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# A generic avian physiologically-based kinetic (PBK) model and its application in three bird species

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ABSTRACT

Physiologically-based kinetic (PBK) models are effective tools for designing toxicological studies and conducting extrapolations to inform hazard characterization in risk assessment by filling data gaps and defining safe levels of chemicals. In the present work, a generic avian PBK model for male and female birds was developed using PK-Sim and MoBi from the Open Systems Pharmacology Suite (OSPS). The PBK model includes an ovulation model (egg development) to predict concentrations of chemicals in eggs from dietary exposure. The model was parametrized for chicken (Gallus gallus), bobwhite quail (Colinus virginianus) and mallard duck (Anas platyrhynchos) and was tested with nine chemicals for which in vivo studies were available. Time-concentration profiles of chemicals reaching tissues and egg compartment were simulated and compared to in vivo data. The overall accuracy of the PBK model predictions across the analyzed chemicals was good. Model simulations were found to be in the range of 22–79% within a 3-fold and 41–89% were within 10- fold deviation of the in vivo observed data. However, for some compounds scarcity of in-vivo data and inconsistencies between published studies allowed only a limited goodness of fit evaluation. The generic avian PBK model was developed following a "best practice" workflow describing how to build a PBK model for novel species. The credibility and reproducibility of the avian PBK models were scored by evaluation according to the available guidance documents from WHO (2010), and OECD (2021), to increase applicability, confidence and acceptance of these in silico models in chemical risk assessment.

## 1. Introduction

Physiologically-based kinetic (PBK) models are effective tools for designing biomedical, pharmaceutical, and toxicological studies and for conducting extrapolations for risk assessments (Krishnan and Peyret 2009). PBK models can fill data gaps and define safe levels of chemicals in ecotoxicological or environmental risk assessment (Paini et al. 2021). In PBK models, the body is represented as a network of interconnected compartments linked via blood flow, as depicted in Fig. 1, to simulate time-concentration curves in target organs. After an oral, dermal or inhalation exposure to a compound, its adsorption, distribution, metabolism, and excretion (ADME) processes are simulated by means of ordinary differential equations (ODE). These modeling approaches are recognized for the crucial role they can play in, for example, predicting the biokinetics of drugs and chemicals in an organism without the need to conduct in vivo experiments.

The principal advantage of PBK models is to evaluate various plausible hypotheses by computer simulation in a fast and efficient way. Application of PBK models provides direction in the design of experimental protocols both for in vivo and in vitro systems, allowing the extrapolation of in vitro responses to in vivo exposures (Stadnicka-Michalak et al. 2014; Bell et al. 2018). In addition, PBK models can be employed to answer a variety of questions related to risk assessments. Examples of such questions include the following: How much of a chemical is present in an egg after exposure of the laying hen to the chemical in its feed? What is the impact of a change in chemical

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structure on the tissue concentration and the toxicokinetic profile? Could chemical residues in a specific organ pose a risk to the development of the offspring? What would be the changes in target organs under time-variable exposure? What would be the impact of changes in physiology (i.e., reflective of life stage or species differences) on the target tissue dose? Could such levels in eggs be a risk for human consumption? Plant protection products (PPP) are formulations which are designed and widely used to protect plants from diseases, insects and weeds (e.g. pesticides). A PPP contains one or more active chemical substances. In order to ensure sufficient efficacy on the one hand and protection of human and animal health and the environment on the other hand an assessment must be carried out before authorization on the EU market. To this end according to the PPP Regulation (EC) No. 1107/2009 a complex assessment involving comprehensive data is required for the authorization of active substances and PPPs. The data requirements for the authorization of PPPs are laid down in Annexes to the Regulation (EC) No 1107/2009, and respective 283/2013. Active substances are first evaluated and assessed by the competent agency, the European Food Safety Authority (EFSA). In a follow up the active substance-containing PPPs are examined and evaluated by the zonal and national authorities of each member state.

Many risk assessments are performed to assess the risk to humans, aquatic organisms and non-target organisms (including wild avian species) posed by contaminants, regulated chemicals and dietary ingredients. Testing in birds is most commonly performed as a requirement for pesticide registration by regulatory authorities (Regulation (EC) No 1107/2009). While requirements vary regionally, the focus of this paper will be in the context of the EFSA risk assessment scheme following EFSA guidance (EFSA 2009). The EFSA assesses the risk of pesticides to birds and mammals using data from acute and reproductive toxicity studies (EFSA 2009).

Avian reproduction studies can be conducted according to Organisation for Economic Co-operation and Development (OECD) TG 206 (1984), typically using bobwhite quail and mallard ducks. Regulatory metabolism and residue studies with birds for the dietary risk assessment are usually conducted in laying chicken hens (Gallus gallus domesticus), according to OECD TG 503 (OECD 2007). The OECD TG223 (OECD 2016) is designed to estimate the acute oral toxicity of substances to birds. The bird species recommended for these studies include bobwhite quail (Colinus virginianus) and Japanese quail (Coturnix japonica) (OECD 2016). The highest dose used in tests does not normally exceed 2000 mg/kg body weight (OECD 2016; EFSA 2009).

Historically, avian acute oral median Lethal Dose (LD50) studies were generally conducted with a minimum of 50 birds (EFSA 2009). The modern OECD guideline TG223 delivers the same endpoints with similar precision using fewer birds (e.g., 12–24 individuals). This is in line with the recent views of the EU Parliament policy goal of further minimizing animal testing in EU (EU Parliament Press Release 16–09-2021)[1]. However, the number of birds needed to conduct reproduction studies is much higher.

A PBK modelling framework that addresses these three species would allow for an integrated assessment of the data of these test systems, e.g., by reproducing or extrapolating chemical concentrations in tissues and eggs. Therefore, PBK modelling simulations may play a pivotal role in reducing and refining such bird toxicity testing, for instance, by informing top dose selection when running an in vivo study, allowing to

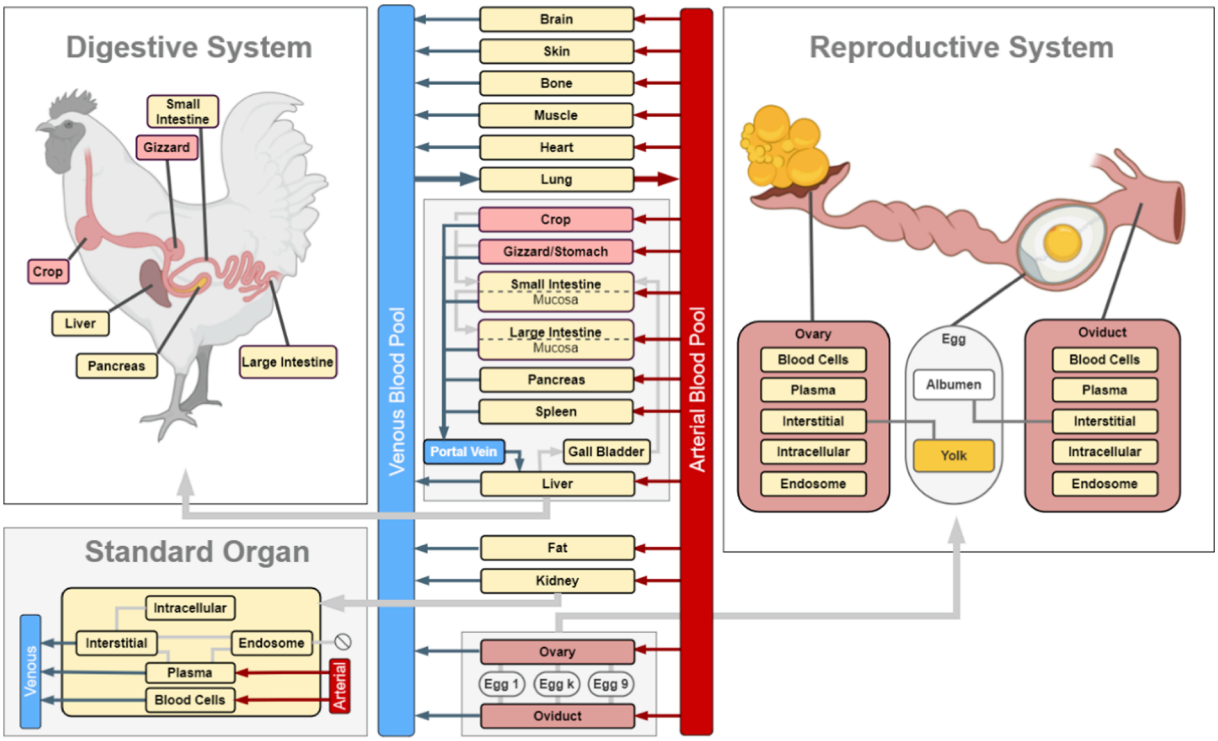


**Fig. 1.** Schematic representation of the avian physiologically-based kinetic (PBK) model, with detail into the hen reproductive system. In the schematic PBK model structure, in the middle of the figure, the boxes in red (Crop, Gizzard/Stomach, and Ovary/Oviduct) are specific to the bird anatomy-physiology and were introduced as new compartments. In addition, a schematic description of the reproductive system and of a standard organ are also displayed. This model is based on the chicken (Gallus gallus) and extended to the mallard duck (Anas platyrhynchos) and bobwhite quail (Colinus virginianus). Egg 1, Egg k, and Egg 9, represent the egg cycle. Eggs grow over a nine-day period, and thereafter one egg will be laid daily (**figure created with BioRender.com and draw.io**).

[1] https://www.europarl.europa.eu/news/it/press-room/20210910IPR11926/meps-demand-eu-action-plan-to-end-the-use-of-animals-in-research-and-testing.

understand saturable kinetic pathways, and to reliably collect and integrate these data without additional satellite animal groups (Tan et al. 2021). However, the number of these models is very limited for birds. A recently published PBK model database, based on a systematic review (Thompson et al. 2021), reports only 14 published chemical-specific PBK models for chicken and two for turkey. In addition, in 2009, a PBK model for midazolam in four avian species was published by Cortright and colleagues (Cortright, Wetzlich, and Craigmill 2009). The most recent work, in which a refined chicken PBK model including an egg compartment was developed by Lautz et al. (2020) and applied to seven chemicals. The limited work available in this area highlights the need for developing a generic avian PBK model including bird species regularly used in ecological risk assessment.

In the present work, a generic avian PBK model for male and female birds (Fig. 1) was developed, using PK-Sim and MoBi from the Open Systems Pharmacology Suite (OSPS). The cross-species whole-body PBK structure was adapted to fit the chicken's physiology. Further, this model was extended to include two species, bobwhite quail and mallard duck in accordance with OECD TG 206 (OECD 1984). To address the import task of understanding the concentration of chemicals reaching the egg, the PBK model included the ovulation model (egg development) to predict egg concentrations of chemicals from dietary exposure (Fig. 1).

To increase acceptance of this new model in the risk assessment process, the generic avian PBK model was developed following a workflow recently published by Schneckener et al. (2020). This "best practice" workflow describes how to build PBK models for novel species, starting from existing PBK models. The avian PBK model was also evaluated for its accuracy in model predictions and credibility was scored using the available guidance documents from WHO (WHO 2010), and OECD (OECD 2021), with the aim to increase applicability, transparency and acceptance of these in silico models.

## 2. Materials and methods

The set-up of the avian PBK model followed the "best practice" workflow that describes how to build PBK models for novel species proposed by Schneckener et al. (2020), (see more in paragraph 2.5). To ensure model reproducibility and to gain confidence in the model predictions, the following methodological section has been compiled following the requirements/elements laid down in several international guidance documents on good modeling practices (WHO 2010; EFSA 2014; Tan et al. 2020; OECD 2021).

### 2.1. PBK model - physiology

The avian physiology analysis was reviewed in a parallel work by Scanes et al., (2022a, 2022b). They concluded that the physiology of the avian species was comparable to mammals, with two major differences: the gastrointestinal (GI) tract and the female reproductive tract. The GI tract in most birds differs from the mammalian GI tract in several ways including the lack of teeth and therefore chewing and the different stomach system (Scanes et al. 2022a, 2022b). Briefly, prior to reaching the gizzard or ventriculus), food transits through the crop (a muscular pouch that is an extension of the esophagus) and the proventriculus (also known as the glandular stomach). Within the crop, food resides for insalivation and storage. It is then ground or "chopped" in the gizzard before transported to the small intestine for final digestion. The small intestine in birds is similar in form and function to mammals. However, fowls have two large caeca for fermentative processes. Additionally, at the end of the colon, the cloaca is located. In contrast to mammals, urine and feces are excreted via the cloaca together. Excreta can pass by retrograde peristalsis up the colon.

