

# Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) in relation to the risk of the presence of sulphonamide residues in eggs resulting from cross-contamination in feed production

## Section of Food Safety and Nutrition

Montaña Cámara Hurtado, María Pilar Conchello Moreno, Álvaro Daschner, Ramón Estruch Riba, Rosa María Giner Pons, María Elena González Fandos, Susana Guix Arnau, Ángeles Jos Gallego, Jordi Mañes Vinuesa, Olga Martín Beloso, María Aránzazu Martínez Caballero, José Alfredo Martínez Hernández, Alfredo Palop Gómez, David Rodríguez Lázaro, Gaspar Ros Berrueto, Carmen Rubio Armendáriz, María José Ruiz Leal, Pau Talens Oliag, Jesús Ángel Santos Buelga, Josep Antoni Tur Marí

## Technical Secretary

Vicente Calderón Pascual

Reference number: AECOSAN-2017-002

Report approved by the Section of Food Safety and Nutrition of the Scientific Committee in its plenary session on 24 May 2017

## Working group

María Aránzazu Martínez Caballero (Coordinator)  
Gaspar Ros Berrueto

## Abstract

Sulphonamides can be administered by adding them to feed within the framework of legal use to treat diseases in animals intended for use in the production of foods, except laying hens. Furthermore, in feed production, cross-contaminations can occur from medicated feed that lead to the appearance of residues of these medicines in animal by-products.

In particular, on some occasions, sulphonamide residues have been detected in eggs resulting from cross-contamination in feed production.

The Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) has assessed the risk to consumer health of the presence of sulphonamide residues in eggs resulting from cross-contamination in feed production. Cases where there was up to 3 % cross-contamination were considered and it was determined that, in this case and based on the disposal model of a residue depletion study in laying hens published in 2015, the estimated daily intake of sulphonamide residues for all foods in the shopping basket including eggs is far lower than the acceptable daily intake established. Therefore, it would not pose a risk to the consumer.

The Scientific Committee holds the opinion that, in any event, good feed manufacturing practice measures must be applied to minimize cross-contamination and the use of antimicrobial medicinal products must be in accordance with Good Manufacturing Practice, reducing the risk of appearance of residues as well as antibacterial resistance.

## Key words

Sulphonamide, residues, feed, cross-contamination.

## 1. Introduction

The use of veterinary medicinal products for therapeutic purposes in the form of medicinal pre-mixes (or premix) for compound feeds intended for animals could pose a risk to animal and human health if they are not subjected to a strictly controlled technological process.

Feed production industries often produce a wide range of compound feeds intended for different species and categories of animals. In this process, certain residual amounts of medicated feeds can be left over in several points throughout the production line, contaminating the resulting batches of feed as they are processed.

It is practically unavoidable that traces of a primary product remain in the production line and are incorporated into the start of production of the following product. This transfer from one production batch to the next is called cross-contamination or carry-over and caused the compound feeds to contain traces of contamination of other substances.

The specific characteristics of all medicinal pre-mixes such as the adhesive strength, particle size and density and electrostatic properties of some active substances, particularly those used in powder form, aggravate the problem of cross-contamination (Anadón, 2009).

The European Commission has proposed a new Regulation of the European Parliament and of the Council on the manufacturing, sale and use of medicated feeds, repealing Council Directive 90/167/EEC (EU, 2014), which states that in order to ensure the safe use of medicated feed, in the case of antimicrobial substances, only 1 % of cross-contamination is permitted.

Pharmaceutical residues in eggs ought not to be a concern since very few medicines are authorised in laying hens. However, these residues can sometimes appear, either because the hens are wrongly medicated or they have been fed with contaminated feeds (cross-contamination).

In order to be able to assess the risk of sulphonamide residues in eggs due to cross-contamination in feed production, data available on pharmacokinetic studies and information relating to the depletion in animal tissues and eggs must be gathered, for the main purpose of finding out or extrapolating the level of each sulphonamide residue in the target tissue of the animal intended for consumption (muscle, liver, kidney and fat) and analyse whether they are sufficiently low so that exposure to the consumer cannot in any case exceed the acceptable daily intake (ADI) of each sulphonamide.

Research on residues in food reveal whether there are cases of:

- illegal sale of veterinary medicinal products,
- the use of medicinal products out with the indications approved (extra-label use) and with improper withdrawal periods, and
- cross-contamination in animal feeds caused by bad manufacturing practice.

The European Food Safety Authority (EFSA) and more specifically the 'Additives and Products of Substances Used in Animal Feeds' Panel (FEEDAP), proposes the maximum residue limits (MRL) for veterinary medicinal products present in tissues intended for human consumptions, a parameter established on the basis of toxicological data, the ADI for men and the residue

depletion data. The MRL for a medicinal product is the marker residue (unaltered compound and/or metabolite) that does not pose any risk to consumer health, which must always be compatible with the ADI value.

