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Can the Use of Coccidiostats in Poultry Breeding Lead to Residues in Vegetables? An Experimental Study

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Supporting Information

ABSTRACT: The aim of this study was to provide information on the dietary exposure of the European public to coccidiostats via vegetable consumption. Five groups of poultry followed a three-phase feeding schedule with feed containing the maximum allowed level of a coccidiostat: monensin, lasalocid A, salinomycin, diclazuril, and nicarbazin/narasin, plus one control group. Vegetables were cultivated on soil amended with manure (10 g of fresh weight/kg of soil) from the treated poultry. To mimic a worst-case scenario, vegetables were also grown on soil spiked with coccidiostats. For each vegetable/treatment combination, samples were harvested, freeze-dried, and analyzed using a validated liquid chromatography−tandem mass spectrometry method. Analysis of the vegetables demonstrated that these plants are capable of taking up these coccidiostats from the soil. However, the results indicate that these low incorporation levels, coupled with food consumption data and acceptable daily intakes, are unlikely to pose a direct threat to public health.

KEYWORDS: *coccidiostats, manure, vegetables, plants, veterinary, residue, contaminant, uptake, incorporation, LC−MS/MS*

INTRODUCTION

Contemporary agricultural practice often involves application of a wide range of veterinary medicines and feed additives to treat and protect animals from a large variety of pathological conditions. Infections (bacterial, parasitic, or viral) account for a large fraction of these diseases. Kools et al. estimated that over 5 million kg of veterinary antibiotics are administered annually in the European Union (EU) alone.[1] Although more and more research is being performed to study the environmental dissemination of these substances, their ecological impact is not well-understood. Boxall et al. state that, when determining the potential of a veterinary medicine to enter the environment, the following factors should be examined: the amounts used, the degree of metabolism and the stability during storage and/or processing of the manure (prior to spreading on the field), and the physicochemical properties of the substance.[2]

Coccidiostats are a group of compounds that act as antiprotozoal agents and have several properties, making them a potential risk to the environment. This risk is based on the large quantities produced, among others. The International Federation for Animal Health (IFAH) estimated that of the 40.7 million tons of feed produced annually in the EU for chickens for fattening, turkeys, and rabbits, approximately 18.3 million tons (45%) contain an added coccidiostat.[3]

The absorption/excretion profile of coccidiostats also makes them prone to dissemination in the environment. Manure from treated animals may contain high amounts of unmodified as well as metabolized compounds as a result of poor absorption. In the manure of treated animals, 98% of the administered dose of the coccidiostat diclazuril is excreted within 10 days; the parent compound accounts for 85−95% of the excreted dose.[4] European Food Safety Authority (EFSA)'s Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) states that coccidiostats have a wide variety of physicochemical characteristics, some of which show potential for bioaccumulation.[5] These factors all contribute to a possibly critical impact of these compounds on the environment in the EU. The relatively high risk that coccidiostats are excreted into the environment was demonstrated theoretically by Boxall et al. and Kools et al.[1,6] These authors developed a prioritization approach to determine the possible threats of veterinary medicines to the environment, specifically regarding soil, water, animals, and fish.[1,6]

On the basis of their chemical structure and main biological activity, the 11 coccidiostats authorized by Commission Regulation No. 1831/2003/EC are divided into two classes: the naturally produced polyether ionophores (monensin, lasalocid, salinomycin, narasin, maduramicin, and semduramicin) and the synthetic coccidiostats (robenidine, decoquinate,

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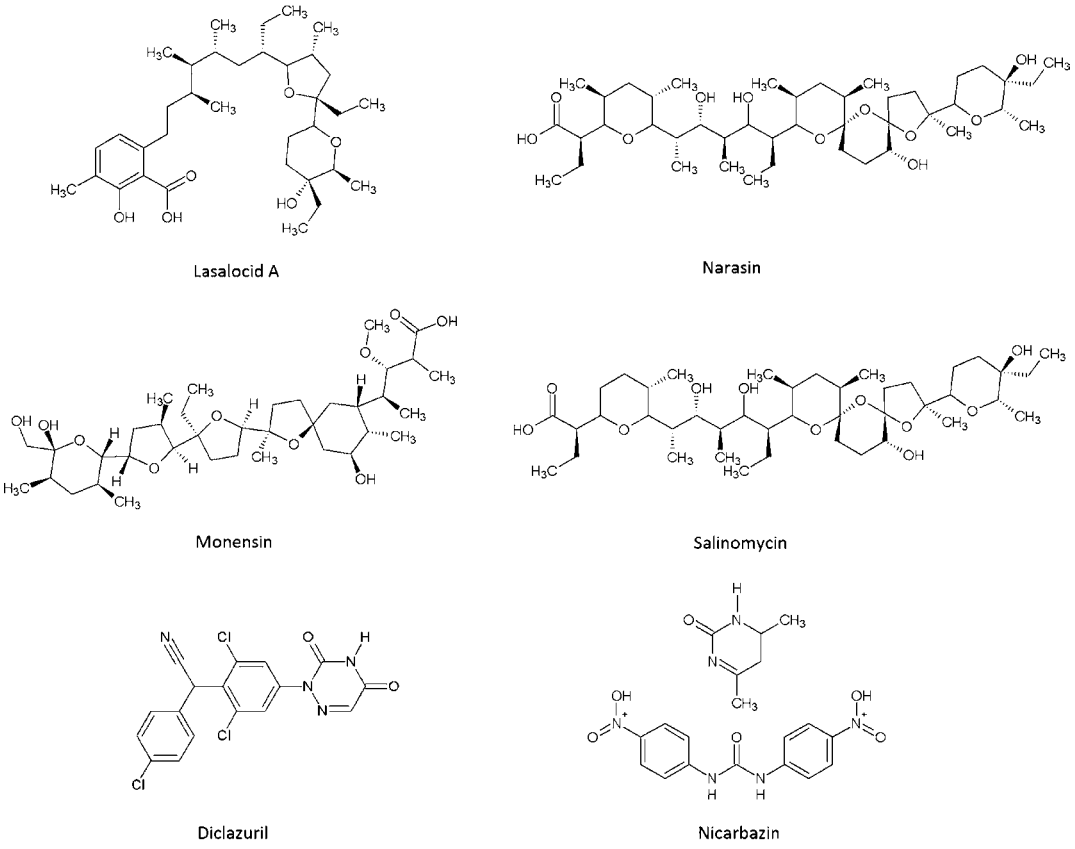