Female birds have both a single ovary and oviduct; the latter being equivalent to the fallopian tubes, uterus, cervix and posterior vagina in mammals. To account for differences between ovulation in birds and mammals, the processes of egg maturation and egg-laying were implemented in the model (as described in 2.3). The ovulation - egg-laying physiology in chicken was described in Hekman and Schefferlie (2011). The egg-laying process for the other bird species was assumed to be similar to the chicken, while the egg physiology (e.g., composition and size) was informed by species-specific parameters (see more detailed explanation in 2.3).

The avian PBK model was structurally based on the mouse model available in PK-Sim (Davies and Morris 1993; Niederalt et al. 2018), following the good model practice approach from Schneckener et al. (2020). To account for species differences, the GI and ovulation tract were re-defined and included, respectively (see Fig. 1). This also allows for the prediction of tissue homogenate concentrations. The crop was additionally implemented and structurally copied from the stomach. Proventriculus and gizzard are combined as one stomach compartment. The intestinal differences were implemented by scaling the existing compartments including duodenum, jejunum, ileum, caecum, colon and the rectum (representing the cloaca). The excreta are represented by the combination of feces and urine.

The following avian-specific organs were not explicitly modeled since chemical distribution to these organs was not expected to contribute to the overall PK or because of a lack of information: membranes and structural tissue within the egg, egg shell, feathers, air sacs, comb, some glands (e.g., shell gland, uropygial gland), saliva, and renal portal system.

### 2.2. PBK model refinement - ovulation model description & assumptions

The reproductive system consists of an ovulation model comprising follicular development as a multistep process over a period of days. When the hen reaches sexual maturity, follicles start to grow and fill up with yolk. After about eight days, the ovum containing yolk separates from the follicular tissue and from the ovary and travels through the oviduct, where albumen and plumping fluid is added within only several hours. In a final step, the membranes and shell are formed in the shell gland (uterus) before the egg is laid. The process in total takes approximately-nine days and this is the timeline implemented in the model.

Technically, the egg cycle can be viewed as starting at a time defined as zero and the first egg is laid after nine days (and then one every day). Therefore, observed data and administration for simulations with egg concentrations need to be shifted by nine days. When the ovulation model is enabled the volume of the eggs is set to 0.1 mL representing small follicles. The egg cycle in the model comprises nine eggs, and after nine days of simulation, an egg is "laid", and its volume is set back to zero before it starts growing again.

Each egg comprises a yolk and an albumen compartment. Passive diffusion processes were allowed between the following model compartments: yolk – ovary-plasma, yolk -ovary interstitial, and albumen - oviduct interstitial. The oviduct was additionally introduced as a new organ. It is structurally copied from the ovary and parameterized with the oviduct-specific organ volume, blood flow rate, and tissue composition.

The ovulation model includes equations to describe the yolk and albumen growth during egg development published previously (Hekman and Schefferlie 2011) and are reported in supplementary material, SM.C.

The development of the avian PBK model is based on several assumptions listed below:

- Although there are different growth phases of the egg parts in vivo (i.e., rapid growth, water reduction (Scanes et al. 2022a, 2022b), the yolk and albumen composition in the PBK model do not change over time, a steady-state in yolk and albumen composition was, therefore, the best approximation.

- The chemical transport into yolk and albumen is based on passive diffusion of unbound drug concentration from ovary and oviduct interstitial space to yolk and albumen, respectively, based on a calculated, composition-based partition coefficient.
- For chemicals likely to bind to lipoproteins, transport into yolk is modelled using an empirical lipoprotein-binding factor and the total ovary plasma concentration multiplied with the yolk surface area.
- A sigmoidal growth shape of the yolk volume based on the model of (Hekman and Schefferlie 2011) is assumed and the surface area between ovary and yolk is implemented on the spherical surface of the volume of yolk.
- Since the oviduct is the site of albumen synthesis, and a high permeability between oviduct interstitial space and albumen is expected, a rapid equilibration between the two compartments is assumed implemented as a large surface area (100 $cm^2$).
- Linear growth is assumed within 10 h (Hekman and Schefferlie 2011).
- No diffusion between yolk and albumen is assumed since the two phases are separated by membranes. Growth duration is set to nine days, as explained in the text.
- One egg per day is laid. It takes almost a day to physically form an egg in the oviduct, this is the maximum amount a chicken can lay, in general around 5–7 eggs are laid per week (Scanes et al. 2022a, 2022b).

### 2.3. PBK model parametrization

#### 2.3.1. Physiological parameters

Information regarding the avian-specific physiology for the three bird species was retrieved and published in Scanes et al. (2022a, 2022b); an extract of these data are available in supplementary material SM.E. Table 1 reports the most relevant physiological parameters used for developing the bird PBK model. Specific organ and tissue weight and volume were obtained for both female and male, mallard duck, and bobwhite quail (study description in SM.A.4). The physiological parameters of the chicken were used as the reference values for the other bird species where no species-specific values were available. Sex-specific values for organ volumes, blood flows, and tissue composition were implemented where available. When bird-specific data was unavailable, the data gap was filled using mouse values.

#### 2.3.2. Case study chemicals

The avian PBK model was developed and evaluated using nine chemicals (Table 2): chloramphenicol (CAP), deltamethrin (DTM), florfenicol (FFC), itraconazole (ITZ), ivermectin (IVM), melamine (MEL), midazolam (MDZ), monensin (MON), salinomycin (SAL). The chemicals were selected based on available in vivo data for the three bird species and used for the development and evaluation of model predictions; to this end, a literature search for chemical-specific in vivo studies was performed. This literature search was carried out by searching the following terms (as a STRING) "in vivo" AND "PK" AND "specific organ: <blood, egg, tissue>" AND "bird species: <chicken, poultry, quail, duck>". The search was performed using Google and different scientific databases such as PubMed and Scopus. This search resulted in 33 studies for the nine chemicals as reported in Table SM.A.2 in supplementary materials.

Table 2 lists the name, CAS number, chemical class and specific ADME processes for each of the selected chemicals along with the different avian species for which in vivo studies were available to evaluate the model predictions (collected and presented in Table SM. A.1). The model was applied using the following exposure routes, oral (Intra-crop, IC, and feed) and intravenous (IV).

**Table 1**
Selected key physiological input parameters for the three avian species.

| Physiological Parameter | Chicken[1] | Duck[2] | Quail[3] |
|---|---|---|---|
| Body Weight (kg) | 1.36 | 0.74 | 0.2 |
| Cardiac Output (l/min) | 0.44 | 0.33 | 0.05 |
| Tissue Volume (l) | | | |
| Plasma | 0.106 | 0.087 | 0.015 |
| Liver | 0.041 | 0.028 | 0.0027 |
| Kidney | 0.005 | 0.009 | 0.0013 |
| Muscle | 0.561 | 0.319 | 0.1119 |
| Fat | 0.181 | 0.029 | 0.0030 |
| Lung | 0.012 | 0.008 | 0.0007 |
| Heart | 0.01 | 0.007 | 0.0008 |
| Blood Flow (l/min/kg organ) | | | |
| Liver | 0.56 | 0.58 | 0.8 |
| Kidney | 3.73 | 1.08 | 3 |
| Muscle | 0.053 | 0.55 | 0.066 |
| Fat | 0.158 | 0.158 | 0.158 |
| Heart | 2.88 | 2.69 | 2.72 |

[1] Chicken – laying hens (Gallus gallus domesticus), [2]Duck- mallard duck (Anas platyrhynchos) and [3]Quail – bob-white quail (Colinus virginianus).

#### 2.3.3. Physicochemical and ADME processes parameters

In order to parameterize the avian PBK model and develop chemical-specific case studies, two types of data were collected: (i) physicochemical (phys-chem), i.e., molecular weight, lipophilicity, or solubility; and (ii) ADME properties, i.e., fraction unbound in plasma, partition coefficients, intestinal permeability (Caco-2), in-vitro metabolism data, transporter activity data. The initial parameters used to calibrate the model are summarized in the supplementary material table SM.A.1. These parameters were retrieved from the literature, from available databases, calculated based on in silico prediction models or in vitro measurements. Table 3 reports the final physicochemical and ADME values (Table 3) obtained after calibration of the PBK model and were used to run the different in vivo exposure scenarios.

When in vitro measurements were applied to parametrize the PBK model, such as clearance values, these were scaled up using the in vitro to in vivo extrapolation (IVIVE - scale up of parameter) approach. This was performed by applying physiological correction factors as established in the OSPS (and are reported in Table SM.A.3). The IVIVE -scale up was performed for MDZ in chicken and bob-white quail, for MON and IVM in chickens (Table in SM.A.3). Species extrapolation to fill in clearance (CL) and fraction unbound (fu) data gap was performed from chicken to mallard duck and from chicken to bob-white quail for CAP, FFC, and MEL. No drug-specific active transport (e.g., P-gp) was included. Renal clearance was modelled by the glomerular filtration rate (GFR) fraction, which was set to 1 for most of the chemicals since these are small molecules, with the exception for MDZ (GFR fraction = 0.67) where a modified value was already available in PK-Sim. The parameters logP/log$K_{ow}$, fu, kcat, and the intestinal permeability (P_int) were fitted with the Monte Carlo method of the OSPS in-built parameter identification (PI) tool using literature data for chicken. Mallard duck and bob-white quail simulations were based on the chicken model with the physiology replaced by the bird-specific parameter values.

For the calculation of the partition coefficients (PCs), five different methods are available in the software: PK-Sim Standard (Willmann et al. 2004); Schmitt (Schmitt 2008); Rodgers & Rowland (RR) (Rodgers and Rowland 2006); Poulin & Theil (Poulin and Theil 2000); Berezhkovskiy (Berezhkovskiy 2004). The five methods use compound-specific (lipophilicity and fraction unbound) and physiology-specific (tissue composition) parameters to derive tissue -specific PCs. In the present work (Table 3), Schmitt and Rodgers & Rowland QSAR -methods were applied due to their applicability to a broad range of chemicals (Utsey et al. 2020).

### 2.4. PBK model– OSP Suite – PK-Sim & MoBi software & code

In the present work, PK-Sim and MoBi from the Open Systems Pharmacology Suite (OSPS) were used to build the PBK models. The OSPS offers a broad spectrum of PBK models for several species. The PBK

