. 4.2.2)
Manufacturer: The R Foundation for Statistical Computing
Place of manufacture: online
Year of manufacture: 2022
Description: A programming language for statistical computing

Name software: DeSolve (tested with v. 1.35)
Manufacturer: Karline Soetaert, Thomas Petzoldt and R. Woodrow Setzer
Place of manufacture: online
Year of manufacture: 2023
Description: Package to solve systems of differential equations
url: https://cran.r-project.org/web/packages/deSolve/index.html

Name software: dplyr (tested with version 1.1.4)
Manufacturer: Hadley Wickham, Romain François, Lionel Henry, Kirill Müller, Davis Vaughan, Ryan Dickerson, Posit Software, PBC
Place of manufacture: online
Year of manufacture: 2023
Description: A fast, consistent tool for working with data frame like objects, both in memory and out of memory.
url: https://cran.r-project.org/web/packages/dplyr/index.html

4 Model applicability

The transfer model presented in this report can be used to simulate the transfer of AFB and AFM to milk, muscle, liver and kidney of lactating cows after exposure to AFB. As such, the model enables comparison of the estimated concentration to regulatory limits of these food matrices. Similarly, the model can be used to estimate the wash-out period needed to comply with regulatory limits in case the concentrations exceeded such regulatory limits.

An example of a model application is given in Figure 2. Exposure to AFB (4.5 µg/kg grass during summer) was simulated for an exposure duration of 66 days. Contaminated grass intake was 7.9 kg per day. After the 66 days of exposure to contaminated feed, an additional 50 days were simulated in which only clean feed (i.e. feed not containing AFB) was provided. Note that the model was fitted for a milk production of 35 kg/day.

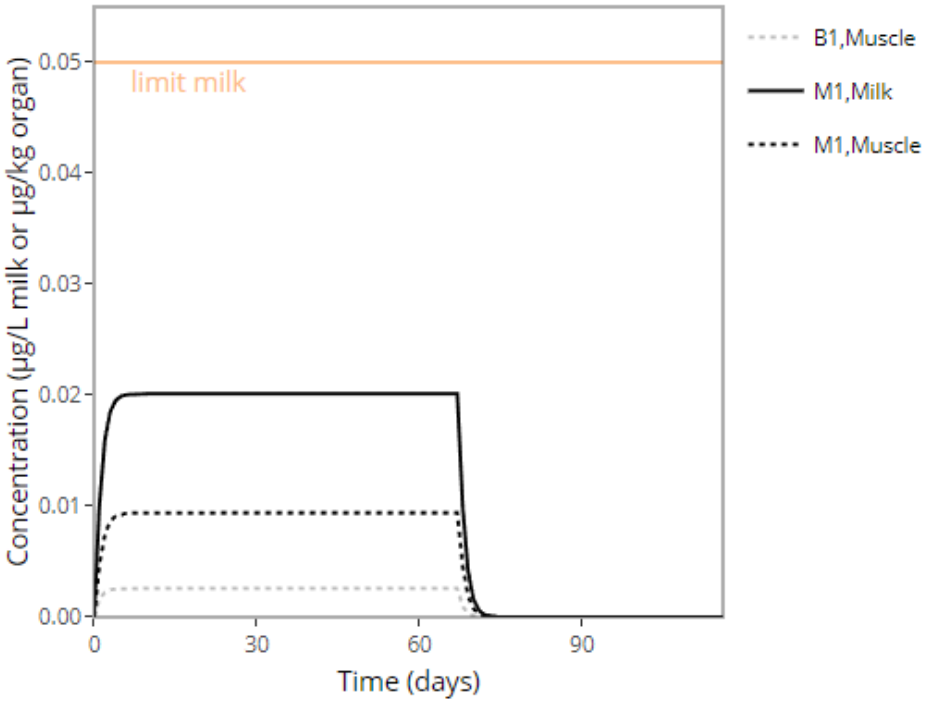

Figure 2 Simulated AFB (B1) and AFM (M1) concentrations in milk and muscle of dairy cows fed grass containing 4.5 µg/kg AFB during 66 days followed by clean feed for 50 days. Dairy cows were fed 7.9 kg grass per day and had a milk production of 35 L/day..

5 References

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Annex I: R-Code

The code below illustrates the basic implementation of the transfer model for aflatoxin B1 in dairy cows v1.1. This code can be used freely and is provided "as is" without warranty of any kind. The National Institute for Public Health and the Environment (RIVM) expressly rejects all liability for the accuracy, completeness, or suitability of the information provided. Use of the information is entirely at your own risk.

Model code (aflatoxin-dairy cow.R):

```
solveModel <- function (pars, tout, state, dosing){#input required for
the solver

  derivs <- function(t, state, parms) { # returns rate of change
    with(as.list(c(state, parms)), {

      dB <- D - (kB + kBM)*B  #
      dM<- kBM * B - (kM + kMm) * M
      dD  <- -D

      Wclean    <- Wlive - Wgimu
      Wmus              <- wrMUS * Wclean
      Cm        <- kMm * M / Wmilk
      CBmus     <-  B / Wmus
      CBliv     <- PBlm * CBmus
      CBkid     <- PBkm * CBmus
      CMmus     <- M / Wmus
      CMliv     <- PMlm * CMmus
      CMkid     <- PMkm * CMmus

      return(list(c(dB, dM, dD),
                  Wclean=Wclean,
                  Wmus = Wmus,
                  Cm = Cm,
                  CMmus = CMmus,
                  CMliv = CMliv,
                  CMkid = CMkid,
                  CBmus = CBmus,
                  CBliv = CBliv,
                  CBkid = CBkid
))

    }) #end with
  } #end derivs

  as.data.frame(ode(y = state, times = tout, events = list(data =
dosing), func = derivs, parms = pars))#

}
```

```
solveResult <- solveModel(pars= c(  Fpar = .4,
                          kB = 12.75,
                          kM = 1.325,
                          kBM       = 4.25,
                          kMm       = .375,
                          Wmilk     = 35,
                          Wlive = 640,
                          Wgimu     = 140,
                          wrMUS     = .4,
                          PMlm = 13.4,
                          PMkm = 125,
                          PBlm = 15,
                          PBkm = 2.9),
           tout  = seq(0, input$tdoseoff+input$tstop, by =
1),#input$tdoseoff+input$tstop
           state= c(B=0, M=0, D=0),#.$dose
           dosing =data.frame(var = c("D") ,
                          time = c(seq(0,input$tdoseoff, by = 1)),
#input$tdoseoff
                          value = c(0.4*input$Cfeed*input$feedIntake),
#Fpar * Cfeed * Ifeed
                          method = c("add"))
    )
```

Running the model (example.R)

```
library(dplyr)
library(ggplot2)
library(tidyr)

### User input ###
input <-c(Cfeed = list(4.5), # ng / kg
        feedIntake = list(7.9), # kg
        tstop = list(116), # days
        tdoseoff = list(66)) # days

### Run model ###
source("aflatoxin-dairy cow.R ")

example_df <- solveResult %>%
  select(time, Cm, CMmus, CBmus) %>%
  pivot_longer(col = -time)

plt_example <- ggplot(example_df) +
  geom_line(aes(time, value, colour=name)) +
  ylab('Concentration (µg/L milk or µg/kg organ)') +
  xlab('Time (days)') +
  ylim(c(0,0.05)) +
  xlim(c(0,90)) +
  scale_colour_manual(values = c("red", "blue", "green"))
```