

Modeling Residue Uptake by Eggs. 1. Similar Drug Residue Patterns in Developing Yolks Following Injection with Ampicillin or Oxytetracycline

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ABSTRACT This study was conducted to model the pattern of antibiotic drug uptake within yolks of developing follicles. In two separate experiments, 16 hens were divided into equal groups ($n = 8$) and injected only once with either 400 mg/kg ampicillin or 200 mg/kg oxytetracycline (OTC: total hens = 32) approximately 1 h after oviposition. Twenty-four hours following injections, hens were euthanized and the ovaries were collected. Yolks were dissected free from the individual follicles with a blunt probe. Individual large yellow yolks (≥ 0.2 g) and a pool of 5 small yellow yolks (< 0.2 g) were collected for determination of ampicillin or OTC content. Samples were prepared and assayed using an agar diffusion microbiological method. Selected parameters were not different ($P > 0.05$) between

Experiments 1 and 2 and the data were combined. Results indicate that short-term drug exposure in hens produced incorporation of drug residues in developing yolks in a specific pattern that does not appear to be drug dependent ($P > 0.05$). These incurred residues are contained in developing yolks that are days to weeks from being ovulated. Drug residues were greater (total microgram content) in some of the less mature yolks vs the largest preovulatory yolk. This may lead to a sequential release of eggs with increasing residue content, even after drug withdrawal. These data were used to construct a model to predict the pattern of incurred residues in formed eggs following a hen's exposure to drugs or other contaminants.

(Key words: drug residues, ampicillin, oxytetracycline, model, eggs)

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INTRODUCTION

Residues in eggs pose human food safety concerns and may adversely effect consumer confidence in this poultry product. Residues in eggs may be produced by exposing laying hens to drugs or contaminants in a number of ways. These include: 1) illegal or extra-label use of drugs, 2) use of feed unintentionally cross-contaminated during feed mixing, 3) use of mislabeled feed, and 4) pesticide, chemical, or heavy metal contamination of feed ingredients or water.

Due to the extended period of egg formation, especially yolk formation, the potential exists for long-term release of eggs containing residues even though hens were exposed to contaminants for only a short period of time. Previous studies have shown release of eggs containing residues even after drug withdrawal (Yoshida *et al.*, 1971; Katz *et al.*, 1973; Archimbault *et al.*,

1978; Roudaut *et al.*, 1987a,b; Nagata *et al.*, 1992; Petz, 1993; Donoghue *et al.*, 1994). Most of these types of studies were conducted by adding the compound of interest to the feed or water and evaluating the residue transfer in a classic depletion strategy. Although these studies provide useful information, they do not identify the mechanisms of action involved in egg residue transfer. It is not possible from this type of study to determine whether the extended pattern of residue release in eggs after drug withdrawal is due to residue uptake in yolks developing during the period of dosing or other variables. The most important of these other variables would usually be the physicochemical properties of selected drugs and their differences in half-life and body accumulation (reviewed Rieviere *et al.*, 1991). For example, drugs that are stored in body fat and released back into the blood long after drug withdrawal would produce residues in eggs for a longer period than drugs without extended body tissue storage.

Partitioning these different factors is necessary to develop a model system capable of predicting the likelihood and extent of residue accumulation in eggs

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following contaminant exposure. As part of this goal, the pattern of drug uptake in yolks developing within the ovary was evaluated. It is known that yolk develops over an extended period of time before ovulation and that the yolks within follicles are in various stages of physiological maturity (reviewed Griffin *et al.*, 1984; Johnson, 1986; Jackson *et al.*, 1993). The majority of yolk development occurs over a 2-wk period before ovulation. During this period, the individual yolks increase in size from approximately 0.2 g to a final mature weight of 17 g (Griffin *et al.*, 1984). In addition, there are approximately 20 small yolks maintained within individual ovarian follicles waiting to enter the 2-wk period of rapid yolk-follicular development. These small yellow yolks develop over about a 1-mo period and weigh approximately 0.1 to 0.2 g each (Griffin *et al.*, 1984). The potential exists that these yolks will accumulate residues and that the pattern of residue accumulation will vary depending upon the developmental stage.

