Assessment and improvement of biotransfer models to cow's milk and beef used in exposure assessment tools for organic pollutants

Article

Accepted Version

Chapter 3 - Assessment and improvement of biotransfer models to cow's milk and beef used in exposure assessment tools for organic pollutants

From a research paper;

Takaki K, Wade AJ, Collins CD (2015) Assessment and improvement of biotransfer models to cow's milk and beef used in exposure assessment tools for organic pollutants. Chemosphere 138: 390-397. DOI: 10.1016/j.chemosphere.2015.04.032

3-1 Abstract

The aim of this study was to assess and improve the accuracy of biotransfer models for the organic pollutants (PCBs, PCDD/Fs, PBDEs, PFCAs, and pesticides) into cow's milk and beef used in human exposure assessment. Metabolic rate in cattle is known as a key parameter for this biotransfer, however few experimental data and no simulation methods are currently available. In this research, metabolic rate was estimated using existing QSAR biodegradation models of microorganisms (BioWIN) and fish (EPI-HL and IFS-HL). This simulated metabolic rate was then incorporated into the mechanistic cattle biotransfer models (RAIDAR, ACC-HUMAN, OMEGA, and CKow). The goodness of fit tests showed that RAIDAR, ACC-HUMAN, OMEGA model performances were significantly improved using either of the QSARs when comparing the new model outputs to observed data. The CKow model is the only one that separates the processes in the gut and liver. This model showed the lowest residual error of all the models tested when the BioWIN model was used to represent the ruminant metabolic process in the gut and the two fish QSARs were used to represent the metabolic process in the liver. Our testing included EUSES and CalTOX which are K_{OW}-regression models that are widely used in regulatory assessment. New regressions based on the simulated rate of the two metabolic processes are also proposed as an alternative to K_{OW}-regression models for a screening risk assessment. The modified CKow model is more physiologically realistic, but has equivalent usability to existing K_{OW}-regression models for estimating cattle biotransfer of organic pollutants.

3-2 Introduction

Biotransfer of organic pollutants to cattle is an important process in quantifying the exposure of humans to toxic chemicals. Surveys in Germany and Canada have demonstrated that over 50% of dioxin and furan exposure to humans was through ingestion of cattle products (Fürst et al., 1990; Birmingham et al., 1989). In 1988, Travis and Arms (1988) proposed simple regressions between the octanol-water partition coefficient (K_{OW}) and a biotransfer factor (BTF) for milk and beef from experimental data, and this model has been incorporated by international regulatory authorities (e.g. European Chemical Agency, California Environmental Protection Agency) into their chemical exposure assessment tools for human health

(European Chemicals Bureau, 2010; Mckone, 1993). These tools are routinely used by regulatory authorities to determine the risk to human health from organic polluted soils; the accuracy of the cattle biotransfer model is therefore critical for robust health risk assessment.

Despite the extensive adoption of the Travis and Arms (1988) model, its validity has been questioned by many authors (Staples et al., 1997; Birak et al., 2001; McKone & Ryan, 1989; McLachlan, 1993). Their criticisms were based on: a limited amount of data, which all relate to persistent chemicals with a narrow K_{OW} range ($3 < \log K_{OW} < 7$), a high residual error in the derived regression equations and, when K_{OW} exceeds $10^{6.5}$, the model has an increase in the BTF but observations show that BTF decreases as K_{OW} increases (Staples et al., 1997; Birak et al., 2001; McKone & Ryan, 1989; McLachlan, 1993). An alternative approach was to generate new K_{OW}-regression models using a larger amount of experimental data, as proposed by MacLachlan and Bhula (2008) and the Research Triangle Institute (RTI) (2005). Hendriks et al.(2007) reported a very weak correlation between K_{OW} and BTF when labile and persistent chemical data were analysed separately (r^2 = 0.35 for labile, 0.02 for persistent). The BTF was more significantly affected by the metabolism of individual chemicals in cattle rather than their hydrophobicity (Staples et al., 1997; Hendriks et al., 2007). Therefore, the widely used K_{OW}-regressions would appear to have a limited theoretical basis. A regression model using the molecular connectivity index (MCI) to characterise the chemical behaviour and metabolism in cattle instead of K_{OW} was proposed by Dowdy et al.(1996), although the USEPA reported that there was no significant difference in performance between this approach and the Travis and Arms(1988) model using their data set (US EPA, 2005).

Mechanistic cattle biotransfer models have also been constructed, for example, ACC-HUMAN (Czub & McLachlan, 2004) based on Mclachlan (1994) model, RAIDAR (Arnot & Mackay, 2008) and OMEGA (Hendriks et al., 2007). All these models are based on mass balance of pollutants between the input, e.g. ingestion of pollutants, and the output, e.g., excretion with milk, faeces, and urine, and metabolism. McLachlan (1994) noted that the metabolic rate and absorption efficiency were the key parameters. However, the specific metabolic rate in cattle for each pollutant needs to be known in all the three models and there are few actual data. To date no simple cattle model has been developed for the metabolism of chemicals based on their chemical properties and this has resulted in the limited applicability of mechanistic models to a broad range of pollutants.

More recently, Rosenbaum et al. (2009) introduced a linear regression of the metabolic rate and K_{OW} into their newly developed model, CKow. When using this approach the model fit to observed BTFs was better than the K_{OW}-regressions of Travis and Arms (1988) and RTI (2005). However, the model accuracy might be limited for a wide range of organic chemicals because of the considerable deviation of the measured from the estimated metabolic rate in their model set (up to two orders of magnitude). Therefore, alternative approaches for deducing the metabolic rate in cattle for various pollutants need to be considered.

In regulatory risk assessment, Quantitative Structure-Activity Relationships (QSARs) are often preferred practically for filling data gaps to reduce costs and prevent animal studies which may have ethical barriers (Cefic & VCI, 2009). Another case for filling the data gaps is using parameter values obtained from other species, called species read-across in this study. For example, Arnot et al., (2010) used the measured metabolic rate in fish as a substitute for avian and mammalian species with the biological explanation of each metabolism. These substitution techniques should also be useful for estimating the cattle metabolism of a wide scope of organic pollutants targeted by the regulatory authorities.

The aim of this study was to assess and improve the accuracy of biotransfer models of organic pollutants to cow's milk and meat for use in human exposure assessment, focusing on the metabolism and the absorption of these contaminants in cattle. This was achieved through QSARs and the species read-across approach, specifically the metabolic rate in cattle was estimated by QSAR biodegradation models of microorganisms (the Biodegradation Probability Program for Windows, BioWIN) and fish (EPI-HL and IFS-HL). The performance of cattle biotransfer estimation using the estimated metabolic rate was then assessed with experimental data and predictions of other existing models.

3-3 Methods

The iterative process for improving performance of cattle biotransfer models was:

1) check the performance of existing models, based on an assessment of the residual error between the simulated and observed BTFs, against a broad range of experimental data;

2) introduce the QSAR and the species read-across approach to these models to deduce the metabolic rate;

3) check the improvement of the model performance following the optimisation of parameters like the absorption efficiency;

4) re-build the model regression using the simulated metabolic rate as a predictor.

The biotransfer of organic pollutants to milk and meat can be expressed in three ways: bio-concentration factor (BCF), biotransfer factor (BTF), and carry-over rate (COR) (Thomas et al., 1999):

$$BCF = \frac{concentration\ in\ meat\ or\ milk\ (mg \cdot kg^{-1})}{concentration\ in\ feed\ (mg \cdot kg^{-1})} \quad (3\text{-}1)$$

$$BTF = \frac{concentration\ in\ meat\ or\ milk\ (mg \cdot kg^{-1})}{daily\ intake\ of\ chemicals\ (mg \cdot kg^{-1})} \quad (3\text{-}2)$$

$$COR = \frac{chemical\ flux\ in\ the\ milk\ (mg \cdot day^{-1})}{daily\ intake\ of\ chemicals\ (mg \cdot day^{-1})} \quad (3\text{-}3)$$

In addition, BTFs to whole milk (BTF_{milk}) and meat (BTF_{meat}) were adopted in this study and other criteria such as BTFs to milk lipid were converted to BTF using values of daily intake of feed (16 kg·day^{-1} for lactating cow, 8 kg·day^{-1} for non-lactating cattle), milk mass flow (23 kg·day^{-1}), and lipid fraction in milk (0.04) and meat (0.25) in manner of previous models (Hendriks et al., 2007; Dowdy et al., 1996; Rosenbaum et al., 2009).

