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Framework for defining pesticide maximum residue levels in feed: applications to cattle and sheep

Zijian Li[a*] and Peter Fantke[b]

Abstract

BACKGROUND: Pesticide residues in animal feed can endanger animal health and compromise the safety of livestock products for human consumption. Even though policymakers such as the European Union and the World Health Organization have established maximum residue levels (MRLs) for pesticides in both human food and animal feed, there is no systematic management of pesticides in animal feed that considers the entire supply chain. In response, we propose a framework for defining consistent MRLs for pesticides in animal feed that assesses the impact of defined MRLs on upstream (*e.g.*, MRLs in feed crops) and downstream (*e.g.*, MRLs in livestock products) sectors of the livestock-product supply chain.

RESULTS: The MRLs determined for the selected pesticides in the feed of cattle and sheep as case study animals indicate that lipophilic pesticides tend to have lower MRLs than hydrophilic pesticides, primarily due to the relatively high toxicity and biotransfer factors of lipophilic pesticides. In addition, we observe that, primarily for lipophilic pesticides, upstream and downstream regulations are not aligned in terms of defining MRLs in feed using current MRLs in crops with relevance to feed and foods of animal origin.

CONCLUSION: Some of the current pesticide regulations in the livestock-product supply chain need to be re-evaluated to ensure that MRLs in the upstream sector (*i.e.*, crops) do not result in unacceptable residues in the downstream sector (*i.e.*, MRLs in livestock products affecting animal and human health). Finally, we provide recommendations for optimizing the derivation of MRLs in feed, including the evaluation of residue fate during feed and food manufacturing processes.

Supporting information may be found in the online version of this article.

Keywords: plant protection products; animal husbandry; livestock health; food safety; pesticide regulation

1 INTRODUCTION

Pesticides are among the most regulated substances because of their intended toxic potency toward living organisms.[1–10] After application to croplands, pesticide residues can be transported *via* the livestock-product supply chain.[11–14] Worldwide, pesticide residues are frequently detected in food products of the livestock-product supply chain, including crops, animal feeds, livestock bodies, and foods of animal origin, particularly for some organic compounds that are amenable to bioaccumulation in livestock products.[5,13,15–21] As pesticides are toxic and designed to kill or control target organisms, pesticide residues remaining in the agricultural product supply chain can also cause health damage to non-target organisms (*e.g.*, livestock and humans) and economic losses in the pastoral industry.[22–24]

Among all major sectors in the livestock-product supply chain (*i.e.*, fodder crop cultivation, feed derivation, livestock farming, raw food production, industrial processing, transportation and retailing, and customer consumption), pesticide regulation in animal feed plays an important role because it can affect the health and quality of livestock products. Efforts have been made to define maximum residue levels (MRLs) of pesticides in feed.[5] The European Union (EU) established a framework for regulating chemical substances in feed, which aims to protect animal health and ensure the quality of foods of animal origin.[25] Most current MRLs for feed are defined using pragmatic approaches (*i.e.*, default or empirical values) rather than science- or risk-based assessment (*i.e.*, mechanism-based residue transfer models), which is mainly due to current data limitations. However, unlike pesticide regulations in crops or foods of animal origin that are directly linked to human consumption, the management of pesticide residues in feed must consider both upstream (*i.e.*, fodder crops) and downstream (*i.e.*, animal health and food safety) sectors in the livestock-product supply chain. In current MRL

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regulations (*e.g.*, EU and Codex Alimentarius), there is a lack of connection among different MRL-related components (*i.e.*, feed materials, animal feed, and foods of animal origin), rendering an impact analysis of pesticide MRLs throughout the livestock-product supply chain difficult.

Thus, the derivation of MRLs in feed is challenging because this process involves the combination of chemical-plant, plant–animal, and animal-region specific migration of pesticides from animal feeds (*e.g.*, in winter [silage] and summer [grass or herbs]) to grazing animals. Detailed information needs to be collected (if not available, modeling approaches should be developed) for at least the most relevant types of fodder crops (*e.g.*, maize, barley, and soybeans), livestock (*e.g.*, ruminants and poultry), and pesticides (*e.g.*, herbicides, insecticides, and fungicides). For instance, hundreds of active ingredients registered for use in agriculture (*i.e.*, feed crops) may be applied at varying rates in different countries. After application, pesticide residues can enter the bodies of livestock and bioaccumulate in their raw products. Consequently, a general framework for describing the fate and transport of commonly used pesticides in major feed crops and livestock products can assist regulatory scientists in regulating pesticide residues in animal feed. However, there is insufficient information to define pesticide MRLs in feed taking into account the effects on or from major sectors of the livestock-product supply chain. Consequently, existing conservative regulatory screening approaches (*e.g.*, defining MRLs as the limit of determination [LOD]) should be complemented by more sophisticated approaches for assessing the impact of pesticide MRLs in feed throughout the livestock-product supply chain for those pesticide-feed combinations that are screened for being problematic based on current conservative screening-level approaches.

To address these challenges in the management of pesticide residues in feed, this study aims to propose a framework that can define pesticide MRLs in feed by considering the influences of major sectors of the livestock-product supply chain. The specific objectives are to: (i) introduce general rules for defining pesticide MRLs in feed, (ii) propose specific modeling approaches for deriving pesticide MRLs in feed, and (iii) apply the proposed framework to cattle and sheep feed in an illustrative case study.

2 MATERIALS AND METHODS

2.1 General MRL definition framework

On the basis of the pesticide's lifecycle through the livestock-product supply chain (Fig. 1), which can be divided into two sectors, namely the upstream and downstream sectors, a modeling framework for defining pesticide MRLs in animal feed was proposed. The defined MRLs for pesticides in animal feed were intended to align pesticide regulations in the upstream and downstream sectors of the livestock-product supply chain. The pesticide regulation in the upstream sector (MRLs in crops) can legally affect the residue levels in animal feed (after feed processing); therefore, the defined MRL in feed should link to that defined in crops in order to align upstream pesticide regulations. Similarly, pesticide MRLs in feed will have legal implications for the downstream sectors of the livestock-product supply chain, such as animal health and the quality of livestock products. Therefore, the defined MRL for feed must account for its impact on the supply chain for livestock products.

The general rules for regulating the MRL of pesticide *i* in feed of livestock *j*, consuming a group of crops *k* and yielding a group of livestock food products *m* ($\mathrm{MRL}_{\mathrm{Feed},i,j,\sum k,\sum m}$, mg kg^{-1}) are illustrated in Fig. 1, which can be described by Eqn (1) as follows:

$$\left\{\overbrace{\mathrm{MRL}_{\mathrm{Feed},i,j,\sum k,\sum m}}^{\text{Feed MRL}}\right\}\subseteq\left\{\overbrace{\mathrm{MRL}_{\mathrm{Crop}\rightarrow\mathrm{Feed},i,j}}^{\text{Feed MRL defined from crop MRL}}\right\}\cap\left\{\overbrace{\mathrm{MRL}_{\mathrm{A\_Health}\rightarrow\mathrm{Feed},i,j,\sum k}}^{\text{Feed MRL defined from animal health}}\right\}$$
$$\cap\left\{\overbrace{\mathrm{MRL}_{\mathrm{Food}\rightarrow\mathrm{Feed},i,j,\sum m}}^{\text{Feed MRL defined from food MRL}}\right\}\cap\left\{\overbrace{\mathrm{MRL}_{\mathrm{H\_Health}\rightarrow\mathrm{Feed},i,j,\sum m}}^{\text{Feed MRL defined from human health}}\right\} \quad (1)$$

where $\mathrm{MRL}_{\mathrm{Crop}\rightarrow\mathrm{Feed},,i,j,\sum k}$ (mg kg^{-1}) is the MRL of pesticide *i* in feed of livestock *j*, composing a group of crops *k* (feed materials), whose derivation is based on MRLs in crops (upstream sector); $\mathrm{MRL}_{\mathrm{A}_{\mathrm{Health}}\rightarrow\mathrm{Feed},,i,j,\sum k}$ (mg kg^{-1}) is the MRL of pesticide *i* in feed of livestock *j*, composing a group of crops *k* (feed materials), whose derivation is based on animal health of livestock *j via* consumption of feed (downstream sector); $\mathrm{MRL}_{\mathrm{Food}\rightarrow\mathrm{Feed},,i,j,\sum m}$ (mg kg^{-1}) is the MRL of pesticide *i* in feed of livestock *j*, producing a group of livestock food products *m*, whose derivation is based on MRLs in livestock products (downstream sector); and $\mathrm{MRL}_{\mathrm{H\_Health}\rightarrow\mathrm{Feed},,i,j,\sum m}$ (mg kg^{-1}) is the MRL of pesticide *i* in feed of livestock *j*, producing a group of livestock products *m* (food), whose derivation is based on human health *via* consumption of livestock products (downstream sector).

