Environmental assessment for Florfenicol FP 10%

Evaluarea riscului pentru mediu a produsului Florfenicol FP 10%

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Abstract

Florfenicol is a 3-fluoro derivative of thiamphenicol. The formulation of Florfenicol FP 10% - oral solution for poultry and pigs, consists of 10% Florfenicol, N-methyl-2-pyrrolidone, propylene glycol and polyethylene glycol 200. The excipients in the formulation will not affect the toxicity or environmental persistence of florfenicol. This description provides a background to determining the true environmental impact of florfenicol in natural environments. A preliminary assessment was made following the Phase I decision tree and the calculated concentration of florfenicol released in the medium as outlined in EMEA (European Medicines Agency), Revised guideline on environmental impact assessment for veterinary medicinal products (CVMP), EMEA/CVMP/ERA/418282/2005-Rev.1). Since direct release into the environment was greater than 100 µg / kg, an environmental risk assessment, Phase II, Steps A and B, was required. This assessment includes taking into account the physicochemical properties of florfenicol, environmental impact studies, acute and chronic environmental effects. Information of florfenicol is used to calculate the Predicted Environmental Concentrations (PECs), the Predicted No Effect Concentrations (PNECs). PECsoil values and PNECs for aquatic and terrestrial organisms were calculated for determination of environmental risk (RQ). Since RQ was greater than 1 for all treated species and zootechnical categories, has passed to the environmental risk assessment of step B. The obtained results confirm that Florfenicol FP 10% does not pose a risk to the environment.

Rezumat

Florfenicolul) este derivatul 3-fluor al tiamfenicolului. Produsul Florfenicol FP 10% - solutie orala pentru galinaceae si suine contine florfenicol, 100 mg/ml si excipientii N-metil-2-pirolidona, propilenglicol si polietilenglicol 200. Excipientii din compozitia Florfenicol FP 10% nu afecteaza toxicitatea sau persistenta in mediu a florfenicolului. Această lucrare oferă un suport pentru determinarea adevăratului impact ecologic al florfenicolului în mediu.. A fost făcută o evaluare preliminară conform arborelui decizional de fază I si nivelului concentratiei calculate de florfenicol eliberat in mediu (Agenția Europeană pentru Medicamente - Ghidul revizuit privind evaluarea impactului asupra mediului pentru medicamentele de uz veterinar (CVMP), EMEA / CVMP / ERA / 418282/2005-Rev.1). Deoarece eliberarea directa în mediu a fost mai mare de 100 µg/kg, a fost necesară o evaluare a riscului pentru mediu, faza a II-a,,treptele A si B. Aceasta evaluare include luarea în considerare a proprietăților fizico-chimice ale florfenicolului, studiile privind impactul asupra mediului înconjurător, efecte acute si cronice asupra mediului. Informațiile despre florfenicol au fost folosite pentru a calcula Concentrațiile predictibile de mediu (PEC), concentrațiile predictibile fără efect (PNEC). Valorile PECsoil si PNEC-urile pentru organismele acvatice si terestre. au fost calculate pentru determinarea riscului de mediu, RQ. Deoarece RQ a fost mai mare decat 1 pentru toate speciile si categoriile zootehnice tratate s-a trecut la evaluarea riscului de mediu treapta B. Rezultatele obtinute confirma ca produsul Florfenicol FP 10% nu prezinta risc pentru mediu.

1. Introduction

Florfenicol (CAS RN 73231-34-2) is the 3-fluoro derivative of thiamphenicol, which is a chloramphenicol analogue in which the p-nitro group on the aromatic ring is substituted with a sulfonylmethyl group (figure 1). Florfenicol is a thiamphenicol derivative. In the fluorophenicol molecule, the hydroxyl group is substituted with a fluorine atom, which results in resistance in the production of bacterial acetyl transferases [5].

Florfenicol is also active against chloramphenicol-resistant bacteria. Florfenicol is a broad spectrum antibiotic that inhibits protein synthesis in the bacterial cell [5, 6].

