CKow – A More Transparent and Reliable Model for Chemical Transfer to Meat and Milk

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The objective of this study is to increase the understanding and transparency of chemical biotransfer modeling into meat and milk and explicitly confront the uncertainties in exposure assessments of chemicals that require such estimates. In cumulative exposure assessments that include food pathways, much of the overall uncertainty is attributable to the estimation of transfer into biota and through food webs. Currently, the most commonly used meat and milk-biotransfer models date back two decades and, in spite of their widespread use in multimedia exposure models few attempts have been made to advance or improve the outdated and highly uncertain K_{ow} regressions used in these models. Furthermore, in the range of K_{ow} where meat and milk become the dominant human exposure pathways, these models often provide unrealistic rates and do not reflect properly the transfer dynamics. To address these issues, we developed a dynamic three-compartment cow model (called CKow), distinguishing lactating and non-lactating cows. For chemicals without available overall removal rates

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in the cow, a correlation is derived from measured values reported in the literature to predict this parameter from K_{ow}. Results on carry over rates (*COR*) and biotransfer factors (*BTF*) demonstrate that a steady-state ratio between animal intake and meat concentrations is almost never reached. For meat, empirical data collected on short term experiments need to be adjusted to provide estimates of average longer term behaviors. The performance of the new model in matching measurements is improved relative to existing models—thus reducing uncertainty. The CKow model is straight forward to apply at steady state for milk and dynamically for realistic exposure durations for meat *COR*.

Keywords: biotransfer factor, *BTF*, carry over rate, *COR*, cow, cattle, dairy, milk, meat, K_{ow}

1   Introduction

Many chemicals formed in combustion and released to air as well as industrial organic chemicals released to air, water, and soil enter humans primarily through food--in particular through meat and dairy products (*1-3*). Agricultural cattle (*Bos taurus,* or "cows") are domesticated ungulates and the dominant food producing animals for meat and dairy products in most of the world (to a lesser extent in Asia and Africa) (*4*). Risk and life-cycle-impact assessments address chemical transfer from cattle diet to food products with default estimates from empirical models. These models are based on incomplete and non-representative experimental data (e.g. in term of dynamics) and have large residual errors. In this paper we revisit and expand on biotransfer experiments to develop a dynamic, pharmacokinetic biotransfer model for lactating and non-lactating cattle. The result is a more precise model, better representative of the biotransfer dynamics in cattle and applicable to a broader range of chemical properties than any existing model, with explicit characterization of uncertainty.

In developing this model, we first review the scientific evidence that supports both the conceptual structure and detail of the model. From this we formulate the model while confronting several significant sources of uncertainty (e.g. variable exposure duration, important loss processes, measurement variability, or interpretation of literature data). We evaluate our modeling approach using residual error analysis applied to measurements and results from other available models. By imbedding

the model performance evaluation into all stages of the model development, we provide users with important insights on the capabilities and limitations of the models and their outputs.

Estimates of human intake through food are often based on residue measurements from market basket surveys or duplicate diet studies (*5*). Although useful for identifying intake, these studies provide little insight about pathways from source to dose, since the corresponding sources and animal exposures cannot be traced back. Chemicals transport from air, soil, and forage into meat and dairy products is the most uncertain among the source-to-dose pathways (*6*). High cost, political motivation, and ethical issues limit the availability of measured cow biotransfer data. This is reflected e.g. in EU's Registration, Evaluation and Authorization of CHemicals (REACH) program. Biotransfer models depend on available literature, which provides experimental biotransfer data on only a limited set of compounds of concern. We have identified 73 non-dissociating, non-ionizing, organic chemicals tested for biotransfer into milk and 42 for meat. We provide these data in detail in the *Supporting Information*. These limited data are also biased towards certain problematic substances, such as PCBs, dioxins/furans and a range of pesticides. These data provide our only current opportunity to develop concepts about the nature of the processes involved, develop and test models to explain these processes, and extend this knowledge to a broader range of chemicals.

Based on measurements for less than 30 substances, Travis and Arms (*7*) (T&A) developed a K_{ow}-based linear regression model, which is still recommended and used widely (*8-11*), e.g. in EU's Technical Guidance Document on Risk Assessment and the related EUSES model used under REACH, to estimate biotransfer factors (*BTF*) for meat and milk. In the many years since their paper was published, only few attempts have been made to improve this approach. Dowdy et al. (*12*) proposed a log*BTF* regression using the molecular connectivity index (MCI), a quantitative structure-activity relationship. According to the authors, the MCI approach provides an estimation model with the lowest available residual errors by avoiding parameter uncertainty of the measured K_{ow}. However, this conclusion has been contested by a US-EPA report where a larger *BTF* dataset was included and no major improvement compared to T&A was found (*11*). MCI requires correction factors for polar

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functional groups that are difficult to apply consistently to *BTF* (*11*), limiting its wide acceptance and use. Birak et al. (*10*) evaluated options to reduce the uncertainty of the T&A equations by adding additional measurements from recent literature and, in collaboration with US-EPA, proposed a new regression model (*9*) using a polynomial K_{ow}-regression (hereafter called RTI model) which is recommended by US-EPA (*11*). They also introduced a correction factor to K_{ow} for dissociating organic acids. But this model severely overestimates the *BTF* for logK_{ow}<5.5 as result of K_{ow}-corrections that inflate at the lower range of the regression. The authors also discuss that their model overestimates biotransfer of highly metabolized chemicals, therefore producing an upper bound estimate for these chemicals (*9*). Hendriks et al. (*13*) proposed K_{ow}-linear correlations that distinguish stable (not or slowly metabolized) and labile (metabolized) chemicals. This approach requires prior knowledge (which is limited for most chemicals) in order to classify chemicals according to their metabolism potential. Their correlation for stable chemicals gives similar results as the RTI approach, while for labile substances *BTF*-estimates are 1-2 orders of magnitude lower.