To evaluate this residue incursion pattern, hens were injected with a single bolus of either ampicillin or oxytetracycline (OTC) and euthanatized 24 h following drug injection and yolks removed from ovarian follicles. This approach eliminated the confounding influences of: 1) variation in drug exposure due to differences in hens feeding or water consumption tendencies, and 2) carry-over effects of drugs with long half-lives or tissue deposition vs drugs without these characteristics. The period of 24 h for drug exposure was chosen as yolk accumulation, advancement of yolks within the follicular hierarchy, and egg laying usually occur on a daily basis (see reviews Griffin *et al.*, 1984; Johnson, 1986).

The data presented in this study are part of the ongoing Egg Residue Modeling Program developed by this laboratory in the Center for Veterinary Medicine, U.S. Food and Drug Administration. The goal of this modeling program is to create a data set capable of reliably predicting residue transfer and content in eggs for a variety of drugs or contaminants.

MATERIALS AND METHODS

Animals

A total of 32 Single Comb White Leghorn hens, either 34 or 36 wk of age (first or second replicate, respectively), were used in this study. Hens had *ad libitum* access to standard laying hen feed and water and were subjected to 14 h of light daily. Hens were individually caged and time of oviposition was recorded.

Experimental Procedure

Experiment 1. Sixteen hens were divided into equal groups ($n = 8$) and injected intramuscularly only once with either 400 mg/kg ampicillin or 200 mg/kg OTC. Prelimi-

nary studies indicated that these dosages would produce drug transfer into yolks, especially the yolks in smaller follicles, at concentrations that would facilitate analysis utilizing a microbiological assay (assay description below; data not shown). Injections were given 1 h after oviposition to synchronize time of injection to yolk and follicular development. Because ovulation occurs within approximately 30 min after oviposition (Johnson, 1986), hens would be dosed at the beginning of the daily phase of yolk accumulation and progression through the follicular hierarchy. Twenty-four hours following injections, hens were euthanatized by cervical dislocation and ovaries were collected. Yolks were dissected free from the individual follicles with a blunt probe. Large yellow yolks (≥ 0.2 g) were individually weighed and diluted 1:3 (wt/vol) with 0.1 M monopotassium phosphate buffer pH = 4.5 or 1% potassium phosphate pH = 6.0 for yolks from hens treated with ampicillin or OTC, respectively. A pool of five small yellow yolks (< 0.2 g) were weighed and similarly prepared. Small yellow yolks had to be pooled to obtain the volume necessary to run six replicate samples by the microbiological assay (see below). Samples were frozen (-80 C) until analysis.

Experiment 2. Approximately 2 wk later, an additional 16 hens were subjected to the treatments described in Experiment 1, serving as a replicate for Experiment 1.

Assay Plate Preparation

Samples were assayed using an agar diffusion microbiological method adapted from Roudaut *et al.* (1987b, ampicillin) or Roudaut *et al.* (1987a, OTC). Petri dishes (100 mm in diameter) were filled with 8 mL of agar and six cylinders (8×10 mm) were evenly placed on the agar. For ampicillin determination, the agar (PM indicator medium) was inoculated with spores of *Bacillus stearothermophilus* ATCC 10149² at a concentration of 1.2×10^6 spores per milliliter of agar. For OTC determination, the agar (antibiotic medium 8)² was inoculated with spores of *Bacillus cereus* ATCC 11778² at a concentration of 5×10^3 spores per milliliter of agar.

Assay Procedure

Standard Curve. A matrix matched standard curve was constructed by spiking control yolk with either ampicillin or OTC. These spiked standards were treated identically to unknown samples. Each standard concentration was pipetted into three plates, three alternate cylinders were filled with the standard (200 μ L) and the other three cylinders were filled (200 μ L) with a reference concentration. Triplicate plate averages for each standard point were corrected to the overall reference concentration. A best fit regression line using the diameter of growth inhibition zones (millimeters: Fisher Zone Reader) was calculated by the method of least squares. The ampicillin assays were incubated 3 h at 64 ± 2 C and the OTC assays incubated 16 to 18 h at 30 ± 1 C. The lower limit

²Difco Laboratories, Detroit, MI 48232.

of assay sensitivity was 6 and 316 ng/g for ampicillin and OTC, respectively.

Sample Preparation. After thawing, samples were centrifuged at $3,000 \times g$ for 30 min at 5 C. The supernatant was decanted and diluted 1:2 (vol/vol) with control yolk extract so that the sample concentration would fall within the range of the standard curve. Each sample was pipetted into two plates, three alternate cylinders were filled with the sample (200 μ L) and the other three cylinders were filled with a reference concentration (200 μ L). Each of the duplicate plates was corrected to the overall reference concentration.