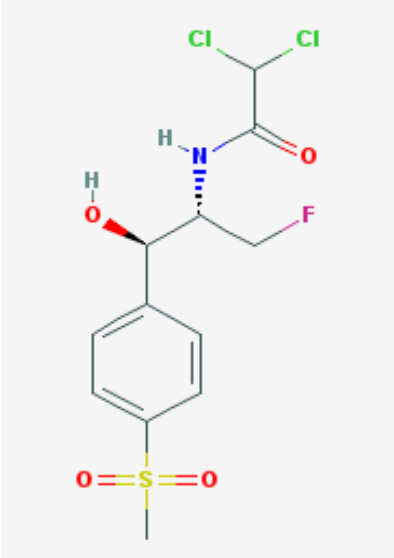

Figure 1. Structure of florfenicol [20].

In the protoplasm, fluorophenol binds to the ribosomal 70S subunit, disrupting the peptidyl transferase enzyme activity. This determines inhibition of protein synthesis from susceptible bacterial cell ribosomes [5].

Florfenicol has a bacteriostatic effect on a wide range of Gram-positive and Gram negative bacteria like:

Pasteurella multocida,
P. haemolytica,
Actinobacillus pleuropneumoniae,
Bordetella bronchiseptica,
Salmonella spp.,
Escherichia coli,
Haemophilus spp.,
Proteus spp.,
Staphylococcus spp.,
Streptococcus spp.,
Shigella spp.,
Klebsiella spp.,
Enterobacter spp., and alike.

After the oral administration, florfenicol is rapidly absorbed and very well distributed in the body High concentrations of active substance reach the lung, kidneys and bile [5]. Important levels are also achieved in the muscles, intestinal tract, cord, liver, spleen and blood serum. Florfenicol suffers a partial biotransformation process in the body; half of the administered dose is removed from the body in its initial form [5].

The pharmacokinetics of florfenicol has been studied in swine treated with oral and intramuscular doses of 15 mg / kg [16].

The bioavailability of florfenicol was similar for oral and intramuscular doses. Florfenicol was rapidly absorbed from the feed and its plasma concentration remained between 2 and 6 µg / ml (over the minimum inhibitory concentration values for pig pathogens) during the three study days [16].

2. Environmental risk assessment of product Florfenicol FP 10% - Phase I

A preliminary assessment was made following the Phase I decision tree - Florfenicol FP 10% :

- is not exempt from regulation;
- is not a natural product;
- is used in animals of economic interest (swine and birds);
- is not intended for use in minor species raised and treated similar to the major species for which an EIA already exists;
- will be used to treat whole systems (not isolated individuals);
- is extensively metabolised to the treated animal;
- will be used to treat terrestrial animals;
- is not an ecto- or endo- parasiticide, is an antibiotic;
- the penetration of the active substance into the terrestrial environment is not prevented;
- animals are grown in intensive systems;
- the PECsol concentration for birds and swine exceeds the limit value of 100 µg / kgbw and further phase II environmental impact assessment is

The product Florfenicol FP 10% oral solution (a Pasteur Romania product) is administered orally, individually or collectively, in drinking water at different doses depending on the

condition and species, required; for hens (broilers, layer replacement) and pigs as follows:

Figure 2. Florfenicol FP 10% oral solution for poultry and swine [INC Pasteur, Romania]

Swine: 1.5 - 2 mg active substance / kg body weight / day for 7 days.

Chickens: (broilers, replacement layer): 20 mg active substance / kg body weight / day for 5 days.

Calculation of the initial PECsol initial for intensively reared animals is dependent on the amount of manure containing the active residue of florfenicol and which can be spread onto land. The nitrogen load of 170 N / ha is the maximum load accepted in EU countries, according to the EUROSTAT database [8].

The PECsoil initial should be calculated using the following equation:

$$PEC_{sol\ iniţial} = \frac{DxAdxBWxPx170xFh}{1500x10000x0{,}05xNyxH} x1000,$$

where:

- PECsoil initial = Predicted Environmental Concentration in soil [$\mu g.kg^{-1}$]
- D = Daily dose of the active ingredient [$mg.kg_{bw}^{-1}.d^{-1}$]
- Ad = Number of days of treatment [d]
- BW = Animal body weight [kg_{bw}] see Table 3
- P = Animal turnover rate per place per year [$place^{-1}.y^{-1}$] see Table 3.
- 170 = EU nitrogen spreading limit [$kg\ N.ha^{-1}$]
- Fh = Fraction of herd treated [value between 0 and 1] see Table 2
- 1500 = Bulk density of dry soil [$kg.m^{-3}$]
- 10000 = Area of 1 hectare [$m^2.ha^{-1}$]
- 0.05 = Depth of penetration into soil [m]
- Ny = Nitrogen produced in one year per place [$kg.N.place^{-1}.y^{-1}$] see Table 3
- H = Housing factor either 1 for animals housed throughout the year or 0.5 for animals housed for only 6 months see Table 3
- 1000 = Conversion factor [1000 $\mu g.mg^{-1}$]

* see EMEA/CVMP/ERA/418282/2005-Rev.1 [7].

Applying this formula, the initial PECsol is calculated for each target species and technological category.

The results for each species were:

PEC soil initial laying hen = 103.619 µg/kg
PEC soil initial replacement = 196.444 µg/kg
PEC soil initial broiler = 886.956 µg/kg
PEC soil initial fattening pig = 82.506 µg/kg
PEC soil initial weaner pig = 121.644 µg/kg
PEC soil initial sow = 29.292 µg/kg

3. Phase II -Tier A Assessment

Phase II assesses the potential of 10% Florfenicol FP to affect non-target species in the environment based on:

- the physicochemical properties of florfenicol,
- studies on the environmental impact,
- acute and chronic effects on the environment,
- the potential of Florfenicol FP 10% to affect non-target species in the environment (figure 3).

Figure 3 Risk Assessment Consistent with World Environment Day [19]

It is not possible to assess the effects of the product on each species in the environment that could be exposed to the product after its administration to the target species. Taxonomic levels tested are intended to serve as surrogates or indicators for the range of species present in the environment [3, 4]. A two-step approach to environmental risk assessment is used.

Physical and chemical characteristics (water solubility, polarity, bonding and adsorption capabilities, photo stability and

biodegradability) of the active substance are very important when considering fate and its transport to the environment. Active substances or metabolites that are soluble in water are more likely to reach surface waters and groundwater [3, 4].

Florfenicol has a low molecular weight, as do its metabolites, which range from 69 to 89% of parent mass. The parent and metabolite solubilities and Kow values differ [11, 12] (figure 4).

The metabolites are markedly more soluble (with solubilities ranging from 49.7 to >500 g/L) and are markedly less lipophilic (i.e. have lower Kow). Theoretically, these factors make the metabolites even more likely than florfenicol to enter and remain in water relative to sediment and not to bioaccumulate in biota [2]. In addition, florfenicol is a non-volatile solid, has an ultraviolet (UV) light absorption maximum at 224 nanometers (nm), and has a melting point of 153–154℃ after the The Merck Index [21].

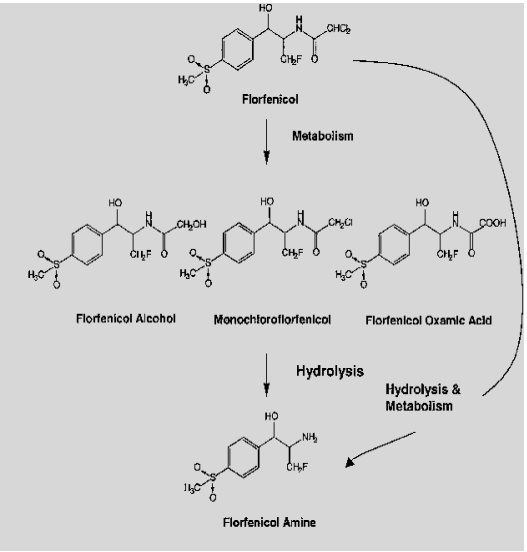

Figure 4. Routes of metabolism of florfenicol

Studies on the susceptibility of florfenicol and its metabolites to photolysis and hydrolysis indicate that these mechanisms are unlikely to play a major role in the degradation of these compounds in the environment [7].

Florfenicol has a partial biotransformation process in the body; half of the administered dose is removed from the body in its initial form [17] (figure 5).

The metabolites of florfenicol, which are amine-metabolite, alcohol-metabolite and oxamic acid-metabolite, ranges from 69 to 89% of parent weight. Metabolites (amine, oxamic acid and alcohol) have very little antimicrobial activity [7, 17, 18].