Figure 1. Chemical structures of ionophores (lasalocid A, narasin, monensin, and salinomycin) and synthetic coccidiostats (diclazuril and nicarbazin). Nicarbazin is an 1:1 equimolar mixture of 4,6-dimethyl-2(1*H*)-pyrimidone (upper structure) and 1,3-*N*,*N*′-bis(4-nitrophenyl)urea [also dinitrocarbanilide (DNC); lower structure].

nicarbazin, diclazuril, and halofuginone).[7] A total of 6 of the 11 authorized coccidiostats were included in our research: monensin, salinomycin, lasalocid, narasin, diclazuril, and nicarbazin, for which the structures are shown in Figure 1. This selection was based on the chemical structure (synthetic and ionophores) and quantities used. Because approximately 75% of the coccidiostats used in Europe are ionophores, we included more ionophores than synthetic coccidiostats in this study. Monensin and lasalocid, although both belonging to the group of ionophores, act differently when they are given to poultry at levels corresponding to cross-contamination levels. For lasalocid, high residue concentrations were found in eggs, liver, and meat. For monensin, however, residue levels were very low (around the detection limit).[8,9] The synthetic coccidiostat nicarbazin was chosen because it is used in rather high concentrations and has a maximum authorized level of 50 mg/kg, the highest allowed concentration of all of the chemical coccidiostats. Furthermore, nicarbazin is a very adhesive substance, which makes it susceptible to cross-contamination. Diclazuril was chosen for its low maximum allowed concentration, the lowest of all authorized coccidiostats.

Coccidiostats can cause a wide range of toxicological effects in humans. The polyether ionophores are lipophilic chelating agents that are known to affect and disrupt the transmembrane ion transport. The synthetic coccidiostats, which represent a more heterogeneous group, have mechanisms of action that are not yet fully understood.[9] Effects may result in neurotoxic disorders, maternal toxicity and fetotoxicity, decreased serum protein levels, and focal degeneration of skeletal muscles.[10] Acceptable daily intake (ADI) levels for humans are situated in the mid to low range (μg kg of body weight^{-1} day^{-1}). Consequently, maximum residue limits (MRLs) have been set for these compounds in several matrices of animal origin. Linking the dietary exposure of the public solely to food of animal origin may be shortsighted, however. It is well-known that plants can incorporate certain compounds, such as pesticides.[11] The uptake of veterinary drugs by plants has recently been demonstrated for several compounds and plant species.[12−14] This phenomenon is due to fertilization with manure from treated animals that may contain high amounts of contaminants. To our knowledge, no data are available regarding coccidiostat uptake or incorporation by plants.

The aim of this study was to provide information on the dietary exposure of the European public to coccidiostats (monensin, lasalocid, salinomycin, narasin, diclazuril, and nicarbazin) via vegetable (carrot, lettuce, potato, tomato, and zucchini) consumption. In this study, broilers were raised and treated with the maximum allowed level of coccidiostats. A fraction of the collected manure (pure excreta) was homogenized, analyzed, and used to grow vegetables in a plant trial. To mimic a worst case scenario, vegetables were also grown on coccidiostat-spiked soil (added as premixture). Following the animal and plant trials, the manure and vegetable samples obtained were analyzed using ultra-performance liquid chromatography−tandem mass spectrometry (UPLC−MS/MS). Additionally, we performed a distribution study (peel versus flesh) and investigated the influence of the most frequently applied food-processing techniques (boiling and frying) on the coccidiostat concentration.

EXPERIMENTAL SECTION

Animal Trial. *Housing.* One-day-old male broiler chicks (Ross 308; Origin: Belgabroed Hatchery, Merksplas, Belgium) were housed in the experimental poultry facility at the Institute for Agricultural and Fisheries Research (ILVO) with 42 male birds in each pen (42 birds times 6 pens, 252 birds in total). The broilers were reared under conventional conditions for lighting, heating, and ventilation. The housing management, feeding, and husbandry conditions were regarded as representative for a modern commercial operation in Europe. The experimental unit was a floor pen with a surface of 2.8 m^2. To prevent cross-contamination, the different treatments were separated by a narrow pathway. The bedding material consisted of wood shavings in a thickness of approximately 10 cm. Extra wood shavings were added during the experiment when the litter became too wet. The trial site was equipped with dynamic ventilation with lateral air inlets at both sides and air extraction at the ceiling. The ventilation rate could vary from 0 m^3/h to the maximum ventilation rate of 25 000 m^3/h depending upon the measured temperature and age of the broilers. The temperature was recorded by means of a minimum/maximum thermometer placed in the service corridor. During the first 10 days, the local temperature was kept as close as possible to approximately 32 °C. After 1 week, no extra local heating was provided by the infrared bulb and the room temperature was gradually decreased to approximately 22 °C on day 25. From day 25 onward, the temperature was gradually decreased by 2 °C/week down to 18 °C. The implemented vaccination program was identical for all animals. The broilers were vaccinated on their first day of age against Newcastle disease (NDW, spray) and infectious bronchitis (Poulvac IB Primer, spray). At day 16, vaccination against NDW was repeated (Clone 30, drinking water). This trial was approved by ILVO's Ethical Commission (2010/142).

Dietary Treatment. Drinking water and feed (in finely ground meal form and depending upon the group with the addition of a coccidiostat premixture) were provided *ad libitum*. A three-phase feeding schedule (starter, 1−13 days; grower, 14−26 days; and finisher, 27−39 days) was implemented. The feeding program consisted of six diets. All pens received a standard diet with either none (control) or the respective coccidiostat supplementation at concentration levels presented in Table 1.