Statistical Analysis

Least squares *t* test comparisons following two-way analysis of variance were used for tests of differences in patterns between: 1) replicate experiments utilizing the same drug (Experiment 1 vs 2), 2) drugs, or 3) each drug vs estimated daily yolk accumulation. Yolk sequence was used as a fixed factor and hen as a random effect. Due to differences in the amount of OTC or ampicillin uptake in yolks, the data were rescaled by using a correction factor (the mean overall drug concentration difference) to normalize drug concentrations in yolks. This transformation maintains the pattern of drug incursion but reduces the influence of drug concentration on transfer. The linear and quadratic components of the drug (either Exp. 1 vs Exp. 2; ampicillin vs OTC; ampicillin (or OTC) vs yolk accumulation) by yolk (yolk sequence) factor interactions were tested in an analysis of variance model. A split-plot model was used to account for the repeated measurements of drug concentration in yolks removed from the same hen. Yolk and yolk squared were treated as covariates. The model, thus, included factors for drug, hen nested in drug (hen (drug)), yolk, yolk squared, and the interactions of the latter two with drug. The pattern of drug transfer was not different ($P \geq 0.05$) between Experiment 1 and 2; therefore the data were combined. The analysis was accomplished using SAS® software (SAS Institute, 1994). A probability of $P \leq 0.05$ was required for statistical significance.

RESULTS

Ampicillin and OTC concentrations (on a microgram per gram basis) in the large developing yolks are depicted in Figure 1. The large yolks are designated Y_1 , Y_2 , Y_3 , etc from the largest (on wet weight basis) and most physiologically mature (closest to ovulation) to smallest, respectively. These yolks are approximately 24 h apart in development with the Y_1 yolk due for ovulation next, the Y_2 yolk approximately 24 h later, the Y_3 yolk 24 h after ovulation of the Y_2 , etc. (Griffin *et al.*, 1984; Johnson, 1986). Developing yolks incorporated approximately two and one-half times more OTC than ampicillin although hens were injected with less OTC than ampicillin (200 vs 400 mg/kg, respectively).

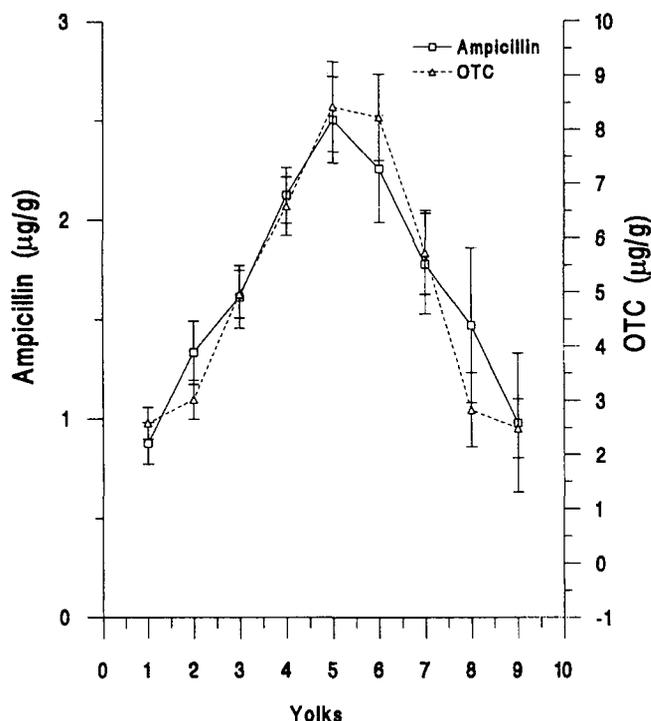


FIGURE 1. Comparison of the pattern of ampicillin or oxytetracycline uptake into larger developing yolks on a microgram per gram basis. Larger preovulatory yolks (0.2 to 17 g) were arranged within the follicular hierarchy designated (Y_1 , Y_2 , etc.). The largest, heaviest yolk closest to ovulation is designated Y_1 , with the Y_2 yolk due for ovulation approximately 24 h later, Y_3 yolk approximately 24 h after the Y_2 , etc. The pattern of drug transfer into developing yolks was not different ($P > 0.05$) between Experiments 1 and 2 (within drug) and therefore the data were combined. The pattern of drug uptake into developing yolks was not different ($P > 0.05$) between drugs as determined by covariate analysis.