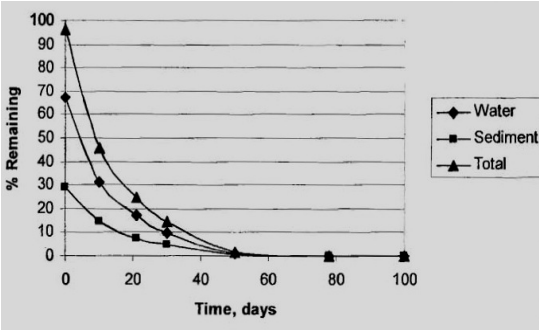

Figure 5. Graphical representation of degradation of florfenicol in sediment and water during the aerobic transformation study [8].

Florfenicol degraded to smaller more polar metabolites which were not persistent. Metabolites were observed to degrade at similar or faster rates than the parent.

The only metabolite collected above 10% was the monochloroflorfenicol labeled with a retention time of 18.4 min. [20].

Degradation of florfenicol in three different sediment-water systems [4, 7]:

Source	Sediment type	% Organic carbon	Degrdation rates for sediment / water Systems (days)		K_d	K_{oc}
			DT_{50}	DT_{90}		
Marine	Loam	3.2	13.0	43.1	0.293	9.1
Freshwater	Loam	2.4	8.4	27.8	0.434	18.1
Freshwater	Sand	0.76	19.4	64.5	0.250	32.9

Photolytic half-lives of florfenicol and its major metabolites:

pH	Florfenicol	Metabolites		
		Amine	Alcool	Acid oxamic
SPAH Code No.	SCH25298	SCH 40458	SCH 45705	SCH 48057
Ph 5	-	-	22.1 d	24.5 d
Ph 7	-	41.2 d	21.0 d	47.9 d
Ph 9	94.8 d	51.4 d	22.8 d	23.9 d
Synthetic humic water	196	NA	NA	NA
Pure water	171	NA	NA	NA

Studies on the susceptibility of florfenicol and its metabolites to photolysis and hydrolysis indicate that these mechanisms are unlikely to play a major role in the degradation of these compounds in the environment [9, 10]. Sorption / desorption characteristics of florfenicol and major metabolites determined in three soil types with $CaCl_2$ [6]:

Parameter	Florfenicol	Metabolites		
		Amine	Alcohol	Oxamic acid
SPAH Code No.	SCH25298	SCH 40458	SCH 45705	SCH 48057
% Sorbed	2-10	23.9-39.9	1.3-8.2	7.5-43
% Desorbed	79-93	86.3-99.8	85.6-161	65-172
Kd	0.07-0.59	1.56-3.35	0.07-0.45	0.41-3.78
Koc range (Geometric mean)	10-27 (18.38)	162-241 (202.28)	7-76.5 (20.16)	36.4-642 (130.40)
Mobilitaty	Very mobile to mobile	Moderately mobile	Very mobile to moderately mobile	Mobile to slightly mobile

The four studies listed lower show **rapid degradation** under different experimental conditions with DT50s (half-lives) ranging from 1.0 to 27.2 days. The mean value of 13.6 days for the sediment / water study is used as the half-life for estimating degradation in water and solids [13, 14]. This is the most appropriate set of experimental conditions for making an estimation of degradation of florfenicol in uneaten feed and excreta from aquaculture facilities. Results of degradation studies:

Principal studies	Matrice / Sistem	Timp de injumătatire (DT_{50}) în zile
Aerobic biodegradation in Manure-Amended Soil	Manure amended soil	3.6 to 27.2
Aerobic degradation in cow manure slumy	Cow manure slury system	2.4 (florfenicol) 3.0 (monochloroflorfenicol metabolite)
Determination of the aerobic transformation of [^{14}C] -Florfenicol in aquatic sediment systems	Sediment / water systems	13.61 (range 8.4 - 19.4)
Anaerobic degradation in pig manure slumy	Pig manure slumy system	1.0 (florfenicol) 2.4 (monochloroflorfenicol metabolite).

