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Transfer kinetics of fipronil into chicken (*Gallus gallus domesticus*) eggs

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ABSTRACT

In 2017–2018, a fipronil contamination occurred in the European chicken egg supply chain due to its illegal use as insecticide to control red lice infestations. Fipronil has the potential to be transferred into eggs of laying hens, thereby entering the food chain. Here, we present a toxicokinetic model to predict the transfer of fipronil in laying hens as a tool for risk assessors and managers. Data from two studies documenting fipronil transfer to eggs were used as a basis to model its kinetic properties. We obtained estimates for fundamental kinetic parameters: the fraction of absorbed fipronil available for transfer 0.44 (CI95 0.41–0.50), the excretion into eggs half-life 11.3 d (CI95 9.9–13.8 d) and the delay of excretion after oral intake. We also calculated the transfer factor at steady state. The parametrized model was evaluated against an independent dataset, showing good correspondence upon visual inspection. The model can be of use in risk assessment and management of fipronil contamination events (repeated exposure) in the egg supply chain and as a starting point for more complex models, i.e. multiple compartments models, which could take in consideration the dynamics of egg-laying.

1. Introduction

Eggs from chickens (*Gallus gallus domesticus*) are an important source of nutrients worldwide. Chicken egg production totals approximately 73.9 million tons per year (in 2016) [1], with around 10% produced in the European Union (EU). Eggs of all animals are a possible excretion pathway of undesirable substances from feed, drinking water, soil or contact with barn materials, for hydrophilic substances (like melamine), lipophilic substances (like polychlorinated biphenyls, PCBs) [2] or substances of surfactant nature (like per- and polyfluoroalkyl substances, PFAS). Such undesirable substances pose a problem in terms of eggs as potentially contaminated food as well as from an ecotoxicological risk assessment point of view because of the maternal transfer of contaminants to the next generation [3]. Many chemicals are never tested on wild animals, so having a toxicokinetic model calibrated for a specific substance and chicken eggs can be the starting point for models for e.g. wild birds by employing allometric scaling and laying performance alterations. In this paper we deal with the substance fipronil and its transfer kinetics into chicken eggs. Transfer is defined following Codex Alimentarius as the “passing of a chemical or biological hazard (including hazardous biotransformation products) from feed of a food-producing animal to an edible product of the animal” [4].

Fipronil is a broad-spectrum, low application rate insecticide and acaricide of the class of phenylpyrazoles, which was released into the market in 1993 [5] and is or has been applied in the EU in seed treatment, biocidal and veterinary products on non-farm animals. It has surfaced as a contaminant in the egg supply chain a number of times in the past. Fipronil affects the nervous system of insects by interference of the chloride ion passage through the γ–aminobutyric acid (GABA) regulated chloride channel [6]. GABA receptor binding is correlated to mammalian toxicity, even though fipronil affinity for mammalian GABA receptors is lower than for insects [7]. Fipronil is acutely toxic in animal experiments when ingested orally, absorbed through the skin or inhaled and is listed in the WHO pesticide classification as moderately hazardous [8]. It has toxic effects on the nervous system in tests with rats, mice, dogs and rabbits, but these effects are reversible in adult animals. Neurotoxicity is observed in the offspring of rats after the mother animals have ingested the substance. Hepatotoxicity has also been observed in rats and mice. According to the current state of scientific knowledge, fipronil is not classified as mutagenic or carcinogenic. Due to the low volatility and dermal absorption potential [9], oral exposure is considered the only relevant exposure pathway. The toxicity of its only relevant plant and livestock metabolite MB46136 (fipronil sulfone) was estimated to be comparable to that of fipronil [9].

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A recent event in 2017/2018 involved a cleaning agent for sanitizing hen houses laced with fipronil as an illegal treatment against red lice (*Dermanyssus gallinae*). Due to the application of the fipronil-containing cleaning agent mainly in the Netherlands and Belgium, but also in a limited number of farms in Germany, laying hens were exposed to fipronil via contaminated feed, dermal contact or by preening (grooming) their feathers, leading to absorption, accumulation in tissues and excretion via eggs and excreta [10–12]. A risk assessment by the German Federal Institute for Risk Assessment came to the conclusion that for the particular fipronil egg contamination event from 2017/2018 [13], only up to 7.8% of the acceptable daily intake (ADI) would be reached for various European consumer groups (based on German and European egg consumption data [14]). Regardless, this contamination event has shown the need for finer quantitative predictions of fipronil transfer, including uncertainty estimations and better predictions of the depuration phase. A toxicokinetic model calibrated specifically with fipronil and laying hens can thus be useful for the assessment and management of human health risks as a consequence of feed and egg contamination. Because of its predictive power, it can potentially be used to set thresholds and maximum levels that are coherent between feed and food.