For $\mathrm{MRL}_{\mathrm{Crop}\rightarrow\mathrm{Feed},,i,j,\sum k}$, the rule is based on the mass balance of the pesticide residue between feed and crops, for which the defined $\mathrm{MRL}_{\mathrm{Feed}}$ (*i.e.*, the theoretical maximum concentration of the pesticide residue in feed) should be equal to or higher than the transformed (feed processing) MRLs in crops (*i.e.*, the theoretical maximum concentration of the pesticide residue in crops). This ensures that the defined $\mathrm{MRL}_{\mathrm{Feed}}$ will not be contradictory to that in crops from the upstream sector of the livestock-product supply chain. For example, if the $\mathrm{MRL}_{\mathrm{Feed}}$ of a pesticide in a fodder made of sorghum is lower than the transformed MRL of the pesticide in sorghum, the defined sorghum $\mathrm{MRL}_{\mathrm{Feed}}$ could be lower than the concentration allowed by the crop MRL from the upstream sector. Notably, most MRLs in crops refer directly to consumption by humans, but some crops (*e.g.*, maize, sorghum and soybean) are also used to produce animal feed; thus, the definition of the related $\mathrm{MRL}_{\mathrm{Feed}}$ should be connected to the respective pesticide MRLs in

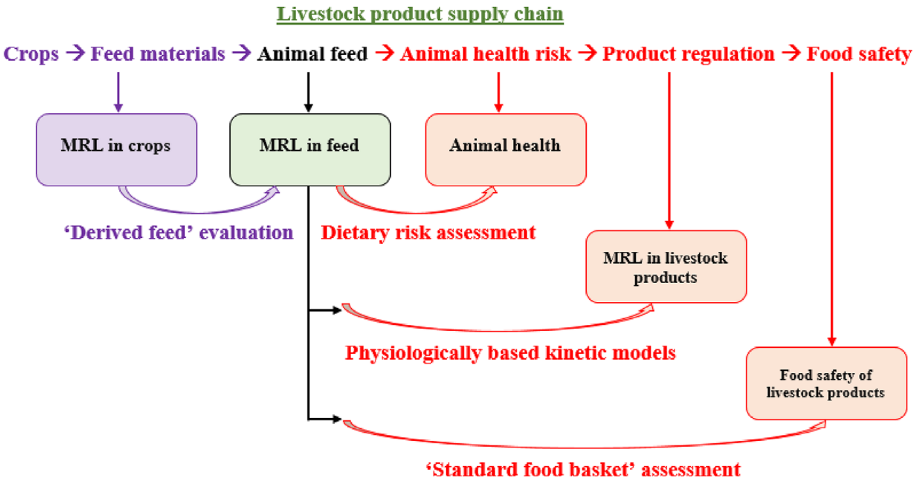

Figure 1. Framework of general rules for consistently defining maximum residue levels (MRLs) in feed.

feed crops. In general, if the crop MRL is unavailable, the $\mathrm{MRL}_{\mathrm{Crop}\rightarrow\mathrm{Feed},,\mathrm{i,j},\sum k}$ can be applied as any positive real number. However, some regulatory agencies (*e.g.*, EU) define crop MRLs as the LOD (*e.g.*, 0.01 mg kg^{-1}) when specific data or field trials are unavailable; then, the $\mathrm{MRL}_{\mathrm{Crop}\rightarrow\mathrm{Feed},,\mathrm{i,j},\sum k}$ can be generated using LODs.

For $\mathrm{MRL}_{\mathrm{A\_Health}\rightarrow\mathrm{Feed},,\mathrm{i,j},\sum k}$, the rule is that the $\mathrm{MRL}_{\mathrm{Feed}}$ should protect animal health, for which a chronic health risk assessment *via* the dietary exposure pathway is conducted. As the toxicological safety value of the acute exposure assessment (*i.e.*, ARfD, mg kg^{-1} day^{-1}) should always be equal to or greater than the acceptable chronic exposure dose (*i.e.*, acceptable daily intake or ADI, mg kg^{-1} day^{-1}),[26] thus, using the chronic risk assessment (*i.e.*, ADI) to define $\mathrm{MRL}_{\mathrm{Feed}}$ can also protect animal health under acute exposure scenarios.

For $\mathrm{MRL}_{\mathrm{Food}\rightarrow\mathrm{Feed},,\mathrm{i,j},\sum m}$, the rule is based on the mass balance of the pesticide residue between feed and foods of animal origin (livestock products), ensuring that the defined $\mathrm{MRL}_{\mathrm{Feed}}$ will be aligned with the respective MRL in livestock products, *i.e.*, MRL Feed will not result in residue concentrations in livestock products that are greater than the respective MRL in livestock products. This needs to consider the pesticide biotransformation in animal bodies (*e.g.*, bioaccumulation, biodegradation, and elimination) as well as the food processing of livestock products (*e.g.*, manufacturing processing and cooking). For example, for pesticides with a biotransfer factor (BTF, defined as the concentration ratio of livestock product (raw) to animal feed) of less than 1, $\mathrm{MRL}_{\mathrm{Feed}}$ should be defined as an upper limit for the back-transformed value from the MRL in livestock products, while for BTF > 1, $\mathrm{MRL}_{\mathrm{Feed}}$ would be defined as the lower limit of that value. In the present study, we applied physiologically based kinetic (PBK) models to link $\mathrm{MRL}_{\mathrm{Feed}}$ to MRLs in livestock products,[27] which generated $\mathrm{MRL}_{\mathrm{Food}\rightarrow\mathrm{Feed}}$.

For $\mathrm{MRL}_{\mathrm{H\_Health}\rightarrow\mathrm{Feed},,\mathrm{i,j},\sum m}$, the rule is that the $\mathrm{MRL}_{\mathrm{Feed}}$ should protect human health, for which typically a dietary risk assessment using the standard food basket is conducted.[28]

2.2 Defining pesticide MRLs in feed

2.2.1 Defining MRLs in feed based on MRLs in crops

As most feed is composed of single or a mixture of crops, the general model linking the crop MRL to $\mathrm{MRL}_{\mathrm{Feed}}$ following the $\left\{\mathrm{MRL}_{\mathrm{Crop}\rightarrow\mathrm{Feed},,\mathrm{i,j}}\right\}$ rule can be expressed as follows:

$$\overbrace{\mathrm{MRL}_{\mathrm{Crop}\rightarrow\mathrm{Feed},i,j}}^{\text{Feed MRL defined from crop MRL}} \geq \frac{\overbrace{\sum_{k=1}^{n}\left(\mathrm{MRL}_{\mathrm{Crop,i,k}}\mathrm{M}_{\mathrm{Fresh,k}}\mathrm{PF}_{\mathrm{Feed,i,k}}\mathrm{I}_{\mathrm{i,k}}(x)\right)}^{\text{Fresh crops}}}{\underbrace{\left(\frac{1}{1-\alpha_{\mathrm{Wet}}}\right)}_{\text{Fresh–to–dry}}\underbrace{\sum_{k=1}^{n}\left(\mathrm{M}_{\mathrm{Dry},k}\mathrm{I}_{\mathrm{i,k}}(x)\right)}_{\text{Dry crops}}} \tag{2a}$$

$$\mathrm{I}_{\mathrm{i,k}}(x)=\begin{cases}0;\textit{pesticide i is not used on crop k}\\1;\textit{pesticide i is used on crop k}\end{cases} \tag{2b}$$

where $\mathrm{MRL}_{\mathrm{Crop,i,k}}$ (mg kg^{-1}) is the MRL of pesticide *i* in crop *k*; $\mathrm{M}_{\mathrm{Fresh,k}}$ (kg) and $\mathrm{M}_{\mathrm{Dry,k}}$ (kg) are the fresh and dry weights of crop *k* at harvest, respectively; $\mathrm{PF}_{\mathrm{Feed,i,k}}$ (dimensionless) is the feed processing factor of pesticide *i* in crop *k*; α_{Wet} (dimensionless) denotes the total moisture content of the feed, which is recommended as a daily ration and applied in the current regulatory process.[25] Therefore, to comply with the pesticide regulation in crops, the derived $\mathrm{MRL}_{\mathrm{Crop}\rightarrow\mathrm{Feed,j}}$ value should be equal to or higher than the transformed value from $\mathrm{MRL}_{\mathrm{Crop,i,k}}$, *i.e.*, the right term in Eqn (2a), which avoids the allowed residue levels in crops that result in exceedance of the MRL in the feed. In case the $\mathrm{MRL}_{\mathrm{Crop,i,k}}$ is unavailable, the $\mathrm{MRL}_{\mathrm{Crop,i,k}}$ can be defined as the LOD (*e.g.*, 0.01 mg·kg^{-1} according to EU regulations). $\mathrm{I}_{\mathrm{i,k}}(x)$ denotes the indicator function as defined in Eqn (2b). Given that pesticides can bioconcentrate in plants through multiple pathways (such as leaf update from spray drift and root uptake from the soil), we assumed that the studied pesticides would be present in all of the selected feed crops, which represents a high-end worst-case, *i.e.* $\mathrm{I}_{\mathrm{i,k}}(x)=1$ for all crops *k*. However, to reflect more realistic cases (*i.e.* most pesticides are used only on certain crops), users can modify $\mathrm{I}_{\mathrm{i,k}}(x)$, *e.g.* according to region-specific field data or regulations. For instance, if pesticide *i* is not registered for use on a certain crop *k*, the respective $\mathrm{I}_{\mathrm{i,k}}(x)$ value can be set to zero.