However, both drugs produced the highest concentrations towards the middle of the order of developing yolks (Y_4 , Y_5 , Y_6) and the pattern of drug incursion was not different between the two drugs ($P > 0.05$).

Although the yolks in mid-development have the highest drug concentrations on a microgram per gram basis (Figure 1), these yolks are smaller (weigh less) and may have less total drug than the larger yolks. These data are expressed in Figure 2 on a total drug transfer basis. When expressed this way, the Y_3 had the greatest drug incorporation for both drugs. Similar to the results obtained on a microgram per gram basis, the pattern of total drug incursion in developing yolks was not different between drugs ($P > 0.05$).

The amount of drug transfer (micrograms per gram and total) into pooled samples of small yolks (< 0.2 g) is shown in Figure 3. Both drugs transferred at a comparable ratio as in the larger yolks.

Figure 4 depicts the comparison of estimated daily yolk accumulation vs ampicillin (Figure 4a) or OTC (Figure 4b) for the large developing yolks. Daily yolk accumulation rates were estimated by subtracting the weights of the next smallest yolk from the next larger yolk (i.e., Y_2 subtracted from Y_1). When total drug

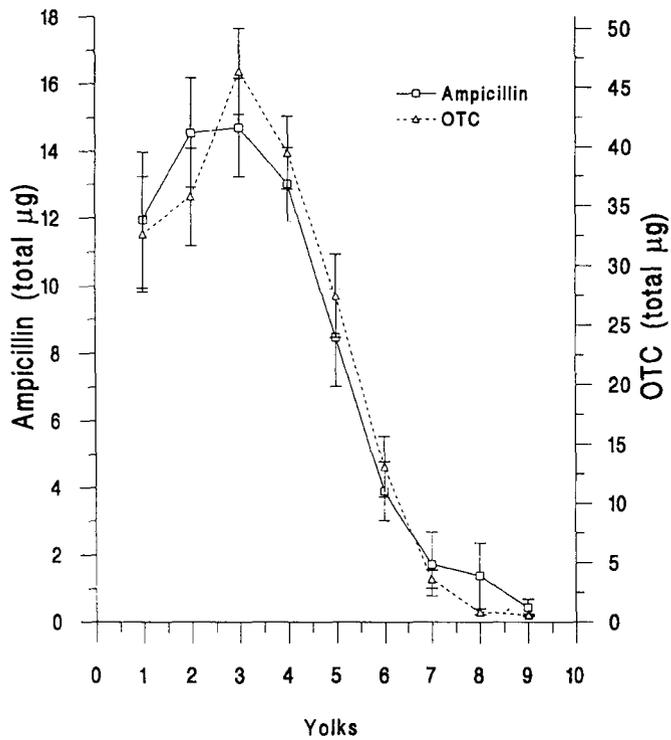


FIGURE 2. Comparison of the pattern of ampicillin or oxytetracycline uptake into developing yolks on the basis of total micrograms content. Larger preovulatory yolks (0.2 to 17 g) were arranged within the follicular hierarchy designated (Y_1 , Y_2 , etc.). The largest, heaviest yolk closest to ovulation is designated Y_1 , with the Y_2 yolk due for ovulation approximately 24 h later, Y_3 yolk approximately 24 h after the Y_2 , etc. The pattern of drug transfer into developing yolks was not different ($P > 0.05$) between Experiments 1 and 2 (within drug) and therefore the data were combined. The pattern of drug uptake into developing yolks was not different ($P > 0.05$) between drugs as determined by covariate analysis.

content vs daily yolk accumulation were plotted against each other (Figure 4a,b), the two patterns were not different ($P > 0.05$).