Table 1. Overview of the Different Treatment Groups, Corresponding Active Ingredient, Product Name, and Theoretical and Measured Concentrations

treatment group	active ingredient	product name	concentration in feed (mg/kg)	measured concentration ± standard deviation (mg/kg) (n = 5)[a]
1	−	−	−	−
2	monensin	Coxidin	120	137 ± 30
3	salinomycin	Sacox	70	80 ± 6
4	lasalocid	Avatec	100	109 ± 28
5	nicarbazin/ narasin	Maxiban	100	46 ± 10 43 ± 5
6	diclazuril	Clinacox	1	1.16 ± 0.00

[a]In fresh material.

Manure Collection. At 35 days of age, 12 birds per treatment were randomly selected, assigned to digestibility cages, and given the same diet as before. Digestibility cages have a steel grid floor to enable collection of the pure excreta. From days 35 to 39, the total excreta for each treatment (±14 kg/treatment) were collected. The excreta were homogenized manually and divided into fractions. One fraction was used to analyze the homogeneity; another fraction was used to determine the coccidiostat concentration in the manure; and the last fraction was mixed with soil to grow vegetables in the plant trial (all fractions were stored at 3 °C before usage).

Plant Trial. *Soil with Manure/Premix Preparation.* The soil for the study was collected from the 0−30 cm depth of a horticultural field and had a grain size distribution of 81.9% sand, 13.4% silt, and 4.7% clay. It had a relatively low organic carbon content (0.77% C) and a normal pH KCl (potassium chloride) (5.42). Plant-available concentrations (measured in ammonium lactate) were high for phosphorus (P) and were relatively low for potassium (K), magnesium (Mg), and calcium (Ca).

The manure samples were analyzed for total content of organic matter, P and nitrogen (N) (results not shown). Manure was mixed at a dose of 10 g of fresh weight/kg of soil, reflecting a field dose of 10 tons/ha. The manure of the blank treatment group was also included in the trial. Chicken manure is rich in P. Because high doses of P can be detrimental to the environment (eutrophication), the use of chicken manure is restricted in the field. Extensive measures were taken to avoid cross-contamination between the treatments during the mixing of the soil and the manure.

Soil samples were also spiked with different coccidiostats to mimic a worst case scenario. The coccidiostats were added as premix to reach homogeneous mixing in the soil without any preferential sorption. The spiked soils were mixed with mineral N, P, and K fertilizers to reach the same level of nutrients as the average dose supplied in the manure-amended soils. Where necessary, mineral fertilizers were supplied during the greenhouse trial to correct for any relevant differences in P or N between the applied poultry manures of the different treatment groups.

For the spiked soil, concentrations equal to the feed in the animal trial were applied (Table 1). The dose of premix added to each soil depended upon the product type, determined by the coccidiostat concentration in the premix as well as the maximum allowed level in the feed. Similar measures were taken as noted above for the manure-amended soils to avoid cross-contamination during the mixing of the soil and the premix.

Growth and Harvest of Vegetables. The selection of vegetables (carrot, lettuce, potato, tomato, and zucchini) was primarily based on consumption and production data from the Food and Agriculture Organization of the United Nations (FAOSTAT). Data were obtained for roots, leaves, or fruits as the consumed part, and vegetables with different chemical characteristics (high water, starch, or lipid contents) and different genera were represented.

Each type of vegetable was grown on soils mixed with each of the six manures obtained during the animal trial, as well as on the soils spiked with the premixture material. Plants were sown in closed containers (i.e., a container with a dish underneath) to prevent losses of coccidiostats by leaching. Care was taken to avoid extensive compaction of the sandy soil in the containers. A bulk density of 1.25 kg/L was used as the target value, comparable to bulk densities of the topsoil of horticultural fields. Carrot [cultivar (cv.) Berlikumer] was sown in 2 L containers; potato (cv. Bintje) was grown in 7 L containers from tubers; and tomato (cv. Pyros), zucchini (cv. Black Beauty), and lettuce (cv. Appia) were planted as small plantlets in 5, 3, and 1 L containers, respectively. The vegetables were grown in a greenhouse under a controlled light and temperature regime and were watered according to the optimal watering regime per type of vegetable. Vegetables were divided between two compartments in the greenhouse: carrots, potatoes and lettuce were grown in a cold compartment (with minimal heating to avoid temperatures beneath 10 °C), and tomatoes and zucchini were grown in a warm compartment (with heating to avoid temperatures beneath 20 °C and natural ventilation at temperatures above 20 °C). When the vegetables were ready for consumption, the yield was determined and the vegetables were harvested, sampled, and freeze-dried.

Analysis Methods. All samples were analyzed according to a quality control scheme, which guaranteed the proper performance of the apparatus during the analysis and the correctness of the results obtained.

Feed. Five feed subsamples of each treatment were analyzed using UPLC−MS/MS. Matrix-matched calibration curves were used for quantification. These curves consisted of eight concentration points, from 0 to double the theoretical coccidiostat concentration in the feed

Table 2. LOD and LOQ (in μg kg^{-1}) for All Compounds in Carrot, Potato, Lettuce, Zucchini, and Tomato

	fresh carrot		fresh potato		fresh lettuce		fresh zucchini		fresh tomato	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
diclazuril	0.75	1.50	1.21	2.42	0.24	0.48	0.32	0.64	0.43	0.86
narasin	0.61	1.22	1.55	3.10	0.26	0.52	0.26	0.52	0.27	0.54
monensin	0.72	1.44	1.38	2.76	0.32	0.64	0.30	0.60	0.29	0.58
salinomycin	0.67	1.34	1.49	2.98	0.29	0.58	0.28	0.56	0.28	0.56
DNC	0.43	0.86	0.86	1.72	0.15	0.30	0.18	0.36	0.18	0.36
lasalocid A	0.73	1.46	1.13	2.26	0.22	0.44	0.31	0.62	0.22	0.44