In rats and mice fipronil toxicokinetics was investigated upon acute and/or repeated exposure in several studies [15,16]. Feeding experiments with dairy cows and laying hens have also been performed [11,17]. All existing studies show that most of the fipronil found in eggs or milk was metabolized to metabolite MB46136. They agreed that fipronil is a substance that reaches a quasi-steady-state slowly and transfer factors for milk and eggs were obtained. Even though there is available data, only general transfer models of lipophilic contaminants exist for farm animals [18,19]. In particular, fipronil was used as an example of a general fat-soluble pesticide in a poultry toxicokinetic model [20].

Toxicokinetic models of feed-to-food transfer in farm animals [21] are becoming more and more important in the consumer health risk assessment and management of chemicals in the feed and food supply chains and their development is at the core of the 3R principle (Replacement, Reduction and Refinement of animal experiments). Our work is part of a wider effort to investigate the feasibility of a computational system biology approach to risk assessment and management of contaminants in the food chain. In this paper we present a toxicokinetic model of fipronil feed-to-egg transfer in laying hens in the context of consumer health assessment of risks arising from contaminated food. Our model was developed focusing on fipronil only and we used different datasets to evaluate the model performance in novel exposure scenarios. We also estimated a confidence interval for the model predictions and provided deeper insight on fipronil toxicokinetics in laying hens.

2. Methods

2.1. Experimental datasets

Two datasets, further referred to as Byrd [12] and Stewart [11], were available from the approval documents of fipronil submitted under Council Directive 91/414/EEC concerning the placing of plant protection products on the European market. The Byrd dataset consists of egg concentration measurements from a transfer experiment: 40 hens (White Leghorn – 62 weeks old) were divided into 1 control and 3 treatment groups of 10 hens each. The treatment groups received an oral nominal dose of encapsulated fipronil amounting to 0.0007 (group 1), 0.0017 (group 2) and 0.0067 mg/kg BW/day (group 3) for a period of 42 days. The eggs were collected twice daily and the concentration of fipronil and its major metabolite MB46136 in whole egg pooled samples (without shell) were measured by gas chromatography with electron capture detector. The Stewart experiment involved 15 hens (Hisex – 22 weeks old) divided into 3 groups of 5 hens receiving an oral nominal ^{14}C-fipronil dose in gelatine capsules containing 0.004 (group 1), 0.174 (group 2) and 0.872 mg/kg BW/day (group 3) for a period of 28 days. Eggs were collected daily and separately analysed for concentration (fipronil + MB 46136 + fipronil sulfinyl) in egg white and egg yolk. Given that at least 95% of the fipronil residues in eggs were in the form of MB46136, we assumed 100% metabolic conversion in eggs and report results in fipronil equivalents (via a molecular mass ratio, i.e. multiply MB46136 amounts by its molecular mass ratio to fipronil).

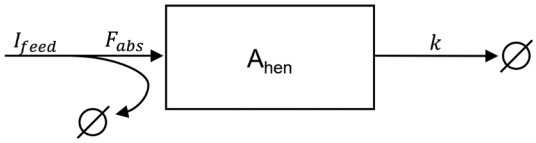

Fig. 1. Schematic representation of the one compartment model. Fipronil is ingested with I_{feed}. Only fraction F_{abs} is available for transfer to eggs (unabsorbed fipronil goes to excreta, indicated by Ø). The variable A_{hen} is the amount of fipronil in the hen that is available for transfer, k is the rate of transfer into eggs and A_{egg} is the cumulative amount transferred into eggs.

2.2. Mathematical model

The laying hen is represented by a single compartment model (Fig. 1) with a constant input representing the fipronil amount absorbed and available to be excreted into eggs. The input consists of the oral fipronil intake I_{feed} multiplied by the parameter F_{abs}, which is the effective parameter for the fraction absorbed and available for transfer into eggs. Unabsorbed and excreted fipronil likely follows multiple routes – i.e. urine excretion to the cloaca and then to the large intestine (where resorption might take place), all of which have been aggregated into 1-F_{abs} (see Table 1). We assume that excretion into eggs follows first-order (mass action) kinetics with a delayed action parameter θ and is formalized by the equation system.

$$\frac{dA_{hen}(t)}{dt} = I_{feed}F_{abs} - kA_{hen}(t-\theta) \quad (1)$$

$$\frac{dA_{egg}(t)}{dt} = kA_{hen}(t-\theta) \quad (2)$$

For description and units of the variables and parameters, see Table 1. For more details, see Supplementary Material "Derivation of the model equations".