3-3-1 Experimental Data.

Experimental data for BTF_{milk} of 133 chemicals and for BTF_{meat} of lactating cows (40 chemicals) and non-lactating cattle (34 chemicals) were gathered from four existing data sets cited by Travis and Arms (1988), RTI (2005), Dowdy et al. (1996), MacLachlan and Bhula (2008), and Rosenbaum et al. (2009). The BTF data for mixtures and for residues including the metabolites, and undefined BTF_{meat} data where there was no differentiation between lactating cows or non-lactating cattle, were excluded. Other BTF data from other references were also included (Gutenmann & Lisk, 1969; Kowalczyk et al., 2013; Kierkegaard et al., 2009). When multiple BTF values for a chemical compound were reported in the references then the geometric mean of BTF values was adopted for the compound as the BTF values are logarithmic (Takaki et al., 2014) (APPENDIX III). The compounds considered covered a wide range of physicochemical

properties including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dioxins and furans (PCDD/Fs), hydrophobic and hydrophilic pesticides and perfluorinated compounds.

3-3-2 Model Descriptions.

Ten existing models for estimating cattle biotransfer of organic pollutants were selected: Travis and Arms model (hereafter referred to as the T&A model) (Travis & Arms, 1988), the MacLachlan and Bhula model (M&B model) (MacLachlan & Bhula, 2008), the RTI model (Research Triangle Institute, 2005), EUSES (Lijzen & Rikken, 2004), CalTOX (Mckone, 1993), the Dowdy et al. model (hereafter MCI model) (Dowdy et al., 1996), RAIDAR (Arnot & Mackay, 2008), ACC-HUMAN (Czub & McLachlan, 2004), OMEGA (Hendriks et al., 2007) and CK_{OW} (Rosenbaum et al., 2009). The first five models listed are K_{OW}-regression based models. The T&A model contains two simple linear regression equations, one for transfer to milk and another for transfer to meat (Travis & Arms, 1988). EUSES and CalTOX adopted these regressions, however, EUSES set an upper and a lower limit of BTF values within the tested range of K_{OW} ($10^3 < K_{OW} < 10^{6.5}$ for milk), and CalTOX set just an upper limit (BTF < 0.1) (Lijzen & Rikken, 2004; Mckone, 1993). The M&B model and RTI model are similar shaped quadratic K_{OW}-regressions, with the RTI model using a single regression for meat and milk and the M&B model using separate equations (MacLachlan & Bhula, 2008; Research Triangle Institute, 2005). The MCI model has a regression for milk and beef (Dowdy et al., 1996). The four mechanistic models RAIDAR, ACC-HUMAN, OMEGA, and CKow simulate the mass flow through cattle i.e. ingestion of food for the input, and for the output faeces excretion, urination, milk excretion and metabolism (Figure 3-1) (Hendriks et al., 2007; Czub & McLachlan, 2004; Arnot & Mackay, 2008; Arnot et al., 2010).

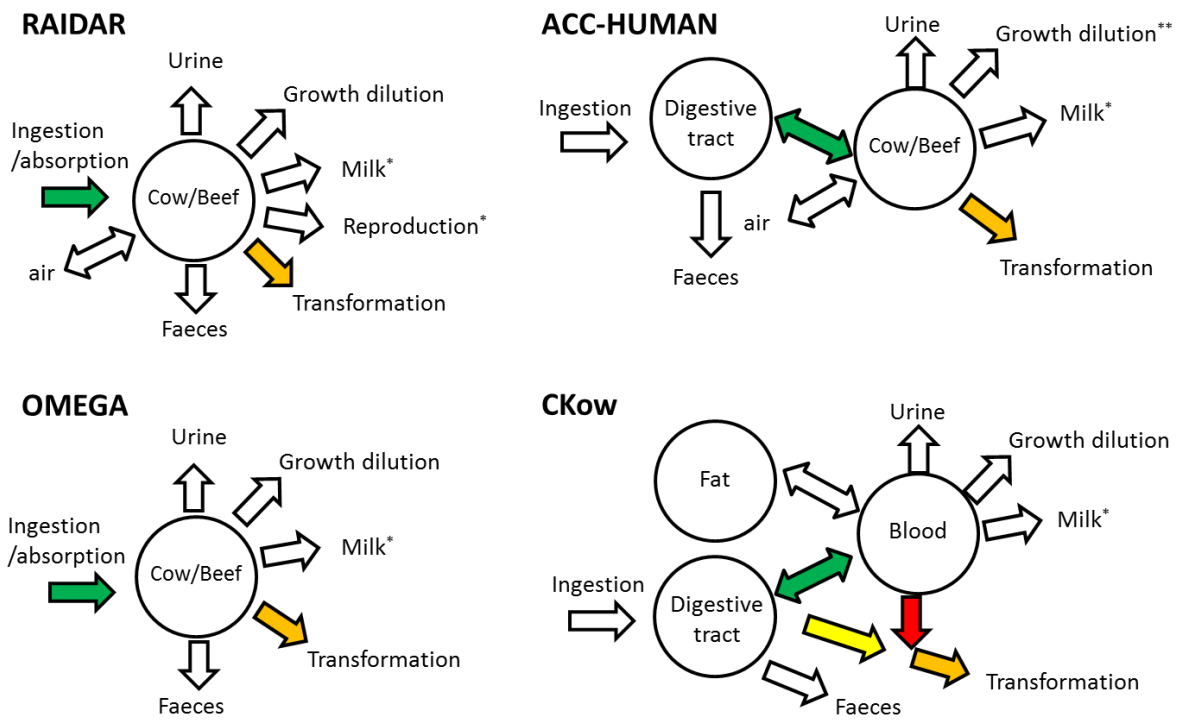


Figure 3-1. The overall structures of the mechanistic cattle biotransfer models used in this study. The arrows represent the mass flow of chemical compounds. Absorption (green) and transformation (orange, red and yellow) are coloured. *Only for lactating cow. **Only for non-lactating beef.

Steady-state conditions are assumed in RAIDAR, OMEGA, and ACC-HUMAN (adopted steady-state version) for both lactating cows and non-lactating cattle while CKow assumes dynamic conditions (Hendriks et al., 2007; Arnot & Mackay, 2008; Rosenbaum et al., 2009; Czub et al., 2011). The averaged exposure duration for each compound measured in the experiments was used as input to CKow in this study (APPENDIX III). When modelling the metabolism in cattle, the chemical specific metabolic rate is needed in RAIDAR while a default value for labile substance is set in ACC-HUMAN and OMEGA. OMEGA classified a labile substance based on the dissociation (pKa < 7), and the transformation (metabolites reported in cattle study) (Hendriks et al., 2007). CK_{OW} estimates both metabolic rate in gut (k_{met}^{gut}) and metabolic rate in cow itself with urine excretion rate (k_{rem}^{cow}) by using the same K_{OW} regression: $\log k_{rem}^{cow/gut} = 1.43 - 0.48 \cdot \log K_{OW}$ ($R^2 = 0.52$). These model equations were described in APPENDIX IV.

3-3-3 The Procedural Models

To assess model performance against experimental data, it was necessary to convert the theoretical models into a consistent working format. This was achieved by coding the models using EXCEL®. The default parameter values for each model were used as a starting point. The physicochemical properties considered, for example K_{OW} and Henry's law constant, were taken from EPI Suite™ developed by U.S. Environmental Protection Agency (U.S.EPA) (US EPA, 2012) except the K_{OW} values of PCBs, PBDEs, and PCDD/Fs, which were derived from Schenker et al. (Schenker, MacLeod, Scheringer, & Hungerbühler, 2005), Wania and Dugani (Frank Wania & Dugani, 2003), and Chen et al. (Chen, Quan, Yazhi, Yan, & Yang, 2001) respectively, because the K_{OW} values of these hydrophobic compounds were very sensitive when the parameterisation of absorption efficiency was undertaken (M S McLachlan, 1994)(Kierkegaard et al., 2009). The BTF value of each compound was then calculated and compared with the literature values.