A limited number of dynamic models for chemical fate in cows are available. Derks et al. (*14*) developed a six-compartment, physiologically based pharmacokinetic (PBPK) model for 2,3,7,8-TCDD in lactating cows based on rodent studies. This model is substance specific, complex, and data intensive, thus limiting its application to a larger set of chemicals. In an effort to address both PBPK mass balance and a broader range of substances, McLachlan (*15*) published a fugacity-based compartment model for the fate of hydrophobic chemicals in a lactating cow. For the limited logK_{ow} range of ~5 to ~8.5, we find this model reproduces steady-state and dynamic measurements reasonably well, but fails to capture well experimental observations for lower K_{ow} values. The same model extended by inhalation, exhalation, and urination has later been used by Czub and McLachlan (*16*) in their ACC-HUMAN describing bioaccumulation of lipophilic organic pollutants from air, water, and soil to humans.

Sweetman et al. (*17*) reviewed several existing approaches for estimating biotransfer in order to recommend appropriate applications and improvements. They observed that biotransfer models need

algorithms to adequately address the process of absorption and specifically a decrease in absorption with increasing K_{ow}, in the gastro-intestinal tract.

The goal of our study is to confront and remedy some of the shortcomings of current modeling approaches, such as the large residual errors of *BTF* regressions and the impact of the application of steady-state assumptions to non-steady-state conditions and data. To achieve this we specifically address the following questions:

- To what extent do assumed steady-state measurements used in these regression models actually correspond to steady-state conditions?

- How can we apply a dynamic model of chemical behavior in cows to make appropriate use of or adjust measurements collected under non-steady-state conditions?

- What are the key elements of a model that determine its validity over a wide spectrum of chemical properties?

- How do we identify and properly characterize the uncertainties in biotransfer estimates based on limited animal experiments?

2   Methods

2.1   Carry Over Rate as the Metric of Biotransfer

The factors typically used to describe the transfer of chemicals from cattle intake to meat and milk are the biotransfer factor (*BTF*) and the carry over rate (*COR*). Under steady-state conditions, both describe the fraction of ingested contaminant actually transferred to animal tissue. The definitions of *BTF* and *COR* are as follows:

$$BTF_{milk\ or\ meat} = \frac{C}{I}$$

(1

$$COR_{milk}(t) = \frac{\int_{o}^{\infty} M^{milk}(\tau) \cdot C^{milk}(\tau) d\tau}{\int_{o}^{\infty} I(\tau) d\tau} \overset{steady\ state}{=} \frac{C^{milk} \cdot \dot{M}^{milk}}{I} = BTF_{milk} \cdot \dot{M}^{milk}; \quad COR_{meat}(t) = \frac{C^{meat}(t) \cdot M^{meat}}{I \cdot t}$$)

where *I* represents the chemical intake of the cow [*kg/day*] per individual exposure pathway or summed over all pathways, *C* is the chemical concentration in milk or in meat [*kg/kg*], $\dot{M}^{milk}$ is the milk

output rate [kg/day]. M^{meat} is a single mass grown, contaminated during a certain exposure duration t, and extracted from the system only once—at the end of the animal's life time. Since *COR* is the output of chemical transferred into milk or meat per unit of chemical intake by the cow, it has a natural limit of 1, which means that a maximum of 100% of input can be transferred into milk or meat.

We use the carry over rate (*COR*) to harmonize comparisons of biotransfer among model and experimental results, using Equation (1) to calculate *COR* on the basis of measured or modeled concentrations applying the following assumptions: For COR_{milk} we assumed an average milking yield of 23kg/day (*18*), and a milk-fat content of 4% (*7, 12*). We calculated COR_{meat} by assuming a meat-fat content of 25% (*7, 12*), an average meat-mass per animal of 440kg (including fat) and an average exposure duration (based on experimental data) for beef cattle of 81$days$.

2.2   Model description

The scarcity of measurements and the low probability that this situation will change in the future call for a deeper understanding and optimal use of these existing data. This can be better provided by mechanistic process-based models--those with explicit representations of chemical mass balance--rather than by linear or polynomial regressions without insight into underlying processes. We believe that interpretation of empirical relationships improves both model reliability and understanding of the relationship of *COR* to chemical properties. Because we cannot repeat the available experiments in a more unified way a large part of the variability and uncertainty remains, but can be reduced by better understanding the available measures.

2.2.1   Base model

We built our model using mass balances and the gut-blood diffusion model by McLachlan (*15*). The proposed CKow model consists of three major compartments--fat, blood, and gastro-intestinal tract (gut). Mass transfers in gut and blood are calculated for quasi-steady-state conditions. Transfers in fat are based on quasi-steady-state transfers between blood and fat, whereas the mass balance in fat is derived dynamically. Milk lipid concentration is assumed to be in equilibrium with blood lipid concentration. For degradation and elimination in blood and gut, empirical literature data have been

used to characterize overall removal rates (metabolic reactions, blood-to-gut/feces, and urine) and are further discussed in the model parameterization section. The model distinguishes lactating and non-lactating cows by a milk output flow, which also allows modeling the meat concentration of lactating cows. Figure 1 gives a conceptual overview of the model.
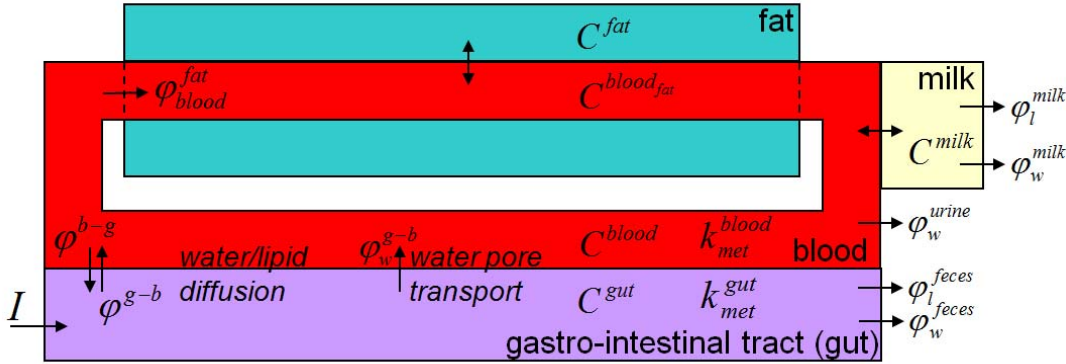