The solution to the equation system (1–2) yields the cumulative amount of fipronil transferred into eggs up to a time point t. We can now calculate the amount of fipronil excreted into eggs (yolk and white) in a given time period Δt by taking the difference between A_{egg} at two given time points, obtaining

Table 1
Variables and parameters legend of the one compartment model.

Variable	Description	Units
A_{hen}	Fipronil amount available for transfer in main compartment	mg
A_{egg}	Cumulative fipronil amount transferred to eggs	mg
I_{feed}	Oral intake of fipronil with feed	mg/d
C_{egg}	Fipronil concentration in egg	mg/g
E	Average egg laying performance	1/d
W_{egg}	Average egg weight	g
$\dot{W}_{feed}$	Average daily feed intake (dry matter)	g/d
Parameter	**Description**	**Units**
F_{abs}	Fraction of absorbed fipronil available for transfer	unitless
k	Egg excretion rate constant	1/d
θ	Fipronil excretion delay	d

$$\Delta A_{egg}(t_1, t_2) = I_{feed} \cdot F_{abs} \left(\Delta t + \frac{e^{-k(t_2-\theta)} - e^{-k(t_1-\theta)}}{k} \right), \quad \Delta t = t_2 - t_1 \tag{3}$$

We use Eq. (3) to estimate the parameters (F_{abs}, k, d) by fitting to the Byrd dataset with $\Delta t = 1$ day.

In order to obtain a prediction of egg fipronil concentration in eggs, which was required for model validation, Eq. (3) was normed by $E \cdot W_{egg} \cdot \Delta t$, which is the mass of whole egg (white and yolk) laid in $\Delta t = 1$ d. The predicted concentration in egg can be calculated as

$$C_{egg}(t) = \frac{I_{feed} \cdot F_{abs}}{E \cdot W_{egg} \cdot \Delta t} \left(\Delta t + \frac{e^{-k(t-\theta)} - e^{-k(t-\theta-1d)}}{k} \right) \tag{4}$$

We used Eq. (4) with $\Delta t = 1$ d to predict the egg concentration for exposure scenarios equivalent to the Stewart datasets and compared the model prediction with the actual experimental data. Eq. (4) can be used in general to predict fipronil transfer into eggs in case of prolonged exposure to fipronil contaminated feed using the optimized parameters shown in Results. The model was implemented in Python3.

2.3. Model fitting to Byrd datasets

We used the Byrd data from groups 2 and 3 to fit Eq. (3) and found an estimate for the model parameters. Data from group 1 were not used, as fipronil was below the level of detection for most egg samples. We calculated the fipronil equivalents amount in eggs by multiplying the concentration measurements by the average egg weight – taken from literature (58.04 g) [22] – and by the laying performance (0.75 eggs/day, calculated from the Byrd sample collection data). We first fitted the Byrd dataset separately for groups 2 and 3 and compared the parameter estimation results. As they were in good agreement, we joined the datasets and to fit a single parameter set to both Byrd groups 2 and 3. To make this possible, we normalized the data by dividing it by the amount of fipronil ingested by the hens of each group – and performed the fit on $\frac{\Delta A_{egg}(t_1,t_2)}{I_{oral}}$. As the objective function for parameter fitting, we used a weighted least squares criterion, using the value of the dependent variable at time t as weight, since we assumed multiplicative Gaussian noise. Parameter estimation was performed using the Levenberg-Marquardt algorithm, implemented in Python3 in the package lmfit [23]. To increase the likelihood of convergence to a unique parameter set, we repeated the fitting procedure 100 times with random initial values.

2.4. Confidence interval

In order to obtain a confidence interval for the model prediction, we performed a resampling of the joint dataset. Given the sparsity of the data set (11 data points for each group), a bootstrap approach was unfeasible. Instead, we used a related leave × out (jack-knife) resampling for × = 1, 2, 3 and 4. We obtained thus $\binom{22}{21} + \binom{22}{20} + \binom{22}{19} + \binom{22}{18} = 9108$ combinations, which were each fitted to the model as described in section 2.3. We then used the resampled curves to compute the 95% and 50% confidence intervals.