2.2.2 Defining MRLs in feed based on animal health

To protect animal health, the chronic health risk assessment *via* the dietary exposure pathway can be applied to define $\mathrm{MRL}_{\mathrm{A_{Health}}\rightarrow\mathrm{Feed,k,j}}$ as follows:

$$\overbrace{\mathrm{MRL}_{\mathrm{A\_Health}\rightarrow\mathrm{Feed,i,j,}\sum k}}^{\text{Feed MRL defined from animal health}} \leq \frac{\mathrm{ADI}_{\mathrm{Animal,i,j}}\mathrm{BW}_{\mathrm{Animal,j}}}{\mathrm{AF}_{\mathrm{Animal,i,j}}\mathrm{IR}_{\mathrm{Feed,j}}F_{\mathrm{Feed,i,j}}} \tag{3}$$

where $\mathrm{ADI}_{\mathrm{Animal,i,j}}$ ($\mathrm{mg{\cdot}kg^{-1}{\cdot}day^{-1}}$) is the acceptable daily intake of pesticide *i* for livestock *j*, $\mathrm{BW}_{\mathrm{Animal,j}}$ (kg) is the body weight of livestock *j*, $\mathrm{AF}_{\mathrm{Animal,i,j}}$ (dimensionless) is the allocation factor of pesticide *i* for livestock *j*, $\mathrm{IR}_{\mathrm{Feed,j}}$ ($\mathrm{kg{\cdot}day^{-1}}$) is the intake rate of feed (the mixture of feed crops) of livestock *j*, and $F_{\mathrm{Feed,i,j}}$ (dimensionless) is the fraction of pesticide *i* in the feed of livestock *j*. The use of $F_{\mathrm{Feed,i,j}}$ takes into account the possibility that certain pesticides may only be applied to specific crops. Similar to the assumption made for Eqn (2b), it was assumed for illustrative purposes that the investigated pesticides were present in all of the selected feed crops, which resulted in the $F_{\mathrm{Feed,i,j}}$ value of 1. Again, users can customize the $F_{\mathrm{Feed,i,j}}$ value according to site-specific conditions. For example, if pesticide *i* is not registered for use or detected in the feed of livestock *j*, the respective $F_{\mathrm{Feed,i,j}}$ value can be set to zero, in which case no related MRL is defined. Alternatively, users may use the detection limit of pesticide *i* to estimate $F_{\mathrm{Feed,i,j}}$ values in accordance with the precautionary principle, which is widely used in current regulatory frameworks.[29]

2.2.3 *Defining MRLs in feed based on MRLs in livestock products*

To comply with pesticide regulations in livestock products, the pesticide MRL in feed should not exceed the corresponding MRLs in foods of animal origin. Therefore, the $\mathrm{MRL}_{\mathrm{Food}\rightarrow\mathrm{Feed,,i,j,}\sum m}$ value can be defined as follows:

$$\overbrace{\mathrm{MRL}_{\mathrm{Food}\rightarrow\mathrm{Feed,,i,j,}\sum m}}^{\text{Feed MRL defined from food MRL}} \leq \mathrm{MIN}\left\{\frac{\mathrm{MRL}_{\mathrm{Food,i,j,}m}}{\mathrm{BTF}_{\mathrm{Feed}\rightarrow\mathrm{Food,i,j,}m}}\right\} \tag{4}$$

where $\mathrm{MRL}_{\mathrm{Food,i,j,}m}$ ($\mathrm{mg\ kg^{-1}}$) is the MRL of pesticide *i* in food product *m* of livestock *j*, and $\mathrm{BTF}_{\mathrm{Feed}\rightarrow\mathrm{Food,i,j,m}}$ (dimensionless, *i.e.*, $\mathrm{mg{\cdot}kg^{-1}}$ pesticide in livestock product per $\mathrm{mg{\cdot}kg^{-1}}$ pesticide in animal feed) is the biotransfer factor of pesticide *i* in food product *m* of livestock *j*, defined as the steady-state concentration ratio of pesticide *i* in food product *m* to that in feed. $\mathrm{MIN}\{\cdot\}$ denotes the minimum function that takes the minimum value of $\frac{\mathrm{MRL}_{\mathrm{Food,i,j,}m}}{\mathrm{BTF}_{\mathrm{Feed}\rightarrow\mathrm{Food,i,j,m}}}$. In the present study, five common livestock products, including muscle, fat, liver, kidney, and milk, were selected; these are regulated in current regulatory jurisdictions. Thus, the defined $\mathrm{MRL}_{\mathrm{Food}\rightarrow\mathrm{Feed,,i,j,}\sum k}$ value in Eqn (4) means that the defined $\mathrm{MRL}_{\mathrm{Feed}}$ should not allow the residue levels in livestock products to exceed any $\mathrm{MRL}_{\mathrm{Food}}$ values (*i.e.*, pesticide MRLs in livestock products).

2.2.4 *Defining MRLs in feed based on human health*

To protect human health, $\mathrm{MRL}_{\mathrm{H\_Health}\rightarrow\mathrm{Feed,,i,j,}\sum m}$ can be defined based on a chronic dietary risk assessment:

$$\mathrm{MRL}_{\mathrm{H\_Health}\rightarrow\mathrm{Feed,,i,j,}\sum m} \leq \frac{\mathrm{ADI}_{\mathrm{Human,i}}\mathrm{BW}_{\mathrm{Human}}}{\mathrm{AF_i}\sum_{m}^{5}\left(\mathrm{IR}_{\mathrm{Food,m}}\mathrm{BTF}_{\mathrm{Feed}\rightarrow\mathrm{Food,i,j,m}}\mathrm{PF}_{\mathrm{Food,i,j,m}}\right)} \tag{5}$$

where $\mathrm{ADI}_{\mathrm{Human,j}}$ ($\mathrm{mg{\cdot}kg^{-1}{\cdot}day^{-1}}$) is the ADI of the pesticide for humans; $\mathrm{BW}_{\mathrm{Human}}$ (kg) is the body weight of humans; $\mathrm{IR}_{\mathrm{Food,i}}$ ($\mathrm{kg{\cdot}day^{-1}}$) is the intake rate of food *i* by humans, estimated using the standard food basket[28]; $\mathrm{AF_i}$ (dimensionless) is the allocation factor of pesticide *i*, which accounts for other possible pesticide exposure pathways; $\mathrm{PF}_{\mathrm{Food,i,j,m}}$ (dimensionless) is the processing factor of pesticide *j* for food product *m* of livestock *j*. Therefore, the defined $\mathrm{MRL}_{\mathrm{H\_Human}\rightarrow\mathrm{Feed}}$ value in Eqn (5) ensures the food safety of livestock products. In summary, the defined $\mathrm{MRL}_{\mathrm{Feed}}$ in Eqn (1) can be expressed as follows:

$$\mathrm{MRL}_{\mathrm{Feed,i,j,}\sum k,\sum m} \geq \underbrace{\frac{\sum_{k=1}^{n}\left(\mathrm{MRL}_{\mathrm{Crop,i,k}}\mathrm{M}_{\mathrm{Fresh,k}}\mathrm{PF}_{\mathrm{Feed,i,k}}\right)}{\left(\frac{1}{1-\alpha_{Wet}}\right)\sum_{k=1}^{n}\left(\mathrm{M}_{\mathrm{Dry,k}}\right)}}_{\text{MRLs in crops}} \tag{6a}$$

$$\mathrm{MRL}_{\mathrm{Feed,\,i,j,}\sum k,\sum m} \leq \mathrm{MIN}\left\{\underbrace{\frac{\mathrm{ADI}_{\mathrm{Animal,\,i,j}}\mathrm{BW}_{\mathrm{Animal,\,j}}}{\mathrm{IR}_{\mathrm{Feed,\,j}}}}_{\text{Animal health}},\ \underbrace{\mathrm{MIN}\left\{\frac{\mathrm{MRL}_{\mathrm{Food,\,i,j,}m}}{\mathrm{BTF}_{\mathrm{Feed}\rightarrow\mathrm{Food,\,i,j,m}}}\right\}}_{\text{MRLs in foods}},\ \underbrace{\frac{\mathrm{ADI}_{\mathrm{Human,\,i}}\mathrm{BW}_{\mathrm{Human}}}{\mathrm{AF_i}\sum_{m}^{5}\left(\mathrm{IR}_{\mathrm{Food,\,m}}\mathrm{BTF}_{\mathrm{Feed}\rightarrow\mathrm{Food,\,i,j,m}}\mathrm{PF}_{\mathrm{Food,\,i,j,m}}\right)}}_{\text{human health}}\right\} \tag{6b}$$

When the derived $\mathrm{MRL}_{\mathrm{Feed}}$ value in Eqn (6a) contradicts that in Eqn (6b), it implies that current pesticide MRLs in crops can theoretically allow the residue level to exceed at least one of the acceptable guidelines in downstream regulatory compartments (*i.e.*, animal health, MRLs in foods of animal origin, and human health).

2.3 Applications to cattle and sheep – an illustrative case study

In the present study, we selected cattle and sheep as example animals to demonstrate the MRL-derivation process, because these two animals are the most common livestock, and their products (*i.e.*, meat, offal, and milk) play a significant role in daily dietary requirements. The proposed framework can also be applied to other livestock animals, such as swine, horses, and deer, by expanding the input animal-specific variables.[27] For poultry, the physiologically based kinetic (PBK) model should be adjusted according to bird-like physiological characteristics.[30]