Figure 1: Conceptual CKow model

Mass balance in gut and transfer from gut to blood

The mass balance in the gastro-intestinal tract can be expressed as:

$$\frac{M^{gut} \cdot dC^{gut}}{dt} = I - \underbrace{\varphi_{rem}^{gut} \cdot C^{gut}}_{\substack{\text{metabolic degradation} \\ \text{and fecal excretion}}} - \underbrace{\varphi^{g-b} \cdot C^{gut} + \varphi^{b-g} \cdot C^{blood}}_{\text{diffusion and water pore blood transport}} \tag{2}$$

where M^{gut} is the mass in gut [kg], C^{gut} is the concentration in gut [kg/kg], I the intake (via food/water/soil) [kg/day], φ^{g-b} and φ^{b-g} the equivalent fluxes of chemical from gut to blood and from blood to gut [kg/day] (Equation (8)), C^{blood} the concentration in arterial blood [kg/kg], φ_{rem}^{gut} is the equivalent removal flux in the gut via degradation and fecal output fluxes [kg/day] calculated as:

$$\varphi_{rem}^{gut} = k_{met}^{gut} \cdot (M_w^{gut} + M_l^{gut} \cdot K_{ow}) + \varphi_w^{fec} + \varphi_l^{fec} \cdot K_{ow} \tag{3}$$

with k_{met}^{gut} the metabolic degradation rate in the gut [$1/day$], M_w^{gut} and M_l^{gut} the mass of water and lipids respectively in gut [kg], K_{ow} the octanol-water partition coefficient [-], φ_w^{fec} and φ_l^{fec} the output fluxes of water and lipid phase respectively through the feces [kg/day].

2.2.2 Calculation of carry over rates and biotransfer factors

We first solved the mass balance in gut, blood and fat compartments at steady state to understand and explain the main processes revealed by experiments (see sections S3-S5 in *Supporting Information* for the development of the base model). For milk, *COR* can be understood as the fraction of chemical absorbed from gut to blood, multiplied by the fraction of chemical transferred from blood to milk:

$$COR_{milk_{ss}} = \underbrace{\frac{\varphi^{g-b}}{(\varphi_{rem}^{gut} + \varphi^{g-b})}}_{\text{fraction absorbed into blood}} \cdot \underbrace{\frac{\varphi^{milk}}{(\varphi_{rem}^{blood} + \varphi^{milk})}}_{\text{fraction excreted into milk}} \tag{4}$$

where φ^{g-b} and φ_{rem}^{gut} are defined in Equations (8) (see also section S3 in *Supporting Information*) and (3), $\varphi^{milk} = \varphi_w^{milk} + \varphi_l^{milk} \cdot K_{ow}$ is the equivalent output flux of milk [kg/day] and φ_{rem}^{blood} is the equivalent removal flux from blood via degradation, urine advection and blood-gut transfer [kg/day] (without milk transfer) as defined in Equation (S6) and discussed in the model parameterization below. For meat, a steady-state assumption is often not valid due to the high bioaccumulation potential in fat and to the punctual extraction of meat from the system. Developed in sections S6 and S7 of *Supporting Information*, the dynamic version of the model characterizes the evolution of concentrations in meat (mostly fat tissues). The corresponding *COR* can be expressed as the fraction of the chemical absorbed in blood that multiplies the residence time of the chemical in fat and a time correction factor that accounts for the duration of exposure:

$$COR_{meat}(t) = \frac{M^{meat} \cdot f_l^{meat} \cdot C^{fat_{ss}} \cdot \left(1 - e^{-k^{fat}t}\right)}{I} \cdot \frac{1}{t} = \underbrace{\frac{\varphi^{g-b}}{(\varphi_{rem}^{gut} + \varphi^{g-b})}}_{\text{fraction absorbed into blood}} \cdot \underbrace{\frac{M^{meat} \cdot f_l^{meat} \cdot K_{ow}}{(\varphi_{rem}^{blood} + \varphi^{milk})}}_{\text{residence time in the fat compartment}} \cdot \underbrace{\frac{\left(1 - e^{-k^{fat}t}\right)}{t}}_{\text{time correction factor}} \tag{5}$$

$$and \ BTF_{meat}(t) = \frac{COR_{meat}(t) \cdot t}{M^{meat}}$$

where $C^{fat_{ss}}$ is the concentration in fat at steady-state, f_l^{meat} is the lipid fraction in meat [-] and k^{fat} is the rate constant for uptake of the chemical in fat [$1/day$]:

$$k^{fat} = \left(\frac{M^{fat} \cdot K_{ow}}{\varphi_{blood}^{fat}} + \frac{M^{fat} \cdot K_{ow}}{\varphi_{rem}^{blood} + \varphi^{milk}}\right)^{-1} \cong \frac{\varphi_{rem}^{blood} + \varphi^{milk}}{M^{fat} \cdot K_{ow}} \tag{6}$$

Since the transfer between blood and fat is rarely a limiting factor, the system dynamic is characterized by the ratio of the overall removal equivalent fluxes from the cow divided by the capacity of the fat reservoir. For milk, one gets a similar dynamic equation:

$$COR_{milk} = COR_{milk_{ss}} \cdot \underbrace{\left(1 - \frac{e^{-k^{fat}t}}{1 + (\varphi_{rem}^{blood} + \varphi^{milk}) / \varphi_{blood}^{fat}} \right)}_{\text{time correction factor}} \qquad (7)$$

2.2.3 Model parameterization

The transfer from gut to blood is a critical step in the model and represents diffusion transfer between gut and blood. McLachlan (*15*) modeled this as a two-film diffusion resistance with the gut wall as a water and an octanol film in series:

$$\varphi^{g-b} = \left(\frac{1}{Q_{AW}} + \frac{1}{Q_{AO} \cdot K_{ow}} \right)^{-1} \qquad (8)$$

with Q_{AO}=0.58*kg/day* as the octanol film diffusion transfer coefficient and Q_{AW}=4,030,000*kg/day* as the water film diffusion transfer coefficient given for a cow (*15*).