## **2. Data relevant for assessing the food safety of residues of veterinary medicinal products**

As a general rule, when the safety for the consumer of residues of veterinary medicinal products in food animal by-products is researched, detailed studies are required, including (FAO/WHO, 1995):

- The identity of the chemical agent and its properties.
- The use and dosage.
- Pharmacokinetic studies, including metabolism and pharmacodynamic studies in laboratory animals, in animals intended for consumption (target-animals) and in humans when available.
- A review of analytical methods to determine the marker residue (unaltered compound and/or metabolite) with a sensitivity  $\leq$  than the MRL value (validated analytical procedures).
- Residues depletion studies in target-animals (from a zero withdrawal period to a time before the recommended withdrawal period).
- Long- and short-term toxicity/carcinogenicity studies, studies on the reproduction and development in test animals and genotoxicity studies.
- Special studies designed to research specific effects, such as toxicity mechanisms, immune responses and covalent DNA binding, among others.
- For compounds with microbial activity, studies designed to assess the possibility that the medicinal product MRL could have an adverse effect on the microbial ecology of the human intestinal tract.
- Studies that provide relevant data on the use and exposure of the medicinal product in humans, including studies on the effects observed after the occupational exposure and epidemiological data in humans after clinical use.

All of this data is fundamental for identifying the danger to public health through establishing the no observed adverse effect level (NOAEL) which, by applying an appropriate safety factor, provides us with the guide value based on health criteria such as the ADI.

## **3. Regulatory framework for sulphonamides**

Sulphonamides, as a group of antimicrobials, are synthetic compounds derived from sulphanilamide, they share a common mode of action but often vary in their chemical characteristics and pharmacokinetic properties (Bishop, 2001). All of the active derivatives have the nucleus para-amino-benzene-sulphonylurea in common. In veterinary medicine, sulphonamides play an important role as efficient medicines for bacterial and protozoan diseases, such as coccidiosis. The antimicrobial activity of sulphonamides increases from their capacity to inhibit the synthesis of folic acid of the microorganism that interferes with the synthesis of

DNA (Botsoglou and Fletouris, 2001). As structural analogues and competitive antagonists of para-aminobenzoic acid (PABA, essential structural element of folic acid), sulphonamides inhibit dihydropteroate synthase, the enzyme that catalyses folic acid synthesis. Sulphonamides inhibit the growth of Gram-positive and Gram-negative bacteria and some species of *Chlamydia*, *Nocardia* and *Actinomyces*. In many cases, to increase their efficiency, sulphonamides are combined with derivatives of diaminopyrimidine, such as trimethoprim and with coccidiostats. In general, sulphonamides are absorbed moderately after oral administration, more quickly in birds than in mammals, they are distributed widely in the tissues, and are biotransformed in the liver through glucuroconjugation or acetylation and are excreted rapidly through the renal route and small amounts through bile and faeces (Frazier et al., 1995) (Botsoglou and Fletouris, 2001). Potential adverse effects in humans include nephrotoxicity, mainly due to the appearance of crystalluria, porphyria and hypersensitivity reactions.

In eggs, sulphonamide residues generally appear and remain longer in the yolk than in the albumen, although initially they are deposited in larger quantity in the albumen (Romvary and Simon, 1992) (Atta and El-zeini, 2001) (Roudaut and Garnier, 2002) (Tansakul et al., 2007). The concentration of sulphonamides in the albumen declines exponentially 24 hours after treatment, but remain in the yolk a lot longer due to the fact that they are deposited in the yolk during different stages of development (Furusawa et al., 1998). However, due to the multiple factors that affect the distribution of sulphonamides in the yolk and albumen, such as pH and/or fat solubility, it is difficult to determine in which part a greater amount of residues may be present (Alaboudi et al., 2013). There is limited pharmacokinetic data regarding sulphonamides in birds. In general, after oral administration, the sulphonamides are distributed widely in the tissues and oral bioavailability is within a range of between 35-80 % (Queralt and Castells, 1985) (Loscher et al., 1990) (Baert et al., 2003). Residues depletion studies suggest that they are slowly eliminated from the eggs (from weeks to months), according to the dose, the route of administration and the duration of treatment (Blom et al., 1975a,b). It has been described that in birds, to obtain residue levels between 10 and 200 µg/kg in eggs, a waiting time or withdrawal period of 7-14 days must be observed (Blom et al., 1975a,b) (Oikawa et al., 1977) (Geertsma et al., 1987). In more recent studies carried out in order to determine sulphamonomethoxine residues in eggs from laying hens treated with a dose of 8 and 12 g/l drinking water using LC-MS/MS (highly sensitive method), levels exceeding the detection limit were detected (1.9 µg/kg at 16-19 days in the yolk and at 37 days in the albumen (Bilandzic et al., 2015)).

Being a very old group of sulphonamide compounds, for most of them there is little toxicological data, mainly genotoxicity and carcinogenicity data. The number of relevant adverse effects described in scientific literature, assessing low levels of exposure of residues, is very little, allergic reactions being the main effects detected in humans, which are not always related to the dose of exposure (Brackett, 2007).

The European Medicines Agency (EMA) has published a list of sulphonamides used in veterinary medicine, in pigs and cattle (sulphachloropyridazine, sulphadiazine, sulphadimethoxine, sulphadimidine, sulphadoxine, sulphaguanidine, sulphamerazine,

sulphamethoxazole, sulphamethoxypyridazine and sulphamonomethoxine) and in chickens for fattening (sulphachloropyridazine, sulphaclozine, sulphadiazine, sulphadimethoxine, sulphadimidine, sulphamethoxazole, sulphamethoxypyridazine, sulphamonomethoxine, sulphaquinoxaline) (EMA, 2016).