**Table 2**
Chemicals used in developing avian PBK model and available validation in vivo sets per species/chemical.

| Compound | CAS # | Chemical class | ADME processes | In vivo data sets* |
|---|---|---|---|---|
| Chloramphenicol (CAP) | 56–75-7 | Drug (antibiotic mainly used in animals) | CAP is rapidly and completely absorbed from GI tract following oral administration (bioavailability 80 %)[2]. CAP is biotransformed in the liver by glucuronidation (90 %), to CAP glucuronate, which is the major metabolic pathway in many species (Chen et al. 2010; Akhtar et al. 1996). However, other metabolites, dehydrochloramphenicol (DH-CAP), nitrophenylaminopropanedione (NPAP), and nitroso-chloramphenicol (CO-CAP) were reported in chickens (A. Anadón et al. 1994). | [1]Chicken – laying hens, Chinese spot-billed ducks |
| Deltamethrin (DTM) | 52918–63-5 | Pesticide (pyrethroid) | DTM intestinal absorption is often incomplete. (Godin et al. 2010) It is metabolized via cytochrome (CYP) and carboxylesterase (CES) enzymes in mammals with known species differences (Hedges et al. 2019). | [1]Chicken – laying hens |
| Florfenicol (FFC) | 73231–34-2 | Drug (antibiotic) | It is well-absorbed and metabolized as well as renally excreted in various mammal species (Arturo Anadón et al. 2008; Dowling 2013). | [1]Chicken – laying hens, Mulard ducks, Muscovy ducks, Bob-white quail, Japanese quail |
| Itraconazole (ITZ) | 84625–61-6 | Drug (antifungal) | Its bioavailability is variable and dependent on the formulation (Tell et al. 2005). The absolute oral bioavailability of ITZ is 55 %, and is maximal when take(Zeng et al. 1998)n with a full meal[4].<br>ITZ is metabolized predominately by the cytochrome P450 3A4 isoenzyme system (CYP3A4) in the liver (University of Washington DB), resulting in the formation of several metabolites, including hydroxyitraconazole, the major metabolite. Fecal excretion of the parent drug varies between 3 and 18 % of the dose. Renal excretion of the parent drug is less than 0.03 % of the dose. About 40 % of the dose is excreted as inactive metabolites in the urine. No single excreted metabolite represents more than 5 % of a dose. | Mallard duck |
| Ivermectin (IVM) | 70288–86-7 | Drug (antiparasitic) | Its bioavailability is poor (Moreno et al. 2015). It is metabolized in liver by CYPs in humans (Zeng et al. 1998). In rats, it was shown to be a P-glycoprotein (P-gp) substrate. (Laffont et al. 2002). IVM and/or its metabolites are excreted almost exclusively in the feces over an estimated 12 days, with less than 1 % of the administered dose excreted in the urine. | [1]Chicken – laying hens |
| Melamine (MEL) | 108–78-1 | Industrial chemical (contaminant and food adulteration, infant formula pet food) | Systemic clearance is via the kidneys.<br>While metabolization occurs only by microorganisms in soil in the environment (Shelton et al. 1997). In addition biodegradation occurs via microorganism in soil and industrial wastewater treatment plants (El-Sayed, El-Baz, and Othman 2006; Takagi et al. 2012).<br>MEL is reported to be a metabolite of cyromazine, a pesticide. | [1]Chicken – laying hens; Mallard duck; Jinding laying ducks, Japanese quails |
| Midazolam (MDZ) | 59467–70-8 | Drug (sedative) | MDZ is rapidly absorbed after oral administration. Due to first pass metabolism, only 40–50 % of the administered oral dose reaches the circulation.[3]MDZ is primarily metabolized in the liver and gut by CYP3A4 to its pharmacologically active metabolite, alpha-hydroxymidazolam (also known as 1-hydroxy-midazolam), and 4-hydroxymidazolam (which makes up 5 % or less of the biotransformation products). Midazolam also undergoes *N*-glucuronidation via UGT1A4 after the process of hepatic oxidation by cytochrome enzymes (Nordt and Clark 1997). | [1]Chicken – laying hens; Bob-white quail |
| Monensin (MON) | 22373–78-0 | Drug (antibiotic) | Metabolized by CYPs (Henri et al. 2008),<br>extensively metabolised by CYP3A in chicken (Dorne et al. 2013) | [1]Chicken – laying hens |
| Salinomycin (SAL) | 53003–10-4 | Drug (antibiotic) | It is metabolized by CYPs in rats (Resham et al. 2015), but no bird-specific information was available. | [1]Chicken – laying hens |

[1] Chicken – laying hens (Gallus gallus domesticus). *More information on in vivo data sets can be found in the supplementary material linked to this paper. [2](https://go.drugbank.com/drugs/DB00446); 3(DailyMed: MIDAZOLAM HYDROCHLORIDE SYRUP CIV (nih.gov)); [4](https://go.drugbank.com/drugs/DB01167).

analyses were performed using qualified installations of the PBK software PK-Sim (version 8.0). R (distribution 3.6) and RStudio (Version 1.2.5) were used in the analyses and for pre- and post-processing of data and model output (Kuepfer et al. 2016).

The PBK birds' model codes are uploaded to the model repository on GitHub (https://github.com/Open-Systems-Pharmacology/Birds-PBK-Model) for general re-use by the Open-Systems-Pharmacology Community and beyond.

### 2.5. PBK model – best practice methodology

Based on the "Best practice" workflow for novel species published by Schneckener et al. (2020), the following steps (Table 4) were employed: i) The template model selected as base to develop the novel bird species PBK model was the mouse. Since bird-specific organ volumes and blood flow rates were available, no allometric scaling was necessary. ii) The exposure routes selected were oral (intra crop) and IV. iii) The GI tract parameters were fixed and calibrated. iv) The other parameters were fixed or calibrated. v) The focus organ in this work was the egg and its sub-compartments yolk and albumen; and in step vi) the focus organ parameters were fixed based on available literature data.

Finally, the PBK models were evaluated by means of comparison with in vivo studies (validation) for the three bird species. In addition, the refinement and parameterization of the GI tract and the specific organs of interest (ovary/egg) were based on the available in vivo bird data physiology. PBK model predictions and evaluation were carried out comparing simulation results to available in vivo data (Table SM1). The avian PBK model was built using physiology information for chicken, mallard duck, and bob-white quail as reported in Scanes et al. (2022a, 2022b). The chemicals were selected based on in vivo PK data availability for birds. The physicochemical properties to parametrize the PBK model are listed in Table 3 (and in Table SM.A.1). Model calibration was conducted on a compound-specific basis depending on data availability.

**Table 3**
Physico-chemical properties used to run the bird PBK model for different scenarios.

| Compound* | Molecular weight (g/mol) | Log Kow (LogP) | Fu | pKa | Clerance (kcat) | Concentration CYP | Solubility | Intestinal permeability | Partition Coefficients Method |
|---|---|---|---|---|---|---|---|---|---|
| Chloramphenicol (CAP) | 323.129 | 1.8 (fitted) | 0.2 (fitted) | 7.49 (acidic) | kcat = 1 1/min (fitted)<br>Km = 50 μmol/l (fixed for linear cl)<br>GFR fraction = 1 | 27 μmol/l | 12500 mg/l (fitted) | 1.17 E-06 cm/min (fitted) | PKSim |
| Deltamethrin (DTM) | 505.2 | 3.5 (fitted) | 0.6 | 6.5 | kcat = 10 1/min (fitted)<br>Km = 50 μmol/l (fixed for lin Cl)<br>GFR fraction = 1 | 29 μmol/l | 50 mg/ml<br>(fitted) | 1.22 E-4 cm/s (calculated) | Rodgers & Rowland |
| Florfenicol (FFC) | 358.21 | 1.62 (fitted) | 0.83 (Afifi and Abo El-Sooud, 1997) | 6.2 | kcat = 7 1/min (fitted)<br>Km = 150 μmol/l (fixed for linear CL)<br>GFR fraction = 1 | 27 μmol/l | 0.5 mg/ml (Wishart et al. 2018) | 9.00E-05 cm/s (fitted) | PKSim |
| Itraconazole (ITZ) | 705.633 | 4.62 | 0.03 (fitted) | 3.91 (base) | kcat = 0.01 (fitted)<br>Km = 0.002 μmol/l<br>GFR = 1 | 29 μmol/l | Km = 0.002 μmol/l (fitted) | 1.61E-04 dm/min (fitted) | Rodgers & Rowland |
| Ivermectin (IVM) | 875.1 | 2.68 (fitted) | 0.06 (fitted)<br>Lipoprotein factor for yolk = 5 (fitted)** | 12.47 | Hep. cl = 3.49 1/min<br>GFR fraction = 1 | Linear clearance | 0.07 mg/l (fitted) | 6.44E-07 cm/min (fitted) | Schmitt |
| Melamine (MEL) | 126.12 | −0.99 (fitted) | 0.69 (fitted) | 4.11 | Renal (partial least-squares) cl = 2 mL/min/kg<br>GFR fraction = 1 | Linear clearance | 3.24 mg/ml | 1.80E-07 cm/min (fitted) | Rodgers & Rowland |
| Midazolam (MDZ) | 325.78 | 2.9 | Chicken: 0.08<br>Quail: 0.16 | 6.2 | Chicken:<br>Km = 2.1 μmol/l<br>Kcat = 1.59 1/min<br>Quail:<br>Km = 3.2 μmol/l<br>Kcat = 4.41 1/min<br>GFR = 0.67 | 27.9 μmol/l | 0.049 (pH 6.5) | 1.554E-4 cm/min | Rodgers & Rowland |
| Monensin (MON) | 670.8 | 4.37 (fitted) | 0.77 | 4.24 | kcat = 2.39 1/min<br>Km = 28.6<br>GFR fraction = 1 | 29 μmol/l | Individual values between 0.5–200 mg/l | 5.24E-3 cm/min (fitted) | Rodgers & Rowland |
| Salinomycin (SAL) | 751.011 | 4.35 (fitted) | 0.8 | 4.45 | CYP3a4:<br>kcat = 2.38 1/min (fitted)<br>Km = 50 μmol/l (arbitrary, fixed)<br>GFR = 1 | 27 μmol/l | 0.3 mg/ml (fitted) | 4.72E-05 cm/min (fitted))<br>Permeability 2.23E-05 cm/min (fitted) | Rodgers & Rowland |

* Values reported in this table are mainly fitted; the original values are listed in the supplementary material, Table SM1. It can be useful to fit certain PBK model parameters for which in vitro data are lacking, IVIVE scaling factors are not well established, or applicability domain of the predicted model to obtain the input parameter (e.g. Log Kow) is not well characterized.
** More than 98% of the bound IVM is bound to lipoproteins (90% bound to HDL, 7% is bound to LDL) while 1.4% is bound to albumin.

### 2.6. PBK model - "goodness of fit" & confidence evaluation

Various terminology, with slight differences in meaning, have been used to assess PBK model credibility, including "qualification", "evaluation", "validation" and "verification". The European Medicines Agency PBPK guidance also differentiated model verification from platform qualification (EMA 2019). In the published standards of the American Society of Mechanical Engineers for establishing the credibility of medical devices the model credibility assessment includes verification, validation, and uncertainty quantification (Zhao, Seo, and Lionberger 2019). In this work, "model evaluation" will be used to refer to the model verification and validation against in vivo data.

To demonstrate the capability of the PBK model to simulate available in vivo time-concentration data, the model predictions were evaluated against available in vivo studies. These studies provided information of chemical concentrations in venous blood and in tissues. When the simulations were within the ≤ 3- to ≤ 10-fold "rule" as established by Lautz et al. 2020. In addition, an in-depth evaluation of the confidence and trust in the avian PBK model and its simulations was also included following the WHO (2010) and OECD (2021) guidance documents and criteria within (this analysis is available in supplementary information Table SM.B1 and SM.B2).

### 2.7. PBK model– sensitivity analysis

A local (one at the time, OAT) sensitivity analysis (SA) was performed by analyzing model parameters of the physiology and physicochemical or biochemical compound properties to identify key parameters that influence the outcome of the three PBK models. Individual parameters were modified without changing the other model

**Table 4**
Steps taken to develop the avian PBK model based on the "Best practice" workflow for novel species published by Schneckener et al. (2020).

| Steps | Schneckener et al., 2020 | Avian PBK model | Comments |
|---|---|---|---|
| 1 | Choose template model<br>Scale model to target species | Mouse Pk-Sim model<br>No scaling was applied as parameters were set explicitly as step. 4 | (Niederalt et al. 2018; Davies and Morris 1993) |
| 2 | Oral route of exposure | IC, intra crop = gavage (oral)<br>IV, intra venous<br>Feed | |
| 3 | Fix GIT parameters | GIT was adapted and parameters were fixed for the three bird species.<br>Rescaling of new compartments, crop and a double stomach were introduced.<br>Transit time data split proportionally among the different GI segments.<br>Calculated based overall GI transit time and length of segment. | The GI tract in birds differs from the mammalian GI tract in several ways including crop compartment and the different stomach system and dimensions of intestine segments.<br>(Davies and Morris 1993; Niederalt et al. 2018; Scanes et al. 2022a, 2022b) |
| 4 | Fix other parameters | As carried out in step 1. Parameters were fixed for bird species where available.<br>Physiology data for avian were collected to allow PBK model scaled to chicken and extended to mallard duck and bobwhite quail. Both sex, male and female, are represented. | Missing values for birds, were kept from the mouse model.<br>The avian PBK model was developed using all relevant in vivo data on the selected bird species, both sexes are taken into account. To test the hypothesis of "concentration reaching the egg" only the female bird model was then used for further analysis. |
| 5 | Focus organ | Ovulation model | Identification and representation of an organ that was not part of the original mouse model but essential to answer the hypothesis tested. |
| 6 | Fix focus organ parameters | Calibration of parameters for this organ was carried out | Done based on literature (Scanes et al. 2022a) |
| 7 | Validate model with available chemicals | Chloramphenicol (CAP), Deltamethrin (DTM), Florfenicol (FFC), Ivermectin (IVM), Melamine (MEL), Midazolam (MDZ), Monensin (MON), Itraconazole (ITZ), Salinomycin (SAL). | For the development and evaluation all chemicals were used. Criteria for selection of chemicals was the availability of in vivo data. |

parameters, which were left at initial values. The SA was set up using the workflow as documented in the OSPS manual documentation, "Sensitivity Analysis - Open Systems Pharmacology (open-systems-pharmacology.org)".