(cf. Table 1). Samples of 5 g were extracted with 25 mL of acetonitrile for 30 min on a shaker. After centrifugation for 10 min at 600*g*, 5 mL of the supernatant was evaporated to dryness at 60 °C. The residue was dissolved in 1 mL of ACN/H_2O (50:50, v/v), and the tube was vortexed for 30 s and placed in an ultrasonic bath for 5 min. The extract was diluted 1000 times for high concentrations (>5 ppm) or 10 times for low concentrations (<5 ppm) and injected after filtration. A second line control sample was also analyzed, with recoveries ranging from 79 to 103%. Although the method developed did not strictly fall within the scope of Commission Decision No. 2002/657/EC (products of animal origin), we used this decision as a guideline for the validation.[15]

Manure. A total of 5 g of manure were spiked and left to stand for 10 min. Sodium sulfate was then added to dry the sample. Subsequently, the manure was extracted by adding 20 mL of acetonitrile, vortexing, 30 min of shaking, and 15 min of centrifugation (4000 rcf). A total of 10 mL of the resulting supernatant was transferred to a new tube and evaporated to dryness. This extract was redissolved in 1 mL of mobile phase, vortexed, filtered, and transferred to a vial for injection into the LC−MS/MS.

Although the method developed did not strictly fall within the scope of Commission Decision No. 2002/657/EC (products of animal origin), we used this decision as a guideline for the validation.[15] Homogeneity of the manure samples was proven using a one-way analysis of variation (ANOVA) analysis (results not shown).

A total of 10 subsamples were taken randomly of each of the manure groups to determine the coccidiostat concentration and assess the homogeneity of the manure samples. Each subsample was analyzed in duplicate using the method described above, resulting in 20 samples being analyzed in a random order for each type of manure.

Vegetables. The freeze-dried vegetable samples were ground and mixed. Samples (0.5 g) were spiked with the internal standards (nigericine, dinitrocarbanilide-D_8, and diclazuril-bis) and were left to equilibrate for 15 min. For the extraction, 10 mL of methanol was used. After vortexing, shaking, and centrifugation, 4 mL of the supernatant was diluted with 24 mL of ultrapure H_2O and loaded onto a preconditioned C_{18} solid-phase extraction (SPE) column (GracePure SPE C18-Max, 500 mg/6 mL). The target analytes were eluted with 2 mL of methanol and, after evaporation, redissolved in 200 μL of MeOH/H_2O (50:50, v/v), ultracentrifuged, and injected into LC−MS/MS. Analysis was performed on an Acquity UPLC−MS Quatro Premier XE with an Acquity UPLC BEH C18, 1.7 μm, 2.1 × 100 mm, column (both purchased from Waters, Milford, MA).

In Table 2, the limit of detection (LOD) and the limit of quantification (LOQ) of the method are given for all compounds in carrot, potato, lettuce, zucchini, and tomato. For more detailed information concerning the instrumentation, development, and validation of this LC−MS/MS method, see ref 16.

Each vegetable and treatment group combination was analyzed in 4-fold, and all samples originated from different plants to account for plant−plant variation. Quantification was based on matrix-matched calibration curves consisting of five concentration points.

Additional Experiments: Distribution Study and Food Processing. Potato is normally heated (for this study, either boiled or fried) prior to consumption. To determine the effect of boiling and frying on the coccidiostat concentration, 7 g of potato slices (thickness of 4−6 mm) of the lasalocid and nicarbazin/narasin premix treatment (an ionophoric and a synthetic coccidiostat treatment) was taken.

Every replicate (n = 5) plus a representative blank sample (obtained from the supermarket and used to construct a calibration curve) was boiled in separate pots of 250 mL of water. All samples were cooked for 3 min (at which time samples were soft). The samples were subsequently removed from the water, left to drain for 10 min, and freeze-dried.

To determine the effect of frying on the coccidiostat concentration in potatoes, these samples and a batch of blank samples were fried separately. A total of 4 mL of olive oil (obtained from a supermarket) was poured into a pan and preheated for 1 min over a Bunsen burner with a constant flame. The slices of potato were then added and left to fry for 4 min (2 min on each side). The samples were then removed from the pan; excess oil was removed by blotting with absorbent paper (obtained from a supermarket); and samples were freeze-dried.

The distribution of these compounds between potato peel and flesh was also studied. The potatoes from the plant trial were peeled, and the two types of samples, namely, the peel (outer 2−5 mm) and the inner flesh, were analyzed using UPLC−MS/MS.

RESULTS AND DISCUSSION

Feed. The coccidiostat concentrations measured in the feed are shown in Table 1. Measured coccidiostat levels in supplemented feeds had to be between 80 and 120% of the expected dose; thus, all prepared feeds were compliant.

Manure. Detected coccidiostat concentrations in manure ranged from 270 ppb (diclazuril) up to 23 ppm (monensin) (Table 3). The ratio of concentrations in manure compared to those in feed varied from 5% (salinomycin) up to 38% (nicarbazin). These results are in accordance with prior research; it is known that salinomycin is rapidly metabolized in the gut and liver to numerous metabolites, mainly mono-, di-, and trihydroxylated derivatives, and that dinitrocarbanilide (DNC), the marker residue for nicarbazin, and metabolites are mainly excreted in the feces (46% unchanged DNC).[17,18]

Table 3. Concentrations of the Different Coccidiostats in Poultry Manure Originating from the Animal Trial

compound	concentration ± standard deviation (μg/kg) (n = 10^2)[a]	coefficient of variation (%)	concentration of manure/ concentration of feed × 100
monensin	23615 ± 5741	24.3	17
salinomycin	3586 ± 327	9.1	5
lasalocid	20891 ± 2741	13.1	21
narasin	6155 ± 956	15.5	12
nicarbazin	18823 ± 1517	8.1	38
diclazuril	278 ± 24	8.6	28

[a]In fresh weight.