Regarding the establishment of the MRLs for sulphonamides in target-tissues of animals intended for use in the production of foods, the Committee of Veterinary Medicinal Products (CVMP) of the EMA considers that a MRL of 100 µg/kg can be applied to all of the pharmaceutical substances of the sulphonamide groups and this maximum limit has been established by Regulation (EC) No 470/2009 (Table 1) (EU, 2009), considering:

- current available toxicological data,
- that the sulphonamide metabolites have the same level of toxicity as the unaltered compound,
- the pharmacokinetic studies currently available, including residue depletion studies,
- that sulphonamide residues can be monitored by HPLC or HPLC/MS-MS with sensitivity lower than the MRL value.

**Table 1.** Maximum residue limits for sulphonamides in the European Union

Pharmacologically active substance	Marker residue	Animal species	MRL	Target tissues	Other provisions (under article 14(7) of Regulation (EC) No 470/2009)	Therapeutic classification
Sulphonamides (all substances that belong to the group of sulphonamides)	Medicinal product	All species intended for food production	100 µg/kg 100 µg/kg 100 µg/kg 100 µg/kg	Muscle Fat Liver Kidney	The total combined residues of all off the substances in the sulphonamide group must not exceed 100 µg/kg. For fish, the MRL in the muscle refers to 'muscle and skin in natural proportions'. The MRLs in the fat, liver and kidney do not apply to fish. They should not be used in animals that produce eggs for human consumption.	Antiinfective/Chemotherapeutic
		Cattle, sheep and goats	100 µg/kg	Milk		

Source: (UE, 2010).

#### **4. Risk assessment of sulphonamides for animal and human health**

There are very limited data on the adverse effects resulting from the use of sulphonamides in non-target animal species and humans. Most come from intoxications in animals and humans. It is known that most sulphonamides produce antithyroid effects in rat, mouse and dog test animals (FAO/WHO, 1961, 1962, 1964, 1965a,b) which generally causes an increase in weight of the thyroids, hyperplasia, loss of colloid and sometimes hyperplasia of thyrotrope cells in the anterior pituitary gland. The antithyroid effects of sulphonamides present identifiable thresholds (FAO/WHO, 1967b); the mechanisms of these effects are the interruption of normal iodine metabolism in the thyroid which reduces the production of thyroxine and increases the secretion of the stimulating pituitary hormone that causes hyperplasia of this gland (FAO/WHO, 1961, 1964). The administration of sulphamethoxazole to rats for prolonged periods causes thyroid carcinoma (FAO/WHO, 1962).

The effects produced by treatment with sulphonamide in the human thyroid function have been described. Sulphamethoxazole (25 mg/kg b.w./day, for 10 days) significantly reduced concentrations of triiodothyronine and bound and free thyroxine in adults. Similar effects were observed with sulphamoxole (12 mg/kg b.w./day, for 10 days) (FAO/WHO, 1968a,b). When it was administered to a group of 49 adult patients, except six adolescents (2-19 years), the sulphamoxole (10 mg) had no effect on the thyroid hormone (FAO/WHO, 1969a). It is admitted that there is little possibility that they present effects on thyroids in human subjects treated with sulphonamides, except when treated with high therapeutic doses. The adverse reactions described in humans are characterised by their haematological effects as well as agranulocytosis and aplastic anaemia on rare occasions (FAO/WHO, 1969b,c, 1970a). Reports on the adverse reactions suggest that sulphamethoxazole, sulphamethizole and sulphamethoxypyridazine are those sulphonamides that presented the adverse effect of aplastic anaemia (in the few cases in which it was presented).

The adverse effects of sulphonamides are mostly hypersensitivity reactions, that are normally manifested in the form of rashes (FAO/WHO, 1970b). They generally start one week after initiating treatment but can appear more quickly in cases of increased sensitivity. Given that there is no evidence that allows the minimum dose that produces these effects to be determined, in order to minimise the appearance of hypersensitivity reaction resulting from the consumption of food of animal origin that contains sulphonamide residues, it is important to monitor residues in food.

#### **5. Risk assessment of the sulphonamide (sulphamethazine)**

Sulphadimidine (or sulphamethazine) is one of the sulphonamides most used for treating a large variety of bacterial diseases in animals and humans; it was also previously used as a growth promotor in food-producing animals, a use which is currently prohibited. The first assessment of this sulphonamide was carried out by JECFA (Joint FAO/WHO Expert Committee on Food Additives) in 1989 when it established a temporary ADI of 0-4 µg/kg b.w./day, based on a NOAEL of 2.2 mg/kg b.w./day for the critical point of follicular cellular hyperplasia in the thyroids of rats, applying a safety factor of 500 (FAO/WHO, 1989). Although complementary studies continued to

be carried out, in a following assessment, in 1991, the same temporary ADI value was maintained (FAO/WHO, 1991).

In 1995, with new genotoxicity, embryotoxicity and teratogenicity studies available, JECFA carried out a reassessment, which is described below (FAO/WHO, 1995).

### 5.1 Toxicological data in animals

*In vitro* and *in vivo* genotoxicity test came out negative.

In a teratogenicity study in rats treated orally with doses of 0, 540, 680 and 860 mg sulphadimidine/kg b.w./day, incidents of visceral abnormalities were observed, identifying a NOAEL of 540 mg/kg b.w./day.