Model predictions of drug residues in eggs following a 1-, 2-, or 3-d drug treatment period are shown in Table 1. This model utilizes the data presented in Figure 2 and 3 (total micrograms only). The initial egg produced after dosing (Day 2) does not contain residues because the yolk had completed formation and was ovulated before drug treatment even though it was still in the reproductive tract. Utilizing total drug microgram content from Figures 2 and 3, residues produced in eggs following a 1-d treatment are estimated by assigning the drug concentration measured in the Y_1 yolk to equal the amount of drug that would be contained in the yolk of the first egg produced, the drug concentration in the Y_2 yolk would equal the amount contained in the yolk of the second egg, etc. The values used are averaged for both drugs and are expressed in percentages with the content of Y_1 yolk set at 100%. The values for the 2- or 3-d drug treatment are generated by addition of the values used for the single day treatment taking into account the ovulation of the Y_1 yolk on each day. For example, during a 3-d dosing period, the first egg with

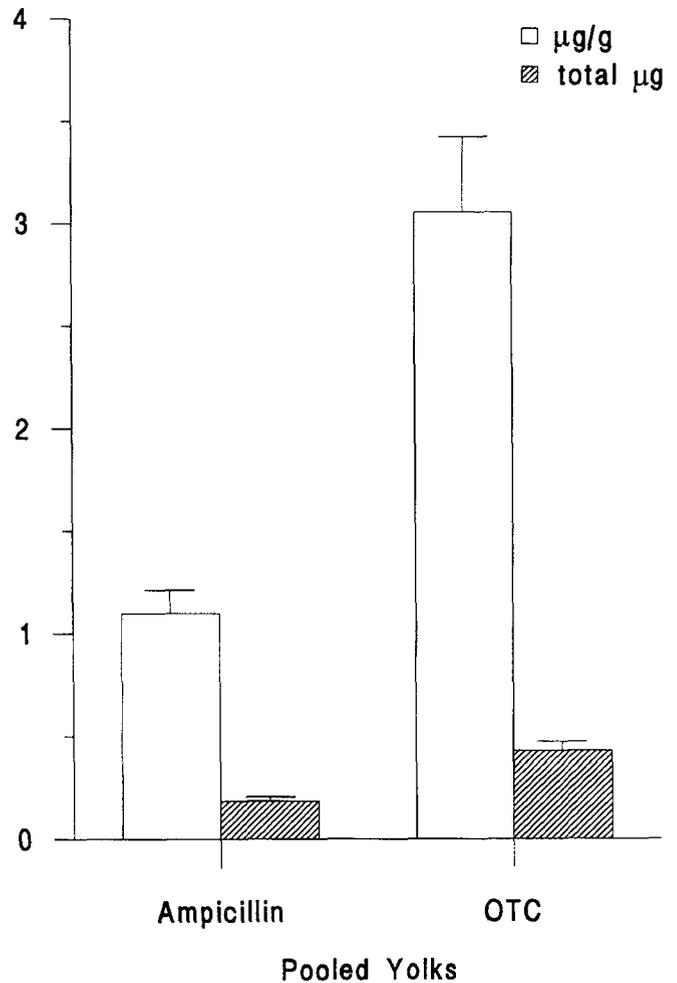


FIGURE 3. Ampicillin or oxytetracycline uptake into pooled small yolks (micrograms per gram and total micrograms content). Smaller developing yolks (0.1 to 0.2 g) were collected and a pool of five yolks was analyzed for drug content. These small yolks represent follicles a week or more away from full development and ovulation.

residues (Day 3) would accumulate drug in egg yolk in the pattern of Y_1 (100%) and then ovulate. The second egg would have Y_2 plus Y_1 ($115.6 + 100 = 215.6\%$) and then ovulate. The third egg [$Y_3 + Y_2 + Y_1$ ($132.5 + 115.6 + 100 = 348.1\%$)], the fourth egg [$Y_4 + Y_3 + Y_2$ ($114.9 + 132.5 + 115.6 = 363.0\%$)], and so on.

DISCUSSION

Results from this study demonstrate that short-term drug exposure produces incorporation of drug residues in developing yolks that are days to weeks from being ovulated (Figures 1, 2, and 3). These drugs were incorporated into developing yolks in a specific pattern that did not differ between the two drugs used in these studies (Figure 1 and 2). Furthermore, these data indicate that drug residues were greater in some of the less mature yolks, potentially producing higher drug concentrations in eggs laid even after drug withdrawal (Figure 2).