A 10 % change in parameter values was chosen, to analyze the effect of a change in parameter on the concentration of the chemicals in blood and in egg. All parameters of organ volumes, specific blood flow rates, tissue composition, metabolism or renal clearance, and compound physico-chemistry were tested for their sensitivity. The threshold cut off for plotted parameters was set to 10 %, reported in Fig. 9 as normalized sensitivity coefficients higher than 0.1, below this value impact is considered neglectable.

The OAT- SA was performed for MEL and FFC, both chemicals have data in the three bird species and SA could be compared between the three PBK models. SA was run in blood and AUC was used as dose metric. The area under the curve (AUC) is a biokinetic dose metric that is representative for a total exposure over time. Whereas Cmax, as the maximum observed concentration, is an exposure metric representing the highest acute exposure in blood or in tissue. Both can be used to compare predictions to observed in vivo data.

## 3. Results

As defined in the introduction, the aim of developing the generic avian PBK model was to predict chemical distribution in tissues with insight into time-concentration profiles in egg. Thus, a comparison of the simulated egg concentrations versus in vivo concentrations was of particular interest and is presented for chicken in Fig. 2 and for duck and quail in Fig. 3. In the present section melamine (MEL) is presented as a case study for which in vivo data of the three bird species was available. While for the other chemicals results on egg concentration are reported in Figs. 2 and 3, the time-concentration curves in other tissue are described in the supplementary material. The PBK model simulations for the chemicals are characterized as time-venous blood/organ concentration curves. The PBK model simulation results from the calibration studies can be found as individual graphs for the chemicals and in vivo scenarios in the supplementary material (Figures SM.D.1 to SM.D.9).

In vivo studies (Table SM.A.2) collected for the three bird species were used to fit to or to evaluate the avian PBK model simulations. The generic avian PBK model was described with 18 compartments plus subcompartments (Fig. 1), the GI tract and the bird reproductive systems were refined and added. The physiological data for the three bird species were collected and curated by Scanes et al., (2022a, 2022b), an extract of the parameters is available in SM.E.

### 3.1. Chicken- laying hen physiologically-based kinetic (PBK) models

The chicken was the "gold standard" species used to develop the avian PBK model, due to the high availability of PBK models (16 in peer review publications) and in vivo studies published, making it a well-characterized bird species. The chicken PBK model was developed for both sexes and was calibrated and evaluated using in vivo data collected for both sexes. Eight chemicals were selected, based on in vivo the availability of published studies: chloramphenicol (CAP), deltamethrin (DTM), florfenicol (FFC), ivermectin (IVM), melamine (MEL), midazolam (MDZ), monensin (MON), and salinomycin (SAL).

Briefly, for CAP, three studies were available, results of non-egg compartment are presented and available in SM.D.1.1. The study from Akhtar (1996), where groups of chickens received multiple intra crop (IC) doses of CAP at 0.5 and 5 mg over a time period of 5 days, was used to fit and predict concentrations of CAP in the eggs (Fig. 2a). The results from this simulation show that the model underestimates the concentration in eggs; the concentration in yolk being comparable with the in vivo data measured. The predicted concentration of CAP in the albumen compartment is an underestimate compared to the in vivo data (Fig. 2a).

There are two published in vivo data sets for DTM sets for chickens; respectively in broiler chickens (Hüyük and Eraslan 2017) and laying hens (MacLachlan 2008). In addition, one in-house data set (M-132448-01-1) from Bayer was available in laying hens.[2]

Simulations of time-concentration curves for DTM in chickens using the avian PBK model versus experimental data are available in SM.D.2. Data from the MacLachlan (2008) in vivo study using laying hen fed 20

[2] The full Bayer study reports can be requested for noncommercial use via the Bayer CropScience transparency initiative (email: cropscience-transparency@bayer.com) by referencing the present study and citing the respective M−Number of the study by Bayer (M-132448-01-1). This registration step is necessary to prevent commercial use of the studies by competitors of the study owner.

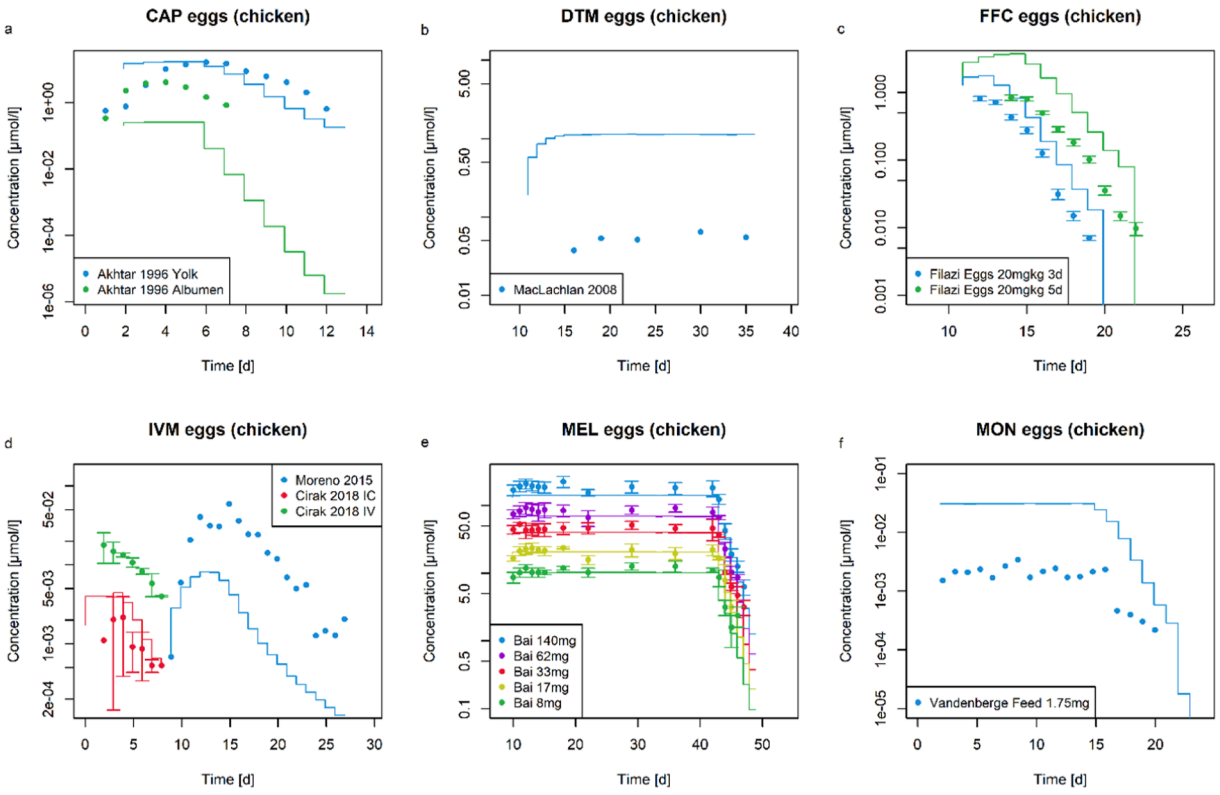


**Fig. 2.** Chicken PBK model egg time (d, days) – concentration (umol/L) profile for six chemicals simulated by the general avian PBK model (continuous line) as compared to in vivo data (dots). Panel a) Simulation of Chloramphenicol (CAP) concentration in egg yolk and albumen evaluated with in vivo data from Akhtar (Akhtar et al. 1996), where laying hens were dosed multiple IC doses of 0.5 and 5 mg of CAP over a time period of 5 days; b) Deltamethrin (DTM) PBK model simulations of egg concentration in time versus the in vivo data from MacLachlan (MacLachlan 2008), where laying hen were fed 20 mg/kg for 28 days (feed consumption of 104 g/d); c). The antibiotic drug Florfenicol (FFC) egg concentrations time profiles simulated using the PBK model and measured from the in vivo study by (Filazi et al. 2014); d) Ivermectin (IVM) in vivo studies were available in laying hens (Moreno et al. 2015; Cirak et al. 2018), as compared to PBK model time concentration simulations in egg yolk; e) Melamine (MEL) PBK Model Egg concentration in time as compared to the in vivo available data from (Bai et al. 2010); f) PBK model simulation of Monensin (MON) egg concentrations in laying hen compared to in vivo data from Vandenberge et al. (Vandenberge et al. 2012).

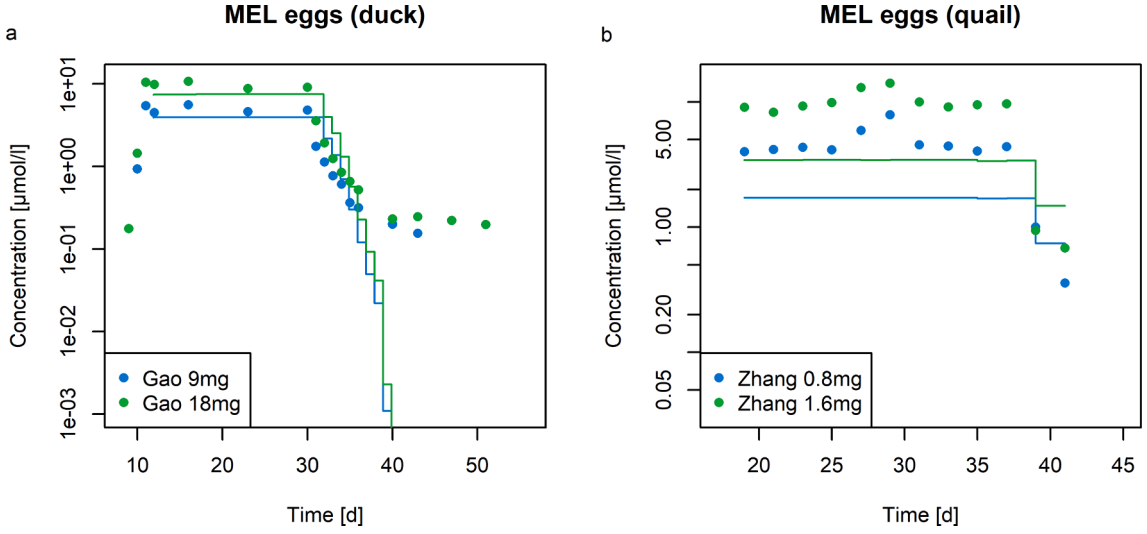


**Fig. 3.** Duck and quail PBK model egg time (in days, d) - concentration (umol/L) profile for Melamine (MEL) chemicals simulated by the general avian PBK model (continuous line) set as laying hen as compared to in vivo data (dots) by Gao et al., (Gao et al. 2010) (duck) and by (Zhang, Guo, and Wang 2012) (quail), respectively.

mg/kg for 28 days (feed consumption of 104 g/d). These were used to simulate whole egg concentration using the avian PBK model for laying hens. The evaluation between the in vivo data and PBK model simulations showed a one order of magnitude overestimate of the actual whole egg concentration (SM.D.2d, Fig. 2b).

The antibiotic drug FFC was the chemical for which the greatest number of in vivo studies in chicken were retrieved (Chang et al. 2010; Afifi and El-Sooud 1997; Ismail and El-Kattan 2009; Liu et al. 2018;

Filazi et al. 2014; Shen et al. 2003; Anadón et al. 2008). Results of non-egg compartment are presented and available in SM.D.3.1. Egg time concentration profiles were well captured by the model but with some, albeit minor, overprediction as reported in Fig. 2c. The simulation in addition shows that up to 22 hr the model predictions matched the in vivo data (Fig. 2c).

IVM in vivo studies were available in laying hens (Moreno et al. 2015; Cirak et al. 2018). Results depicted in Fig. 2d show concentration–time simulations in different organs together with yolk. The model parameterized the clearance process g in vitro data (IVIVE-scale up approach). This improved the model results. However, underestimation of the concentration in yolk (Fig. 2d) was noted. Additional results of time concentration tissue compartment are presented in SM.D.4.

Three studies assessed the in vivo distribution of MEL (Poapolathep et al. 2015; Bai et al. 2010; Dong et al. 2010) in laying hens. The laying-hen PBK model for MEL was calibrated and fitted to an in vivo study in which MEL was administered i.v. (5.5 mg/kg) (Poapolathep et al. 2015) and blood concentrations assessed in blood (Figure SM.D.5.1a). A parallel study via oral administration showed good agreement with the in vivo data (Figure SM.D.5.1b). In the other two in vivo studies (from (Dong et al. 2010; Bai et al. 2010)) the laying hens were fed using a different exposure scenario as reported in Table SM.A.2. The simulations were in good agreement with in vivo data in the first 40/45 days after which a rapid decrease of the PK profile was observed. Model simulation were underestimating the in vivo data (figure SM.D.5.1, panels c to i). Egg concentrations were in good agreement when compared to the in vivo available data (Fig. 2e) from Bai et al (Bai et al. 2010).