In a similar study on rabbits with doses of up to 1 800 mg sulphadimidine/kg b.w./day, no abnormalities were observed, but with higher doses there were incidents of fetal deaths in litters. The established NOAEL for the embryotoxicity was 1 200 mg/kg b.w./day.

In short-term toxicity studies in rats, a dose of 600 mg sulphonamide/kg b.w./day, produced an increase in the weight of thyroids, decrease in the concentration of tri-iodothyronine (T3) and thyroxine (T4) thyroid hormones, and an increase in the thyroid stimulating hormone (TSH); these changes were accompanied by hypertrophy and hyperplasia of the follicular cells of the thyroids. In these studies, a NOAEL of 5 mg/kg b.w./day was identified.

In another study carried out on pigs, sulphadimidine in doses of 0, 5, 10, 20, or 40 mg/kg b.w./day, produced the same effects, the NOAEL also being 5 mg/kg b.w./day. It was concluded that tumours observed with the highest doses of sulphadimidine are due to an increased hormonal stimulation of the thyroid gland caused by high levels of TSH produced by the sulphadimidine, not by their direct action.

In light of all the information available, JECFA established a ADI of 0-50 µg/kg b.w./day, based on a NOAEL of 5 mg/kg b.w./day, observed in rats and pigs for changes in the morphology of thyroids, and applying a safety factor of 100 (FAO/WHO, 1995).

### 5.2 Toxicological data in humans

Although it is recognised that primates (including humans) are less susceptible to the antithyroid effect of sulphonamides than rats and pigs, JECFA considers the possibility that in the case of sensitivity to sulphonamides, hypersensitivity reactions can appear resulting from the intake of sulphonamide residues in food of animal origin.

### 5.3 Residue data

Only data from the assessment carried out by JECFA are available (FAO/WHO, 1989). Data were obtained from a study carried out by the FDA (Food and Drug Administration) and the USDA (United States Department of Agriculture) in which 110 mg sulphadimidine [<sup>14</sup>C]/kg of food was administered to pigs. The sulphadimidine was rapidly absorbed and eliminated. The concentration of residues was more rapidly reduced when the sulphadimidine was administered intramuscularly than when it was administered in feed or in drinking water. Sulphadimidine was metabolized

in the pig. To three metabolites, namely *N*-acetylsulphadimidine, *N*-glucosesulphadimidine and desaminosulphadimidine. The *N*-acetyl derivative was also observed in farm animals, rodents and humans. Less information is available on the metabolism in other species. The unaltered compound and the three metabolites account for more than 80 % of the extractable residues in the pig at 8 hours withdrawal period; however, as withdrawal period increased to 2, 5 and 10 days, there was a gradual increase in the percentage of non-extractable <sup>14</sup>C-labelled residues. At 10 days withdrawal period, the non-extractable residues marked <sup>14</sup>C were mainly from the unaltered compound and the concentrations were less than 0.06 mg/kg, which corresponds to 38 % in muscle, 76 % in liver, 65 % in kidney and 60 % in fat. Table 2 includes the estimated maximum daily intake of sulphonamide residues based on food intake values. An ADI of 0.004 mg/kg would be equivalent to a daily intake of 0.24 mg of sulphadimidine for a person weighing 60 kg. This value is higher at 8 hours and at 2 days withdrawal period, but not at 5 days withdrawal time or longer. These data confirm that in this study on pigs, to reach an MRL of 100 µg/kg, a long withdrawal period must be observed before the animal is slaughtered.

**Table 2.** Estimated maximum daily intake of sulphadimidine residues in the tissues of pigs treated with 110 mg [<sup>14</sup>C]sulphadimidine/kg of feed

Withdrawal period	Estimated maximum daily intake (mg of parent drug) based on total residues
8 hours	2.43
2 days	0.78
5 days	0.17
10 days	0.01

**Note:** It is assumed that 80 % of the residues are extractable and that 90 % of the extractable residues have a toxicological potency equivalent to that the parent drug.

In milk cows, sulphadimidine is rapidly eliminated after intramuscular injection or intramammary administration. Concentrations of residues in milk decline rapidly and after a waiting time or withdrawal period of 3 days, the levels in milk are less than 0.1 mg/litre (average concentrations reached values less than 0.05 mg/litre).

In birds, which received 1 or 2 g of sulphadimidine/litre of drinking water, for 5 days, sulphadimidine residues were found in eggs:

- on the fifth day of treatment (i.e. at a withdrawal time of zero days), levels of 84 mg/kg were detected,
- at 2 days of withdrawal time, these levels of residues remained,
- at 8-9 days withdrawal period, the levels found were less than 0.1 mg/kg.

#### 5.4 Maximum residue limit

Due to the lack of information on possible reactions of hypersensitivity resulting from the intake of food (of animal origin) containing sulphonamides, the European Union (EU) recommends that the

MRL must be as low as possible, in accordance with good practice and use of veterinary medicinal products, and for this reason, it is recognised that these concentrations fall below the levels considered to be significant from the microbiological point of view. JEFCA and the EU establish for the sulphadimidine value of 100 µg/kg in muscle, liver, kidney and fat, and 25 µg/kg for milk (for milk, the EU 100 µg/kg). The MRL for eggs was not established (FAO/WHO, 1995) (EU, 2010).