A second challenge is to determine the values for the two removal rates in blood and gut. Hendriks et al. (*13*) provided a review of empirical overall removal rates from cow for 36 chemicals covering a broad range of K_{ow} values and discussing their relation to K_{ow}. Since the transfer in milk is considered separately, we need here to calculate the removal rate excluding the transfer in milk. Section S8 of *Supporting Information* shows that these removal rates are negatively correlated with K_{ow} ($\log k_{rem}^{cow_{no\_milk}} = 1.42 - 0.48 \cdot \log K_{ow}$; R^2=0.52). While empirical removal rates will be used in priority when available, we use this correlation to estimate the removal for other chemicals. The removal flux in blood is given by:

$$\varphi_{rem}^{blood} = k_{rem}^{cow_{no\_milk}} (M_w^{cow} + M_l^{cow} \cdot K_{ow}) \qquad (9)$$

with M_w^{cow} and M_l^{cow} the available masses of water and lipid in the cow. Regarding the relatively short duration of the experiments, the cow is not at steady state for persistent lipophilic chemicals (see results section below) and only a fraction of the total mass is available for degradation during the experiment.

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Thus, the empirical removal rate should only be applied to the available fraction of the total fat during the removal rate experiment: $M_l^{cow} \cong f_l^{available} \cdot M^{fat}$. Introducing these experimental removal rates in the model, we propose a value of $f_l^{available} = 0.35$ that minimizes the total residual error between modeled and empirical carry over rates (see additional details in section S8). Regarding the uncertainty of this parameter, a sensitivity study is presented in the Discussion to test its influence on the model outputs. For metabolism and removal mechanisms in gut, there is little operational information available. We therefore pragmatically approximated the degradation rate in the gut as a function of K_{ow} using the same correlation as the removal rate in blood ($\log k_{rem}^{gut} = 1.42 - 0.48 \cdot \log K_{ow}$) to test the model's ability to represent changes in *COR* while using simplified correlations. Table S1 in *Supporting Information* summarizes all factors required to calculate the *COR* of Equations (4) and (5) and provides values for all used parameters.

2.3 Defining an appropriate model evaluation process

Models are used to organize and explore scientific premises and to inform decisions to regulate, monitor, or further investigate a broad range of environmental problems. A model performance evaluation provides information on the suitability of a model for these tasks. According to the US-EPA, model evaluation is the process for generating information over the whole project duration that helps to determine whether a model and its analytical results are of a quality sufficient to serve as the basis for a decision (*19*). Model quality has meaning only within the context of a specific model application. For estimating *COR* it is important to establish that our model has captured the relevant processes; is based on a broad and representative set of observations, makes predictions that match observations, while having an explicitly characterized and improved (lower) residual error with respect to observations. It is also important to examine and discuss uncertainties and sensitivities. Finally, we must consider how well the model performs its goal of estimating *COR* based on chemical properties.

To address these issues, we focus the model performance evaluation on the ability of the model to match measured *COR* values. In addition to the data used by Travis and Arms (T&A) (*7*) and Dowdy et

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al. (*12*) we added new data from 17 recent publications reflecting either feeding or mass balance measurements based on identifiable individual animals (see Tables S6 and S7 in *Supporting Information*). These studies covered a much wider range of K_{ow} than earlier studies, especially in its upper range, revealing different biotransfer behavior than observed by T&A and Dowdy et al. Where possible we considered dynamic concentration curves to compare with our dynamic model, made use of data for individual animals, rather than averaging between several animals, and took into account the animal-specific exposure duration in our dynamic model. There are no data representing a prolonged period that could reveal a convergence towards steady-state concentrations over time and provide insight as to whether and when steady state was reached during the experiment. Furthermore, for a correct interpretation of experimental data it is useful to have information about the age of the animals (especially for meat), which can vary greatly but in many cases was not given. Such inter-individual variability between the cows and variations in experimental design, dosing, handling of the animals, background residues, etc. contribute to the large variability observed among the individual measurements. The resulting experimentally derived carry over rates are provided in Tables S6 and S7 of the *Supporting Information*.

3   Results

We compare the ability of our proposed and other models to capture observations of *COR/BTF* for milk and meat.

3.1   Comparison between measured and modeled *COR*

Figure 2 illustrates how our CKow model, the T&A model (*7*) and the RTI model (*9*) compare to measured *COR* data as K_{ow} varies. Milk (Figure 2a) and meat (Figure 2b) follow very similar patterns. The horizontal dashed line marks the physical limit of *COR*=1 meaning 100% biotransfer, and the vertical dashed line marks the K_{ow} value where the T&A model surpasses this physical limit on *COR*. The decrease in uptake for $\log K_{ow} > 6$ is directly linked to the resistance of the water film in the gut as described by Equation (8). The high variability in measured *COR* in combination with the high

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uncertainty of K_{ow} measurements in the upper K_{ow} range appears consistent with but does not necessarily confirm this decrease. In the low K_{ow} range, the RTI model seems driven by a correction to account for very-low-K_{ow} organic acids that were included in the model's training set. At low K_{ow}, it results in a low decrease in *COR* and the RTI model overestimates the experimental *COR* by more than two orders of magnitude. The downward trend of *COR* for logK_{ow}<6.5 is best represented by the CKow model leading to a reasonably good fit. Variations between individual points predicted by the CKow model around a given K_{ow} show that empirical removal rates can influence *COR* by one order of magnitude compared to the K_{ow} extrapolation.