To evaluate the predictions of monensin (MON) concentrations, comparisons were made to in vivo studies with Ross Chickens (Henri et al. 2009) and Hubbard female chickens (Atef et al., 1993a) (figure in SM.D.7). In addition to in vivo blood PK studies, one study reporting egg concentrations of MON in laying egg was available (Vandenberge et al. 2012). When comparing the in vivo data to the model predictions, in the early phase, simulated egg concentrations are overpredicted but within an order of magnitude, while at around 20 days the simulated MON concentrations in eggs dropped (Fig. 2f); this being part of the washout phase (Fig. 2f).

One study for MDZ the PBK model was calibrated and fitted to venous blood data (SM.D.6). Clearance in vitro data was applied using the IVIVE-scale up approach but did not improve the modeling simulations. For this chemical, no egg concentrations were available, thus, the model was not used to simulate MDZ egg concentrations. The same holds for SAL, two in vivo studies using 75 Hubbard chickens (Atef et al. 1993) and 132 female and male Ross Chicken (Henri et al. 2012) using different route of exposure (IC and i.v.) and at different doses were found (SM.D.9). None of the published studies measured concentration of SAL in egg, thus, no comparison of concentrations in eggs was performed.

### 3.2. Mallard duck physiologically-based kinetic (PBK) models

Mallard duck simulations were produced using the same avian PBK model structure that was applied for the chicken, but taking into account differences in the mallard duck physiology (Table 2). The evaluation of the duck PBK model was done using in vivo data comparison, goodness of fit, for the following chemicals: CAP, FFC, MEL, and ITZ. Simulated time-concentration profiles are reported in the SM, for CAP, in Figure SM.D.1.2; For FFC in SM.D.3.2; and for ITZ, in figure SM.D.8.

The only compound with egg data in ducks was MEL, where there are two studies (Gao et al. 2010; Suknikom et al. 2016). Gao et al., (2010) reported in vivo data that were used to evaluate PBK model predictions for MEL. An in vivo study available in Jinding laying ducks provided information on concentrations in eggs (Gao et al. 2010) that were used in the evaluation of the simulations. The simulated egg concentrations were in very good agreement with the in vivo data, although at day 40 a sharp decrease was simulated concentration which was not observed in vivo (Fig. 3a).

### 3.3. Bob white quail physiologically-based kinetic (PBK) models

For bobwhite quail three chemicals (FFC, MDZ, and MEL) had in vivo data published in the literature. For FFC (SM.D.3.3) and for MDZ (SM.D.6.2) no egg time concentration profiles were available in the literature, more description of the PBK model simulations is available in SM. A robust in vivo study was available for Melamine (MEL). The study included 600 Japanese quails, that were exposed to different doses of MEL via the diet (2, 10, 50 and 100 mg/kg diet for 30 days, (Zhang, Guo, and Wang 2012). MEL PBK model simulations resulted in underestimation of the in vivo egg concentration reported in both studies (Fig. 3b).

### 3.4. PBK model evaluation

In order to assess the overall performance of the models, we made an analysis of the overall data points against the model predictions as a "goodness of fit" criterium, as described in Lautz et al. (2020), where models were evaluated within a range of $\leq$ -3 and $\leq$ -10 fold to account for variability from the in vivo studies. The performance analysis was conducted for each chemical by comparing all (including all tissue-concentrations) observed in vivo data by species.

#### 3.4.1. Chicken-laying hen physiologically-based kinetic (PBK) models

The performance analysis for DTM (Fig. 4a) resulted in 23 (38 %) predicted data points out of total 60 points were within the 3-fold; 20 datapoints were between 3-fold and 10- fold. In total almost 72 % of the data point simulated with the DTM chicken PBK model were within the 10-fold and 17 data points (28 %) were greater than the 10-fold deviation.

While the performance analysis for IVM (Fig. 4b) showed that 55 (61 %) predicted data points out of 90 points were within the 3-fold; 18 datapoints where between 3-fold and 10- fold. In total almost 87 % of the data point simulated with the chicken IVM PBK model were within the 10-fold threshold and 12 data points (13 %) were higher 10-fold deviation, due to the discrepancy from the yolk predictions.

For MON (Fig. 4c) the results showed that 31 predicted data points out of total 196 points were within the 3-fold and 29 data points were between the 3-fold and 10- fold range. In total 31 % of the data points simulated with the chicken MON PBK model were within the 10-fold range, of which 16 % where less than 3-fold. Sixty-nine percent of the data points (136) where higher that the 10-fold deviation.

For SAL (Fig. 4d), the goodness of fit analysis showed that 71 (32 %) predicted data points out of total 220 points were within the 3-fold; 59 datapoints were between 3-fold and 10- fold. In total, 59 % of the data point simulated with the chicken SAL PBK model were within the 10-fold threshold and 90 data points (41 %) were greater than the 10-fold deviation.

The goodness of fit analysis for CAP (Fig. 5a) showed that 36 (42.9 %) predicted data points out of total 84 points were within the 3-fold and 23 datapoints were between 3-fold and 10- fold. In total, 70.2 % of the data point simulated with the chicken CAP PBK model were within the 10-fold threshold and 23 data points (41 %) showed a deviation that was greater than 10-fold.

In the case of MDZ (Fig. 5c), the analysis showed that 11 (61 %) predicted data points out of total 18 points where within the 3-fold and one data-point was between 3-fold and 10- fold. In total, 67 % of the data points simulated with the chicken MDZ PBK model were within the 10-fold threshold and 6 data-points (37 %) where greater than 10-fold deviation.

Fig. 6a summarizes the results of the goodness of fit analysis for FFC, where 28.5 % were less than 3-fold (90 out of total 316 points), and 56 data points were within the 3- to 10-fold range, resulting in a total of 46 % datapoints less than the 10-fold threshold and 56 % greater. Fig. 7a, reports the analysis for MEL with 198 (67 %) out of total 296 points, below the 3-fold threshold and a total of 77 % of the data points below

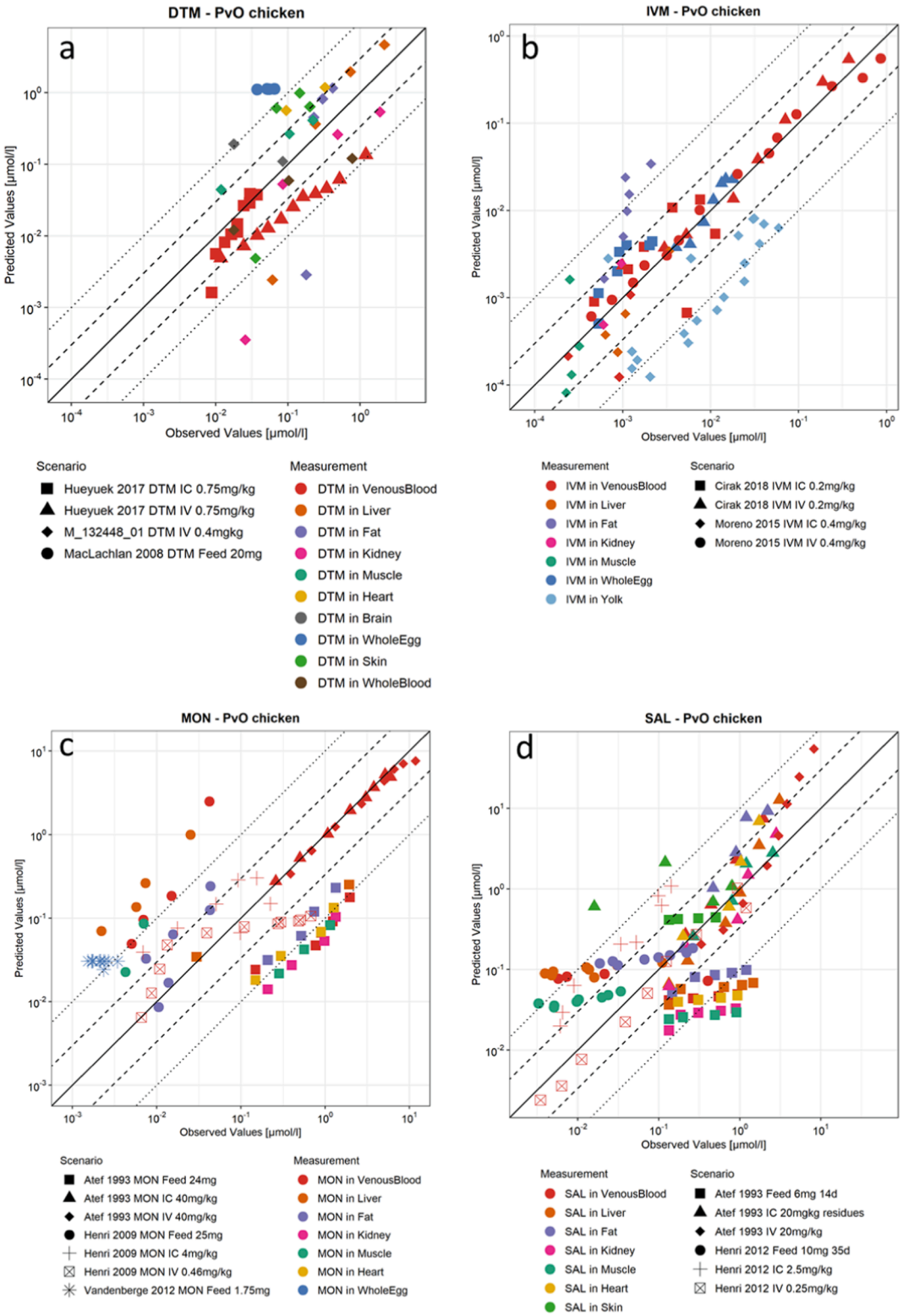


**Fig. 4.** Comparison between quantities measured in chicken in vivo studies and PBK model predictions for four chemicals (Deltamethrin, DTM; Ivermectin, IVM; Monensin, MON; Salinomycin, SAL) in various organs. Dotted lines represent 3-fold (dashed) and 10-fold changes (dotted). Organs and experimental dataset references are indicated in legends: colors and shapes represent organs and studies, respectively.

the 10-fold threshold (227 out of total 296 points).