We note that cattle and sheep that graze in rangelands (particularly in summer) are free from consuming the derived feed. In the present study, we assume that the feed is the only food source for cattle and sheep, for the following reasons: (i) in some cold regions during winter, feeds are the major food source for grazing animals; (ii) to achieve a high commercial value of livestock products, animals are fed using commercial fodder; and (iii) for regulatory purposes, it is an effective means to evaluate whether pesticide regulation in feed can protect animal health and ensure food safety of livestock products. The common crop-made silage for cattle and sheep can be expressed as follows:

$$M_{\mathrm{Silage}} = M_{\mathrm{Dry,Silage}} + M_{\mathrm{Water}} + M_{\mathrm{Others}} \approx M_{\mathrm{Dry,Silage}} + M_{\mathrm{Water}} \tag{7a}$$

$$M_{\mathrm{Dry,Silage}} = M_{\mathrm{Dry,Barley}} + M_{\mathrm{Dry,Maize}} + M_{\mathrm{Dry,Rye}} + M_{\mathrm{Dry,Sorghum}} + M_{\mathrm{Dry,Soybean}} \tag{7b}$$

where the total mass of silage (M_{Silage}, kg) is equal to the sum of the dry mass of silage ($M_{\mathrm{Dry,Silage}}$, kg), the mass of water (M_{Water}, kg), and the mass of other ingredients (M_{Others}, kg), which is approximately equal to the sum of $M_{\mathrm{Dry,Silage}}$ and M_{Water}. In the present study, five common fodder crops were selected (*i.e.*, $k = 5$) for cattle and sheep (*i.e.*, vector j is fixed), including maize, soybean, rye, barley, and sorghum, for which the corresponding dry masses (M_{Dry}, kg) are expressed in Eqn (7b). Using the mass percentage of M_{Silage} and combining Eqns (7a) and (7b), we obtain the following equation:

$$M_{\mathrm{Silage}} = \overbrace{\left(\underbrace{\alpha_{\mathrm{Wet}}}_{\text{Wet mass percentage of feed}} + \underbrace{\sum_{k=1}^{n=5} \alpha_{\mathrm{Dry},k}}_{\text{Dry mass percentage of feed}} \right)}^{\text{Sum of mass ratios equals 1}} M_{\mathrm{Silage}} \tag{8a}$$

$$\sum_{k=1}^{5} \alpha_{\mathrm{Dry},k} = \alpha_{\mathrm{Dry,Maize}} + \alpha_{\mathrm{Dry,Soybean}} + \alpha_{\mathrm{Dry,Rye}} + \alpha_{\mathrm{Dry,Barley}} + \alpha_{\mathrm{Dry,Sorghum}} \tag{8b}$$

where $\alpha_{\mathrm{Dry},k}$ denotes the percentage of the dry mass of crop k of M_{Silage}. Thus, by varying $\alpha_{\mathrm{Dry},k}$ values, we can obtain silage with different combinations of fodder crops. The defined $\mathrm{MRL}_{\mathrm{Feed}}$ values in Eqn (6a) can be further expressed using $\alpha_{\mathrm{Dry},k}$ as follows:

$$\begin{aligned}
&\overbrace{\underbrace{\mathrm{MRL}_{\mathrm{Feed},\,i.\sum 5}}_{\text{Defined MRL in feed}} \times \underbrace{M_{\mathrm{Silage}}}_{\text{Fresh mass of feed}}}^{\text{Defined residue mass in feed}} \\
&\geq \overbrace{\sum_{k=1}^{5} \left(\underbrace{\mathrm{MRL}_{\mathrm{Crop},\,i,k}}_{\text{Existing MRL in feed crop}} \times \underbrace{\frac{M_{\mathrm{dry},k}}{1-\theta_{\mathrm{water},\,k}}}_{\text{Fresh mass of feed crop}} \times \mathrm{PF}_{\mathrm{Feed},\,i,k} \right)}^{\text{Theoretical residue mass in feed crops}} \\
&\mathrm{MRL}_{\mathrm{Feed},i.\sum 5} M_{\mathrm{Silage}} \geq \sum_{k=1}^{5} \left(\mathrm{MRL}_{\mathrm{Crop},i,k} \frac{\alpha_{\mathrm{Dry},k} M_{\mathrm{Silage}}}{1-\theta_{\mathrm{water},k}} \mathrm{PF}_{\mathrm{Feed},i,k} \right) \\
&\mathrm{MRL}_{\mathrm{Feed},i.\sum 5} \geq \sum_{1}^{5} \left(\frac{\mathrm{MRL}_{\mathrm{Crop},i,k} \alpha_{\mathrm{Dry},k}}{1-\theta_{\mathrm{water},k}} \mathrm{PF}_{\mathrm{Feed},i,k} \right)
\end{aligned} \tag{9}$$

where $\theta_{\mathrm{water},k}$ is the water content of fresh feed crop k. Thus, when the silage is made from a single crop, the defined $\mathrm{MRL}_{\mathrm{Feed}}$ value of pesticide i using feed crop k in Eqn (9) can be simplified as $\mathrm{MRL}_{\mathrm{Feed},i,k} \geq \mathrm{MRL}_{\mathrm{Crop},i,k} \frac{1-\alpha_{Wet}}{1-\theta_{\mathrm{water},k}}$. Then, for the feed (silage) for cattle and sheep that is prepared from a single crop, the defined MRL in feed can be transformed from Eqn (6a) as follows:

$$\mathrm{MRL}_{\mathrm{Feed},i.\sum 5} \geq \mathrm{MAX} \left\{ \mathrm{MRL}_{\mathrm{Crop},i,k} \frac{1-\alpha_{Wet}}{1-\theta_{\mathrm{water},k}} \mathrm{PF}_{\mathrm{Feed},i,k} \right\} \tag{10}$$

The $\mathrm{MRL}_{\mathrm{Feed}}$ in Eqn (10) can ensure that the defined pesticide MRL in cattle and sheep feed is compatible with all MRLs in crops with fodder relevance.

The $\mathrm{ADI}_{\mathrm{Animal}}$ values should be specified for cattle and sheep to define the $\mathrm{MRL}_{\mathrm{A\_Health} \rightarrow \mathrm{Feed}}$ value in Eqn (3); however, the $\mathrm{ADI}_{\mathrm{Animal}}$ values for many ruminant animals are not available. Thus, we obtained the $\mathrm{ADI}_{\mathrm{Animal}}$ values for cattle and sheep from the chronic 'no observed adverse effect levels' (NOAEL, $\mathrm{mg{\cdot}kg^{-1}{\cdot}day^{-1}}$) of experimental animals using the dose-by-factor method (*i.e.*, allometric scaling by body weight and surface area) as follows[31]:

$$\mathrm{ADI}_{\mathrm{Animal}} = \mathrm{NOAEL} \times \underbrace{\left(\frac{\mathrm{BW}_{\mathrm{Test}}}{\mathrm{BW}_{\mathrm{Animal}}} \right)^{1-\frac{2}{3}}}_{\text{Inter-species factor}} \times \underbrace{\frac{1}{\mathrm{UF}}}_{\text{Intra-species factor}} \tag{11}$$

where $\mathrm{BW}_{\mathrm{Test}}$ (kg) is the body weight of the tested animal and UF is the uncertainty factor considering the intra-species uncertainty, for which a default value of 10 was applied. The NOAELs were obtained from regulatory agencies[32–37]; in case the NOAELs were unavailable, the NOELs (No observed effect levels) were applied.

The $\mathrm{BTF}_{\mathrm{Feed} \rightarrow \mathrm{Food},i}$ values of pesticides for cattle and sheep were obtained from Li *et al.*[27] and were solved by matrix algebra that transforms the fate of pesticide residues in animal bodies into first-order kinetics. The physiological parameters and derivation process of the PBK model can be referred from Li *et al.*[27] In the present study, we assume that the steady state of pesticide distribution between tissues and feed is achieved because slaughter typically occurs several years after the birth of cattle and sheep, which is longer than the time it takes for residue distribution in animal bodies to reach the steady state. The parametric models for predicting the $\mathrm{BTF}_{\mathrm{Feed} \rightarrow \mathrm{Food},i,j,m}$ values of pesticides for cattle and sheep are provided in the Supplementary File.

The ADI values of pesticides for humans were obtained from the EU pesticide database[38] and the World Health Organization.[39]

2.4 Pesticides

In the present study, we used the framework to define MRLs in feed for a total of 24 pesticides, which were selected based on their extensive use in agriculture, high detection frequencies in crops, and their occurrence in feed materials that may affect livestock health and the quality of livestock food products.[40] Difenzoquat metilsulfate and kasugamycin were omitted from the priority list of pesticides proposed by Klüche *et al.*[40] for the analysis because the EU has not defined any MRLs for these two pesticides in agricultural commodities. For these two pesticides, whose MRLs are less straightforward than pesticide-specific MRLs, users can either directly set the default value of 0.01 mg $\mathrm{kg^{-1}}$ as their feed MRLs or back-calculate the feed MRLs using 0.01 mg $\mathrm{kg^{-1}}$ as MRLs in crops and livestock products. The selected pesticides with their ADI and log K_{OW} values are provided in Table 1; their BTFs, current MRLs in crops and foods of animal origin, and the defined MRLs in the feed are provided in the Supplementary Database.

Table 1. Summary of the selected pesticides and their derived ADI values for cattle and sheep

Pesticides	CAS No.	Class	Log K_{OW}	NOAEL (or NOEL)	Test animal	ADI derived for cattle	ADI derived for sheep	ADI for humans (EU)
Amitraz	33089-61-1	Insecticide	5.5	0.25	Dog	6.0 E-03	1.2E-02	0.003
Amitrole	61-82-5	Herbicide	−0.97	0.025	Rat	1.6 E-04	3.3E-04	0.001
Captan	133-06-2	Fungicide	2.5	300	Dog	7.2 E+00	1.5E+01	0.1
Chlormequat chloride	999-81-5	Plant growth regulator	−3.47	75	Rat	4.9 E-01	9.9E-01	0.04
Chlorothalonil	1897-45-6	Fungicide	2.94	5.1	Dog	1.2 E-01	2.5E-01	0.015
Cyhexatin	41083-11-8	Acaricide	4.84	0.34	Rat	2.2 E-03	4.5E-03	0.003
Cyromazine	66215-27-8	Insecticide	0.069	0.75	Dog	1.8 E-02	3.7E-02	0.06
Diquat dibromide	85-00-7	Herbicide	−4.6	0.5	Dog	1.2 E-02	2.4E-02	0.002
Dithianon	3347-22-6	Fungicide	3.2	1	Rat	6.5 E-03	1.3E-02	0.01
Ethephon	16672-87-0	Plant growth regulator	−1.89	—	—	1.4 E-02	2.9E-02	0.03
Fenbutatin oxide	13356-08-6	Acaricide	5.15	5.2	Rat	3.4 E-02	6.8E-02	0.05
Fentin acetate	900-95-8	Fungicide	3.43	—	—	1.9 E-04	3.8E-04	0.0004
Fentin hydroxide	76-87-9	Fungicide	3.43	—	—	1.9 E-04	3.8E-04	0.0004
Fluazifop	69806-50-4	Herbicide	3.18	0.74	Rat	4.8 E-03	9.7E-03	0.01
Fluazifop-P	79241-46-6	Herbicide	3.18	0.74	Rat	4.8 E-03	9.7E-03	0.01
Folpet	133-07-3	Fungicide	3.02	10	Dog	2.4 E-01	4.9E-01	0.1
Fosetyl aluminum	39148-24-8	Acaricide	−2.1	250	Dog	6.0 E+00	1.2E+01	3
Glufosinate	51276-47-2	Herbicide	−3.96	6.3	Rabbit	1.0 E-01	2.1E-01	0.021
Glyphosate	1071-83-6	Herbicide	−3.2	30	Rat	1.9 E-01	3.9E-01	0.5
Haloxyfop	69806-34-4	Herbicide	4.2	0.03	Dog	7.2 E-04	1.5E-03	0.00065
Haloxyfop-P	95977-29-0	Herbicide	0.27	0.065	Dog	1.6 E-03	3.2E-03	0.00065
Maleic hydrazide	123-33-1	Plant growth regulator	−1.83	25	Dog	6.0 E-01	1.2E+00	0.25
Mepiquat chloride	24307-26-4	Plant growth regulator	−3.55	58.4	Dog	1.4 E+00	2.9E+00	0.2
Paraquat dichloride	1910-1942-5	Herbicide	−4.5	0.45	Dog	1.1 E-02	2.2E-02	0.004