Vegetables. Monensin and narasin/nicarbazin premix application in the soil resulted in phytotoxic properties toward carrot growth/germination in some of the treatments. This phytotoxicity was based on a visual assessment of the reduction

Table 4. Concentrations (μg kg^{-1}) of the Different Coccidiostats in Fresh Vegetables (n = 4)[a]

	compound	potato	carrot	courgette	tomato	lettuce
M	monensin	<LOD	<LOD	<LOD	<LOD	0.94 ± 0.72
	salinomycin	<LOD	<LOD	<LOD	<LOD	<LOQ
	lasalocid	<LOD	<LOD	<LOD	<LOD	<LOD
	narasin/nicarbazin	<LOD	1.11 ± 0.10	<LOD	<LOD	<LOQ
	diclazuril	<LOD	<LOD	<LOD	<LOD	<LOQ
P	monensin	47.1 ± 25.3	NA	<LOD	<LOD	2.06 ± 0.41
	salinomycin	<LOD	<LOD	<LOD	<LOD	<LOD
	lasalocid	2.80 ± 0.61	1.75 ± 1.20	<LOD	<LOD	<LOD
	narasin/nicarbazin	15.7 ± 8.4	NA	1.22 ± 0.44	<LOD	<LOD
	diclazuril	<LOD	<LOD	<LOD	<LOD	<LOD

[a]LOD and LOQ values can be found in Table 2. M, manure-amended soils; P, premix treatments; NA, not available (because of phytotoxic properties of the treatment).

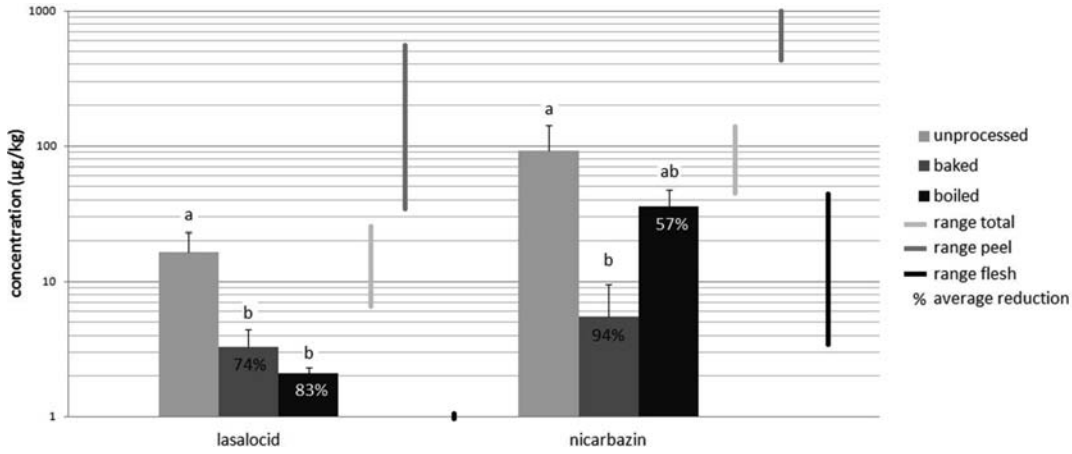

Figure 2. Overview of potato samples from the lasalocid and nicarbazin/narasin premix treatments before and after baking and boiling (n = 5) as well as a distribution study (total, peel, and flesh) in the unprocessed samples (n = 5).

of germination (carrots) or shoot formation (potatoes). The monensin premix exhibited a similar but less profound phytotoxicity in the potato plants. Additional tests indicated that this effect was related to the concentration of the active pharmaceutical ingredient and not the presence of fertilizers and/or premixes (results not shown).

Analysis of the vegetable samples revealed the capability of some of these plants to incorporate coccidiostats in their tissues following uptake from soil (Table 4). The detected concentrations are relatively low (<LOQ, 47 μg/kg) and mostly originated from vegetables grown on soil spiked with premixes (as a worst case scenario; the concentration levels used in these treatments are unlikely to be found in practice). Within the manure-amended soils, nicarbazin was taken up in carrots and monensin was taken up in lettuce (both with an average concentration of ±1 μg/kg). Tomato was the only vegetable for which no concentrations higher than the LOD could be detected. This could be due to the edible parts being situated above ground (contrary to potato and carrot) and the relatively low amount of lipids (unlike zucchini), which makes it more difficult for coccidiostats to be incorporated into the fruits. Several coccidiostats (diclazuril, salinomycin, and narasin) did not show uptake above the LOD in the vegetables included in the study.

Several research groups have observed a link between plant uptake and the octanol–water partitioning coefficient (log K_{ow}) of the concerning compound.[19−23] The results of the present study could be partially explained by some of the models/trends that they observed earlier. Compounds that were incorporated into plants (lasalocid, monensin, and nicarbazin) have the lowest log K_{ow} values of the six, all situated around a value of 2−3. The log K_{ow} values of the compounds that showed no uptake were situated around 4−5. This can be explained by the greater tendency of lipophilic compounds to partition into plant root lipids than hydrophilic chemicals. If an organic pollutant has a log K_{ow} value lower than 0.5, it is generally too hydrophilic to pass membranes and does not become incorporated into plant tissues. Organics with log K_{ow} values higher than 3 will be retained by the lipid bilayers of the membrane and will not enter cell fluids. Furthermore, the soil sorption coefficient is linearly related to the octanol–water partition coefficient. As the log K_{ow} value increases, sorption to the soil will increase and smaller amounts will be found in the soil water. Because uptake by the roots is related to the partitioning of the component in the soil water, too high of log K_{ow} values would result in less uptake by the plant.[24]

Several lettuce samples from the salinomycin, nicarbazin/narasin, and diclazuril manure treatments also appeared to contain monensin (same mass spectrometric transitions, retention times, and relative ion intensities), but these levels of contamination never exceeded the LOQ. Lettuce samples originating from the monensin treatments (manure as well as premix) had levels well above the LOQ. This gives a good indication that the monensin detected in the lettuce grown on