### 3.4.2. *Mallard duck physiologically-based kinetic (PBK) models*

For CAP (Fig. 5b), the goodness of fit analysis of the duck PBK model showed that 6 (60 %) predicted data points out of total 10 points were within the 3-fold; one datapoint was between 3-fold and 10- fold. In total, 70 % of the data point simulated with the duck CAP-PBK model were within the 10-fold threshold and 3 data-points (30 %) showed higher than 10-fold deviation. Fig. 6b reports the results of the goodness of fit analysis for FFC in ducks, where 23 % was below 3-fold (11 out of

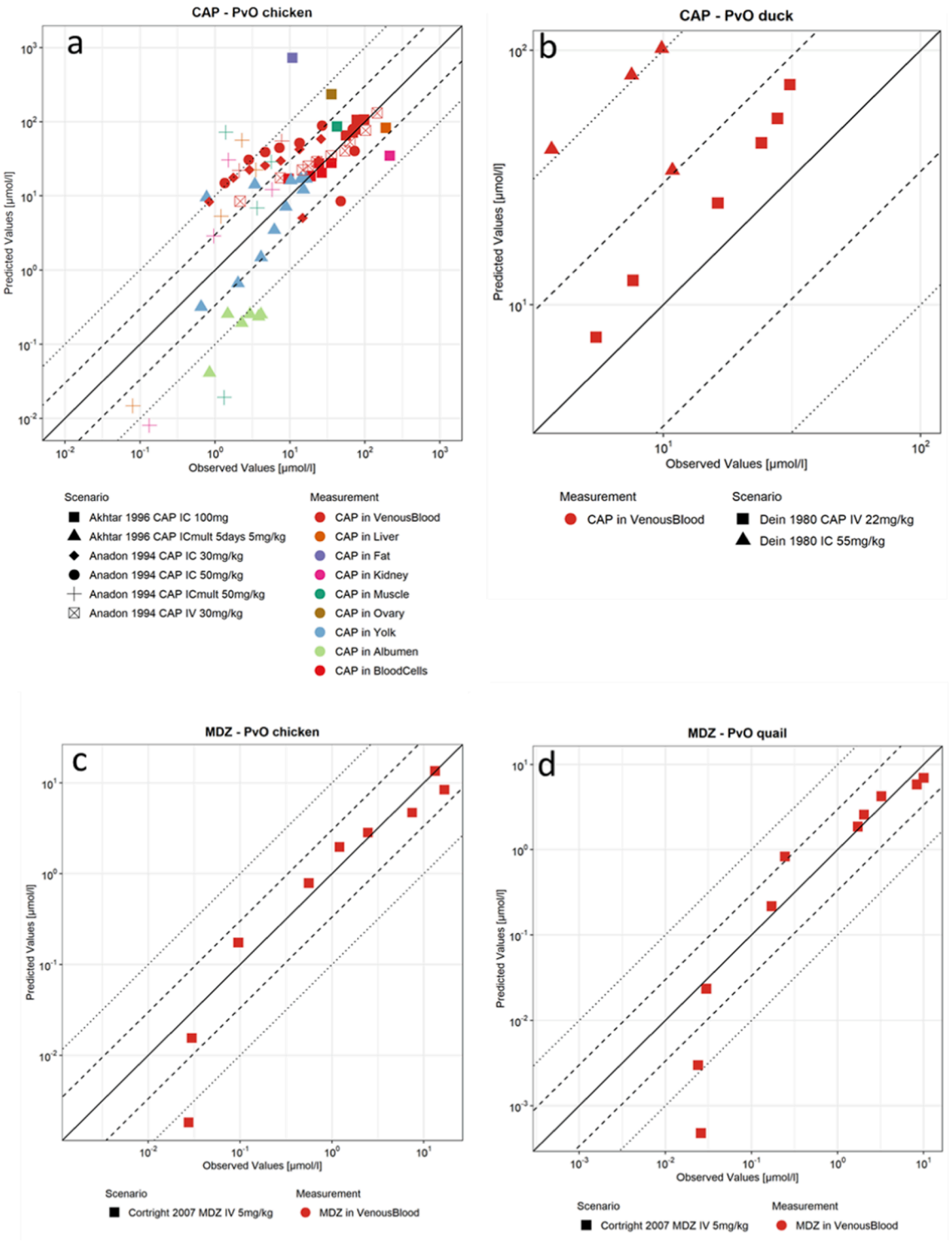


**Fig. 5.** Comparison between quantities measured in avian in vivo studies and PBK model predictions for Chloramphenicol (CAP) and Midazolam (MDZ) chemicals in various organs for three species (chicken, duck, quail - as specified on the plots). Dotted lines represent 3-fold (dashed) and 10-fold changes (dotted). Organs and experimental dataset references are indicated in legends: colours and shapes represent organs and studies, respectively.

total 46 points), while 4 data-points were within the 3-fold to 10-fold range, resulting in a total of 33 % data-points below the 10-fold threshold. Fig. 7b, reports the analysis using the duck PBK model for MEL with 40 (38.5 %) out of total 104 points below the 3-fold threshold and a total of 55 % of the data-points below the 10-fold threshold (57 out of total 104 points). In the case of ITZ (Fig. 8), the analysis showed that 7 (32 %) predicted data points out of a total of 22 points were within the 3-fold and 8 data-points were between 3-fold and 10- fold. In total, 68 % of the data point simulated with the duck PBK model for ITZ were within the 10-fold threshold and 7 data-points (32 %) showed a deviation higher than 10-fold.

### 3.4.3. *Bobwhite quail physiologically-based kinetic (PBK) models*

The same goodness of fit analysis was performed for the bobwhite quail PBK model. In the case of MDZ (Fig. 5d), the analysis showed that 7 (70 %) predicted data points out of total 10 points where within the 3-

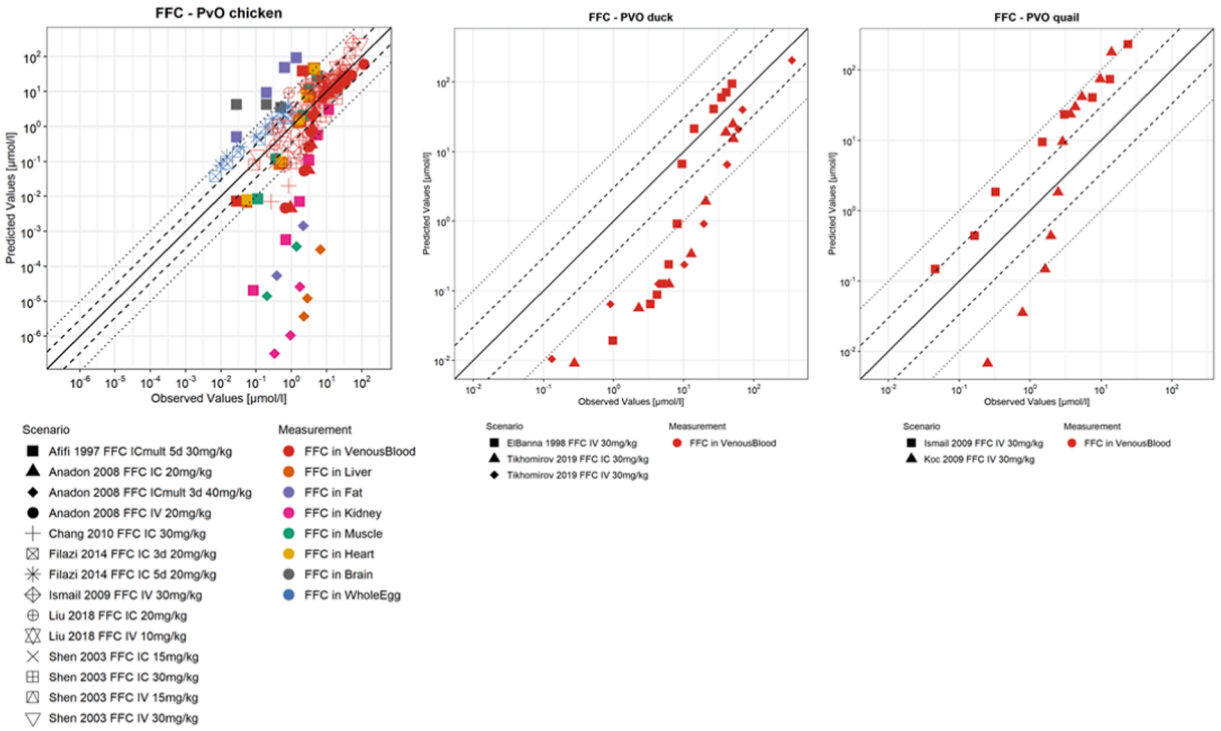


**Fig. 6.** Comparison between quantities measured in avian in vivo studies and PBK model predictions for Florfenicol (FFC) in various organs for three species (chicken, duck, quail - as specified on the plots). Dotted lines represent 3-fold (dashed) and 10-fold (dotted) changes. Organs and experimental dataset references are indicated in legends: colors and shapes represent organs and studies, respectively.

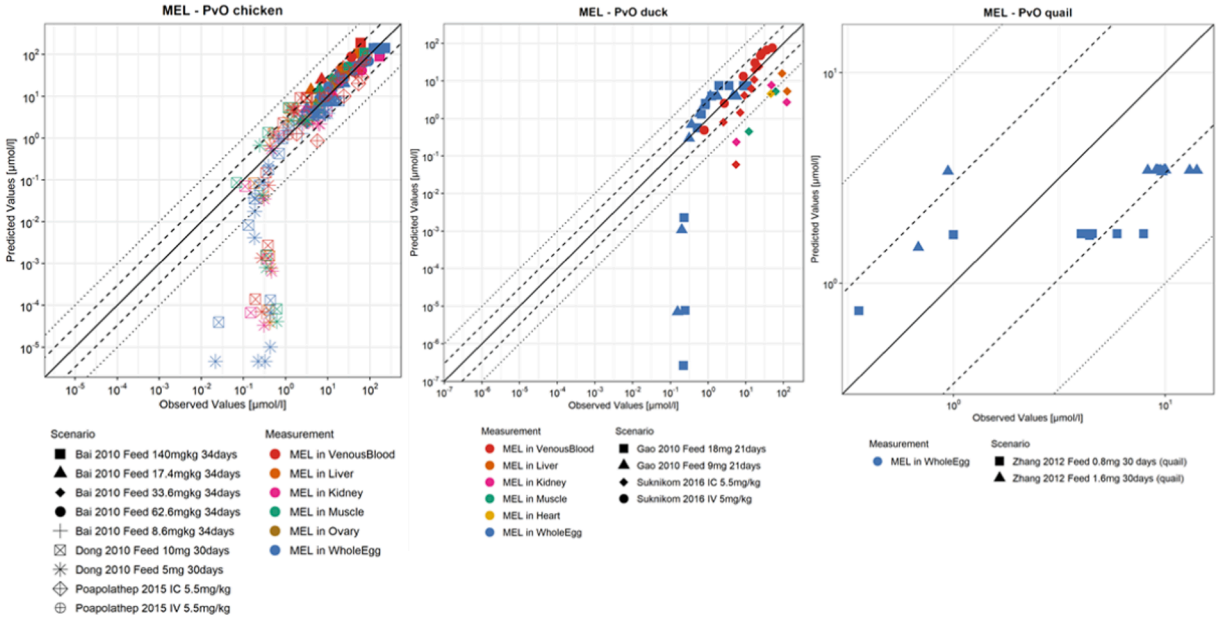


**Fig. 7.** Comparison between quantities measured in avian in vivo studies and PBK model predictions for Melamine (MEL) in various organs for three species (chicken, duck, quail - as specified on the plots). Dotted lines represent 3-fold (dashed) and 10-fold (dotted) changes. Organs and experimental dataset references are indicated in legends: colours and shapes represent organs and studies, respectively.

fold; 2 data-points were between 3-fold and 10-fold, so in total 90 % of the data points simulated with the quail MDZ-PBK model were within the 10-fold threshold. 3 data-points (10 %) were higher 10-fold deviation. Fig. 6c, reports the results of the goodness of fit analysis for FFC in quail, where 11 % was below 3-fold (4 out of total 38 points), while 17 data points were within the 3–10-fold range, resulting in a total of 55 % datapoint below the 10-fold threshold. Fig. 7c, reports the analysis for MEL with 19 (79 %) out of total 24 points, below the 3-fold threshold and a total of 100 % of the data points below the 10-fold threshold (24 out of total 24 points).

### *3.4.4. PBK model sensitivity analysis*

Sensitivity analysis (SA) was performed using the melamine (MEL) and florfenicol (FFC) specific PBK models to identify the parameters that

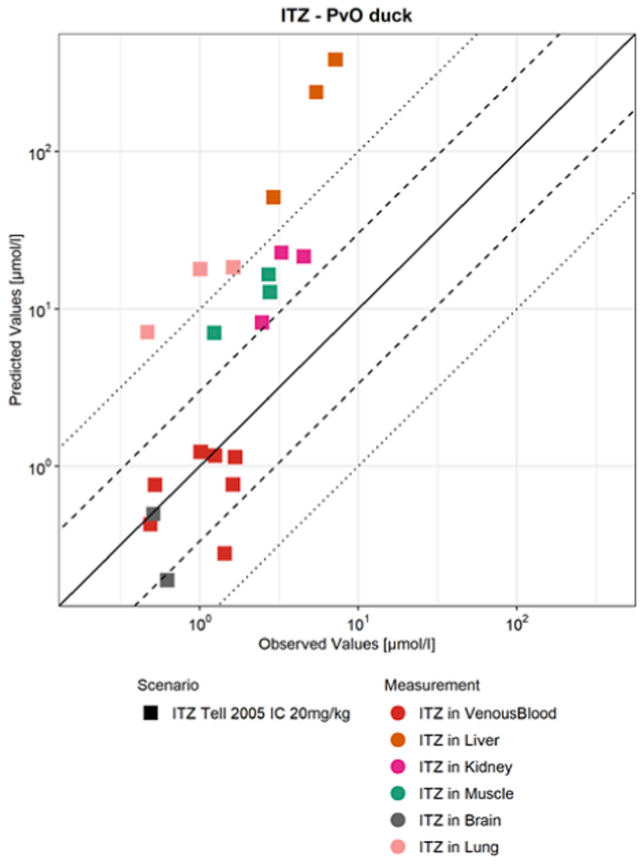


**Fig. 8.** Comparison between quantities measured in avian in vivo studies and PBK model predictions for Itraconazole (ITZ) in various organs in duck. Dotted lines represent 3-fold (dashed) and 10-fold (dotted) changes. Organs and experimental dataset references are indicated in legends: colors and shapes represent organs and studies, respectively.

can influence the outcome of the chemical concentration in blood. These two chemicals were selected since are models were developed in the three bird species. The scenario set for MEL - SA was 5 mg/kg as an IV injection and simulated at 24 h after administration; while the scenario set for FFC was 30 mg/kg via IV injection and simulated at 24 h after administration. The AUC was used as dose metric representative of the overall exposure in both analyses.