The body weights of dog (beagle), rat, and rabbit (test animals) were considered as 8.0, 0.15,[31] and 2.5 kg,[41] respectively; while the body weights of cattle (dairy cow) and sheep were considered as 600 and 70 kg, respectively.

The NOAELs for ethephon, fentin acetate, and fentin hydroxide were unavailable; their ADI values for cattle and sheep were estimated using the ADI values for humans (the dose-by-factor method).[31]

2.5 Application context

2.5.1 *Point estimate approach*

The point estimate approach, using fixed values of inputs, was applied to define MRLs in animal feed. This approach can be used when certain information (*e.g.*, PF) is lacking or a simple conservative and screening-level calculation is needed. The model inputs for the point estimate approach are provided in Supporting Information, Table S1.

2.5.2 *Uncertainty analysis*

As some model inputs (*e.g.*, AF, PF, and BTF) are determined by various factors (*e.g.*, temperature, human exposure patterns, and cooking techniques) that cannot be precisely predicted, resulting in variations in the defined MRLs of pesticides in animal feed, the uncertainty analysis was conducted to evaluate the impact of inputs on the simulation results (Monte Carlo sensitivity test). The tested model inputs are listed in Supporting Information, Table S2. Probabilistic distributions of model inputs were used to conduct the uncertainty analysis, and it was assumed that the input variables are mutually independent to simplify the simulation. The parameters of probabilistic distributions of the model inputs are provided in Supporting Information, Table S2. The Excel add-in (@Risk Industrial version [Palisade], Ithaca, USA) was used to perform the simulation. Such approach is relevant if distributions are needed beyond a simple screening-level context.

2.5.3 *Empirical approach*

In addition to the mechanism-based models (such as the BTF model) used for defining feed MRL, we proposed an empirical approach using available field or estimated data for AF, PF, and BTF. To revise or update MRLs for pesticides in feed, the empirical method can leverage existing databases and expert judgment. Sometimes, the regulatory process for pesticides favors a conservative, straightforward approach, for which empirical data can assist regulatory scientists in making decisions. Consequently, the empirical method can serve as an alternative to mechanism-based models, especially when such models are unavailable. To illustrate the MRL definition process using the empirical approach, one pesticide from the priority list made by Klüche *et al.*,[40] namely kasugamycin, was used as modeling examples. The calculation method is described in the Supplemental File (Supporting Information, Section S5).

3 RESULTS AND DISCUSSION

3.1 Case study of Codex MRLs for chlormequat chloride

In this section, we presented a case study of Codex MRLs for chlormequat chloride to illustrate the proposed regulatory process using the point estimate approach. Chlormequat chloride is a widely used plant growth regulator and is frequently detected in livestock feeds, threatening animal and human health.[42,43] Chlormequat chloride was used to evaluate Codex MRLs because the Codex defines a large number of MRLs in agricultural commodities for chlormequat chloride [MRLs of chlormequat (chlormequat cation)],[39] which is greater than the MRLs of other selected pesticides.

The Codex defined chlormequat chloride in two of the selected fodder crops, *i.e.*, barley (2.0 mg·kg^{-1}) and rye (20 mg·kg^{-1}); we considered that these two MRLs were defined for fresh crops at harvest (*i.e.*, a default moisture content of 0.15 g·g^{-1}). Under the $\{\mathrm{MRL}_{\mathrm{Crop}\rightarrow\mathrm{Feed}}\}$ rule, the defined MRLs in the corresponding livestock feed should prevent the legally-permitted residue levels in crops from exceeding the MRLs in feed. Thus, according to Eqn (2a), the defined MRLs of chlormequat chloride in the barley- and rye-based feed (single-crop silage) should be greater than 2.1 and 20.7 mg·kg^{-1}, respectively. The MRLs in feed based on the $\{\mathrm{MRL}_{\mathrm{Crop}\rightarrow\mathrm{Feed}}\}$ rule are very close to those in the original crops, because of the similar moisture contents between crops at harvest and the derived feed.

Under the $\{\mathrm{MRL}_{\mathrm{A}_{\mathrm{Health}}\rightarrow\mathrm{Feed}}\}$ rule, the defined MRLs in feed should protect livestock health. According to the dietary risk assessment using Eqn (3), the defined MRLs in feed should not exceed 16.2 and 32.9 mg·kg^{-1} for cattle and sheep, respectively. We noted that the ADI values for cattle and sheep were extrapolated from the NOAEL values of tested animals (*i.e.*, rats, dogs, and rabbits), and we added an uncertainty factor of 10 to consider inter-species variations. This approximation approach for estimating the ADI values of livestock is similar to that for humans; thus, the derived ADI values for cattle and sheep could be conservative. However, from a regulatory perspective, this approach could protect animal health against pesticide residues in feed.

Under the $\{\mathrm{MRL}_{\mathrm{Food}\rightarrow\mathrm{Feed}}\}$ rule, the defined MRLs in feed should not allow pesticide residue levels to exceed the MRLs in livestock products. We applied the parametric BTF models to link the residue levels in feed to those in livestock products, which can provide the upper limit of the defined MRLs in feed according to Eqn (4). Figure 2(A) illustrates the defined MRLs of chlormequat chloride in cattle and sheep feed based on the MRLs in their common products. Overall, the defined MRLs in feed based on pesticide regulations in livestock products are extremely high compared with 'common' MRLs in plant or animal communities because chlormequat chloride has a very low log K_{OW} value of −3.5, which leads to extremely low BTF values in cattle and sheep products. Therefore, the allowable MRLs of chlormequat chloride in cattle and sheep feed can be very high without avoiding MRLs in cattle and sheep products. Among the common cattle and sheep products, the MRLs of chlormequat chloride in feeds based on fat are lowest, *i.e.*, 6153 and 5650 mg·kg^{-1} for cattle and sheep

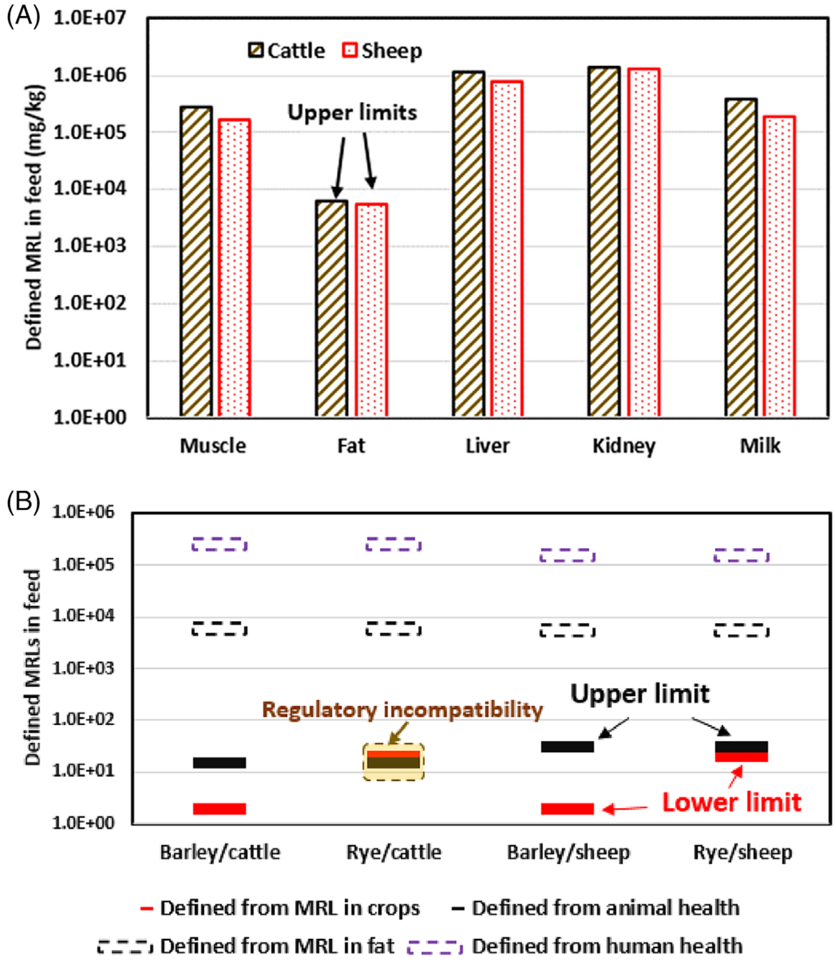

Figure 2. (A) Defined maximum residue limits (MRLs) of chlormequat chloride in feed based on the MRLs in livestock products for cattle and sheep. (B) The lower and upper limits for defined MRLs of chlormequat chloride in cattle and sheep feeds based on barley and rye.

feed, respectively, which can serve as the upper limits for the defined MRLs in feed according to the $\{MRL_{Food \rightarrow Feed}\}$ rule.