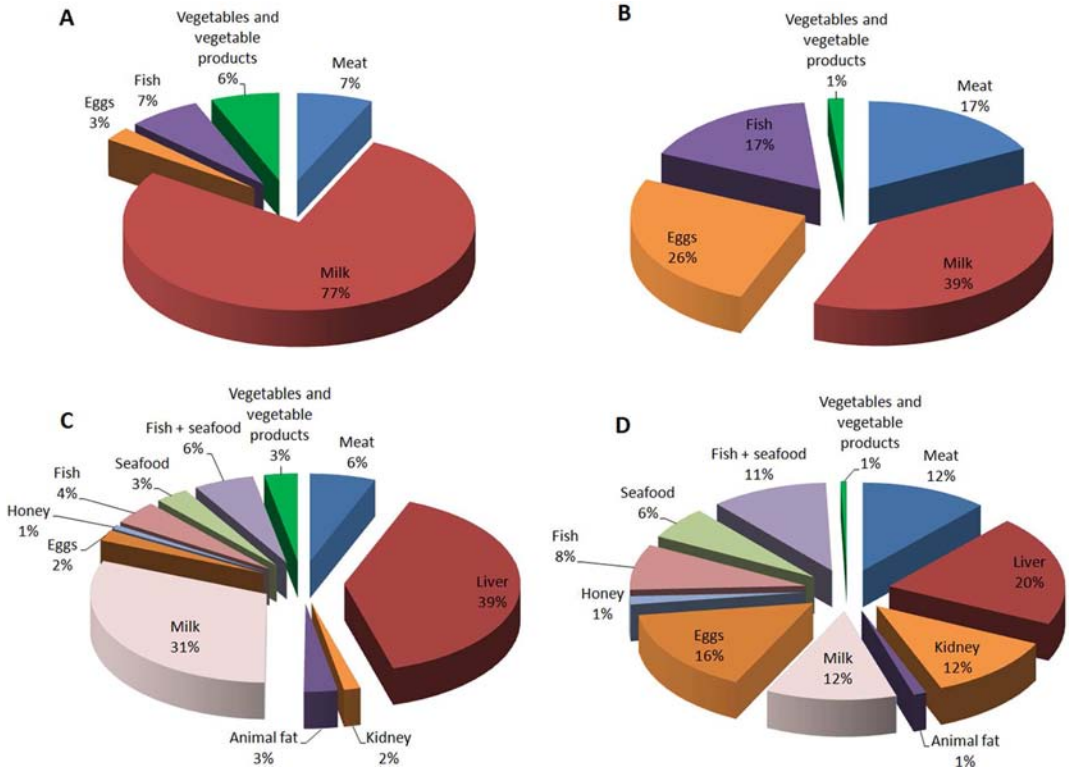

Figure 3. Relative shares of the consumed matrices with regard to the acute intake (95th percentile) of coccidiostats as described by EFSA's "Guidance for establishing the safety of additives for the consumer". Calculations are for the highest consumer in the category, with coccidiostat concentrations based on findings in this study (vegetables and vegetable products) and MRLs in legislation (other food commodities): (A) monensin for toddlers, (B) nicarbazin for toddlers, (C) monensin for adults, and (D) nicarbazin for adults.

the monensin-containing manure-amended soil is originating from the uptake from this soil, while the detected peaks in the other treatments (below LOQ) could be caused by contamination, interfering compounds, or perhaps *in situ* formation (monensin is produced by *Streptomyces cinnamonensis*, predominantly found in soil and decaying vegetation[25]). Because this was not further investigated, we stress the hypothetical nature of these possible causes.

The results of this study provided information on the uptake of veterinary medicines, particularly coccidiostats, into plants from soil amended with manure from poultry treated against coccidiosis or from soil spiked directly with coccidiostats. This information may be used to estimate the exposure of the consumer to these compounds via vegetable consumption (see the section below).

Additional Experiments: Distribution Study and Food Processing. Results of the UPLC–MS/MS analysis of the samples originating from the additional experiments (boiling, frying, and distribution study) are shown in Figure 2.

The data concerning food processing demonstrate that a portion of the contaminants is removed or destroyed during potato processing. Frying resulted in an average reduction of 75% of lasalocid and 95% of nicarbazin. Boiling the samples resulted in an average reduction of 85% of lasalocid and 55% of nicarbazin. Nicarbazin (DNC) is more efficiently removed by frying, not surprising given the usage of fat as a heat-transfer medium and considering that nicarbazin has the highest log K_{ow} value of the two compounds. Lasalocid, the more hydrophilic of the two compounds, is more efficiently removed by boiling.

For both compounds, the peel appears to contain much higher levels of the contaminant than the potato as a whole. The concentrations of coccidiostats in the inner part of the potato were drastically lower. The lasalocid concentration in potato flesh was below the LOD. The concentration of nicarbazin measured in the flesh was above the LOQ but was still 3–10 times lower than the potato samples analyzed as a whole.

Higher concentrations in the outer regions can be explained by the assumption that almost every organic contaminant is incorporated into plants by passive diffusion.[26] With the exception of hormone-like chemicals, there is no evidence of active uptake and transport for xenobiotic chemicals.[27] The peel, which is in direct contact with the soil, will therefore exhibit higher concentrations of these contaminants.

Exposure and Toxicity Assessment for Vegetables. The use of coccidiostats is strictly regulated, and MRLs have been set for several matrices of animal origin. The goal of this study was to assess the public exposure via vegetable consumption. These compounds are used in high amounts and frequency, are likely to contaminate the environment, and have been shown to exert toxic effects in humans.

To assess the risk of the public to the coccidiostat concentrations at the incorporation levels determined in vegetables in this study, the above-mentioned results need to be combined with food consumption data. The resulting exposure data can then be compared to values derived from toxicological studies, e.g., ADIs.