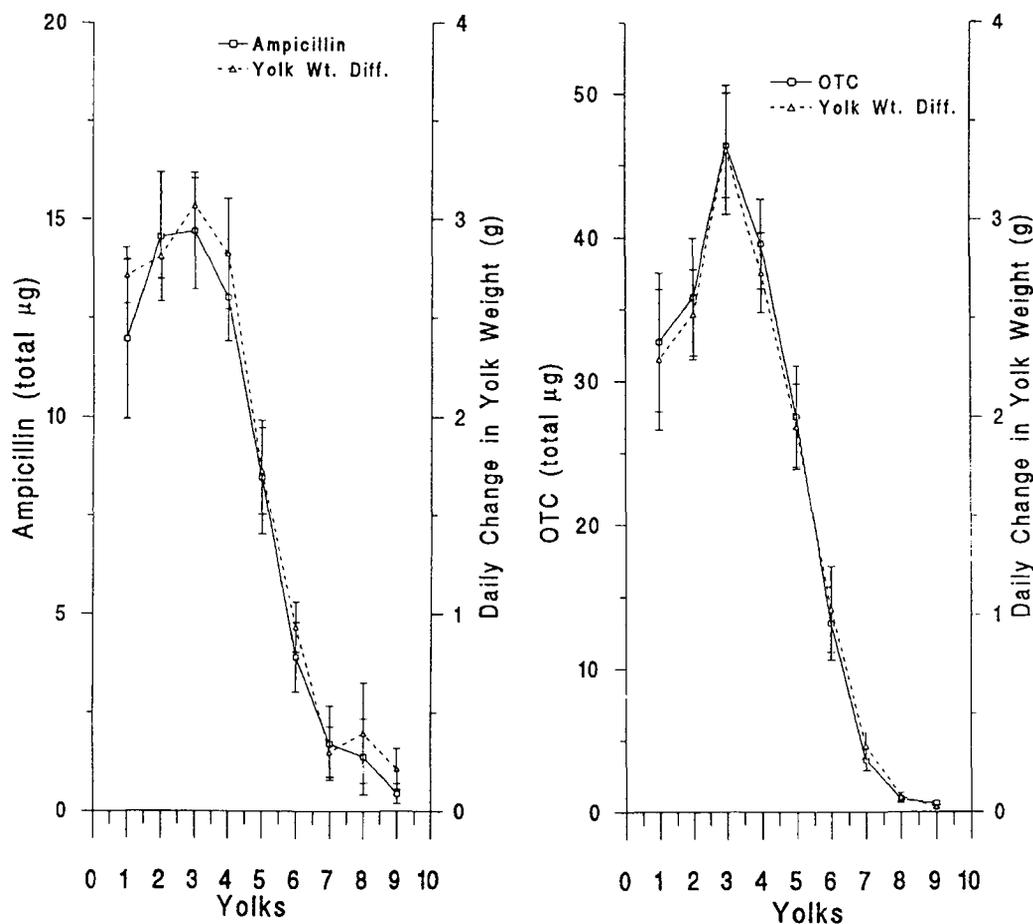


FIGURE 4. Comparison of the pattern of estimated daily yolk accumulation vs total ampicillin (Figure 4a) or oxytetracycline (Figure 4b) content (total micrograms) in the larger developing yolks. Daily yolk accumulation rates in large preovulatory yolks (0.2 to 17 g) were estimated by subtracting the weights of next smallest yolk from the next larger yolk (i.e., Y_2 subtracted from Y_1). The postovulatory yolk weight was used to calculate yolk accumulation for the Y_1 yolk (i.e., Y_1 subtracted from ovulated egg yolk). When total drug content versus daily yolk accumulation was plotted against each other (Figure 4a,b), the two patterns were not different ($P > 0.05$) as determined by covariate analysis (see statistical section). Data between experiments were combined as described in Figure 2.

These results have significant human food safety implications. Even with only a 24-h period of drug exposure, hens incorporated drug residues in yolks that were not slated for ovulation for days or weeks. Potentially, this would expose the consumer to egg containing drug residues for an extensive time period. Somewhat surprisingly, the smaller Y_3 yolk had the greatest total drug content, even more than the larger Y_1 and Y_2 yolks. Even without additional drug transfer, the Y_3 yolk will contain more total drug residues and, dependent upon the drug's stability, will result in the yolk in the third egg produced containing a greater content than the first or second egg produced after drug exposure. This result leads to the unusual phenomenon of drug residues actually increasing in eggs following termination of drug exposure to the hen. In addition, our results indicate that the pattern of drug incursion in developing follicles is similar even though two dissimilar classes of drugs were used in this study (Figures 1, 2), which suggests the possibility that the pattern of residue uptake in developing yolks detected in the