Overall, the CKow models reflect well experimental observations over the whole range of 2<logK_{ow}<9. The model is mainly driven by the three substance-specific properties K_{ow} and overall removal in blood and gut, but does not always accurately capture metabolic degradation since empirical data are scarce. For COR_{meat} the remaining variation between model and experimental results is partially due to its high sensitivity to the exposure duration and the lack of information on the actual age of the animals in the experiments.

a)
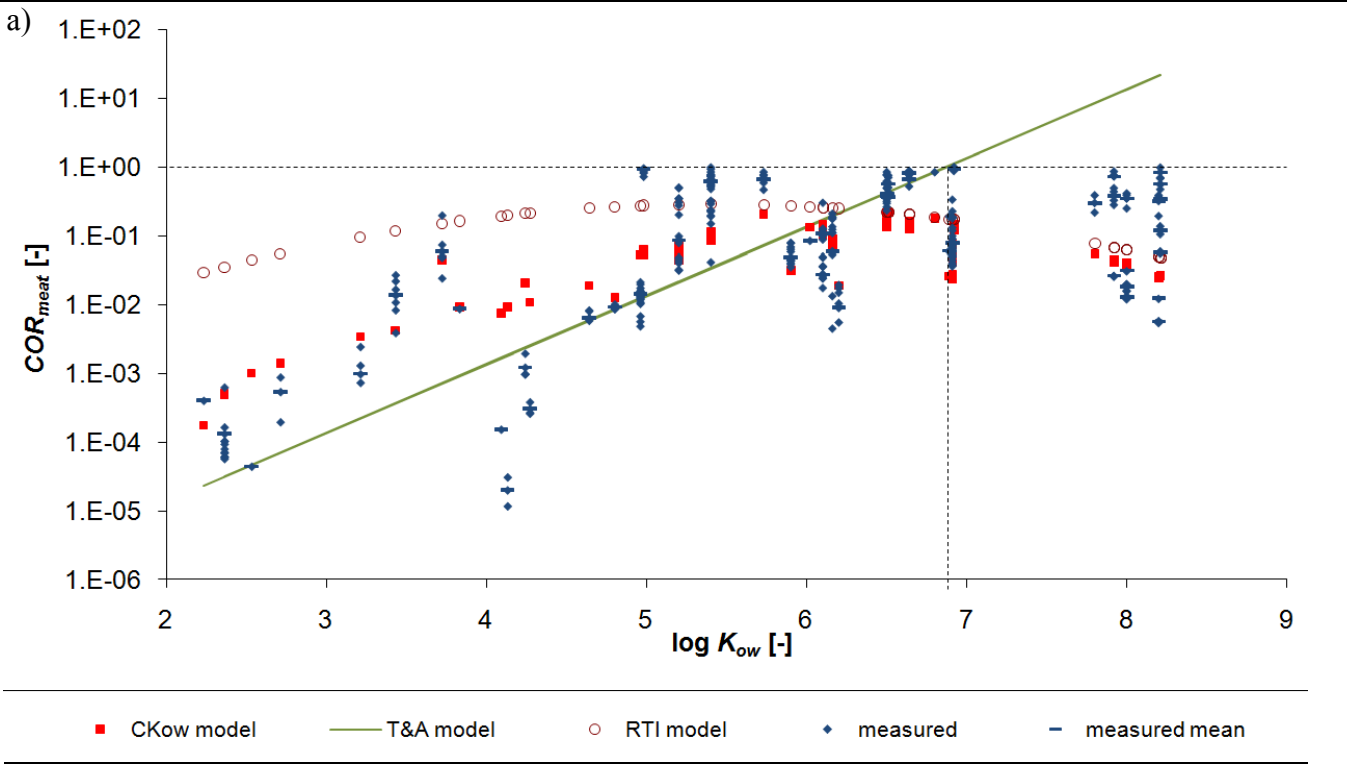
b)
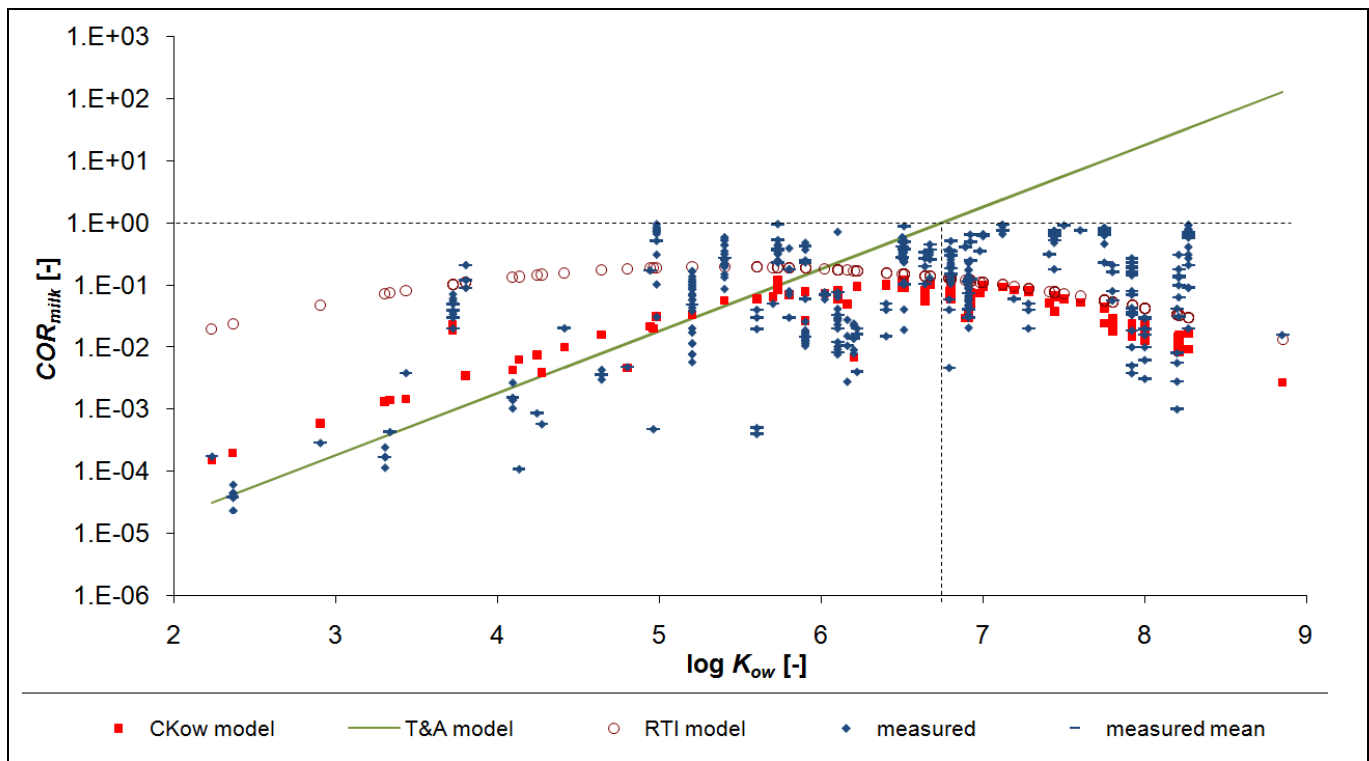
Figure 2: a) COR_{meat} and b) COR_{milk} plotted against $\log K_{ow}$: Comparison of measured data for 73 substances with the CKow model (dynamic with actual experimental exposure duration), the T&A model (*7*) and the RTI model (*9*)