Under the $\{MRL_{H\_Health \rightarrow Feed}\}$ rule, the defined MRLs in feed should protect human health. Therefore, we conducted a dietary risk assessment using a standard food basket according to Eqn (5) to define the MRLs of chlormequat chloride in the feed, yielding the defined MRL values of 2.6×10^5 and 1.6×10^5 mg·kg^{-1} for cattle and sheep feeds, respectively. The extremely high MRLs in feed defined based on human health are due to the low BTFs of chlormequat chloride in cattle and sheep products.

Figure 2(B) shows the lower and upper limits of the defined MRLs of chlormequat chloride in the feed, which were generated by combining the above four rules [see Eqns (6a) and (6b)]. The lower limit of the defined MRLs was derived based on the MRLs in crops, and the upper limit was derived based on animal health. As chlormequat chloride has extremely low BTFs in cattle and sheep products, the upper limits derived from the MRL in livestock products and human health are much higher than those derived from animal health. The results show that regulatory values need to be better aligned when defining the MRL of chlormequat chloride in rye-based feed for cattle. According to Rule 1 (MRLs in crops), the lower limit of the defined MRLs in feed is 20.7 mg·kg^{-1}, whereas according to Rule 2 (animal health), the upper limit of the defined MRLs in feed is 16.2 mg·kg^{-1}. This indicates that the regulations of chlormequat chloride in the upstream (MRLs in crops with feed relevance) and downstream (animal health) sectors of the livestock-product supply chain need to be revised; otherwise, the MRL of chlormequat chloride in rye may lead to a residue level in cattle feed that adversely affects cattle health. For other types of silage, the ranges of defined MRLs for chlormequat chloride in feed are 2.1–20.7 mg·kg^{-1} for barley-based cattle feed, 2.1–32.9 mg·kg^{-1} for barley-based sheep feed, and 20.7–32.9 mg·kg^{-1} for rye-based sheep feed. The Codex defined the MRLs of chlormequat chloride in barley- and rye-based fodders as 50 and 20 mg·kg^{-1}, respectively. The Codex MRL for chlormequat chloride in rye-based fodder is acceptable because the value can be considered to fall within the simulated range, considering that the moisture content of rye may vary. However, the Codex MRL for chlormequat chloride in barley-based fodder is higher than the upper limits of the defined MRL in cattle and sheep feeds, indicating that the current regulation of chlormequat chloride in feed can cause adverse health effects in livestock. The Codex does not define MRLs for chlormequat chloride in maize, sorghum, soybean, and fodders; therefore, the lower limits of the defined MRLs in the feed derived from these three crops are all zero.

3.2 Defining MRLs in feed based on EU regulations

The EU defines nearly all MRLs of the selected 24 pesticides in crops and livestock products; however, no MRLs in feed have been introduced.[38] Therefore, based on the available MRLs, we defined the lower and upper limits of MRLs in feed for the selected pesticides based on Eqns (6a) and (6b) using the point estimate approach, which are illustrated in Fig. 3. The results showed that in general, the simulated lower and upper limits of the defined MRLs in feed decrease with increasing log K_{OW} values of the selected pesticides. This is because pesticides with low log K_{OW} values tend to have lower toxicity,[44] which could lead to relatively high defined MRLs in crops or foods of animal origin. For example, glyphosate, a hydrophilic herbicide with a log K_{OW} of −3.2, has relatively high defined MRLs in crops (*e.g.*, 20 mg·kg^{-1} in barley, soybean, and sorghum) due to its relatively low toxicity (*i.e.*, the ADI value for humans is 0.5 mg·kg^{-1}day^{-1})[38] compared with other selected pesticides; thus, the lower limit of the defined MRL of glyphosate in cattle and sheep feeds according to Eqn (10) is 20.7 mg·kg^{-1}. In contrast, amitraz, a lipophilic insecticide with a log K_{OW} value of 5.5, has MRLs in crops much lower than those of glyphosate (*e.g.*, 0.05 mg·kg^{-1} in all selected crops) due to its relatively high toxicity (*i.e.*, the ADI value for humans is 0.003 mg kg^{-1}day^{-1})[38]; thus, the lower limit of the defined MRL of amitraz in cattle and sheep feeds is 0.05 mg·kg^{-1}. As for the lower limit of the defined MRL in the feed, the simulated upper limit according to Eqn (6b) also follows a similar trend, indicating that stricter MRLs in the feed should be defined for lipophilic pesticides.

As the EU does not define any MRLs of pesticides in feed, the simulated ranges of MRLs in feed can help regulatory agencies establish pesticide MRLs in feed. However, some issues across the relevant regulatory definitions were found for the selected pesticides, where simulated lower limits of the defined MRLs in feed are higher than the corresponding upper limits. These issues indicated that pesticide regulations in the upstream sector (*i.e.*, crops with fodder relevance) of the livestock-product supply chain theoretically allow the residue levels to exceed the acceptable limits of pesticides in downstream sectors (*i.e.*, MRLs in livestock products or safety limits for animals and humans). Regulatory incompatibility occurred in ten and nine of the selected pesticides for defining MRLs in cattle and sheep feeds, respectively. For three cases, we found issues in regulatory requirements not being aligned for pesticides with a log K_{OW} value lower than 0.0 for both cattle and sheep product supply chains, whereas all cases were recorded for pesticides with log K_{OW} values higher than 0.0 for cattle and sheep, respectively. This is because, in general, lipophilic pesticides are more toxic to animals and have high BTF values in livestock products, resulting in low upper limits of the defined MRLs in feed. For most lipophilic pesticides with regulatory requirements not being aligned in the defined MRLs in feed, the upper limits were simulated based on Rule 3 (*i.e.*, complying with MRLs in livestock products), indicating that the EU MRLs for these pesticides in fodder crops could lead to the residue levels exceeding the MRLs in cattle and sheep products. For most hydrophilic pesticides (*i.e.*, log $K_{OW} < 0$) where regulatory requirements are not currently aligned for the MRLs in feed, the upper limits were simulated based on Rule 2 (*i.e.*, protecting animal health). However, as there is currently a lack of toxicological information for livestock mammals, in the present study, we considered inter- and intra-species uncertainties in the derivation of ADI values for livestock. This ADI-derivation process is similar to that for humans; hence, overestimation of the dietary risk for livestock could occur when defining MRLs in feed for animal health. Therefore, we suggest that the EU evaluate the current MRLs for pesticides in the livestock-product supply chain and establish approaches to assess the health risks to livestock.

3.3 Uncertainty analysis

To evaluate the impacts of model inputs on the defined pesticide MRL in animal feed, an uncertainty analysis was conducted using the probabilistic approach (Sections S3 and S4 in the Supplementary File). We applied cyromazine to perform the simulation exercise because it has relatively moderate lipophilicity (*i.e.*, log $K_{OW} \sim 0$) among the selected pesticides. Using the EU's MRLs in

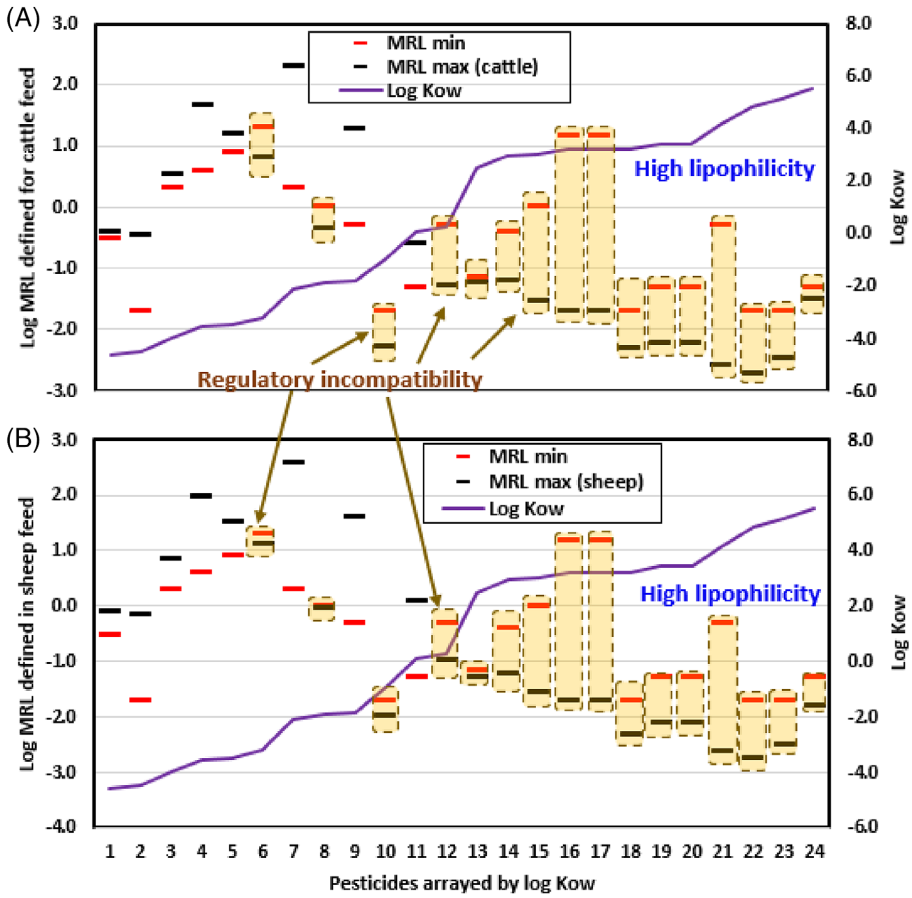

Figure 3. Lower (MRL min) and upper (MRL max) limits of defined MRLs in cattle (A) and sheep (B) feeds for 24 selected pesticides. Pesticides were arrayed by their log K_{OW} values (from minimum to maximum values). Pesticides arranged by log K_{OW} values in ascending order: diquat dibromide, paraquat dichloride, glufosinate, mepiquat chloride, chlormequat chloride, glyphosate, fosetyl aluminum, ethephon, maleic hydrazide, amitrole, cyromazine, haloxyfop-p, captan, chlorothalonil, folpet, fluazifop, fluazifop-p, dithianon, fentin, acetate, fentin hydroxide, haloxyfop, cyhexatin, fenbutatin oxide, and amitraz.