present study may also apply to other drugs or contaminants. If true, then other types of residue exposure (i.e., drugs, pesticides, or heavy metals) may also produce an extended period of release of contaminated eggs, even after only a 1-d exposure period. It should be emphasized that even if the pattern of residue uptake by developing yolks is similar for different drugs, the actual residue pattern in laid eggs will probably differ between drugs, because other factors may interact to influence residue patterns in laid eggs (see model assumption, below). For example, residues detected in eggs after drug withdrawal may be due to a combination of drug uptake occurring during the dosing or postdosing periods due to persistent drug plasma levels associated with a drug with a long half-life. Our laboratory's strategy to euthanize the hens 24 h after dosing prevented additional drug uptake by the ovary and eliminated day to day confounding influences. This approach allowed partitioning and identification of the daily pattern, and only the daily pattern of drug uptake in developing yolks. The possibility that other drugs

TABLE 1. Model predictions of the pattern of residues in the yolks of eggs following a 1-, 2-, or 3-d dosing period¹

Days	Total yolk residues		
	1 d	2 d	3 d
	(%)		
1	0.0	0.0	0.0
2	0.0	0.0	0.0
3	100.0	100.0	100.0
4	115.6	215.6	215.6
5	132.5	248.1	348.1
6	114.9	247.3	363.0
7	77.5	192.3	324.9
8	36.4	113.9	228.8
9	12.8	49.2	126.7
10	7.1	19.9	56.3
11	2.8	9.9	22.7
12	1.4	4.2	11.3
13	1.4	2.8	5.6
14	1.4	2.8	4.2
15 to 30	1.4	2.8	4.2

¹This model utilizes the data presented in Figures 2 and 3 (total micrograms only). Hens would be dosed starting on Day 1, following oviposition. The egg laid after initial dosing (Day 2) would not contain residues because the yolk had completed formation and was ovulated before drug treatment. The residues produced in eggs following a 1-d treatment are generated by assigning the concentration detected in the Y₁ yolk to equal the amount contained in the yolk of the first egg with residues, the concentration in the Y₂ yolk to equal the amount contained in the yolk of the second egg with residues, etc., utilizing total microgram content from Figures 2 and 3. The values used are averaged for both drugs and are expressed in percentages with the content of Y₁ yolk set at 100%. The values for the 2- or 3-d drug treatment are generated by addition of the values used for the single day treatment taking into account the ovulation of the Y₁ yolk on each day. For example, during a 3-d dosing period, the first egg with residues (Day 3) would accumulate drug in egg yolk in the pattern of Y₁ (100%) and then ovulate. The second egg would have [Y₂ + Y₁ (115.6 + 100 = 215.6%)] and then ovulate. The third egg [Y₃ + Y₂ + Y₁ (132.5 + 115.6 + 100 = 348.1%)], the fourth egg [Y₄ + Y₃ + Y₂ (114.9 + 132.5 + 115.6 = 363.0%)], etc. This model makes a number of assumptions regarding drug characteristics, drug treatment, hen performance, and the physiological state of the hen.

transfer into developing yolks in a similar daily pattern and the contribution this pattern of transfer has on overall residue content in laid eggs is currently under investigation.

The pattern of drug uptake in developing yolks is probably related to the physiological pattern of daily yolk accretion within the ovary. Daily yolk accumulation rates for each of the developing yolks within the follicular hierarchy were estimated by subtracting the weights of the next smallest yolk from the next larger yolk (i.e., Y₂ subtracted from Y₁). The pattern of drug content was similar to the pattern of daily yolk accumulation rate (Figure 4a,b). Apparently, the pattern of residue deposition is due to: 1) proportional co-migration of residues with yolk, and 2) different quantities of yolk being deposited dependent upon the maturity of the developing follicles. This conclusion is supported by the seminal work of Warren and Conrad (1939). These researchers injected the fat-soluble dye Sudan III and evaluated the deposition of daily dye

bands (also called ring structures) in ovulated yolks. They concluded that for yolks 1 to 9 d from ovulation secretion of yolk material and incorporation of dye residues increases rapidly until 3 d before ovulation and then declines until ovulation. These results are consistent with our results for the pattern of daily yolk accumulation and incorporation in developing yolks (Figure 4).

Both the results presented in this study and those of Warren and Conrad (1939) indicate a similar pattern for drug or contaminant uptake in yolks in various stages of development. This information may be used to model or predict the pattern of residue uptake in eggs for any compounds of interest following any number of days of exposure to the laying hen. To this end, a prediction pattern model (Table 1) for incorporation of residues in eggs following a 1-, 2-, or 3-d treatment period is presented utilizing the data in Figure 2 for the large yolks and Figure 3 (total micrograms, only) for the small yolks.