3.2 Dynamic behavior: from experiment duration to steady state

For carry over into meat the influence of time (i.e. exposure duration) on BTF_{meat} is studied by applying the CKow dynamic to calculate BTF for an exposure duration equal to the average beef-cattle lifetime of ~500*days* in order to get realistic exposure estimates. Figure 3a compares this value with i) BTF calculated using reported individual exposure duration for the corresponding experiment and ii) for steady-state calculations. An implicit assumption here is that BTF_{meat} is a cohort-based parameter not allowing interpretation about individual animals. Figure 3a shows that using the reported experimental exposure duration in the dynamic CKow model tends to underestimate the beef-cattle-lifetime biotransfer factor in the high bioaccumulation range by up to one order of magnitude. On the contrary, the steady-state assumption overestimates BTF_{meat} in its high range compared to the typical ~500*days* lifetime.

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When we conduct the same comparison for BTF_{milk} (Figure 3b), we find that the reported experimental duration also demonstrate an underestimation of BTF_{milk}, but to a lesser extent than for meat, due to the faster removal of lipids with milk. The milk excretion flux leads to a quicker turnover as reflected in Equation (4) and steady state is fully reached before $500 days$ as demonstrated by the quasi-perfect accord between the 500-$days$ and steady-state calculations. This suggests that steady state is an acceptable assumption for milk biotransfer.

Thus, it is apparent that two adjustments are necessary to adequately represent real exposure situations: i) use of dynamic modeling for meat, avoiding short-term exposure or steady-state calculations, ii) use of the model to correct the data points from the experimental exposure duration to the average beef-cattle lifetime of $\sim500 days$ in order to get realistic exposure estimates. For this latter adjustment, the experimental BTF and COR for meat need to be corrected by the time correction factor of Equation (5) applied to the growth duration (t_{growth}) divided by the time correction factor applied to the experimental exposure duration ($t_{experiment}$): $f_{correction}^{BTF} = \left(1 - e^{-k^{fat} t_{growth}}\right) \Big/ \left(1 - e^{-k^{fat} t_{experiment}}\right)$.
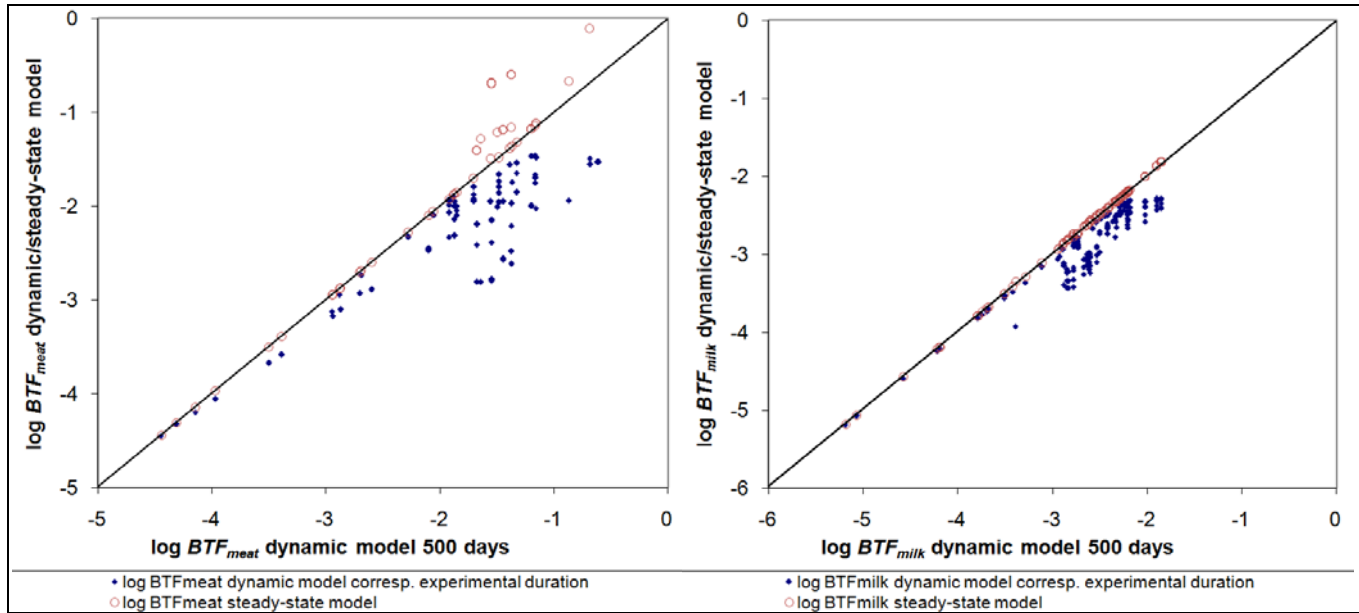

Figure 3: Modeled a) BTF_{meat}, and b) BTF_{milk} from the dynamic CKow model for $500 days$ exposure compared to the dynamic CKow model for individual observed experimental duration and at steady state