3-3-4 Estimation of Cattle metabolism

The metabolic rate in cattle was estimated using three biodegradation models: BioWIN 4 (Boethling et al., 1994), EPI-HL (Arnot et al., 2009) and IFS-HL (Brown et al., 2012). These were chosen because only the simple structural information of the targeted compound is required for estimating their biodegradation. These data are also easy to access because BioWIN 4, EPI-HL and IFS-HL are all available online (US EPA, 2012; Brown et al., 2012). BioWIN calculates the probability of biodegradation of chemicals by microorganisms. There are seven types of BioWIN, but BioWIN 1, 2, 5, 6 and 7 were excluded since their estimations are not quantitative. BioWIN 3 and 4 are semi-quantitative models for ultimate and primary biodegradation respectively and are based on expert survey scores for the degradation of 200 chemicals as follows: 5 = hours; 4 = days; 3 = weeks; 2 = months; 1 = longer (Boethling et al., 1994). In this study focusing on primary biodegradation, BioWIN 4 was adopted and the scores were converted to the half lives and then to the metabolic rate in microorganism ($k_{met\ BioWIN}$) where the scores 5 to 1 were given half lives of 1/24, 1, 7, 30, 365 (day) respectively in accordance with the expert scores and estimated exponentially ($r^2 = 0.999$, Figure 3-2). Therefore $k_{met\ BioWIN}$ were deduced:

$$k_{met\ BioWIN} = \frac{ln(2)}{3200 \cdot e^{-2.2 \cdot (BioWIN4\ score)}} \quad (3\text{-}4)$$

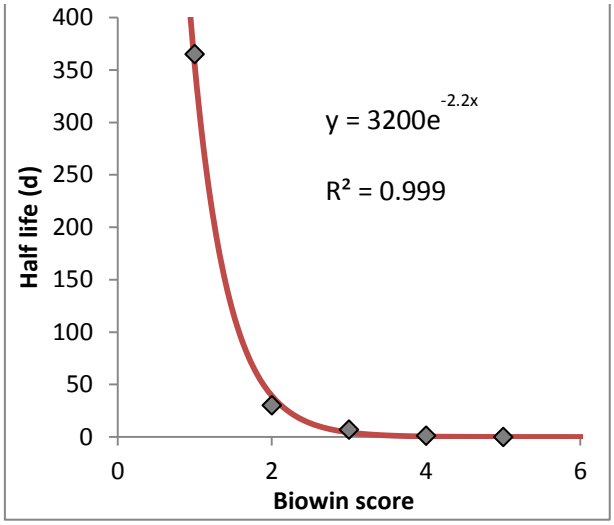


Figure 3-2 The relationship between BioWIN score and half life in the environment. Exponential regression and the coeffici-ent of determination were shown in the chart.

The mean value of the metabolic rate in fish ($k_{met\ Fish}$) from EPI-HL and IFS-HL was adopted for the predictions. Each metabolic rate was then converted to the metabolic rate in cattle being multiplied by the interspecific correction factor (ICF) based on Arnot et al. (J. a Arnot et al., 2010) which previously assumed the cattle metabolic rate 5 times faster than the fish metabolic rate.

$$k_{met\ cattle} = k_{met\ BioWIN\ (or\ Fish)} \cdot ICF \qquad (3\text{-}5)$$

The ICF was set as an integral number in each model for minimising the residuals between simulated and observed BTF values. CKow has two metabolic stages: in the gut and the following absorption into the bloodstream, thus CKow introduced the combination of $k_{met\ BioWIN}$ (in the gut) and $k_{met\ Fish}$ (after the absorption).

3-3-5 Model Parameterisation

Model parameterisation was considered for determining absorption efficiency (AE) known to be the other key property after metabolic rate for cattle transfer (McLachlan, 1994). RAIDAR, ACC-HUMAN and CKow have adopted AE from McLachlan (1994) parameterisation. However, other AE data, whose values deviated from the Mclachlan (1994) regression, have also been obtained (Thomas et al., 1999; Kierkegaard et al., 2009). Gut metabolism was not considered in McLachlan's parameterisation. The parameters related to AE were therefore re-optimised in this study adding additional data sets and

considering the metabolism in gut. This parameterisation could only be done in CKow since the metabolism in gut was not included in other models. AE was described in CKow as:

$$AE = \frac{I - \varphi_{fae}}{I} = \frac{\varphi_{g-b} + \varphi_{met}^{gut} - \varphi_{b-g}}{\varphi_{g-b} + \varphi_{fae} + \varphi_{met}^{gut} - \varphi_{b-g}} \quad (3\text{-}6)$$

$$\varphi_{g-b} = \left(\frac{1}{Q_{AW}} + \frac{1}{Q_{AO} \cdot K_{OW}}\right)^{-1} \quad (3\text{-}7)$$

Where:

I = chemical intake

φ_{g-b}, φ_{b-g} = transport flux from gut to blood, and blood to gut respectively (kg/day)

φ_{met}^{gut} = removal flux via metabolism in gut (kg/day)

φ_{fae} = removal flux via faeces respectively (kg/day).

Q_{AO}, Q_{AW} = octanol and water film diffusion transfer coefficient respectively (kg/day)

Q_{AO} and Q_{AW} are the main parameters of the transport fluxes above and were set to be variables for McLachlan's parameterisation.

In this study, the values of those two variable parameters (Q_{AO} and Q_{AW}) were optimised by minimising the residuals between the observed and simulated logAE for 58 chemicals (APPENDIX III). After the parameterisation for AE, the parameter for the fraction of the chemical in the total lipid mass that is available for degradation during the experiment ($f_l^{available}$) was set. This value was previously set to 0.35 for CKow to minimise the simulated and observed COR residues (Rosenbaum et al., 2009). Since $f_l^{available}$ was considered to be low due to its hydrophobicity in CKow, we assumed the value of $f_l^{available}$ to decrease linearly with increasing $\log K_{OW}$ instead using a fixed value. The slope and the intercept were then determined by minimising the residual errors.

3-3-6 Goodness of Fit Statistics

Two approaches to goodness of fit tests were chosen for evaluating the accuracy of the models against the experimental data: the residual sum of squares (RSS) as an indication of absolute differences between observed and estimated values (Eqn. 3-8), and the standard errors (S_e) for normalising the differences using the number of samples in each experiment (Hendriks et al., 2007);

$$RSS = \sum_{i=1}^{N} (m_i - r_i)^2 \quad (3\text{-}8)$$

$$S_e = \sqrt{\sum_{i=1}^{N} (m_i - r_i)^2 / (N - k)} \quad (3\text{-}9)$$

where N =sample number, m = simulated logBTF, r = observed logBTF, k = 1 (non-linear model), 2 (regression-based model). Another typical standard tests of model performance, the coefficient of determination r^2, was excluded because it is an inadequate measure for the goodness of fit in nonlinear models (Spiess & Neumeyer, 2010).

3-4 Results and Discussion

3-3-5 Comparison of Existing Cattle Biotransfer Models

The predictions of the models under assessment for milk transfer of organic pollutants from feed were compared against experimental observations using $logK_{OW}$-$logBTF_{milk}$ charts (Figure 3-3). In addition, the goodness of fit tests between the model estimation and the observed data were performed (Table 3-1).