One way to represent food consumption data is to take the 95th percentile consumption of the vegetables considered here. This value can be derived from EFSA's Comprehensive European Food Consumption Database.[28] This database is comprised of several surveys. When the exposure is determined,

the conservative approach is to base the calculations on the survey with the highest consumption. For "starchy roots and tubers", the data consulted were from an Irish survey for adults (8.0 g kg of body weight^{-1} day^{-1}) and a survey from Finland on toddlers (12−35 months of age) (16.9 g kg of body weight^{-1} day^{-1}). For "vegetables and vegetable products", a Spanish survey showed the highest consumption for adults (7.1 g kg of body weight^{-1} day^{-1}) and an Italian study showed the highest consumption on toddlers (21.6 g kg of body weight^{-1} day^{-1}).

By combining the results from our study with the consumption data from EFSA's Comprehensive European Food Consumption Database, we calculated the exposure to coccidiostats by vegetable consumption. When vegetables grown on premix soils are not regarded and, thus, the manure-amended treatment is the sole focus, the exposure that accounts for the largest fraction of the respective ADI (thus, the highest risk) of the vegetable/coccidiostat combinations is monensin in lettuce for toddlers. This exposure to monensin by consumption of lettuce is less than 1% of the corresponding ADI. With the premix treatments (the worst case scenario), additional combinations of coccidiostats and vegetables lead to uptake and higher concentration levels could be seen. For vegetables of the premix treatment, the exposure to monensin via potato for toddlers (95th percentile) is more or less 4 times smaller than its corresponding ADI. However, these high concentrations are unlikely to be encountered outside of this study because these were vegetables grown on soil samples spiked directly with different coccidiostats.

Exposure and Toxicity Assessment for Other Intake Routes. Additional data relevant to a risk assessment were used to compare the exposure of coccidiostats through vegetable consumption with other matrices that contribute to this exposure (meat, eggs, milk, etc.). This approximated the intake of coccidiostats via vegetables and allowed us to compare this to other intake routes and assess the relative risk that vegetables present in the general European diet. This comparison is solely based on data resulting from the manure-amended soils, given the unlikely uptake of coccidiostats in a worst case scenario, such as a premix treatment.

A first approach to assess this relative risk was to use a "daily food basket" as described by the European Medicines Agency Committee for Medicinal Products for Veterinary Use (EMA CVMP).[29] This model is included in EU legislation and is widely used by other European and international risk assessment bodies. EFSA's FEEDAP states that it has limitations, however.[29] It only reflects chronic intake, only addresses adults, fixes a consumption pattern, and assumes that all adults are consumers of each food item. As an alternative, values can be derived from the EFSA Comprehensive European Food Consumption Database representing the high intake (95th percentile) of consumers only for each food item listed in the table and differentiating between chronic and acute intake.

These acute intake values for toddlers and adults (95th percentile) are given in Table 5. Statistical analysis revealed a very low likelihood that the same consumer will choose to eat large amounts of more than two food groups at the same time.[30] For risk assessment, the intake of both consumer groups (adults and toddlers) should be calculated for all food items (Table 5). The sum of the two highest values is then taken as the total intake. Furthermore, if the ADI is based on a pharmacological effect, the acute intake data should be taken for the calculation following the procedure above.

Table 5. Default Values of EU Food Consumption for High Consuming Adults and Toddlers (g/day)

	chronic intake[a]		acute intake[b]	
	toddlers[c]	adults[d]	toddlers	adults
meat[e]	90	290	135	390
liver		60		170
kidney		15		100
animal fat		30		40
milk[f]	1050	1500	1500	2000
eggs	35	70	50	130
honey		30		50
fish	65	125	130	280
seafood		75		200
fish + seafood		165		360

[a]Chronic intake is the 95th percentile of the distribution of average individual consumption levels (over the survey period) for consumers only from all available EU national surveys. [b]Acute intake is the 95th percentile of the distribution of daily consumption levels (all days considered as independent) for consuming days only from all available EU national surveys. [c]Toddlers = 1−3 years of age, 12 kg of body weight. [d]Adults = 18−65 years of age, 60 kg of body weight (presently under consideration by EFSA). [e]Meat including processed meat products. [f]Milk including dairy products.

In Figure 3, the results derived from acute intake values (95th percentile) are given for toddlers and adults and their exposure to monensin and nicarbazin via different intake routes. According to the above-described risk assessment, the total intake is equal to the sum of the two highest values. Vegetables do not belong to the second highest class in any of the situations in Figure 3. The partial exposure via vegetables varies from 1 to 6% of the total intake.

These data indicate that the direct public health risk represented by the intake of coccidiostats via vegetable consumption is minimal. However, the questions of possible allergy and resistance problems still need to be addressed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Photos of the animal and plant trials. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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REFERENCES

(1) Kools, S. A.; Moltmann, J. F.; Knacker, T. Estimating the use of veterinary medicines in the European union. *Regul. Toxicol. Pharmacol.* **2008**, *50* (1), 59−65.

(2) Boxall, A. B.; Fogg, L. A.; Kay, P.; Blackwel, P. A.; Pemberton, E. J.; Croxford, A. Prioritisation of veterinary medicines in the UK environment. *Toxicol. Lett.* **2003**, *142* (3), 207−218.

(3) International Federation for Animal Health (IFAH). *IFAH-Europe Report to the European Commission on the Future of Coccidiostats and Histomonostats*; IFAH: Brussels, Belgium, 2007

(4) European Medicines Agency Committee for Medicinal Products for Veterinary Use (EMA CVMP). *Clazuril Summary Report*; EMA CVMP: London, U.K., 1998; EMEA/MRL/371/98-FINAL.

(5) European Food Safety Authority (EFSA)'s Panel on Additives and Products or Substances used in Animal Feed (FEEDAP).. Scientific opinion on the safety and efficacy of Coxidin® (monensin sodium) as a feed additive for chickens reared for laying. *EFSA J.* **2011**, *9* (12), 2442.

(6) Kools, S. A.; Boxall, A.; Moltmann, J. F.; Bryning, G.; Koschorreck, J.; Knacker, T. A ranking of European veterinary medicines based on environmental risks. *Integr. Environ. Assess. Manage.* **2008**, *4* (4), 399−408.