3.3   Quantification of the precision of all compared models

To quantify precision for each model against measurements we employ the residual error (RE). The RE and its use in such context is discussed by McKone (20). The squared geometric standard deviation ($GSD^2=10^{2RE}$) represents the geometric factor capturing the two-standard-deviation prediction interval, i.e. the 95% confidence interval (geometric mean divided/multiplied by GSD^2). We determined RE and GSD^2 for our model, in both steady-state and dynamic (for the exposure duration corresponding to the respective experiment) modes, and for the T&A and the RTI models against all individual measurements and the arithmetic mean of individual cows per substance. Figure 4 compares the GSD^2 for $\log COR_{meat}$ and $\log COR_{milk}$ from the respective models. Further details are given in section S10 of *Supporting Information*.
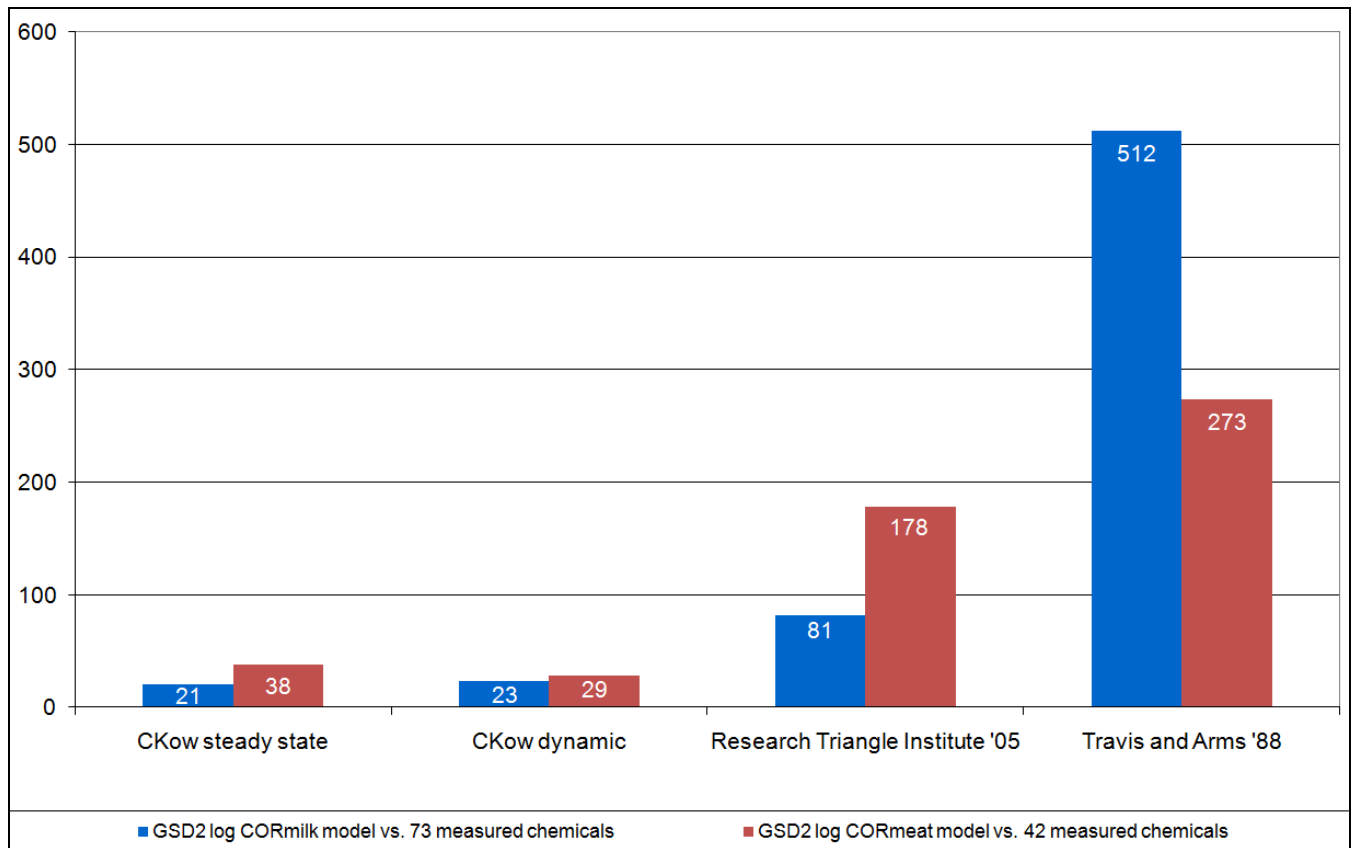
Figure 4: Comparison of GSD^2 for COR_{meat} and COR_{milk} from the CKow models (steady-state and dynamic), the RTI (2005) model, and the T&A (1988) model, plotted against measured COR_{meat} from 42 chemicals and measured COR_{milk} from 73 substances

A clear reduction in uncertainty is demonstrated for the steady-state and dynamic CKow model for both meat and milk biotransfer compared to the other models. The reduction in precision of the steady-state assumption for COR_{meat} is reflected by the GSD^2. The dynamic and the steady-state CKow model significantly reduce the 95% confidence interval to two orders of magnitude, clearly performing better than the other models.

4   Discussion

We introduce the CKow model and demonstrate that it provides a more scientifically defensible basis and increased precision in biotransfer modeling for meat and milk. This is important when modeling multimedia fate and exposure measures such as the intake fraction (*iF*)—the fraction of an emission taken in by the population (*1*). When changing from T&A to the simplified CKow model we found differences in total ingestion *iF* of up to a factor five for $\log K_{ow} > 6$ (see section S11 in *Supporting Information*). By expressing *COR* as a multiplication of interpretable factors, we identified key model elements and captured a wide spectrum of K_{ow} values while retaining a model that is transparent and easy to use. Proposing an empirically-based correlation for the removal rate as a function of K_{ow} makes the CKow model a practical improvement for assessment of human exposure through meat and milk. Our approach also offers more insight for the processes involved in biotransfer and their influence—something that is not available from linear regressions and polynomial fits to measurements.

The CKow approach also avoids current problems with regression models. For example, for $\log K_{ow} > 6.7$, the commonly-used Travis and Arms (*7*) relationship gives a *COR* that implies that an animal transfers more chemical to milk or tissue than it consumes, meaning that, for e.g. dioxin, the cow violates conservation of mass. EU's TGD avoid this by truncating the regressions at the maximum and minimum K_{ow} used in the underlying data (*8*), resulting in a constant *COR* outside the K_{ow} range of T&A's training set. Similarly, Bennett et al. (*1*) reported this problem and suggested an upper limit of *BTF*=0.1. In both cases, our results indicate that the resulting *COR* are too high. Because Bennett et al. (*1*) observe that meat and milk intake are the dominant pathways (i.e. >50% of overall intake) for $\log K_{ow} \geq 6$ (Figure S2 in *Supporting Information*) it is important to assure reasonable model performance

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for high K_{ow}-ranges. The physically impossible transfer rates obtained from the T&A model in that K_{ow}-range reveals the problem of using a model outside its calibrated range.