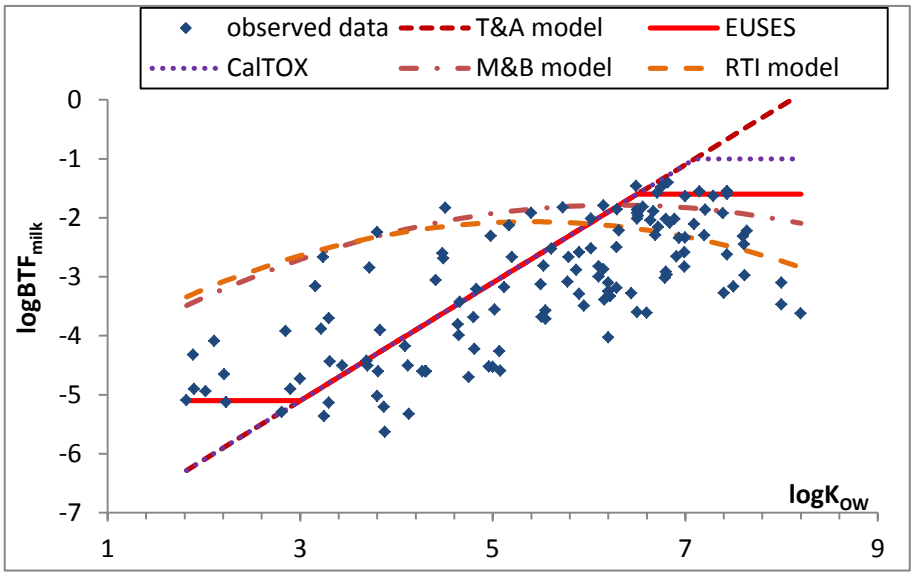
TABLE 3-1. Results of the goodness of fit tests for estimated versus measured $logBTF_{milk}$ and $logBTF_{meat}$ (original models)

Model	$logBTF_{milk}$ (n=129)		$logBTF_{meat}$ (n=93)	
	RSS	***S_e***	***RSS***	***S_e***
regression based model				
T&A model	196	1.24	166	1.35
EUSES	117	1.00	114	1.12
CalTOX	165	1.19	119	1.14
M&B model	271	1.53	341	1.94

RTI	240	1.44	268	1.72
MCI model	101	0.89	98	1.04
mechanistic model				
RAIDAR	518	2.00	485	2.30
ACC-HUMAN (persistent)	542	2.16	405	2.10
(labile)	188	1.27	167	1.35
OMEGA (persistent)	332	1.60	406	2.10
(labile)	173	1.16	138	1.22
CK_{OW}	77	0.77	83	0.95

Goodness of fit of the models is characterized by the residual sum of squares (RSS) and the standard errors (Se).

(a)



(b)

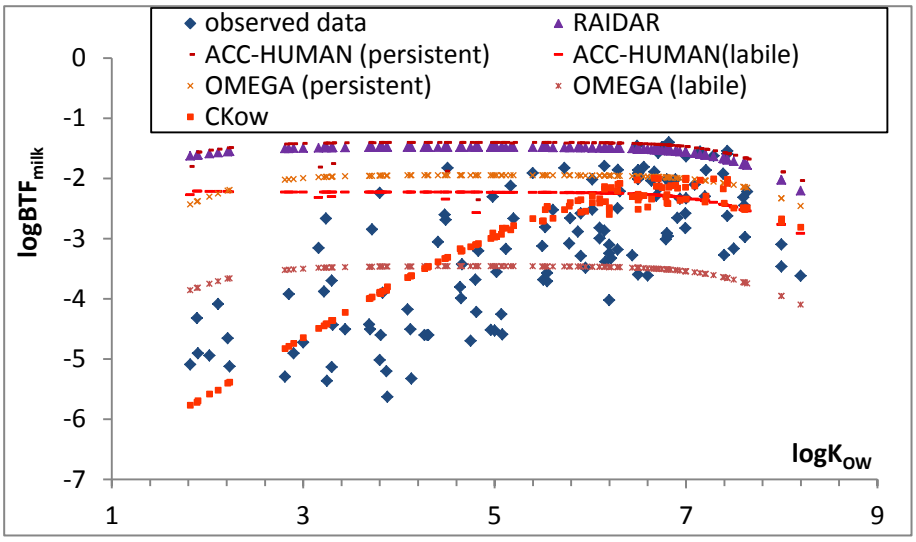
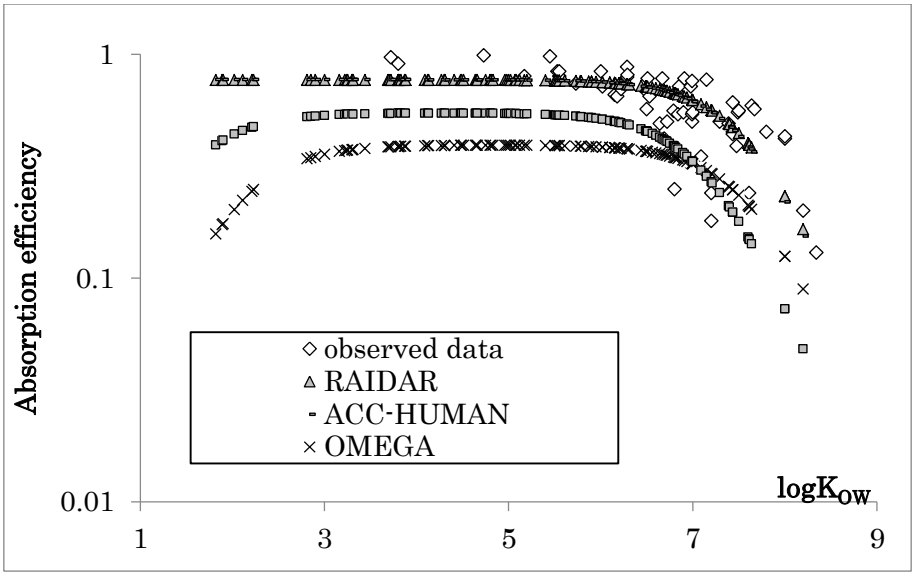


Figure 3-3. The results of model predictions for milk transfer models shown on $logK_{OW}$-logBTF chart with experimental data ((a): regression-based models, (b): mechanistic models).

The observations expressed high variability of BTF values irrespective of K_{OW}, therefore the goodness of fit of the K_{OW}-regression model was consequently low; no models had a standard error less than 1.00. EUSES demonstrated a lower residual error (*RSS* = 116.9, S_e = 1.00) than that of the other K_{OW}-regression models for milk transfer, including the T&A model (*RSS* = 195.5, S_e = 1.24). This was because EUSES adopted a maximum and minimum BTF value outside the tested range ($10^3 < K_{OW} < 10^{6.5}$ for milk). The M&B model and RTI model overestimated BTF values across the K_{OW} range because these models were built focusing on persistent chemicals in their data sets. The MCI model, based on a regression between logBTF and polar corrected MCI instead of K_{OW}, showed better estimation than K_{OW}-regressions (*RSS* = 100.5, S_e = 0.89). This result has been reported previously (Dowdy et al., 1996; US EPA, 2005). Mechanistic models, whose metabolic rates were fixed, had three phases in accordance with K_{OW} changing. The BTF has a fixed value in RAIDAR and ACC-HUMAN where $\log K_{OW} < 2$, the BTF of OMEGA decreased linearly with K_{OW} in this range. The three models then estimated the BTF values to be constant within the $\log K_{OW}$ range of approximately 2 to 7. The BTF decreased with increasing K_{OW} when $\log K_{OW} > 7$. These trends were consistent with those of the absorption efficiency (Figure 3-4), yet, did not follow those of observed data (Figure 3-3). Meanwhile, CK_{OW}, which contains the K_{OW}-based regression for the metabolic rate (Figure 3-4), showed a linear increase of BTF with increasing K_{OW} as for the regression-based models when $\log K_{OW} < 6$. The slope reduced with $\log K_{OW} > 6$ due to a decline in the absorption efficiency. This combination of estimation between the metabolic rate and the absorption efficiency resulted in the best fit (*RSS* = 76.7, *Se* = 0.77) of all models. These results supported the importance of the metabolic rate and the absorption efficiency in cattle for estimating the biotrasfer.