crops and cattle food products, probabilistic distributions of the defined cyromazine MRLs in cattle feed for four proposed rules were generated. Under the $\{MRL_{Crop \rightarrow Feed}\}$ rule, the PF of cyromazine in animal feed had a substantially larger impact on the simulated cyromazine MRL in feed compared to water contents in feed and crops, indicating that the residue reduction during feed processing cannot be neglected. This phenomenon was also observed in the derived cyromazine MRL in feed under the $\{MRL_{H\_Health \rightarrow Feed}\}$ rule because the PFs of pesticides in feed or livestock food products typically have large uncertainty and variability,[45] depending on processing conditions, manufacturing methods, cooking techniques, and other factors. In addition, the PFs are multiplication factors of theoretical residue concentrations (*i.e.*, MRL) in feed or food products, of which the values directly affect the residue transport to the downstream sectors of the livestock food product chain. Also, the derived cyromazine MRL in animal feed based on human health had a large uncertainty (*e.g.*, the 5th and 95th percentiles of the distribution are 21 mg kg^{-1} and 506 mg kg^{-1}, respectively), which was due to the large uncertainty of human behavior variables including AF and IR. This wide uncertainty interval indicated that regional or individual exposure assessments of pesticides needed to be considered, particularly for vulnerable population groups (children and elderlies) in the regions where pesticides are intensively used.

Moreover, the uncertainty of the interaction between pesticides and animals substantially affected the derived pesticide MRL in animal feed. For example, the toxicity and biotransfer potential of cyromazine determined the derived cyromazine MRLs in cattle feed under $\{MRL_{A\_Health \rightarrow Feed}\}$ and $\{MRL_{Food \rightarrow Feed}\}$ rules, respectively. However, due to information limitations, the ADI and BTF values of cyromazine for cattle were estimated using the screening approaches. Compared to the point estimate approach, the probabilistic simulation (Table S3) generated large uncertainty intervals of the derived cyromazine MRLs in cattle feed, indicating that the pesticide-and-livestock interaction needed to be fully understood in order to improve the MRL definition in animal feed.

3.4 Study limitations and recommendations for future research

The framework proposed in this study includes four rules for defining pesticide MRLs in feed, which considers the potential impacts of the defined MRLs on both upstream and downstream sectors of the livestock-product supply chain. However, due to data limitations, some assumptions were made to facilitate the regulatory process. We believe that the following recommendations (see Fig. 4) can help regulatory agencies optimize pesticide regulation in animal feed.

For Rule 1 (defining MRLs in feed from MRLs in fodder crops), we suggest conducting a comprehensive evaluation of feed

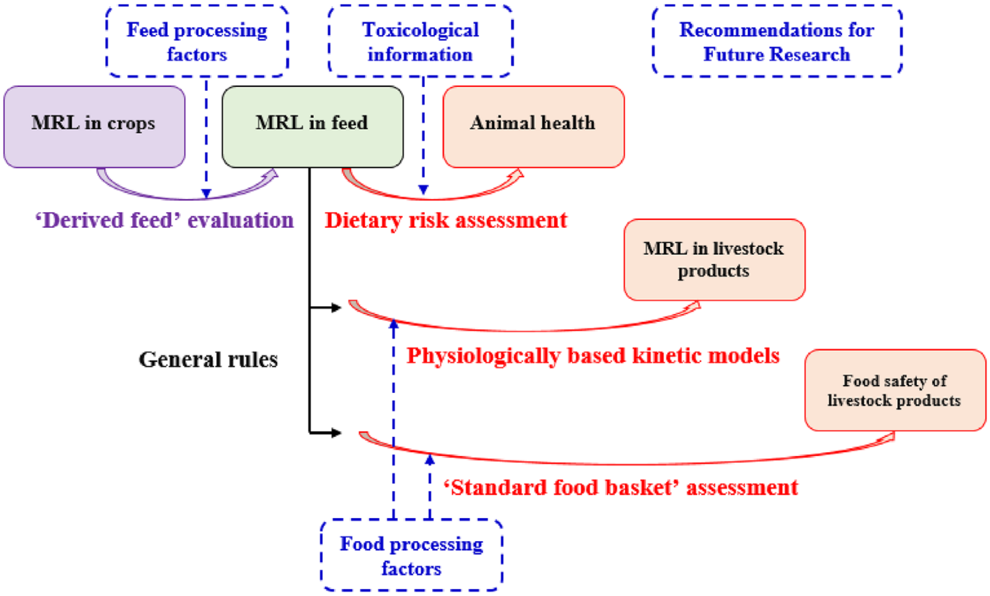

Figure 4. Recommendations for future research about improving the framework for defining pesticide maximum residue levels in animal feeds.

processing factors for pesticide residues. Feed manufacturing methods vary for different crops, livestock, and manufacturers' technologies, which include physical (*e.g.*, grinding and extrusion), chemical (*e.g.*, acidification), and biological processes (*e.g.*, fermentation). Therefore, the feed processing factors for the pesticides should be evaluated for each feed manufacturing process. In addition, before feeding livestock, crops and fodder undergo storage or transportation, for which the fate and distribution of pesticide residues should be evaluated. For example, the dissipation half-life of pesticides in plants can vary significantly for different pesticides and plant species under different storage conditions,[46–48] which should be considered when defining MRLs in feed based on MRLs in fodder crops. Notably, pesticides are used on all fodder crops, but not always the same pesticides are applied to all fodder crops. This leaves a certain pesticide only being applied to certain fodder crops. The information on which pesticide is used on which fodder crop(s) is a user input, since this varies by region, year, and farmer. As a result, we added the indicator function to Eqn (2a), whose value (0 or 1) is set by the user.

For Rule 2 (defining MRLs in feed for protecting animal health), we suggest that a standard procedure for deriving ADI values of livestock be established. Using livestock mammals to conduct toxicological tests for hundreds of pesticides requires immense costs—using currently available data from other animals (*e.g.*, rats) is recommended. The procedure for deriving ADI values for livestock mammals from those of test animals should consider the following aspects: (1) the guidelines for defining acceptable health risks of livestock, which can help determine pesticide MRLs in feed, and (2) guidelines for defining inter- and intra-species uncertainty factors, which can help protect common breeds of livestock. The ADI-derivation process for livestock may not require the method to be as conservative as that for humans; however, the safety and health of livestock are important for the quality of livestock products and sustainable development of the pastoral industry.

For Rule 3 (defining MRLs in feed from MRLs in livestock products) and Rule 4 (defining MRLs in feed for protecting human health), we suggest considering a comprehensive evaluation of food processing factors for pesticides in foods of animal origin. In the present study, we applied the BTF approach to link the residue levels of pesticides in feed to those in raw animal products due to data limitations regarding the fate of pesticides during food processing and cooking processes. Although using a processing factor of 1.0 in our model is acceptable for regulatory practice when essential data are missing, this conservative approach could overestimate human health risks, particularly for meat products that usually undergo high-temperature cooking processes. Studies have shown that pesticide residues undergo thermal degradation, and cooking techniques (*e.g.*, boiling and frying) can substantially reduce pesticide residue levels in meat products.[49,50] However, it must be noted that some food manufacturing processes (*e.g.*, concentrating and drying processes) could increase pesticide residue levels, for which a processing factor above 1.0 should be applied.[49] A flexible empirical approach with AF, PF, and BTF was proposed to account for the aforementioned factors (Supporting Information, Section S5 of the Supplementary File), which can help regulatory scientists update simulation results with available information and assist users in modifying model inputs for any scenario of interest. In addition, the toxicity of parent compound metabolites should be considered in the dietary risk assessment of livestock products because some metabolites have similar or higher toxicity than their parent compounds. Thus, a comprehensive evaluation of the fate of pesticides as well as the toxicity of metabolites is needed to define MRLs in feed based on the quality and safety of livestock food products.