## **6. Risk assessment of sulphonamide residues in eggs by cross-contamination**

Given that there is no established MRL of sulphonamide in eggs because its use is not authorised in laying hens because an excessively long waiting time would have to be established which could not be observed from a production and economical point of view, in order to be able to carry out a risk assessment by cross-contamination we can define different scenarios, assuming that we use as a reference the so-called “food basket” or shopping basket intake described in Directive 2001/79 (EU, 2001) and based on the sulphamonomethoxine residue depletion study carried out by Bilandzic et al. (2015).

Analysing the study done on laying hens by Bilandzic et al. (2015), we have:

- Laying hens treated with doses of sulphamonomethoxine of 8 and 12 g/l via drinking water, (648.11 mg/kg of feed and 973.60 mg/kg of feed, respectively) for 7 days, equivalent to 43.6 mg/kg b.w./day and to 65.5 mg/kg b.w./day, respectively.
- Sulphamonomethoxine is determined by LC-MS/MS; the validation criteria of the technique used are the following:
  - LOQ= 7.4 µg/kg; LOD= 1.9 µg/kg; precision <8.5 %; recovery= 94-106 %.
- With a waiting time or withdrawal period of zero, i.e. when treatment was finished, sulphamonomethoxine residues differed between the egg yolk and white:
  - For the egg yolk, values of up to 5 358 µg/kg were reached for a dose of 43.6 mg/kg b.w. and up to 8 101 µg/kg for a dose of 65.5 mg/kg b.w.
  - And for the egg white, values of up to 4 737 µg/kg were reached for a dose of 43.6 mg/kg b.w. and up to 6 018 µg/kg for a dose of 65.5 mg/kg b.w.
- With a waiting time or withdrawal period of 3 days, the sulphamonomethoxine residues in the yolk and the egg white are the following:
  - For the egg yolk, values of up to 6 521 and 7 329 µg/kg were reached for a dose of 43.6 mg/kg b.w. and 65.5 mg/kg b.w., respectively.
  - For the egg white, values of up to 1 370 and 1 539 µg/kg were reached for a dose of 43.6 mg/kg b.w. and 65.5 mg/kg b.w., respectively.
- Concentrations of sulphamonomethoxine in eggs decrease gradually, values below 100 µg/kg being observed between 11 and 13 days of withdrawal for the egg yolk, and between 19 and 22 days for the egg white.
- For the two treatments, concentrations of sulphamonomethoxine decreased to values below the LOQ of 7.4 µg/kg after a waiting or withdrawal period of 16 days in the egg yolk and 25 days in the egg white.

- However, concentrations of sulphamonomethoxine exceeding the LOD (1.9 µg/kg) were detected in the white at 37 days after administration of a dose of 65.5 mg/kg b.w.
- Using a linear regression, it was observed that sulphamonomethoxine residues are retained longer in the white than in the yolk, with a biological half-life of 8.04 and 9.94 days in the white for the two doses studied. The biological half-life for the yolk is 4.58 and 5.37 days.

In conclusion, Bilandzic et al. (2015) demonstrate that both doses cause a prolonged retention of sulphamonomethoxine residues in eggs. This work justifies the prohibited use of sulphamides in laying hens, where the applying a waiting or withdrawal period is not viable.

Using this data from Bilandzic et al. (2015), the potential residues by a cross-contamination of 1 % can be calculated by extrapolation, following the proposal of the new Regulation of the European Parliament and of the Council on the manufacturing, sale and use of medicated feeds, repealing Council Directive 90/167/EEC (EU, 1990), which states that in order to ensure the safe use of medicated feed, in the case of antimicrobial substances, only 1 % of cross-contamination is permitted. However, more recently, the Committee on Agriculture and Rural Development of the European Parliament (AGRI) recommends adopting a limit of 3 % of cross-contamination until EFSA establishes the specific limits for each active ingredient (AGRI, 2016).

### 6.1 Scenario: cross-contamination of 1 % for antimicrobial substances

Based on the experimental work of Bilandzic et al. (2015), 1 % cross-contamination would be equivalent to a dose of 0.655 mg/kg b.w. equivalent to 9.74 mg/kg of feed:

- In a waiting or withdrawal period of zero, for the dose of 65.5 mg/kg p.c. (973.60 mg/kg of feed), the sulphamonomethoxine residues in egg yolk are 8 101 µg/kg (Bilandzic et al., 2015). Using a dose-dependent linear elimination model, we would find that a dose of 9.74 mg/kg of feed (0.655 mg/kg b.w.) (1 % of the dose, coming from cross-contamination), in zero waiting time would cause residual levels of sulphamonomethoxine in egg yolk of 81.01 µg/kg (1 % of 8 101 µg/kg), equivalent to 81.01 µg/day of daily intake (81.01 µg/kg x 0,1 kg, intake of eggs (yolk) in the food basket\*).
- In a waiting or withdrawal period of zero, for the dose of 65.5 mg/kg p.c. (973.60 mg/kg of feed), the sulphamonomethoxine residues in the white or albumen of egg are 6 018 µg/kg (Bilandzic et al., 2015). Using a dose-dependent linear elimination model, we would find that a dose of 9.74 mg/kg of feed (0.655 mg/kg b.w.) (1 % of the dose, coming from cross-contamination), in zero waiting time would cause residual levels of sulphamonomethoxine in egg albumen of 60.18 µg/kg, equivalent to 6.018 µg/day of daily intake (61.08 µg/kg x 0,1 kg, intake of eggs (whites) in the food basket\*).
- The total residues of the estimated daily intake (µg/day) in eggs would be: 8.101 + 6.018= 14.12 µg/day, together with the sum of the rest of the residues of the estimated maximum daily

\*Daily human food intake (corresponding to meat in the form of muscle (0.3 kg), liver (0.1 kg), kidney (0.05 kg), fat (0.05 kg), milk (1.5 kg) and egg (0.1 kg), or “food basket”) (EU, 2001).

intake (food basket\*) we would have:  $87.5 + 14.12 = 101.62 \mu\text{g/day}$ , which corresponds to only 3.38 % of the established ADI of  $3\,000 \mu\text{g/day}$ , which would not pose a risk to humans.