(b)

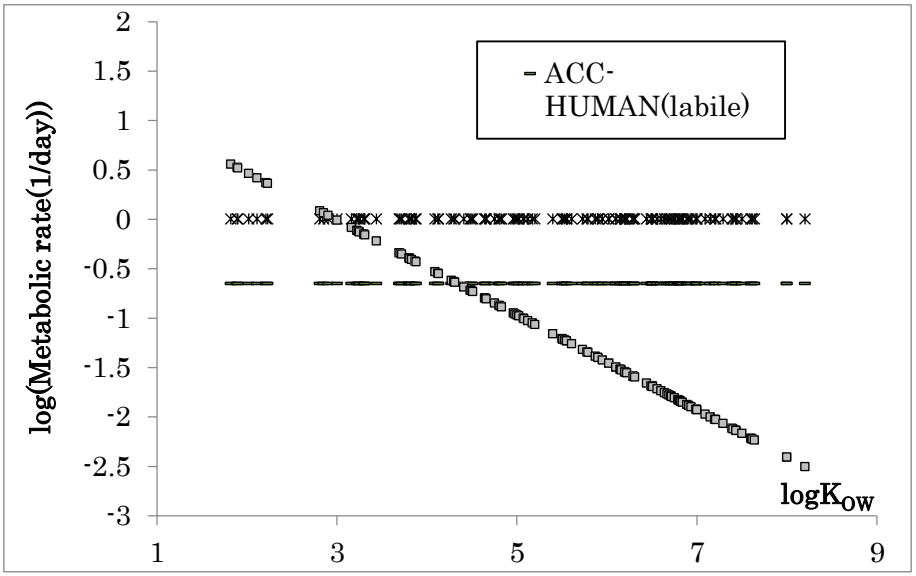
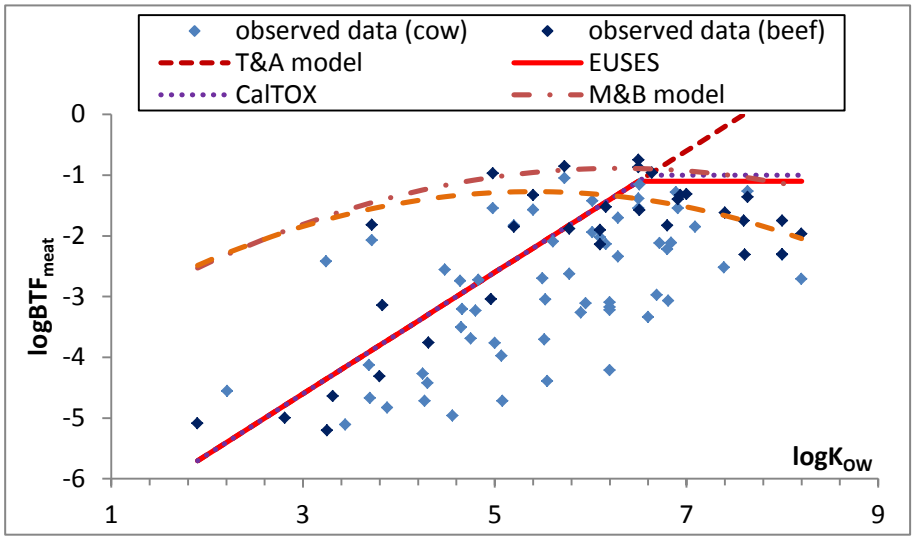


Figure 3-4 Model comparison for (a)absorption efficiency and (b)metabolic rate.

The model predictions for the biotransfer to meat in both lactating cow and non-lactating beef are shown in Figure 3-5 and the results of goodness of fit tests are presented in Table 3-1. The overall trend was same as that of BTF_{milk}. CKow showed the lowest residues of all (*RSS* = 83.3, *Se* = 0.95), MCI model was the second lowest (*RSS* = 98.3, *Se* = 1.04), and EUSES the third (*RSS* = 114.4, *Se* = 1.12).

(a)



(b)

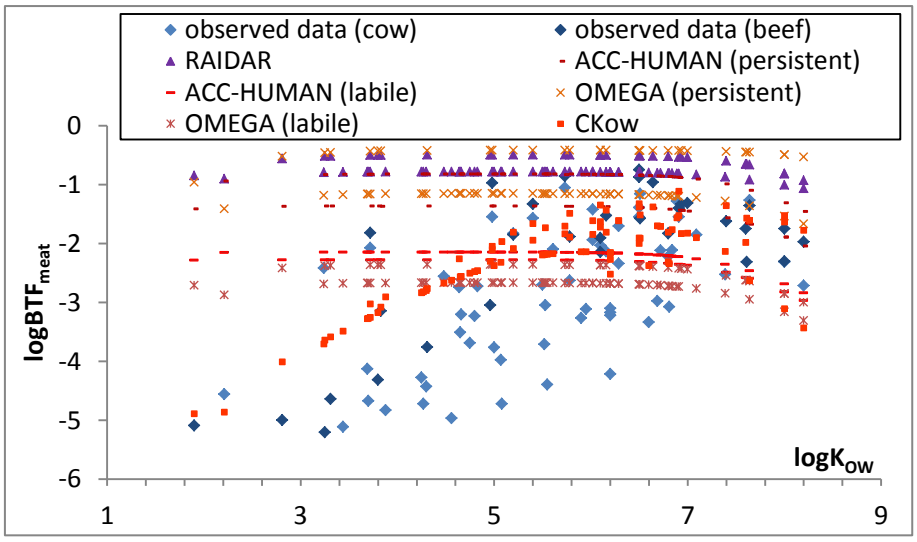


Figure 3-5. The results of model predictions for beef transfer models shown on logK$_{OW}$-logBTF chart with experimental data ((a): regression-based models, (b): mechanistic models).

3-3-6 Introduction of Simulated Metabolic Rate

The terms $k_{met\ BioWIN}$, and $k_{met\ Fish}$ were introduced to the mechanistic cattle biotransfer models described above as simulated metabolic rates in cattle. The modified model estimations were compared against experimental data and the results of the goodness of fit tests were shown in Table 3-2.

TABLE 3-2. Results of the goodness of fit tests for estimated versus measured $logBTF_{milk}$ and $logBTF_{meat}$ (Introducing QSAR for estimating the metabolism)

Model	Method of estimating metabolism	$logBTF_{milk}$ (n=129)			$logBTF_{meat}$ (n=93)		
		ICF	***RSS***	S_e	***ICF***	***RSS***	S_e
RAIDAR	BioWIN	4	94	0.85	7	84	0.96
	Fish	2	73	0.75	3	62	0.82
ACC-HUMAN	BioWIN	6	91	0.89	8	88	0.98
	Fish	3	71	0.78	4	65	0.84
OMEGA	BioWIN	5	86	0.82	14	77	0.91
	Fish	2	72	0.74	7	55	0.78
CKow	BioWIN	1	82	0.80	2	74	0.90
	Fish	1	177	1.17	1	86	0.97
	BioWIN (gut)	1	64	0.71	1	55	0.77
	Fish (liver)						
CKow (parameterised)	K_{OW} regression	-	75	0.76	-	69	0.87
	BioWIN (gut)	1	54	0.65	1	41	0.67
	Fish (liver)						

Goodness of fit of the models is characterized by the residual sum of squares (RSS) and the standard errors (Se). Metabolic rate is deduced by K_{OW} regression from CKow model, BioWIN4, or Fish QSAR biodegradation models (EPI-HL, IFS-HL). The interspecific correction factor (ICF) was set as an integral number in each model for minimising the residues between simulated and observed BTF values

RAIDAR, ACC-HUMAN, and OMEGA, which have one metabolic pathway (Figure 3-1), all showed reduced residual errors following incorporation of $k_{met\ BioWIN}$ or $k_{met\ Fish}$. Particularly, the introduction of the term $k_{met\ Fish}$ enabled the three models to produce better estimates for BTF than CKow, which had showed the lowest residual errors for both BTF_{milk} and BTF_{meat} (Table 3-1 and 3-2). The estimation of the CKow was further improved when $k_{met\ BioWIN}$ and $k_{met\ Fish}$ were incorporated for the metabolic rate in gut and the metabolic rate after the absorption respectively (Tables 3-1 and 3-2). It is well known that the metabolism in the cow gut is caused mainly by microorganisms in the process of rumination, and the metabolism after the absorption occurs in the liver, as is the case for metabolism in fish. It was, therefore, reasonable to estimate the metabolism in the gut using BioWIN, and the metabolism after the absorption by fish

biodegradation models. These assumptions produced the best fit to the experimental cattle biotransfer data. ICF values showed a different trend between CKow and the other three. While the ICF values of CKow were 1 in most cases, the other three had larger values (Table 3-2). One possible reason was that CKow included two metabolic processes but the others had only one, and they amplified their metabolisms using larger ICF values. Their ICF values for fish QSARs ranged 2 to 7 that was consistent with the previous study using RAIDAR (ICF = 5, Arnot et al., 2010).