4 CONCLUSION

In this study, we proposed a framework for defining pesticide MRLs in animal feeds, which was developed, based on four general rules considering the impacts from upstream and downstream sectors of the livestock-product supply chain. These four general rules are defined by mathematical equations in which users can modify placeholder variables (such as allocation factor, indicator function, and residue fraction) to undertake site-specific regulatory management of pesticides in animal feed. The essential role of pesticide MRLs in feed is to protect animal and human health and ensure compatibility with MRLs in fodder crops and livestock products. The results for the selected pesticides indicated that current pesticide MRLs in the livestock-product supply chain (*i.e.*, crops, feeds, and animal products) need to be evaluated to ensure compatibility of MRLs across upstream and downstream sectors and the health of livestock and humans. In addition, we provided recommendations for the rules of the proposed framework, including the evaluation of pesticide fate in the feed manufacturing process, the suggested procedures for deriving toxicological data for livestock (*e.g.* evidence-based approaches), and the evaluation of food processing factors and pesticide metabolites, which can help decision makers to optimize the process for deriving MRLs for animal feed.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Pietrzak D, Kania J, Malina G, Kmiecik E and Wątor K, Pesticides from the EU first and second watch lists in the water environment. *Clean* **47**:1800376 (2019).
2 Fantke P and Jolliet O, Life cycle human health impacts of 875 pesticides. *Int J Life Cycle Assess* **21**:722–733 (2016).
3 Maggi F, Tang FHM, Black AJ, Marks GB and McBratney A, The pesticide health risk index—An application to the world's countries. *Sci Total Environ* **801**:149731 (2021).
4 Tang FHM, Lenzen M, McBratney A and Maggi F, Risk of pesticide pollution at the global scale. *Nat Geosci* **14**:206–210 (2021).
5 Handford CE, Elliott CT and Campbell K, A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. *Integr Environ Assess Manage* **11**:525–536 (2015).
6 Raherison C, Baldi I, Pouquet M, Berteaud E, Moesch C, Bouvier G *et al.*, Pesticides exposure by air in vineyard rural area and respiratory health in children: a pilot study. *Environ Res* **169**:189–195 (2019).
7 Regrain C, Zeman FA, Guedda M, Chardon K, Bach V, Brochot C *et al.*, Spatio-temporal assessment of pregnant women exposure to chlorpyrifos at a regional scale. *J Expo Sci Environ Epidemiol* **32**:156–168 (2021).
8 Legind CN, Kennedy CM, Rein A, Snyder N and Trapp S, Dynamic plant uptake model applied for drip irrigation of an insecticide to pepper fruit plants. *Pest Manage Sci* **67**:521–527 (2011).
9 Kosnik MB, Hauschild MZ and Fantke P, Toward assessing absolute environmental sustainability of chemical pollution. *Environ Sci Technol* **56**:4776–4787 (2022).
10 An Q, Wu Y, Li D, Hao X, Pan C and Rein A, Development and application of a numerical dynamic model for pesticide residues in apple orchards. *Pest Manage Sci* **78**:2679–2692 (2022).
11 Năstăsescu V, Mititelu M, Goumenou M, Docea AO, Renieri E, Udeanu DI *et al.*, Heavy metal and pesticide levels in dairy products: evaluation of human health risk. *Food Chem Toxicol* **146**:111844 (2020).
12 Rosenbaum RK, McKone TE and Jolliet O, CKow: a dynamic model for chemical transfer to meat and Milk. *Environ Sci Technol* **43**: 8191–8198 (2009).
13 Gómez-Pérez ML, Romero-González R, Martínez Vidal JL and Garrido Frenich A, Analysis of veterinary drug and pesticide residues in animal feed by high-resolution mass spectrometry: comparison between time-of-flight and Orbitrap. *Food Addit Contam, Part A* **32**: 1637–1646 (2015).
14 Dorca-Preda T, Fantke P, Mogensen L and Knudsen MT, Towards a more comprehensive life cycle assessment framework for assessing toxicity-related impacts for livestock products: the case of Danish pork. *Sci Total Environ* **815**:152811 (2022).
15 Romero-González R and Garrido Frenich A, *Application of HRMS in Pesticide Residue Analysis in Food From Animal Origin, Applications in High Resolution Mass Spectrometry: Food Safety and Pesticide Residue Analysis*. Elsevier (2017).
16 Juraske R, Fantke P, Ramírez ACR and González A, Pesticide residue dynamics in passion fruits: comparing field trial and modelling results. *Chemosphere* **89**:850–855 (2012).
17 Kong Z, Li M, Chen J, Bao Y, Fan B, Francis F *et al.*, Processing factors of triadimefon and triadimenol in barley brewing based on response surface methodology. *Food Control* **64**:81–86 (2016).
18 Fantke P, Charles R, Alencastro LF d, Friedrich R and Jolliet O, Plant uptake of pesticides and human health: dynamic modeling of residues in wheat and ingestion intake. *Chemosphere* **85**:1639–1647 (2011).
19 Rein A, Legind CN and Trapp S, New concepts for dynamic plant uptake models. *SAR QSAR Environ Res* **22**:191–215 (2011).
20 Romera-Torres A, Romero-González R, Martínez Vidal JL and Frenich AG, Study of the occurrence of tropane alkaloids in animal feed using LC-HRMS. *Anal Methods* **10**:3340–3346 (2018).
21 Li Z, PBCLM: a top-down causal modeling framework for soil standards and global sustainable agriculture. *Environ Pollut* **263**:114404 (2020).
22 Ramón F and Lull C, Legal measures to prevent and manage soil contamination and to increase food safety for consumer health: the case of Spain. *Environ Pollut* **250**:883–891 (2019).
23 Tango I and Ezemonye L, Human health risks associated with residual pesticide levels in edible tissues of slaughtered cattle in Benin City, southern Nigeria. *Toxicol Rep* **2**:1117–1135 (2015).
24 Fantke P, Friedrich R and Jolliet O, Health impact and damage cost assessment of pesticides in Europe. *Environ Int* **49**:9–17 (2012).
25 European Commission, (2002).
26 Zarn JA and O'Brien CD, Current pesticide dietary risk assessment in light of comparable animal study NOAELs after chronic and short-termed exposure durations. *Arch Toxicol* **92**:157–167 (2018).
27 Li Z, Xiong J and Fantke P, Screening of pesticide distributions in foods of animal origin: a matrix-based approach for biotransfer factor modeling of grazing mammals. *Environ Sci Process Impacts* **24**: 609–624 (2022).
28 European Commission, Brussels, Belgium (2003).
29 Drogué S and DeMaria F, Pesticide residues and trade, the apple of discord? *Food Policy* **37**:641–649 (2012).
30 Lautz LS, Nebbia C, Hoeks S, Oldenkamp R, Hendriks AJ, Ragas AMJ *et al.*, An open source physiologically based kinetic model for the chicken (Gallus gallus domesticus): calibration and validation for the prediction residues in tissues and eggs. *Environ Int* **136**:105488 (2020).
31 Nair A and Jacob S, A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* **7**:27 (2016).
32 USEPA, Integrated Risk Information System, 2021. https://www.epa.gov/iris [17 August 2021].
33 WHO, Internationally Peer Reviewed Chemical Safety Information, 2021. http://www.inchem.org/#/search [17 August 2021].
34 European Chemicals Agency, ECHA Database—Registered substances, 2021. https://echa.europa.eu/hr/information-on-chemicals/registered-substances [17 August 2021].
35 JMPR, The Joint FAO/WHO Meeting on Pesticide Residues (JMPR), 2021. http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/jmpr/en/ [17 August 2021].
36 Lewis KA, Tzilivakis J, Warner DJ and Green A, An international database for pesticide risk assessments and management. *Hum Ecol Risk Assess* **22**:1050–1064 (2016).

37 New Zealand Government, (2019).
38 European Commission, EU Pesticides database, 2021. https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=search.pr [28 July 2021].
39 WHO, Codex Pesticides Residues in Food Online Database, 2021. http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/en/ [19 August 2021].
40 Klüche M, Bikker P, van Egmond HJ, Hoffmans Y, and JGJ Mol, Potential presence of pesticide residues in feed, requiring single residue methods (SRM): Probability of exceedance of EU legal residue limits in feed materials imported from non-EU countries, due to differences in legislation between the EU and third countries (2020).
41 Zhang L, Huang T, Bi J, Zheng Y, Lu C, Hui Q *et al*., Long-term toxicity study of topical Administration of a highly-stable rh-aFGF Carbomer 940 hydrogel in a rabbit skin wound model. *Front Pharmacol* **11**:58 (2020).
42 Xiagedeer B, Hou X, Zhang Q, Hu H, Kang C, Xiao Q *et al*., Maternal chlormequat chloride exposure disrupts embryonic growth and produces postnatal adverse effects. *Toxicology* **442**:152534 (2020).
43 Dreassi E, Mancini A, Corbini G, Botta M, Tarchi F and Francardi V, Bioaccumulation of deltamethrin, tebuconazole and chlormequat chloride in *T. molitor* larvae and risks associated with their human consumption. *J Insects Food Feed* **6**:457–465 (2020).
44 Yang L, Sang C, Wang Y, Liu W, Hao W, Chang J *et al*., Development of QSAR models for evaluating pesticide toxicity against Skeletonema costatum. *Chemosphere* **285**:131456 (2021).
45 Ferrier H, Shaw G, Nieuwenhuijsen M, Boobis A and Elliott P, Assessment of uncertainty in a probabilistic model of consumer exposure to pesticide residues in food. *Food Addit Contam* **23**:601–615 (2006).
46 Fantke P and Juraske R, Variability of pesticide dissipation half-lives in plants. *Environ Sci Technol* **47**:3548–3562 (2013).
47 Jacobsen RE, Fantke P and Trapp S, Analysing half-lives for pesticide dissipation in plants. *SAR QSAR Environ Res* **6**:325–342 (2015).
48 Fantke P, Gillespie BW, Juraske R and Jolliet O, Estimating half-lives for pesticide dissipation from plants. *Environ Sci Technol* **48**:8588–8602 (2014).
49 Zhang J, Li M, Kong Z, Bai T, Quan R, Gao T *et al*., Model prediction of herbicide residues in soybean oil: relationship between physicochemical properties and processing factors. *Food Chem* **370**:131363 (2022).
50 Albaseer SS, Factors controlling the fate of pyrethroids residues during post-harvest processing of raw agricultural crops: An overview. *Food Chem* **295**:58–63 (2019).