Considering the MRLs ( $\mu\text{g/kg}$ ) set in each one of the tissues of the food basket (muscle 100; liver 100; kidney 100; fat 100; milk 25) the estimated maximum daily intake of sulphamonomethoxine residues, for all of these foods included in the food basket, would be  $87.5 \mu\text{g/day}$  (without counting the intake of egg) and  $101.62 \mu\text{g/day}$  (counting the intake of egg, overestimated taking into account consumption of 0.1 kg white and 0.1 kg yolk). This is significantly less than the established ADI value of  $3\,000 \mu\text{g/day}$  for a person with a body weight of 60 kg and therefore does not pose a risk to humans.

## 6.2 Scenario: cross-contamination of 3 % for antimicrobial substances

Based on the experimental work of Bilandzic et al. (2015), 3 % cross-contamination, would be equivalent to a dose of  $1.965 \text{ mg/kg b.w.}$  equivalent to  $29.21 \text{ mg/kg}$  of feed:

- In a waiting or withdrawal period of zero, for the dose of  $65.5 \text{ mg/kg b.w.}$  ( $973.60 \text{ mg/kg}$  of feed), the sulphamonomethoxine residues in egg yolks are  $8\,101 \mu\text{g/kg}$  (Bilandzic et al., 2015). Using a dose-dependent linear elimination model, we would find that a sulphamonomethoxine dose of  $29.21 \text{ mg/kg}$  of feed ( $1.965 \text{ mg/kg b.w.}$ ) (3 % of the dose, coming from cross-contamination), in zero waiting time would cause residual levels of sulphamonomethoxine in egg yolk of  $243.03 \mu\text{g/kg}$  (3 % of  $8\,101 \mu\text{g/kg}$ ), equivalent to  $24.303 \mu\text{g/day}$  of daily intake ( $243.03 \mu\text{g/kg} \times 0.1 \text{ kg}$ , intake of eggs (yolk) in the food basket\*\*).
- In a waiting or withdrawal period of zero, for the dose of  $65.5 \text{ mg/kg b.w.}$  ( $973.60 \text{ mg/kg}$  of feed), the sulphamonomethoxine residues in the white or albumen of eggs are  $6\,018 \mu\text{g/kg}$  (Bilandzic et al., 2015). Using a dose-dependent linear elimination model, we would find that a sulphamonomethoxine dose of  $29.21 \text{ mg/kg}$  of feed ( $1.965 \text{ mg/kg b.w.}$  or 3 % of the dose, coming from cross-contamination), in zero waiting time would cause residual levels of the medicinal product in egg white of  $180.54 \mu\text{g/kg}$ , equivalent to  $18.054 \mu\text{g/day}$  of daily intake ( $180.54 \mu\text{g/kg} \times 0.1 \text{ kg}$ , intake of eggs (yolk) in the food basket\*\*).
- The total residues of the estimated daily intake ( $\mu\text{g/day}$ ) in eggs would be:  $24.303 + 18.54 = 42.843 \mu\text{g/day}$ , together with the sum of the rest of the residues of the estimated maximum daily intake (food basket\*\*) we would have:  $87.5 + 42.843 = 130.343 \mu\text{g/day}$ , which corresponds to only 4.34 % of the established ADI of  $3\,000 \mu\text{g/day}$ , which would not pose a risk to humans.

Taking into account the MRLs ( $\mu\text{g/kg}$ ) set in each one of the tissues of the food basket (muscle 100; liver 100; kidney 100; fat 100; milk 25) the estimated maximum daily intake of sulphamonomethoxine residues, for all of these foods included in the food basket, would be  $87.5 \mu\text{g/day}$  (without counting the intake of egg) and  $130.343 \mu\text{g/day}$  (counting the intake of egg, overestimated taking into account consumption of 0.1 kg white and 0.1 kg yolk). This is significantly less than the established ADI value of  $3\,000 \mu\text{g/day}$  for a person with a body weight of 60 kg and therefore does not pose a risk to humans.

\*\*Daily human food intake (corresponding to meat in the form of muscle (0.3 kg), liver (0.1 kg), kidney (0.05 kg), fat (0.05 kg), milk (1.5 kg) and egg (0.1 kg), or "food basket") (EU, 2001).

## **Conclusions of the Scientific Committee**

The studies available in the literature on the kinetics and depletion of sulphonamide residues show that high concentrations of sulphonamides can remain in the eggs of laying hens treated with these antimicrobial medicinal products. The study carried out by Bilandzic et al. (2015) on laying hens treated with 973.60 mg of sulphamonomethoxine/kg of feed, shows that at a withdrawal period of zero, sulphamonomethoxine levels in eggs reached values of 8 101 µg/kg in the yolk and 6 018 µg/kg in the white, concentrations that are unacceptable from the point of view of food safety. Considering these studies, the control and monitoring of antimicrobial medicated feeds for non-target species is recommended, especially for laying hens.