3-3-7 Model Parameterisation

The absorption efficiency (AE) parameter in CKow was recalculated from the new data set and the metabolic process in gut considered rather than using the values of the Mclachlan regression(M S McLachlan, 1994). As described in the Methods section, the values of the two variable parameters, the octanol and the water-film diffusion transfer coefficient (Q_{AO}, Q_{AW}, kg·day^{-1}), were determined by minimising the residual error between the observed and simulated logAE (Q_{AO} = 0.58 → 0.65, Q_{AW} = 4030000 → 13000000, Figure 3-6).

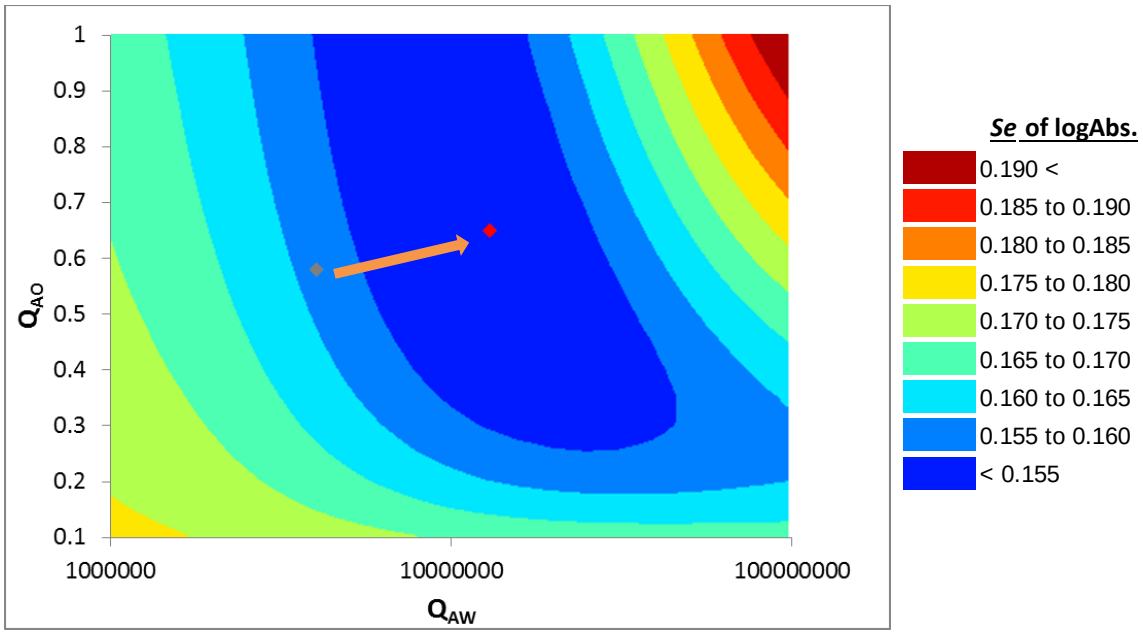


Figure 3-6 Parameterisation of the Absorption efficiency (Abs.) to minimise the standard error between observed and estimated logAbs. The red and grey dots represent calibrated and original Q_{AO} and Q_{AW}.

The fraction of the chemical in the total lipid mass that is available for degradation during the experiment ($f_l^{available}$) was determined by calibration by minimising the residual error between observed and simulated logBTF. The calibrated $f_l^{available}$ was determined for CKow using K_{OW}-regression for estimating metabolic rate (CKow, original), and CKow using $k_{met\ BioWIN}$, and $k_{met\ Fish}$ (CKow, modified) separately (Figure 3-7). The use of calibrated AE and $f_l^{available}$ in CKow improved the score of the goodness of fits further (Table 3-2).

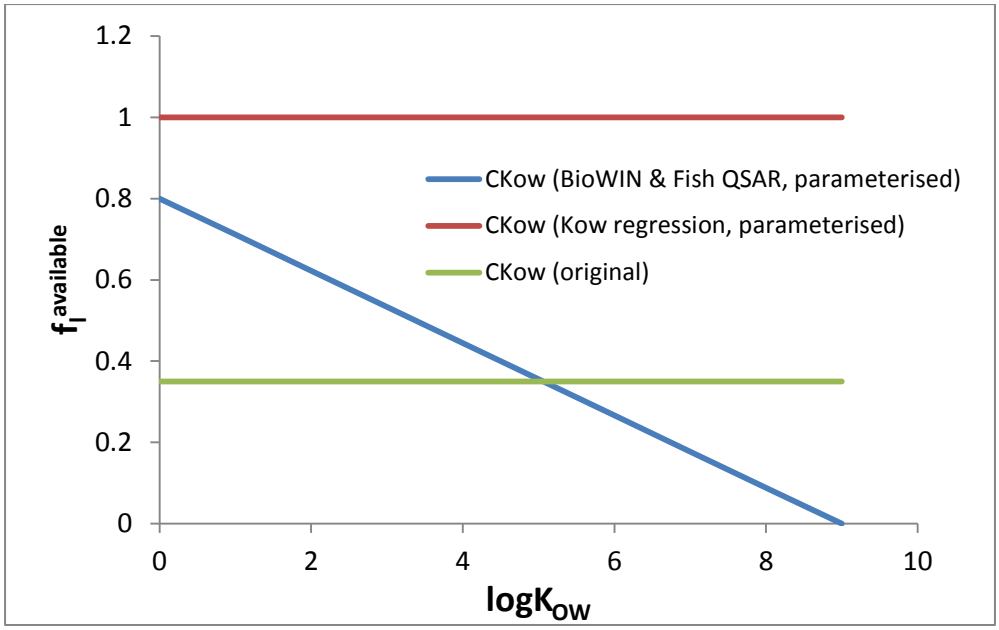


Figure 3-7 Parameterisation of $f_l^{available}$ to minimise the standard error between observed and estimated logBTF.

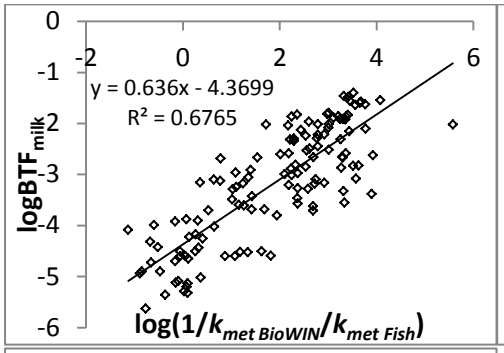
3-3-8 New Regressions for Estimating BTF

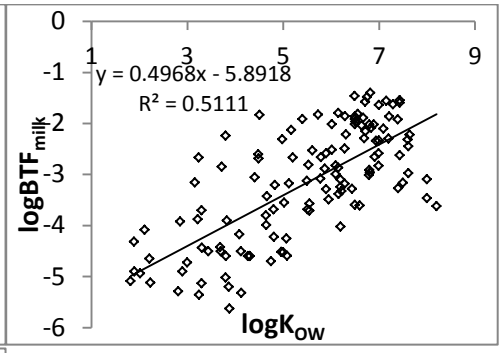
As described above, metabolic rate was a controlling factor of BTF estimation. In this section, to make cattle transfer models simpler, new regression models of BTF estimation using metabolic rate were proposed instead of the complex mechanistic models, like CKow. Assuming that increasing metabolic rate decreases logBTF, $\log(k_{met\ BioWIN} \cdot k_{met\ Fish})^{-1}$, when considering whole metabolism in cattle, was set as a predictor for logBTF. Adding the existing two predictors for the regression, $\log K_{OW}$ and logarithm polar-corrected molecular connectivity indices (log ($^1X_{pc}$)) used in MCI model, three predictors for new regressions were selected. Four responses for logarithmic biotransfer factors were set: to milk ($\log BTF_{milk}$), to meat including lactating cow and non-lactating beef ($\log BTF_{meat}$), to lactating cow meat ($\log BTF_{cow}$), and to non-lactating beef $\log BTF_{beef}$. The results of the goodness of fit tests comparing estimated and observed logBTF values are shown in Table 3-3. $\log((k_{met\ BioWIN} \cdot k_{met\ Fish})^{-1})$ expressed the best correlation with all four responses. $\log K_{OW}$ and log ($^1X_{pc}$) showed considerably weaker correlation than $\log((k_{met\ BioWIN} \cdot k_{met\ Fish})^{-1})$. The relationships between BTF and the predictors are shown in Figure 3-8.