2.5. Model evaluation using Stewart dataset

The model performance was evaluated on the Stewart dataset using Eq. (4) with the estimated values for parameters F_{abs}, k and θ. The average laying efficiency E and the average egg weight W_{egg} came from the experimental observations for the Stewart dataset, as did I_{feed}, which corresponds to the nominal daily oral dose (g1 − 0.007/g2 − 0.3045 3 − 1.526 mg/d). The confidence interval was calculated as described in section 2.4. We then compared the plot with the experimental data for each hen of each group in the Stewart study.

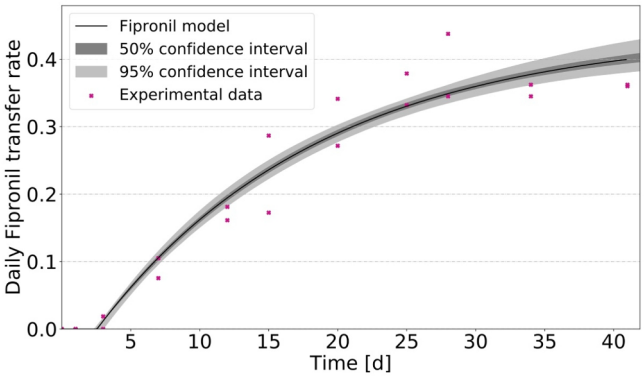

Fig. 2. Model simulations (solid black line) using final parameter sets and data from Byrd groups 2 and 3 (purple crosses). The dark and light grey areas are the 50% and 95% prediction intervals obtained from the resampling procedure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.6. Transfer rate and transfer factor

The transfer rate (TR) is the ratio of the fipronil equivalents amount excreted into eggs to the fipronil amount ingested. At steady state, the transfer rate tends towards $TR_{SS} = F_{abs}$ (Supplementary equations S8-S9). The transfer factor is the ratio of concentration in egg (in fipronil equivalents) to the fipronil concentration in feed in dry matter (Supplementary equation S10-S11). At steady state, the transfer factor tends to

$$TF_{ss} = \frac{F_{abs} \cdot \dot{W}_{feed}}{E \cdot W_{egg}} \tag{5}$$

where $\dot{W}_{feed}$ is the average daily feed intake (dry matter). In our model, $\dot{W}_{feed}$ was obtained from the Byrd experiment report.

3. Results

We fitted the modelled amount of fipronil excreted into eggs in $\Delta t = 1$ day (Eq. (2)) to the Byrd groups 2 and 3 datasets as described in Materials and Methods. The resulting fit (Fig. 2) yielded parameters $F_{abs} = 0.4413$ (CI95 0.4093–0.4968), $k = 0.06$ (CI95 0.05–0.07) d^{-1} and $\theta = 2.05$ d (CI95 1.85–2.5 d) for the toxicokinetics of fipronil. See also Supplementary Material "*Concise description of the toxicokinetic model and its constants*". The model gives a fractional absorption upon oral intake (represented by the parameter F_{abs}) equal to 44.13%, confirming the high bioavailability shown by experimental data (fipronil sulfone recovered in excreta amounted to 41%/53%/34% of administered dose (Stewart experiment)). The value obtained for the elimination rate constant k was used to calculate the fipronil excretion half-life in laying hens ($ln2/k$), which amounts to 11.27 d. According to the model, the transfer rate at steady state tends towards $TR_{SS} = F_{abs}$ independent of the fipronil dose. The transfer factor (Eq. (5)) for the Byrd dataset in dry matter feed basis is 0.91 (group 2) and 1.11 (group 3). We also estimated the time to quasi-steady-state (5 time the half-life) obtaining 56.4 days, almost twice the previous estimate [11].

The fitting itself robustly converged towards the optimum (independent of initial values) and yielded consistent results across all resampled datasets. The confidence interval for the model predictions was relatively narrow (grey areas in Fig. 2), indicating the stability of the model prediction.