Cross-contamination of antimicrobial medicinal products in feed for non-target animal species can produce unacceptable residue levels in edible animal tissues.

The elimination model used to calculate residual levels in eggs of laying hens (Bilandzic et al., 2015), to assess a hypothetical cross-contamination of sulphonamides in feed, revealed that 1 % cross-contamination (equivalent to 9.74 of mg/kg of feed) produced a level of 81.01 µg/kg in egg yolk and 6.018 µg/kg in egg white. The estimated maximum daily intake of sulphonamide residues for all of the foods of the food basket, including egg (yolk and white) is 101.62 µg/day, significantly less than the established ADI value of 3 000 µg/day for a person with a body weight of 60 kg and therefore does not pose a risk to humans.

The elimination model used to calculate residual levels in eggs of laying hens (Bilandzic et al., 2015), to assess a hypothetical cross-contamination of sulphonamides in feed, revealed that 3 % cross-contamination (equivalent to 29.21 of mg/kg of feed) produced a level of 24.303 µg/kg in egg yolk and 18.054 µg/kg in egg white. The estimated maximum daily intake of sulphonamide residues for all of the foods of the food basket, including egg (yolk and white) is 130.343 µg/day, significantly less than the established ADI value of 3 000 µg/day for a person with a body weight of 60 kg and therefore does not pose a risk to humans.

Cross-contamination of sulphonamides for the non-target, food-producing species, the laying hen, up to a level of 3 % that could be equivalent to 29.21 mg sulphamide/kg of feed (a typically used dose of 65.5 mg/kg b.w. equivalent to 973.60 mg/kg of feed) produces an intake of up to 42.843 µg sulphamide/day in eggs, which would not pose as a risk to the consumer.

To control this risk, the proposal of the Regulation of the European Parliament and of the Council on the manufacturing, sale and use of medicated feeds, repealing Council Directive 90/167/EEC establishes that if there are no specific limits transfer by cross-contamination of an active ingredient (as is the case of sulphonamide), for antimicrobial active substances, a limit of 1 % of the active ingredient of the last batch of medicated feed or intermediate product produced before the manufacture of feeds intended for other non-target animals shall be applied.

The Scientific Committee holds the opinion that, in any event, good manufacturing practice measures must be applied to minimize cross-contamination and the use of antimicrobial medicinal products must be in accordance with Good Manufacturing Practice, reducing the risk of appearance of residues as well as antibacterial resistance.

## References

- AGRI (2016). Committee on Agriculture and Rural Development. 2014/0255(COD)-05/04/2016 Committee report tabled for plenary, 1st reading/single reading. Available at: <http://www.europarl.europa.eu/oeil/popups/summary.do?id=1430343&t=e&l=en> [accessed: 9-05-17].
- Alaboudi, A., Basha, E.A. and Musallam, I. (2013). Chlortetracycline and sulfanilamide residues in table eggs: prevalence, distribution between yolk and white and effect of refrigeration and heat treatment. *Food Control*, 33, pp: 281-286.
- Anadón, A. (2009). Seguridad alimentaria en piensos y su repercusión en la cadena alimentaria. Capítulo 7. In book: *Seguridad Alimentaria e Higiene de los Alimentos*. Instituto Tomás Pascual Sanz para la Nutrición y la Salud. pp: 147-157.
- Atta, A.H. and El-zeni, S.A. (2001). Depletion of trimethoprim and sulphadiazine from eggs of laying hens receiving trimethoprim/sulphadiazine combination. *Food Control*, 12, pp: 269-274.
- Baert, K., De Baere, S., Croubels, S. and De Backer, P. (2003). Pharmacokinetics and oral bioavailability of sulfadiazine and trimethoprim in broiler chickens. *Veterinary Research Communications*, 27, pp: 301-309.
- Bilandzic, N., Bozic, D., Kolanovic, B.S., Varenina, I., Cvetnic, L. and Cvetnic, Z. (2015). Distribution of sulfamonomethoxine and trimethoprim in egg yolk and white. *Food Chemistry*, 178, pp: 32-37.
- Bishop, Y. (2001). In book: *The Veterinary Formulary*, 5th ed., Pharmaceutical Press, London.
- Blom, L. (1975a). Plasma half-lives and the excretion into egg-white and -yolk of three sulphonamides and pyrimethamine after medication of laying hens. *Acta Pharmacologica et Toxicologica*, 37, pp: 79-93.
- Blom, L. (1975b). Residues of drugs in eggs after medication of laying hens for eight days. *Acta Veterinaria Scandinavica*, 16, pp: 396-404.
- Botsoglou, N.A. and Fletouris, D.J. (2001). In book: *Drug Residues in Food*. Marcel Dekker, Inc., New York. pp: 692.
- Brackett, C.C. (2007). Sulfonamide allergy and cross-reactivity. *Current Allergy and Asthma Reports*, 7 (1), pp: 41-48.
- EMA (2016). European Medicines Agency. Defined daily doses for animals (DDDvet) and defined course doses for animals (DCDvet). EMA/224954/2016. Available at: [http://www.ema.europa.eu/ema/pages/includes/document/open\\_document.jsp?webContentId=WC500205410](http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500205410) [accessed: 9-05-17].
- EU (2001). Commission Directive 2001/79/EC of 17 September 2001 amending Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition. OJ L 267 of 6 October 2001, pp: 1-26.
- EU (2009). Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council. OJ L 152 of 16 June 2009, pp: 11-22.
- EU (2010). Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 51 of 20 January 2010, pp: 1-63.
- EU (2014). Proposal for a Regulation of the European Parliament and of the Council on the manufacture, placing on the market and use of medicated feed and repealing Council Directive 90/167/EE, 2014/0255 (COD).
- FAO/WHO (1961). Food and Agriculture Organization/World Health Organization. Evaluation of the carcinogenic hazards of food additives. Fifth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 220. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- FAO/WHO (1962) Food and Agriculture Organization/World Health Organization. Evaluation of the toxicity of a number of antimicrobials and antioxidants. Sixth report of the Joint FAO/WHO Expert Committee on Food