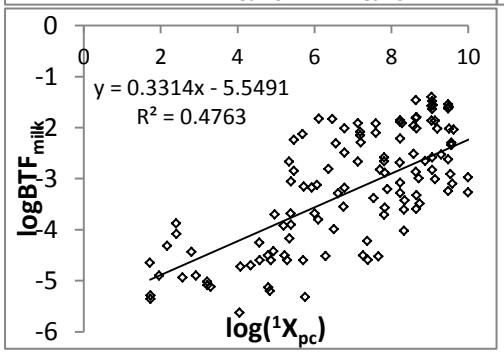
TABLE 3-3. The new regressions with a new predictor on the metabolism using the BTF data set of this study, and the results of the goodness of fit tests for estimated versus measured logBTF

predictor x	regression equation	N	r^2	estimated versus measured logBTF	
				RSS	S_e
Biotransfer to milk					
$\log K_{OW}$	$\log BTF_{milk} = 0.5x - 5.89$	129	0.51	76	0.78
$\log({}^1X_{pc})$	$\log BTF_{milk} = 0.33x - 5.55$	129	0.48	81	0.80
$\log((k_{met\ BioWIN} \cdot k_{met\ Fish})^{-1})$	$\log BTF_{milk} = 0.64x - 4.37$	129	0.68	51	0.63
Biotransfer to meat					
$\log K_{OW}$	$\log BTF_{meat} = 0.57x - 5.88$	93	0.41	81	0.95
$\log({}^1X_{pc})$	$\log BTF_{meat} = 0.32x - 5.07$	93	0.33	94	1.01
$\log((k_{met\ BioWIN} \cdot k_{met\ Fish})^{-1})$	$\log BTF_{meat} = 0.78x - 3.95$	93	0.67	45	0.70
Biotransfer to cow meat					
$\log K_{OW}$	$\log BTF_{cow} = 0.5x - 5.72$	61	0.30	52	0.94
$\log({}^1X_{pc})$	$\log BTF_{cow} = 0.24x - 4.71$	61	0.16	63	1.03
$\log((k_{met\ BioWIN} \cdot k_{met\ Fish})^{-1})$	$\log BTF_{cow} = 0.66x - 4.12$	61	0.65	26	0.67
Biotransfer to beef					
$\log K_{OW}$	$\log BTF_{beef} = 0.58x - 5.61$	32	0.55	24	0.90
$\log({}^1X_{pc})$	$\log BTF_{beef} = 0.92x + 0.29$	32	0.65	19	0.80
$\log((k_{met\ BioWIN} \cdot k_{met\ Fish})^{-1})$	$\log BTF_{beef} = 0.96x - 4.35$	32	0.73	15	0.70

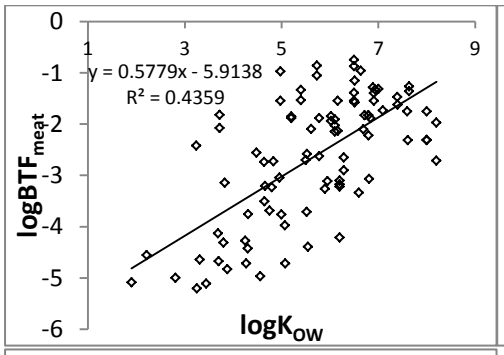
(Biotransfer factor into milk)

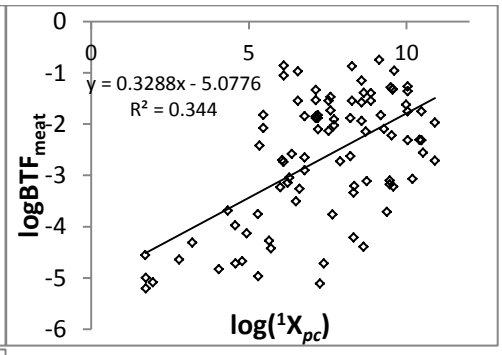


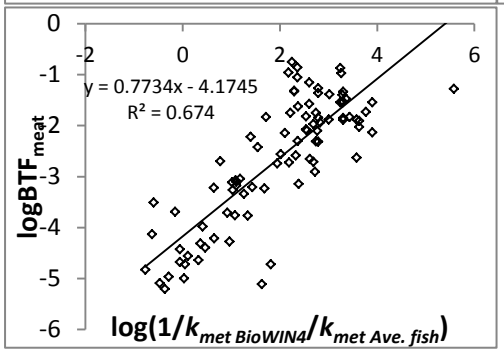




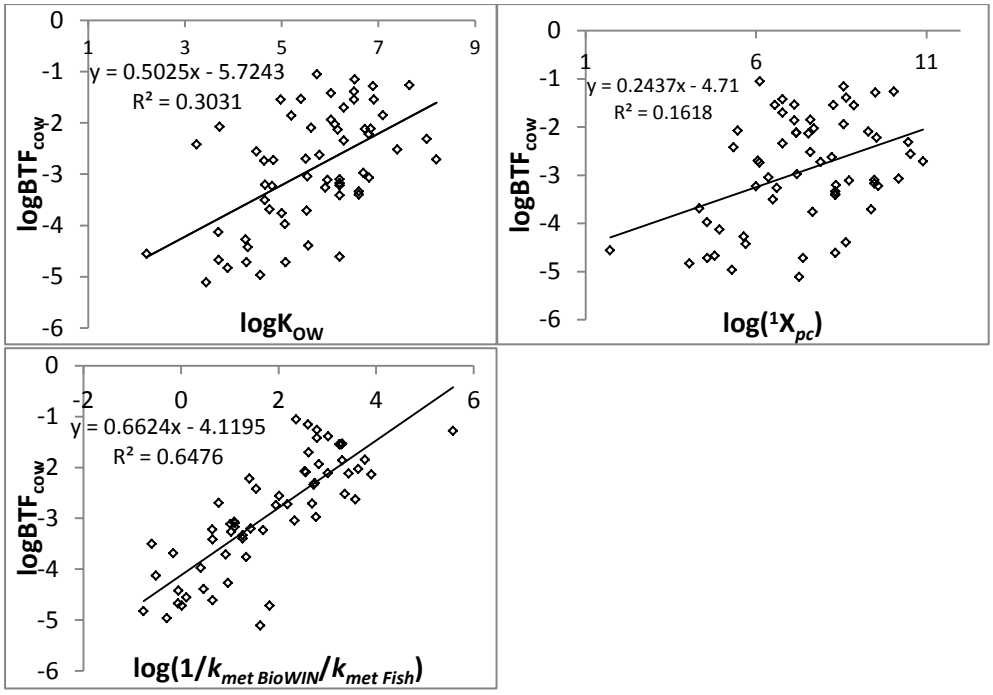
(Biotransfer factor into meat)







(Biotransfer factor into dairy cow)



(Biotransfer factor into non-lactating beef)

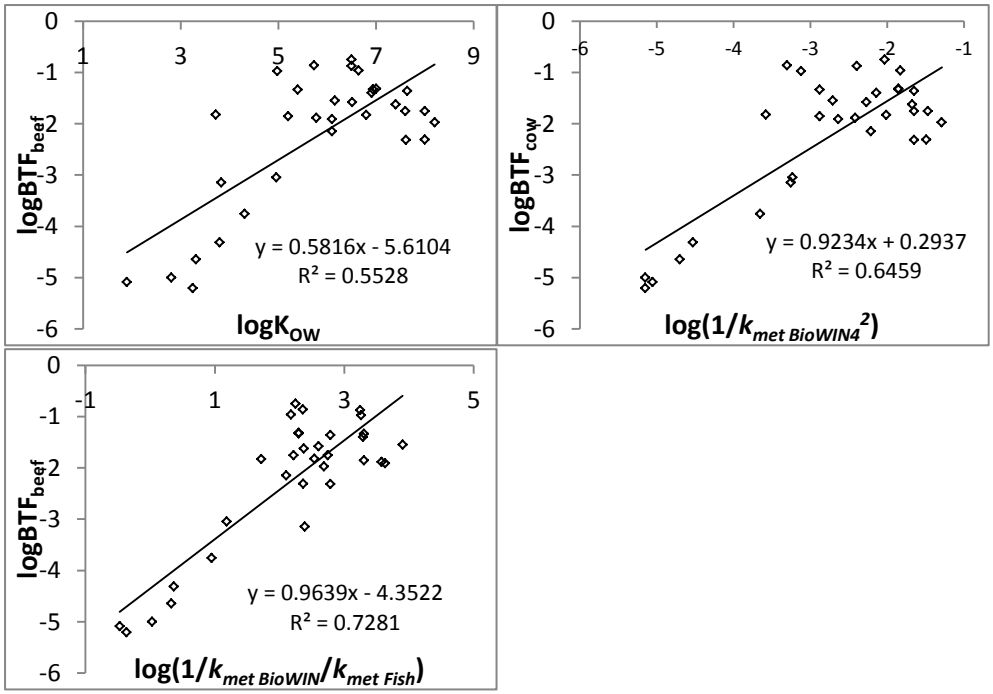


Figure 3-8 Relationship between observed BTF and the predictors. The predictor used was described on the chart.