Fig. 9 displays all parameters of the SA with a sensitivity score above 10 %. Normalized sensitivity coefficients were calculated for all parameters but only parameters that had a normalized sensitivity coefficient higher than 0.1 are displayed. Fig. 9a shows the parameters with a major impact on the model predictions of MEL concentrations in blood; these were in decreasing order following the chicken PBK model outcome: the kidney volume, fraction unbound, kidney plasma clearance, volume of muscles, GFR and blood flow rate to kidney; also volume of fat, bone, and skin can influence the values of MEL in blood. Fig. 9b shows the parameter with a major impact on the model predictions of FFC blood concentration; these are in decreasing order (of the chicken model): plasma clearance, kidney plasma clearance, fraction unbound (plasma), kidney volume, liver volume, kidney specific blood flow rates, muscle volume, fat volume, bone volume, venous blood volume, skin volume and oviduct volume.

### 3.5. *What makes a PBK model credible for regulatory applications?*

A generic avian PBK model was developed and used to assess the absorption, distribution, metabolism and excretion of nine chemicals, primarily pharmaceuticals in three bird species. To evaluate the PBK model performance, the confidence in the use of PBK models arises from different criteria, the biological basis of these models and their ability to simulate dose metrics of relevance to risk assessment question and allowing characterization of the sources of PK uncertainty (WHO 2010). To this end, to understand the scale of confidence of the avian PBK model the WHO/OECD (WHO 2010; OECD 2021) confidence matrix was used (Table 5) based on the criteria/scoring from (WHO 2010) see Table SM.B.1) and also following OECD (2021) (Table SM.B.2).

The criteria are PBK model: i) biological basis, how good does the model represent the biology of the species; ii) model simulations of data, how well does the model reproduces the PK kinetics data and curve shape; iii) uncertainty in input parameters and model outputs sensitivity analysis, model goodness of fit. Based on these criteria the overall PBK model assessment scored a medium to high level of confidence based on the available information and documentation.

## 4. Discussion

The present work aimed to develop a generic avian PBK model to represent the adult male and female bird, the latter including the reproductive system represented by the ovulation model and compartments. The PBK model was developed following the generic best practice workflow to develop a PBK model for a novel species proposed by Schneckener et al. (2020). Thus, the model structured use in this work was established on the published mouse PBK models (Davies and Morris 1993; Niederalt et al. 2018). Accurate physiologically-based models must represent, to the best of the current knowledge, the physiological attributes of the simulated individual (Edginton, Schmitt, and Willmann 2006). To this end, comprehensive research on bird physiology was conducted and published in Scanes et al. (2022a, 2022b) and used as the foundation of the model.

When developing the generic avian PBK model, limitations and assumptions were made: i) The physiology was compiled from various data sources. The bird GI tract was included but tissue compositions (needed for partition coefficient predictions) were not comprehensive as information was found only for mallard duck and bobwhite quail (Scanes et al. 2022a, 2022b). ii) It was observed that the required relevant gene/protein expression data in birds was not available (e.g. enzymes and transporters) making the in vitro-in vivo extrapolation (IVIVE) – scale up- for parameters challenging, or impossible. iii) often, in vitro data such as fraction unbound in plasma, clearance/metabolism, and involved transporters/enzymes) were collected from in vitro studies in other species, limiting IVIVE – scale-up of parameters. iv) The chemical information, physico-chemistry, solubility in different media and characterization of lipoprotein binding were missing for certain chemicals, thus fitting was necessary. v) The lipoprotein factor used for yolk uptake, could not be predicted and more data are needed for a rule-based prediction (i.e., a regression (QSAR) function or model).

While keeping in mind the underlying limitations and assumptions the avian PBK model was specifically calibrated and used to simulate male chickens and female-laying hens and extrapolated to mallard duck and bobwhite quail. These two species were selected since they are primarily used in testing of chemicals to comply and to inform ecotoxicological/environmental risk assessment. The avian PBK model, developed for chicken, duck, and quail, can be applied to predict concentrations in blood, tissues and egg as time-concentration profile curves. These predictions can be used downstream for risk assessment in the hazard characterization phase and provide valuable insight for the analysis of residues in the ecotoxicological/environmental exposure assessment.

Literature data on egg concentration was limited and this could impact the model performance and evaluation, restricted to these few in vivo studies, as discussed next. The generic avian PBK model was developed and evaluated using the following chemicals: chloramphenicol (CAP), deltamethrin (DTM), florfenicol (FFC), itraconazole (ITZ), ivermectin (IVM), melamine (MEL), midazolam (MDZ), monensin

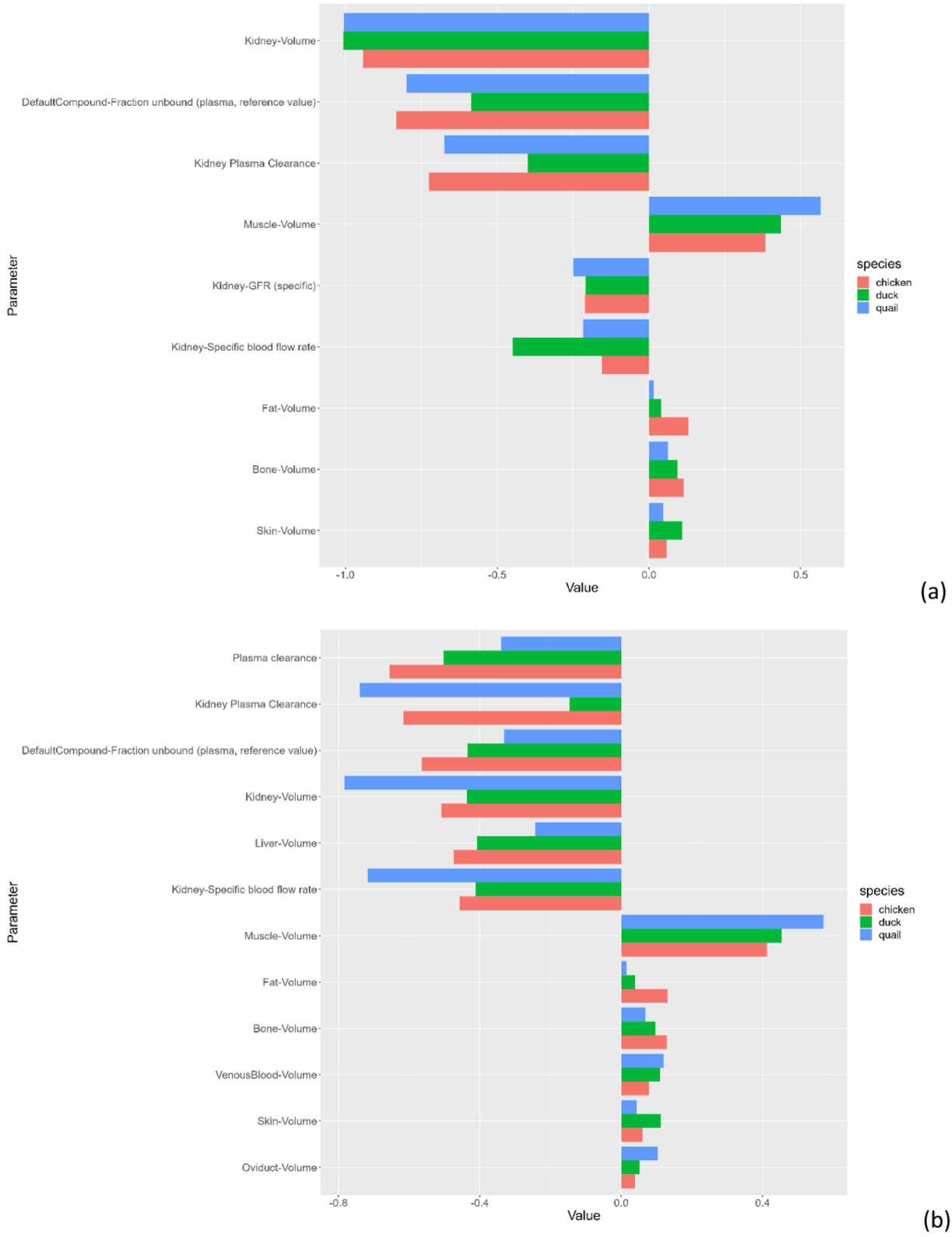


**Fig. 9.** (a) Sensitivity analysis of the MEL PBK model applied to the three bird species, at an I.V. dose of 5 mg/kg The chicken PBK model is represented by red bars, the duck is represented by green and the blue bars represents the quail. (b) Sensitivity analysis of the FFC PBK model applied to the three bird species, at an I.V. dose of 30 mg/kg. The chicken PBK model is represented by red bars, the duck is represented by green and the blue bars represents the quail. Both graphs report several parameters that were evaluated and are plotted by decreasing impact following the chicken PBK model outcome. In both graphs the values represents the normalized sensitivity coefficients with a cut of 10 % (as explained in the methodology and results sections).

**Table 5**
Schematic representation of the scale of confidence and resulting score for the avian PBK model (following WHO 2010 and OECD 2021 GDs).

| | Level Of Confidence | | |
|---|---|---|---|
| | None/ Low | Medium | High |
| Biological Basis | | | The model structure was very well documented and followed available knowledge of the species, with high biological basis and is consistent with available input parameters for each bird species and sex. The model was parameterised using all available information in the literature. |
| model simulations of data | | The model reproduces consistently some of the kinetics data and shape for most of the nine chemicals in the test species. For FFC, the kinetics in vivo showed a 2-phase shape kinetic process which was not reproduced by the model. | . |
| uncertainty in input parameters and model outputs Sens Analysis | | | Model simulations were compared to in vivo data in relevant biological matrix for the species and exposure route of relevance to toxicity/risk assessment and matched the in vivo data within 3 and 10-fold – goodness of fit analysis; a OAT sensitivity analyses was performed |

(MON), and salinomycin (SAL). Eight of these substances were used to characterize the chicken, four for the mallard duck and three for the bobwhite quail. Two chemicals (FFC and MEL) were predicted using all three bird species, but only MEL egg concentration were available for all species.

Melamine was the chemical with in vivo data available for the three bird species, chicken, mallard duck, and bobwhite quail. The predictions of MEL in duck and quail were based on the respective bird physiology and the identified parameters from the chicken model, and no additional fitting for these species was performed. The PBK model showed good simulation results in chicken, with around 70 % of the simulated data within a factor of 3. This was also reflected when the PBK model was used to predict time-concentration profiles in agreement with the available in vivo data for the other two species. Although, the goodness of fit exercise for the duck and quail was less promising as compared to chicken, with 33 % and 79 %, respectively, to be within a factor of 3, the in vivo concentration in egg were found to be similar among the three bird species, however, PBK model simulations for the quail were slightly underestimating the in vivo data (Fig. 7). This could be the result of different exposure scenarios in the respective studies or processes within the bird that are not known, e.g. a different fraction unbound in plasma.

A second very interesting case study was ivermectin (IVM). To the best of our knowledge this is the first time PK time concentration profiles for IVM were made using an avian PBK model. In vitro data for informing the clearance were used, thus, in vitro to in vivo extrapolation (IVIVE-scale up) was performed (Table SM.A.3). This extrapolation improved model predictions. IVM is highly bound to lipoproteins, which are known precursors for the egg yolk (Scanes et al. 2022a, 2022b). This was in agreement with the simulation results where an additional transport into the yolk was necessary to reach the observed egg concentration. However, the yolk concentrations are still slightly underpredicted by the model, whereas the simulated whole egg concentrations exceed the observations, indicating a slightly different distribution of IVM between albumen and yolk than predicted.