- Additives. WHO Technical Report Series, No 228. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- FAO/WHO (1964). Food and Agriculture Organization/World Health Organization. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents. Seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 281. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- FAO/WHO (1965a). Food and Agriculture Organization/World Health Organization. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants. Eighth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 309. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- FAO/WHO (1965b). Food and Agriculture Organization/World Health Organization. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings. Report Series No 38A. WHO/Food Add./24.65.
- FAO/WHO (1966a). Food and Agriculture Organization/World Health Organization. Specifications for identity and purity and toxicological evaluation of some food colours, FAO Nutrition Meetings. Report Series No 38B. WHO/Food Add/66.25.
- FAO/WHO (1966b). Food and Agriculture Organization/World Health Organization. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases. Ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 339. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- FAO/WHO (1967a). Food and Agriculture Organization/World Health Organization. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No 40A,B,C. WHO/Food Add/67.29.
- FAO/WHO (1967b). Food and Agriculture Organization/World Health Organization. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances. Tenth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 373. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- FAO/WHO (1968a). Food and Agriculture Organization/World Health Organization. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents. Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 383. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- FAO/WHO (1968b). Food and Agriculture Organization/World Health Organization. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No 44A. WHO/Food Add/68.33.
- FAO/WHO (1969a). Food and Agriculture Organization/World Health Organization. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No 44B. WHO/Food Add/69.31.
- FAO/WHO (1969b). Food and Agriculture Organization/World Health Organization. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics. Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 430. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].

- FAO/WHO (1969c). Food and Agriculture Organization/World Health Organization. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Report Series, No 45A. WHO/Food Add/69.34.
- FAO/WHO (1970a). Food and Agriculture Organization/World Health Organization. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 445. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- FAO/WHO (1970b). Food and Agriculture Organization/World Health Organization. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No 46A. WHO/Food Add/70.36.
- FAO/WHO (1989). Food and Agriculture Organization/World Health Organization. Evaluation of certain veterinary drug residues in food. Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 788. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- FAO/WHO (1991). Food and Agriculture Organization/World Health Organization. Evaluation of certain veterinary drug residues in food. Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 815. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- FAO/WHO (1995). Food and Agriculture Organization/World Health Organization. Evaluation of certain veterinary drug residues in food. Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 851. Available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/> [accessed: 9-05-17].
- Frazier, D.L., Jones, M.P. and Orosz, S.E. (1995). Pharmacokinetic considerations of the renal system in birds: Part II. Review of drugs excreted by renal pathways. *Journal of Avian Medicine and Surgery*, 9, pp: 104-121.
- Furusawa, N., Tsuzukida, Y. and Yamaguchi, H. (1998). Decreasing profile of residual sulphaquinolaxine in eggs. *British Poultry Science*, 39, pp: 241-244.
- Geertsma, M.F., Nouws, J.F.M., Grondel, J.L., Aerts, M.M.L., Vree, T.B. and Kan, C.A. (1987). Residues of sulphadimidine and its metabolites in eggs following oral sulphadimidine medication of hens. *The Veterinary Quarterly*, 9, pp: 67-75.
- Loscher, W., Fassbender, C.P., Weissing, M. and Kietzmann, M. (1990). Drug plasma levels following administration of trimethoprim and sulfonamide combinations to broilers. *Journal of Veterinary Pharmacology and Therapeutics*, 13, pp: 309-319.
- Oikawa, H., Nakamoto, K., Hirota, K. and Katagiri, K. (1977). Clearance of sulfamethoxazole in eggs and tissues of chickens. *Poultry Science*, 56, pp: 813-821.
- Queralt, J. and Castells, I. (1985). Pharmacokinetics of sulfamethoxazole and trimethoprim association in hens. *Poultry Science*, 64, pp: 2362-2367.
- Romvary, A. and Simon, F. (1992). Sulfonamide residues in eggs. *Acta Veterinaria Hungarica*, 40, pp: 99-106.
- Roudaut, B. and Garnier, M. (2002). Sulphonamide residues in eggs following drug administration via the drinking water. *Food Additives and Contaminants*, 19, pp: 373-378.
- Tansakul, N., Niedorf, F. and Kietzmann, M. (2007). A sulfadimidine model to evaluate pharmacokinetics and residues at various concentrations in laying hen. *Food Additives and Contaminants*, 24, pp: 598-604.