Another regression for example, log($k_{met\ BioWIN}$) only or log($k_{met\ Fish}$) only, or the multiple regression of log($k_{met\ BioWIN}$) and log($k_{met\ Fish}$), did not produce better r^2 and S_e values compared to using log(($k_{met\ BioWIN}$ ·

$k_{met\ Fish}$)$^{-1}$) (data not shown). The new regression of the metabolic rate reproduced the observed BTF as well as calibrated CKow (modified) in the previous section (S_e = 0.63 (logBTF_{milk}), S_e = 0.70 (logBTF_{meat}), S_e = 0.67 (logBTF_{cow}), and S_e = 0.70 (logBTF_{beef})) (Table 3-3). These new regression models for estimating milk and beef transfer were found to be more accurate and are more physiologically realistic model with equivalent simplicity and usability for providing lower residual error than existing K_{OW}-regression models and were based on metabolic rate, a controlling factor of BTF, rather than K_{OW}.

3-3-9 Limitations of the modified cattle transfer models

The model prediction of the calibrated CKow (modified) and new regressions still displayed a certain degree of deviation from the experimental data (S_e = 0.63 - 0.70). Twenty two classes of chemical compounds with simulated values of BTF by calibrated CKow (modified) deviated from the observed values for over one order of magnitude (Table 3-4).

Possible causes of the deviation regarding the limitation of the biodegradation models (BioWIN4, EPI-HL, and IFS-HL) may be due to:

1) a lack of recognition of congener differences in QSAR models;

2) current limitations of EPI-HL (Arnot et al., 2009) to represent either acids or bases, or compounds that have a fragment with a large negative coefficient value and many fragment counts for the same descriptor, and current limitations of BioWIN4 (Boethling et al., 1994) to represent compounds that have carbonate fragments cycloalkane rings and two-nitrogen heteroaromatic rings;

3) an estimation of the half-live of biodegradation that deviated from the observed values by over one order of magnitude in EPI-HL (Arnot et al., 2009);

4) compounds that have low structural similarity to those chemicals used to build IFS-HL (Brown et al., 2012).

TABLE 3-4. Chemical list with residual error between measured and predicted logBTF by calibrated CKow (modified) larger than ± 1

Chemical name	CAS NO.	logK_{OW}	logBTF observed	logBTF predicted	Residual error	Possible cause

Biotransfer to milk						
Terbacil	5902-51-2	1.89	-4.32	-5.49	1.17	2(a,b,d),4
Bromacil	314-40-9	2.11	-4.08	-5.88	1.79	2(a,b,d),4
PFHxS (Perfluorohexanesulfonic acid)	355-46-4	3.16	-3.16	-4.81	1.65	2(a,c),4
Epoxiconazole	106325-08-0	3.44	-4.51	-3.29	-1.22	2(c)
PFOS	1763-23-1	4.49	-2.69	-4.61	1.92	2(a,c),4
Indoxacarb	173584-44-6	4.65	-3.99	-5.44	1.45	2(b,d)
Chlorpyrifos	2921-88-2	4.96	-4.52	-3.50	-1.02	2(a,b)
Propargite	2312-35-8	5.00	-4.53	-3.38	-1.15	2(d)
Dicofol	115-32-2	5.02	-3.55	-2.49	-1.07	2(a),4
Methoxychlor	72-43-5	5.08	-4.59	-2.97	-1.62	4
PCB 18	37680-65-2	5.55	-3.57	-2.53	-1.04	1
PCB 28	7012-37-5	5.55	-3.71	-2.39	-1.32	1
chlordane	12789-03-6	6.16	-3.39	-2.27	-1.12	2(d)
PCB 110	38380-03-9	6.22	-3.32	-2.16	-1.17	1
Biotransfer to cow meat						
epoxiconazole	106325-08-0	3.44	-5.11	-2.68	-2.42	2(c)
tebufenozide	112410-23-8	4.25	-4.27	-3.21	-1.06	2(a)
indoxacarb	173584-44-6	4.65	-3.51	-4.83	1.32	2(b,d)
methoxychlor	72-43-5	5.08	-4.72	-2.19	-2.53	4
BDE-49	243982-82-3	6.29	-2.90	-1.71	-1.19	1
Biotransfer to beef						
2,4-D	94-75-7	2.81	-5.00	-3.97	-1.03	2(a,b)
HCB	118-74-1	5.73	-0.86	-1.95	1.10	3,4
23478-PeCDF	57117-31-4	6.50	-0.75	-1.85	1.10	1

1 = congener difference, 2 = (a)acid/base(EPI-HL), (b)large negative coefficient value (EPI-HL), (c)numerous fragment count of same descriptor (EPI-HL), (d)carbonate, cycloalkane rings, or two-nitrogen heteroaromatic rings (BioWIN4), 3 = large deviation from training set value (EPI-HL), 4 = low similarity to training data set (IFS-HL).

Five of the 22 compounds considered in this study were PCBs, BDEs, and PCDD/Fs, which have been reported to have congener differences for cattle biotransfer (Table 4). Another 7 compounds were acids or bases, which might affect not only the EPI-HL estimation listed above, but also the absorption and transfer in cattle because the dissociation changes the partition between the flux in water and lipid irrespective of K_{OW}. Perfluorinated compounds (PFHxS, PFOS) also showed poor prediction. Adding to the possible causes listed e.g. acid or base, the lack of consideration of the fluoride fragment in IFS-HL would be another cause of the large deviation. Similarly, epoxiconazole, which showed the second highest residual error, was reported to be metabolised through the cleavage of the oxirane ring (European Food Safety

Authority, 2012), but this reactive fragment was not considered in QSAR biodegradation models. The deviations described could be improved with improving the accuracy of QSAR biodegradation model.

The estimation of the BTF for methoxychlor gave the highest residual error. Methoxychlor has been found to accumulate particularly in fish and this error might come from different mechanism of metabolism for methoxychlor between cattle and fish; the degrading enzyme (cytochrome P450) in fish has been found not to respond to mammalian P450 inducers (Stuchal et al. 2006). These examples illustrate the limitation of the method for introducing fish biodegradation to estimate the metabolism in cattle.

3-5 Conclusion

Introducing biodegradation models for estimating metabolic rate in cattle was confirmed to be an effective approach to improve the model accuracy, i.e. reducing the residual error, for all mechanistic cattle biotransfer models, particularly for CKow, which could reproduce the two-stage metabolism. The accuracy was improved further when the other sensitive parameter, the absorption efficiency, was optimised. Furthermore, new regressions using the simulated metabolic rate were then proposed. These showed equivalent scores in the goodness of fit tests to the calibrated CKow (modified) model while these have much simpler model structures. The K_{OW} regression model approach has been used for over two decades as it is simple and easy to use in spite of a lack of a mechanistic basis(Lijzen & Rikken, 2004). However, the modified CKow and the new regressions developed here were not only more accurate, but had equivalent usability to the original K_{OW} regression; it is therefore recommended they are subsequently used in the current screening chemical risk assessment models.