Midazolam (MDZ) was only used to obtain PK profiles in blood for chicken and bobwhite quail, which resulted in a very good model performance with a goodness of fit of 60–70 % within the 3-fold range. For this chemical, the IVIVE approach was applied to scale up microsomal fraction to the total organ to parametrize the PBK model (IVIVE- scale up for model parametrization). The IVIVE scale up of the MDZ parameters was based on in vitro microsomal clearance measurements (Cortright and Craigmill 2006), but no value for the microsomal protein content (MPPGL) was included. As a surrogate, this parameter was taken from a different study (Henri et al. 2012). Moreover, no value for the MPPGL in bobwhite quail was available, and the chicken value was used. This could explain the low extrapolated clearance versus the higher fitted value. It is known that there are multiple enzymes involved in MDZ metabolism in mammals, but little information on avian MDZ metabolism was available. After 8 h, a plateau in the in vivo plasma concentrations was found for both species, indicating a possible binding partner. A comparison of dose-normalized plasma concentrations showed that MDZ concentrations in birds are lower than in humans; suggesting further differences in metabolism in avian species, which might not be captured in the in vitro measurements or the model.

For the antibiotic chloramphenicol (CAP), concentrations in blood from Atef et al. (1991) study in chicken were found to be higher than those reported in other studies (Anadón et al. 1994) and these data were therefore excluded for model evaluation. CAP tissue-concentrations in ducks were lower as compared to those in chickens (SM.D.1.1 and SMD.1.2). Notably, the duck data comes from only one study (Dein, Monard, and Kowalczyk 1980), which used different analytical methods than was used for the chicken data, hampering a reliable judgment. Additionally, the deviation in in vivo PK measurements after oral administration among studied animals was very large, possibly due to incomplete dissolution of the formulation or reduced bioavailability. Bioavailability could be reduced due to intestinal metabolism and first-pass effects, but this explanation cannot be confirmed without reliable data on the metabolism of the drug as well as the protein abundances in birds. The tissue distribution is unexpectedly high, and a low terminal clearance, especially in muscle, is observed. This behavior is unexpected for the hydrophilic nature of CAP and might indicate a binding partner in the process, but no further information is available to confirm this interpretation. Sex differences were employed in the model for CAP, simulations were conducted and evaluated also for male chicken (SM.D.1.1a,b,d, and e), these showed similar predictions to the laying hen (data not shown). However, in physiology there is a marked difference in lipid content, e.g., in the liver, between female and male, with about 17 % higher concentration in females (Scanes et al. 2022a, 2022b) and these could influence the PBK model performance.

Deltamethrin (DTM) is a highly lipophilic compound (LogKow = 6.20), thus the limit of the applicability domain of the partition coefficient models could be reached. Predictions based on high lipophilicity tend to overestimate tissue partitioning significantly. When a lower (fitted) logP/LogKow was applied (LogKow = 3.5), model predictions were more than 70 % of all data points within range of the 3- to 10-factor, with only 30 % under the 3- factor. Additionally, the lack of data on fraction unbound, metabolism, and transporters governing the ADME processes of DTM in birds impedes model development and refinement. Moreover, data between in vivo studies (from Hüyük and Eraslan (2017), MacLachlan et al., (2008) and Bayer – Transparency initiative (in house data – M-132448-01-1), was found to be highly variable (plots in figure SM.D.2). Known species differences like CYPs and carboxylesterase (CES) activity, which are known DTM metabolizing enzymes in mammals (Godin et al. 2007) suggest that the metabolism may not be

properly reflected in the model due to a lack of bird-specific information.

Although there is a large variety of in vivo PK data available in chickens, there is little information on florfenicol (FFC) metabolism for any of the bird species. Thus, metabolism parameters could only be fitted. The data suggest a high volume of distribution for FFC in chickens compared to other species (https://www.vetpharm.uzh.ch/wir/00007323/1342_03.htm). Moreover, there may be an unknown mechanism involved in the FFC ADME processes that were not captured in the present analysis, e.g. enterohepatic recirculation (Afifi and El-Sooud 1997). As reported in the assessment by Lautz et al. (Lautz et al. 2020), deviations between predicted concentrations and experimental data for FFC may be explained by the lack of quantitative information to model efflux transporters in the PBK model, since such data are scarce in the literature (Lautz et al. 2020).

Literature data from bird species for itraconazole (ITZ) was rather sparse and only one study was available using oral and sub-cutaneous (SC) administration in ducks (Tell et al. 2005). Predictions were based on data from oral administration as no dedicated absorption module for SC administration is available in PK-Sim. Additionally, ITZ absorption is known to be strongly pH-dependent, and solubility/ formulation effects influenced the PK in this study. Therefore, the bioavailability could be overpredicted. Metabolism data for ITZ in birds was not available in the literature, thus, parameters could only be fitted to a single dataset. The value for the fu in ducks that was identified by parameter optimization was comparable to the reported value in penguins (Bunting et al. 2009).

The observed data for monensin (MON) shows a plateau in plasma concentrations after a quick distribution phase ((Henri et al. 2009); IV 0.46 mg/kg) which is not seen in other data sets. For the oral administration, solubility is the limiting factor for absorption. Due to the lipophilic nature of MON, its water solubility is low, limiting the bioavailability. Bioavailability is enhanced in alcohol vehicle which was used for the IV and IC studies (Henri et al. 2009). The administered dose in the feeding studies might be biased by ad libitum feeding making it difficult to estimate the precise exposure dose. In addition, the only two in vivo studies available had conflicting data. The two in vivo IV datasets show inconsistencies that hamper the identification of the true parameters, and a perfect match of the Cmax, as well as the clearance, is not to be expected. However, the metabolic clearance was successfully extrapolated by IVIVE from microsomal measurements (Henri et al. 2008) and the model reproduced most datasets.

The results for salinomycin (SAL) were based on fitted in vivo data for the chicken, since metabolism and clearance data were not available. The simulations for chickens after an IV administration were in very good agreement for both studies (Henri et al. 2012, 2017; Atef et al. 1993), while, when intra crop exposure was used in the same studies, the Cmax was overpredicted. The available feeding studies, given the uncertainties in effective dose, compound solubility, and dosing timepoints gave a reasonable prediction range with slight underprediction of the Atef dataset (Atef et al. 1993). Unfortunately, there were no in vivo studies reporting SAL concentrations in eggs, so there were no means to compare the simulated results to observed in vivo data.

For chemicals lacking PK time concentration profiles, e.g., missing in vivo time egg concentration profile, like SAL, MDZ and ITZ, an alternative would be to evaluate model predictions by applying a read across approach by using the in vivo information (e.g., measured in eggs) from a “source” - data rich - chemical to inform the “target” - data poor - chemical (Paini et al. 2021; Ellison 2018; Ellison and Wu 2020), but such an in-depth analysis is beyond the scope of the current study.

The overall accuracy of the model predictions across the chemicals analyzed was found to be species- and compound-specific. To this end it was concluded that PBK models were often best informed for only one bird species (mainly chicken based on available literature data). Thus, when extrapolating to the other species (quail and duck) the system-specific compound data, (e.g., fraction unbound), were from the reference bird (chicken). In some cases, there are also marked PK differences between the three avian species. However, the underlying mechanisms are still unknown; e.g., ADME defining properties such as plasma protein binding (e.g., as observed for MDZ). Overall, when developing the avian PBK model, a high variability in input data (dose-normalized plots, data not shown) was observed, hinting at a great need for improved experimental and analytical consistency.

The most recent work in this area is from Lautz (Lautz et al. 2020), in which the development and optimization of a PBK model in chickens for the pharmacokinetics and tissue residues of seven chemicals was reported. In the current paper, nine chemicals were assessed and physiological scales for three different avian species were collected and used. The results were analyzed using goodness of fit (GOF) criteria (% within 3- or 10-fold), as reported in Lautz (Lautz et al. 2020). Lautz and the team excluded some data points from the GOF analysis due to the limit of detection and quantification of these data observed points, while in this analysis we have included all data points. A more detailed egg structure is proposed in the current paper, while no distinction between egg albumen and yolk was reported in the Lautz et al. (Lautz et al. 2020) model. While this differentiation might not be important for assessing human safety from dietary egg exposure; applications in ecotoxicological/environmental risk assessment might benefit from this detailed representation.

The OAT-SA for MEL using the three birds PBK models revealed that the major impact was from the volume of organs (kidney, muscle, fat, bone, and skin) and kidney-specific key parameters such as GFR and clearance. On the other hand, in the FFC model the most sensitive parameter in all three bird species was the (kidney) plasma clearance followed by fraction unbound and finally organ volumes. The differences in the sensitivities among the three species might be due to differences in relative body composition and organ volumes. Further, the lower specific blood flow rate for the kidney in the duck limits the renal clearance and renders this parameter more sensitive for the renally cleared compound MEL. The apparently less sensitive fu of MEL in ducks is due to its direct impact on the renal clearance (only unbound drug can be cleared) and resembles therefore a similar sensitivity score as the kidney plasma clearance. The SA is more informative when it is set up in the context of a real scenario with a concrete problem formulation, and can be used to determine parameters of the PBK model which have little influence on model outputs so that they can be fixed to improve overall outcome (Lautz et al., 2020).

The PBK model was evaluated using available guidance documents. To assess model credibility and score confidence in the PBK model predictions (Table 5) the confidence matrix reported in WHO (WHO 2010) and OECD (OECD 2021) was used. Using the available guidance and the credibility matrix the model scored medium to high. Therefore, application of the PBK model predictions could be used as an additional line of evidence in hazard characterization when performing an ecotoxicological risk assessment.

When developing these PBK models gaps and limitations were identified; these were postulated as recommendations for future work. Improvements of the PBK models for bird species will rely on (i) better in vitro characterization of the bird “in vitro” ADME(T) and mass balance, to provide good PBK model estimates to be used in Chemical Risk Assessment. (ii) integration of gene and protein expression data specifically from birds; (iii) further species-specific high-quality physiology and anatomical data, e.g., knowledge related to organ volume and tissue composition could be measured and collected in specific databases. (iv) addressing uncertainties associated with predictions for PBK model evaluation with inclusion of more chemicals could be established.

In addition, addressing mixtures (as compared to a single substance) will be another area of relevance for future applications and development of the generic avian PBK model. PBK models should address chemicals interactions to be more representative of a real-life aggregate exposure scenario. Finally, the publication of the open source generic avian PBK model on the OSPS community framework on GitHub will allow a transparent further development of the model to expand it to new and other applications and will lead to a continuous re-qualification

of its applicability for various intended-use case scenarios.

With this work, a chicken (Gallus gallus), a bobwhite quail (Colinus virginianus), and mallard duck (Anas platyrhynchos) PBK model for both genders (male and female) were established. The female bird models contain an ovulation module (egg-development) to predict egg concentrations of ingested substances. Residue measurements in blood/egg/tissues in avian reproduction studies could help with validating PBK models and supplementing the use of a PBK model to answer a specific risk assessment question. These PBK models can currently be used to refine in vivo experiments by providing a quantitative prediction of internal chemical exposure and could be further leveraged in the future to support reduction of avian in vivo testing. In the near future, in line with (EFSA 2009), these PBK models could be used in support of current practice in the ecotoxicological risk assessment to further reduce and ultimately replace bird in vivo testing studies.

## Author statement/contribution

**Vanessa Baier**, conceived, developed, and executed the generic avian PBK model, participated in the writing and review of the manuscript; **Alicia Paini**, reviewed results, model evaluation, drafted and prepared the manuscript and revisions; **Stephan Schaller**, coordination, conceptualization, participated in the writing and review of the manuscript; **Colin G. Scanes**, conceptualization, reviewed model during its development, participated in the writing and review of the manuscript; **Audrey Bone**, conceptualization, reviewed model during its development, participated in the writing and review of the manuscript; **Markus Ebeling**, conceptualization, reviewed model during its development, participated in the writing and review of the manuscript; **Thomas G. Preuss**, conceptualization, reviewed model during its development, participated in the writing and review of the manuscript; **Johann es Witt**, conceptualization, reviewed model during its development, participated in the writing and review of the manuscript; **David Heckmann**, coordination, conceptualization, reviewed model during its development, participated in the writing and review of the manuscript.

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## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The project was conducted by Bayer Crop Science division. Some of the authors are employed at Bayer or were paid for their work by Bayer. Bayer produce and sell pharmaceuticals and agrochemicals. All the authors have the interest to get PBPK/PBK model accepted for regulatory purposes.

## Data availability

The PBK model Code will be published in Github

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107547.

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