4. Discussion

The model quantifies the fipronil excretion half-life to 11.32 d (9.9–13.8 d) and shows that a quasi–steady–state will be reached after

about 8 weeks of continuous exposure. This is similar to the half-life of 8 d found by MacLachlan in 2008 [20] but about 30% longer. The model also confirms the high bioavailability of the substance, having a transfer rate of more than 44%. We also found that the transfer factor (whole egg and dry feed matter) at steady-state (repeated exposure scenario) in the Byrd experiment amounts to 1.01 (calculated using Eq. (5) and taking the average between TF of groups 2 and 3). This value can be used when assessing long-term exposure scenario and represents a more trustworthy estimate that the mean TF calculated in the EFSA report (0.65 for group 2 and 0.77 for group 3), which is based on the average of only three values (likewise whole egg and dry feed matter). This is because our calculations are based on a toxicokinetic model, thus are informed by all data points and not only three as in the mean TF calculated in the report. The difference in values is probably due to the fact that while we calculated the transfer factor at steady state (limit operand), the EFSA estimates are derived by actual data points on days in which the real steady state was not yet reached, thus resulting in an underestimation. Overall, the kinetic properties found here can be of use in the risk assessment and management of fipronil feed-to-egg transfer in laying hens. The model provides better understanding of the plateau phase and of the transfer kinetics when steady state is reached. We also provide a simple Python tool to generate egg contamination predictions similar to Fig. 3 as part of the Supplementary material.

It should be mentioned that most of the fipronil was excreted (as fipronil sulfone) into egg yolk and a minor amount into egg white. The yolk-to-white concentration ratio is time-dependent, starting at a value of around 2 in the beginning of the experiment and increasing asymptotically to around 25–35 near the steady state, as calculated from the Stewart data (not shown). This is consistent with our knowledge of the chemical composition of the egg, the physicochemical properties of fipronil sulfone [24] and the process of egg formation. As fipronil sulfone is lipophilic and about a quarter of the egg yolk consists of fatty acids [25], we expect it to accumulate there. The change in concentration ratio may be explained by the yolk growth process: yolk formation is long (up to 60 days) and in the last 6–14 days the oocyte diameter changes from 8 mm to full size (around 40 mm) [25]. In the initial phase of the exposure, the yolks in the ovary are already almost fully formed and have little time to be in contact with the still relatively low blood concentration of fipronil. Fully formed yolks also have a much smaller surface area to volume ratio, meaning that diffusion will cause the fipronil concentration to change at a slower rate compared to the small oocytes. As the blood concentration rises and the younger oocytes grow, there is both more fipronil diffusing into them and more exposure time. This could explain the observed yolk-to-white fipronil concentration ratio pattern and would hint to the need of more complex models to separately predict white and yolk concentrations. The present model, however, predicts fipronil concentration in whole egg and disregards the physiology of oocyte growth.

We evaluated model performance by generating a prediction of egg contamination (Eq. (4) with the optimized parameters shown in Results) corresponding to Stewart experiments 1–3 and then comparing it with the experimental egg concentration results from the corresponding dataset (Fig. 3). Fig. 3A simulates Stewart experiment 1, where the fipronil dose levels were similar to Byrd dataset 2 and 3. Our model performs well, predicting the experimental data satisfactorily. Fig. 3B and C simulate Stewart data sets 2 and 3 respectively. The model here tends to slightly overestimate the fipronil concentration in eggs. The overestimation of C_{egg} compared to the experimental values becomes more pronounced with higher feed concentrations; an explanation would be a saturation mechanism in the absorption. This is in line with the experimental data, which document decreasing absorption with increasing dose levels (fipronil recovered in excreta of Stewart hens was 9%, 27% and 51% of administered dose). This implies a dose-dependent absorption, which is not currently incorporated in the model and would be a feature to add. Nevertheless, the model appears robust and performs well in predicting contamination levels for very different dose levels (1:43:218). The confidence interval obtained with the resampling procedure is relatively narrow, partly because of the method (leave × out cross validation) employed. The narrowness suggests that the model prediction is not heavily influenced by any specific data point and has somewhat high confidence. Note that the confidence interval pertains to the confidence we have that the data used for fitting produce the given model. The confidence interval has no bearing on, among other things, the biological variability of the concentration of individual eggs (cf. data in Fig. 3 vs the narrow average prediction confidence intervals).