To address the discrepancy caused by interpreting meat biotransfer experiments as representing life-span or steady-state conditions (as done by all existing models), we recognize and account for chemicals that may never attain steady-state levels in a beef cow in its 1-2 years lifespan. Contrasting actual experimental conditions, our results show that the optimum exposure duration in cattle to predict average intake by humans, is the average beef-cattle lifetime of ~500 *days*. There is thus a need to find an appropriate assumption and correction factor for re-interpreting *COR* for the existing results of different experiments. This also applies to all existing models as these currently predict COR_{meat} for very short exposure of several weeks only, due to their dependency on the experimental measurements, thus likely underestimating long-term BTF_{meat}. As a clear step ahead from current K_{ow}-correlation-based models, these corrected data points can serve as a more robust basis for a new correlation model. Steady state should be assumed with great caution for meat biotransfer and might only be appropriate for specific cases, e.g. dairy cows with a considerably longer life span and almost constant chemical excretion flow via milk, or for chemicals with a high metabolic rate. However, for milk it was confirmed to be appropriate in most cases.

The fact that experimental data show a stronger decrease in *COR* with decreasing K_{ow} than explained by the RTI model has several potential explanations. The increase of removal rates with decreasing K_{ow} makes excretion the dominant loss process at lower K_{ow}. Another, perhaps complementary or even alternative, interpretation is a faster metabolism for most hydrophilic substances. The obtained accuracy is sufficient in the low range for most applications, since it is likely to be an exposure pathway of secondary importance in that range. US-EPA proposes a metabolism factor for bis(2-ethylhexyl)phthalate to correct the RTI model resulting in a *BTF* closer to experimental observations, but recognizing that the lack of empirical data prevents the development of such factors for other substances (*11*). While the CKow model represents well the general trend of empirical observations and fulfills the respective modeling recommendation by Sweetman et al., (*17*) an important limitation is the

measuring and modeling of metabolism, whose enormous variability among individual animals sets an ultimate limit to model reliability.

Model evaluation demonstrated increased precision for both the dynamic and steady-state CKow models, reducing uncertainty (GSD^2) by factor ~24 for COR_{milk} and ~9 for COR_{meat} compared to the T&A model. The remaining variability is harder to reduce as it is, among other factors, due to measurement uncertainty, which can be very large for data dating back 50 years, and remains important for recent measurements especially for high K_{ow}. Other important sources of variability and uncertainty are differences between individual animals, parameter uncertainty for K_{ow}, and transformation factors as well as model uncertainty not captured by RE. Another uncertain parameter is the fraction of the total fat mass available for degradation during the experiment. A sensitivity study was performed regarding its influence on RE: there is limited variation in GSD^2 when varying this parameter within a plausible range of 0.15 to 0.5, the GSD^2 varying from 29 ($f_l^{available} = 0.35$) to between 39 and 36 for meat and from 23 to between 25 and 31 for milk, thus an increase of maximum 35% in GSD^2. The influence of this parameter on the output is however not negligible and further investigation is required, e.g. using the dynamic model to better analyze and understand the individual data reported on removal rates.

One can argue that in the K_{ow} range, relevant for meat and milk exposure, the RTI model is acceptable because the overestimation in the lower K_{ow} range is not a significant issue. However, we believe that both scientific value of understanding and representing well the lower K_{ow} range in one harmonized model and the need to avoid bias when comparing or ranking hydrophilic to lipophilic compounds (as done in comparative risk or life cycle assessment) favors the CKow approach. For example Bennett et al. (1) demonstrated that for $\log K_{ow} < 6$ and less volatile compounds, grain and produce provide the dominant food exposure pathways. Since cow manure might be used as natural fertilizer, this can significantly contribute to the soil and ultimately the plant concentrations for certain chemicals and thus enter the human food chain via this pathway and should hence be realistically modeled.

Both CKow models are limited to non-dissociating, non-ionizing organic chemicals, the substance class of all measured COR used for model evaluation. Due to inherent averaging and assumptions the

CKow approach is applicable only to herds but not to single animals, which is in line with observations by Sweetman et al. (*17*). Because *COR* measurements are available only in the range of $2 \leq \log K_{ow} \leq 9$, the CKow models were only evaluated in this range and should not be applied to chemicals outside this interval. As with any model, whenever measured data of sufficient quality are available, these should be used in preference over the model.

From a policy and decision making perspective, the evidence presented here on existing correlations, specifically the T&A model, calls into question their use for very lipophilic chemicals. The discrepancy at high K_{ow} between the importance of meat/milk exposure pathways for human health and available knowledge, calls for increased research in order to better understand and reduce possible risks.

Further research should address questions such as better measuring bioavailability in the gut and options for using chemical-specific measurements of metabolic degradation. Interpreting clearing curves showing the decrease in concentration over time after exposure ends could provide valuable insights into metabolic degradation. Furthermore, K_{ow} is an uncertain parameter, specifically in its upper range and, as a measured property, always accompanied by parameter uncertainty, which could be avoided by replacing it with a more stable parameter, such as a QSAR metric, as attempted by Dowdy et al. (*12*). The selected QSAR metric should be substance specific, derived from little or no experimental measurements, well correlated with K_{ow} and/or *COR/BTF,* and easily obtained. *COR* for other chemical classes, such as dissociating organics, is clearly a subject of further investigation, and current models (for generic organics) should not be applied to these other substances.

Acknowledgements

Supporting Information Available

Tables of all measured data including *BTF* and *COR* calculations and the references, the derivation of mass balances, differential equation systems and solutions, a list of symbols, additional figures, the parameters used in the CKow models, and further discussions on model performance evaluation and on the effect of improved *COR* on a multimedia exposure estimate (intake fraction) can be found therein. This information is available free of charge via the Internet at http://pubs.acs.org.

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Brief

A dynamic three-compartment cow model that distinguishes lactating/non-lactating cows and confronts key uncertainties increases the precision and transparency of models for biotransfer into meat and milk.