Toxicity

The environmental risk assessment includes data on the acute and chronic effects of florfenicol and its metabolites, where known, for microorganisms, fish, aquatic invertebrates and terrestrial and aquatic and terrestrial plants [1, 4, 9-14].

The data are then used to calculate the PNECs for each species. Florfenicol exhibits activity against a wide spectrum of prokaryotic microorganisms with minimum inhibitory concentration (MIC) values ranging from 0.25 mg/L for *Pasteurella multocida* to >1,000 mg/L for *Trichoderma viridae* and *Aspergillus niger* [21].

The MIC of florfenicol was determined to be 65 and 2.5 mg a.i./L for *Nitrobacter* sp. and *Nitrosomonas europaea*, respectively [8].

It is notable that *Anabaena flos-aquae* was more sensitive than the other species. This is not unexpected, as A. flos-aquae is

more appropriately classified with the cyanobacteria rather than the green algae and other aquatic plants, and florfenicol is an anti-bacterial compound [8].

The acute toxicity of florfenicol and its major metabolites was determined for two freshwater species, rainbow trout (*Oncorhynchus mykiss*) and bluegill sunfish (*Lepomis macrochirus*), in GLP studies conducted under static conditions following FDA Guidance 4.11 (Freshwater Fish Acute Toxicity) [8].

The results indicate that florfenicol is not toxic to either freshwater fish species with LC_{50} values >780 and >830 mg/L, respectively.

While the metabolites were not tested at the same concentrations, no mortalities were observed in either species when exposed to concentrations up to 20, 15, and 25 mg/L in the case of the amine, alcohol, and oxamic acid metabolites, respectively.

The data support the concept that neither florfenicol nor its degradation products are likely to cause toxic effects to fish species which may be exposed at estimated environmental concentrations (i.e., PECs) [11,12].

Florfenicol was found to have a transient effect on the microbial transformation of nitrogen when added to soils at concentrations of 0.1, 0.5, and 2.5 mg/kg [4] in the Soil Microorganisms: Nitrogen Transformation Test While the nitrate concentrations were similar to those in controls throughout the study, the ammonium levels rose significantly in soils treated at 0.5 and 2.5 mg/kg, before the rates returned to the control level by Day 28. Florfenicol does not pose a risk to the environment and is considered safe for use in the treatment of PAC (*Piaractus mesopotamicus*) due to low acute toxicity and RQ <1 [3].

Experimental studies on the absorption of florfenicol in the roots of carrot and lettuce leaves showed the presence thereof in concentrations representing about 10% of the daily allowable values (ADI), which indicates that there is no evidence of appreciable risk [2].

Data on florfenicol toxicity to plants shows that the lowest concentration at which 50% of its maximum effect is observed, the lowest EC50 (0.25 mg / kg dry soil) was found for B. napus biomass. The lowest NOEC values (<0.06 mg / kg dry soil) were found for S. lycopersicum [4].

Florfenicol has a molecular weight of 358.21, a water solubility of 1.32 grams per liter (g / l) at pH 7 and an octanol-water partition coefficient (log Kow) of 0.37, the latter indicating a low bioaccumulation potential according to criteria presented in Phase II of VICH / CVM where substances with a log Kow <4.0 are not considered to be bioaccumulative.

PNECs for fish species, invertebrates and aquatic species (Tier A) [3, 10, 12]:

Species	EC_{50} sau LC_{50} (mg/l)	AF	PNEC (mg/l)
Oncorhynchus mykiss	>780	100	**7.8**
Lepomis macrochirus	>830	100	**8.3**
Daphnia magna	>330	100	**3.3**
Navicula pelliculosa	61	10	**6.1**
Pseudokirchneriella subcapitata	1	10	**0.1**
Lemna gibba	0.76	10	**0.076**
Anabaena flos-aquae	0.23	10	**0.023**

PNECs for invertebrates and terrestrial plants (Tier A) [1, 9,13]**:**

Specia	EC_{50} / NOEC (mg/kg)	AF	PNEC (mg/kg)
Earthworm NOEC reproduction	1.56	10	**0.156**
Cress EC50 weight	0.5	100	**0.005**
Mustard EC50 weight	1.7	100	**0.017**
Wheat EC50 weight	6.7	100	**0.067**
Cress EC50 weight	>1	100	**>0.01**
Cabbage EC50 weight	0.859	100	**0.009**
Mustard EC50 weight	0.705	100	**0.007**

For terrestrial plant studies, the most sensitive toxic result for a particular species is used in hazard characterization.