A model parameter that has a large influence on the outcome is the laying performance, which is expected given the single compartment and excretion route. Implementing a multiple compartment model might attenuate this issue, especially if the ovary is compartmentalized. The oocyte (yolk) would then come in equilibrium with the bloodstream, reaching a certain concentration level, which would correspond to most of the fipronil excreted through the egg. However, parameters may not be uniquely identifiable in such an elaborate multi-compartment model. A possible approach to address such identifiability problem could be to integrate the model with data from other bird species and/or use data from *in silico* methods. It would also be interesting to discretize the egg growth excretion process, which would better represent the biology of the animal. One could include a stochastic element and have the hen model excrete either 0, 1 or 2 eggs per day, as it happens in the roost. We could then obtain a distribution of the fipronil levels in eggs which could be a better predictor for contamination. Including an additional excretion route (from the bloodstream to the intestine) could also improve the model performance. The model equations could also incorporate more physiological processes, especially the oocyte growth dynamics discussed before, which would probably be better represented by delay differential equations, which have a "memory" of the system at previous time points. In our simple ODE model, we represent this aspect with the parameter θ, which summarizes the time shift between the first oral administration and the

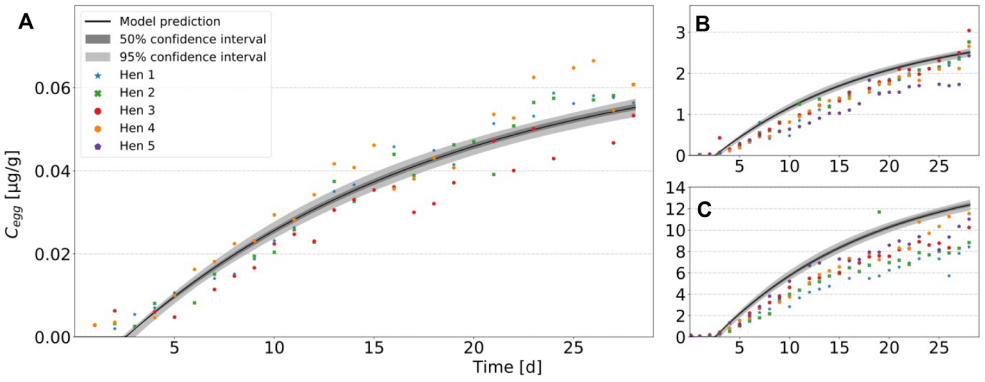

Fig. 3. Model predictions using the final parameter sets (solid black line) for concentration C_{egg} (fipronil equivalents) and comparison to the evaluation data set (different hens in the Stewart experiments; coloured markers). The model prediction and confidence interval are shown in black/grey. A) Model evaluation on data from Stewart experiment group 1, which received a nominal dose of 0.004 mg/kg BW/d. B) Model evaluation on data from Stewart experiment group 2, which received a nominal dose of 0.174 mg/kg BW/d. C) Model evaluation on data from Stewart experiment group 3, which received a nominal dose of 0.872 mg/kg BW/d.

first observation in eggs.

5. Conclusions

The model we developed (Eq. (4) with optimized parameters in Results section) showed good predictive power for egg contamination following oral intake. The model performed well on the evaluation datasets, on very different dose levels. As it is, it can be used to estimate feed-to-food transfer for fipronil in whole egg (in cases of repeated exposure). We propose a new value for fipronil half-life in laying hens of about 11 d (9.9–13.8 d), corresponding to a time to steady state of about 8 weeks (6–9 weeks), or twice the previous estimate [11]. We also estimated the TF for repeated exposure at steady state to be 1 and the fraction of absorbed fipronil available for transfer to be 44% (41–50%). This is an advance in current knowledge about fipronil kinetic properties in laying hens. Additionally, the model could be used as a reference point when developing wild animal toxicokinetic models aimed to improving the ecotoxicological risk assessment of fipronil. While in this paper we focused on a model created to inform human risk assessment related to contamination of chicken eggs with fipronil, it remains to be investigated whether the model is scalable to other egg-laying species for which no data are available.

As mentioned in the discussion, more complex models can be developed, and the first step would be to use delayed differential equations to better represent the biological processes underlying fipronil distribution and excretion. It would be interesting to combine this approach with a stochastic discrete model that would better simulate the dynamics of egg laying. More work is needed to develop and evaluate such models in order to provide solid systems biology tools to risk assessors and risk managers. At last, in order to help the development of models that can distinguish between egg white and yolk, we would like to encourage experimenters to take separated measurements instead of analysing pooled egg samples.

CRediT authorship contribution statement

Pietro Gerletti: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Max Von Kleist:** Methodology, Formal analysis, Writing - review & editing. **Hans Mielke:** Methodology, Formal analysis, Writing - review & editing. **Thomas Kuhl:** Resources, Writing - review & editing. **Robert Pieper:** Writing - review & editing, Supervision. **Monika Lahrssen-Wiederholt:** Writing - review & editing, Supervision, Funding acquisition. **Jorge Numata:** Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.comtox.2020.100131.

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