This model predicts release of eggs containing residues for weeks following either a 1-, 2-, or 3-d treatment period. However, eggs released during the 1st wk will contain the highest concentrations of residues. By the end of the 2nd wk, eggs will be laid that contain only 1.4, 2.8, or 4.2% of the total contained in the first egg produced during or after dosing for the 1-, 2-, or 3-d dosing treatment, respectively. This lower concentration represents the extent of drug transfer into the numerous yolks in the small yolk phase of development. Although these yolks contain only a small percentage of the drug content of the larger yolks, dependent upon the drug or contaminant, this percentage may represent a significant human food safety concern. As would be expected, the drug content and pattern of eggs containing residues is elevated and extended after a longer duration of drug exposure by the hen. These data can be extrapolated to predict the percentages and pattern of egg residues for any number of days of treatment. In addition, if the residue content can be quantitated for one egg following drug exposure, and the length of time hens were exposed to contaminants is known, it is possible to use this model to calculate predicted residue concentrations for the other eggs produced. Of course, this model is presented as a working paradigm and the its efficacy can only be assessed by testing different xenobiotics under various conditions.

These model predictions are supported, in part, by the results obtained by Warren and Conrad (1939). They reported incorporation of dye residues in specific yolk bands or rings occurring during daily yolk accretion. Detection of yolk ring formations has also been reported after staining (Grau, 1976) or noninvasive detection by magnetic resonance imaging (Hutchison, 1992). Although the research of Warren and Conrad (1939) was directed at understanding the physiology of yolk

formation, and not residue accumulation *per se*, they did observe a specific pattern of dye residues in formed eggs. This pattern is consistent with our model predictions for residue incursion in formed eggs.

This model may be used for regulatory decision making. For example, if an egg sample is determined to contain drug residues, this model predicts there will be a number of eggs, either having already been produced or that will be produced by the hen, that will contain residues. This, even after only a single dosage episode. These predictions can be either conservative or nonconservative. The conservative estimate speculated that an identified egg was produced later in sequence after dosing and, therefore, there were a number of eggs with higher residue contents produced earlier. The nonconservative approach assumes that an identified egg contains the highest quantity of residues possible (for the 1-d dosage this would equal the third egg) and calculates the lower residue content of other eggs produced by those hens.

This model makes a number of assumptions; 1) there is no drug carry-over from day to day treatment or after drug withdrawal, 2) the drug does not transfer back out of the follicles, 3) there is 100% egg production, 4) the pattern of residue transfer is consistent even for different periods of drug exposure (excluding enhanced contaminant metabolism after extended exposure), 5) there is insignificant drug transfer to albumen, and 6) the pattern of drug transfer is similar in healthy vs diseased state. In practice, depending upon the drug, dosing, route of administration, age of hens, rate of egg production, and health of the hens, the pattern of residue transfer in eggs could be higher or lower. Patterns of higher residues in eggs or an extended pattern of residue transfer would produce the following scenarios: 1) hens that have more skip days (lower egg production) would release yolks with incurred drug residues over a longer period of time or 2) the use of drugs with long half-lives, or drugs with a propensity to be stored in body tissues (such as the fat or liver) and released back to the blood stream will enhance daily "carry-over" accumulation of drugs in developing yolks. Many drugs used in feed or water will not be cleared from the body within 24 h. A pattern of lower residues would be produced if, during extended treatment, the hen increased elimination of the drug from the body by increasing metabolism or excretion. Drug residues may also decline if the drug has limited stability and breaks down while stored in the ovary. It is also possible that drugs may transfer out of the follicle, although there is no evidence known to the authors to support this concept. And finally, if drugs are used in sick vs healthy animals, it would be possible for residue transfer into yolks to either increase or decrease depending on the illness and its severity. These caveats, however, do not limit the usefulness of this model. Considerable information is available pertaining to these conditions such as

drug half-life, stability, and metabolism (Rieviere *et al.*, 1991). The producer can also calculate egg production and estimate drug or contaminant uptake in feed or water dependent upon their given situation. Incorporation of available information into the particular situation at hand should allow extrapolation from our proposed model to predicted egg residues.

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