PNECwater = PNEC $_{Anabaena\ flos\text{-}aquae}$ = 0,023 mg/l
PNEC$_{soil}$ = (0,1176 + 0,01764 x K_{oc}) x PNEC$_{water}$ [16]
PNEC$_{soil}$ = (0,1176 + 0,01764 x 18,38) x 0,023 = 0,01016mg/l
PNEC$_{sed}$ = (0,783 + 0,0217 x K_{oc}) x PNEC$_{water}$
PNEC$_{sed}$ = (0,783 + 0,0217 x 18,38) x 0,023 = 0,02718mg/ml

Risk characterization [5]:

Compartiment	PEC$_{sol\ purcei}$ (mg/kg, mg/l)	PNEC (mg/L)	RQ (PEC/ PNEC)
Soil	0,121	0,01016	RQ_{sol} = 11,9
Ground water	0,100	0,023	$RQ_{apa\ subt}$ = 4,34
Surface water	0,091	0,023	$RQ_{apasupraf}$ =3,95
Sediment	0,230	0,02718	$RQ_{sediment}$ = 8,46

Compartiment	$PEC_{sol\ broileri}$ (mg/kg, mg/l)	PNEC (mg/L)	RQ (PEC/ PNEC)
Soil	0,886	0,01016	RQ_{sol} = 87,2
Ground water	0,736	0,023	$RQ_{apa\ subt}$ = 32
Surface water	0,667	0,023	$RQ_{apasupraf}$ = 29
Sediment	1,681	0,02718	$RQ_{sediment}$=61,84

Rafinement risk characterization [5,14,15]:

Criteriu de perfectionare	PEC pig soil rafinement mg/kg	PNEC, mg/kg	$RQ_{perfectionat}$ (PEC / PNEC)
Metabolism	0,060	0,01016	5,90
Degradation in manure	0,019	0,01016	1,87
Degradation in soil	0,121	0,01016	11,9

Criteriu de perfectionare	PEC broiler sol rafinement mg/kg	PNEC, mg/kg	$RQ_{perfectionat}$ (PEC/ PNEC)
Metabolism	0,443	0,01016	43,6
Degradation in manure	0,182	0,01016	17,9
Degradation in soil	0,887	0,01016	87,3

RQ> 1, the Tier B risk assessment is performed (the effects determined in the long-term exposures).

4. Phase II - Tier B Assessment

The Tier B risk characterization considers the effects determined in long-term exposures, typically regarded as chronic effects, upon the aquatic and terrestrial receptors. The data on chronic effects are used with standard assessment factors from the VICH/CVM guidance to determine the PNECs [7].

The algal and cyanobacterial growth inhibition studies that were conducted [11] can be used to assess both acute and chronic effects, although different test endpoints and assessment factors are used in Tier B (chronic effects) as compared to Tier A (acute effects). For the invertebrates, data from a *Daphnia* life-cycle study [12], a rotifer reproduction study, and a 28-day benthic midge study [3] are available. For fish, an early life-stage study provides data for Tier B assessment [1, 4, 9, 13].

5. Conclusions

- Florfenicol is unlikely to degrade by hydrolysis or photolysis and has a low tendency to sorbed in the soil.
- The degradation of florfenicol and the monochloro metabolite is rapid in soil, sediment / water systems, aerobic and anaerobic digestion.
- Bacteria and cyanobacteria are the most sensitive organisms which is not unexpected given the antibacterial activity of florfenicol.
- Aquatic plants (algae) are an additional group of organisms that are relatively sensitive to florfenicol.
- The available data indicate that florfenicol was algistatic, and not algicidal, meaning that populations of algae were inhibited but not killed.
- PNEC values presented for cyanobacteria and algae are based on inhibition of growth, not mortality.
- Thus it can be expected that when the stressor is removed, populations that were inhibited from growth in the presence of the stressor are able to recover.
- Florfenicol does not pose a risk to the environment and is considered safe for use.

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