(7) European Union.. Commission Regulation No. 1831/2003/EC. On additives for use in animal nutrition. *Off. J. Eur. Communities: Legis.* **2003**, 29−43.

(8) Kennedy, D. G.; Hughes, P. J.; Blanchflower, W. J. Ionophore residues in eggs in Northern Ireland: Incidence and cause. *Food Addit. Contam.* **1998**, *15* (5), 535−541.

(9) Vandenberge, V.; Delezie, E.; Huyghebaert, G.; Delahaut, P.; Pierret, G.; De Backer, P.; Croubels, S.; Daeseleire, E. Transfer of the coccidiostats monensin and lasalocid from feed at cross-contamination levels to whole egg, egg white and egg yolk. *Food Addit. Contam., Part A* **2012**, *29* (12), 1881−1892.

(10) Dorne, J. L.; Fernández-Cruz, M. L.; Bertelsen, U.; Renshaw, D. W.; Peltonen, K.; Anadon, A.; Feil, A.; Sanders, P.; Wester, P.; Fink-Gremmels, J. Risk assessment of coccidostatics during feed cross-contamination: Animal and human health aspects. *Toxicol. Appl. Pharmacol.* **2011**.

(11) Juraske, R.; Vivas, C. S.; Velásquez, A. E.; Santos, G. G.; Moreno, M. B.; Gomez, J. D.; Binder, C. R.; Hellweg, S.; Dallos, J. A. Pesticide uptake in potatoes: Model and field experiments. *Environ. Sci. Technol.* **2011**, *45* (2), 651−657.

(12) Boxall, A. B.; Johnson, P.; Smith, E. J.; Sinclair, C. J.; Stutt, E.; Levy, L. S. Uptake of veterinary medicines from soils into plants. *J. Agric. Food Chem.* **2006**, *54* (6), 2288−2297.

(13) Jones-Lepp, T. L.; Sanchez, C. A.; Moy, T.; Kazemi, R. Method development and application to determine potential plant uptake of antibiotics and other drugs in irrigated crop production systems. *J. Agric. Food Chem.* **2010**, *58* (22), 11568−11573.

(14) Dolliver, H.; Kumar, K.; Gupta, S. Sulfamethazine uptake by plants from manure-amended soil. *J. Environ. Qual.* **2007**, *36* (4), 1224−1230.

(15) European Union.. Commission Decision No. 2002/657/EC. *Off. J. Eur. Communities: Legis.* **2002**, 8−36.

(16) Broekaert, N.; Van Peteghem, C.; Daeseleire, E.; Sticker, D.; Van Poucke, C. Development and validation of an UPLC−MS/MS method for the determination of ionophoric and synthetic coccidiostats in vegetables. *Anal. Bioanal. Chem.* **2011**, *401* (10), 3335−3344.

(17) European Food Safety Authority (EFSA)'s Panel on Additives and Products or Substances used in Animal Feed (FEEDAP).. Scientific opinion on the safety and efficacy of Koffogran (nicarbazin) as a feed additive for chickens for fattening. *EFSA J.* **2010**, *8* (3), 1551.

(18) Scientific Committee for Animal Nutrition (SCAN). *Report of the Scientific Committee for Animal Nutrition on the Use of Salinomycin Sodium in Feedingstuffs for Pigs*; SCAN: Brussels, Belgium, 1991.

(19) Trapp, S. Modelling uptake into roots and subsequent translocation of neutral and ionisable organic compounds. *Pest Manage. Sci.* **2000**, *56*, 767−778.

(20) Briggs, G. G.; Bromilow, R. H.; Evans, A. A. Relationship between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pestic. Sci.* **1982**, *13*, 495−504.

(21) Sicbaldi, F.; Sacchi, G. A.; Trevisan, M.; Del Re, A. A. M. Root uptake and xylem translocation of pesticides from different chemical classes. *Pestic. Sci.* **1997**, *50*, 111−119.

(22) Hsu, F. C.; Marxmiller, R. L.; Yang, A. Y. S. Study on root uptake and xylem translocation of cinmethylin and related compounds in detopped soybean roots using a pressure chamber technique. *Plant Physiol.* **1990**, *93*, 1573−1578.

(23) Burken, J. G.; Schnoor, J. L. Predictive relationships for uptake of organic contaminants by hybrid poplar trees. *Environ. Sci. Technol.* **1998**, *32*, 3379−3385.

(24) Wen, Y.; Su, L. M.; Qin, W. C.; He, J.; Fu, L.; Zhang, X. J.; Zhao, Y. H. Linear and non-linear relationships between soil sorption and hydrophobicity. *SAR QSAR Environ. Res.* **2012**, *23* (1−2), 111−123.

(25) Haney, M. E., Jr.; Hoehn, M. M. Monensin, a new biologically active compound. I. Discovery and isolation. *Antimicrob. Agents Chemother.* **1967**, 349−352.

(26) Collins, C.; Fryer, M.; Grosso, A. Plant uptake of non ionic organic chemicals. *Environ. Sci. Technol.* **2006**, *40* (1), 45−52.

(27) Trapp, S. Model for uptake of xenobiotics into plants. In *Plant Contamination: Modelling and Simulation of Organic Chemical Processes*; Trapp, S., McFarlane, J. C., Eds.; Lewis Publishers: Boca Raton, FL, 1995.

(28) European Food Safety Authority (EFSA).. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. *EFSA J.* **2011**, *9* (3), 1970.

(29) European Medicines Agency Committee for Medicinal Products for Veterinary Use (EMA CVMP). *Note for Guidance on the Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin*; EMA CVMP: London, U.K., 2001; EMEA/CVMP/187/00-FINAL.

(30) European Food Safety Authority (EFSA)'s Panel on Additives and Products or Substances used in Animal Feed (FEEDAP).. Guidance for establishing the safety of additives for the consumer. *EFSA J.* **2012**, *10* (1), 2537.