Divergent position on a revised CVMP opinion on an Article 35 referral for

HIPRALONA ENRO-S and its generics indicated for use in rabbits

Enrofloxacin: How it works.

Active substance: HIPRALONA ENRO-S and its generics contain, as an active substance, enrofloxacin, a fluoroquinolone carboxylic acid derivative. Fluoroquinolones are concentration-dependent antimicrobials that are part of the quinolone group of synthetic antibiotics. It is the halogenated chemical groups (fluorine) that, when compared to quinolones, confer the increased effectiveness of enrofloxacin. This entails a broader spectrum of bactericidal activity across Gram + and Gram – bacterial types, including pathogenic bacteria causing animal disease, but also commensal bacteria within the same treated animals; this combined effect encompasses a high selection pressure in terms of exposure and dissemination of antibiotic resistance.

Pharmaceutical form: HIPRALONA ENRO-S and its generics are administered via drinking water to rabbits for five (5) days. The withdrawal period for meat and offal is two (2) days.

Rabbit production: Rearing conditions which contribute to a high risk of resistance selection, development, and dissemination

Rabbits exhibit natural coprophagic behavior and at the physiological level, this contributes to a caecotrophic effect since rabbits are hindgut fermenters. In terms of antimicrobial resistance pressure, this normal behavior is expected to contribute to a cycling of sub-therapeutic enrofloxacin re-absorption in and amongst rabbits. Such cycling is considered as a complicating factor in this species for assessment of veterinary drug use. How it specifically impacts on resistance selection, development, and dissemination is, however, not clear.

Rabbit in contrast to poultry production: Rabbits are mammals in close contact with their off-spring during the farrowing and lactating/suckling periods. Maternal contact facilitates vertical and horizontal transmission of bacteria (pathogenic and/or commensal). Does and finishing rabbits can be affected clinically by pasteurellosis; therefore, oral treatments are potentially administered to both does and finishing rabbits. The commensal flora of does, where treated with enrofloxacin, would undergo selection pressure. Does may then transmit resistant bacteria to their off-spring. Though such resistance transmission has not been studied specifically in rabbits, it is documented in swine production (Belloc *et al*., 2005) and expected to be present in rabbits since both swine and rabbits rear their young in close contact over a period of several weeks.

Potential use of enrofloxacin in nursing/lactating does creates other complicating factors. The ease of penetration of enrofloxacin into milk should be considered when treating lactating does that continue to nurse (Fraile LJ, Martinez C, Aramayona JJ, *et al.* 1997). Therapeutic concentrations of enrofloxacin are reached in milk following a dose of 7.5 mg/kg bodyweight (Aramayona JJ, Mora J, Fraile LJ, *et al.* 1996). Furthermore, elimination of enrofloxacin is significantly less in neonates until at least sixteen (16) days of age compared with that in adult rabbits. The long-term effects on these neonates have not been fully assessed including the dissemination of resistant strains.

Once the off-spring is separated from the does, treatments with enrofloxacin administered at the finishing stage may result in a specific selection of resistant bacteria population present in the digestive tract of rabbits. In contrast, a vertical transmission of bacteria in poultry production, though it can occur, is limited. Different generations are physically separated and good hatching practices keep the egg, developing offspring and the mother hen independent of each other.

Lastly, food rabbit farming is organized differently from chicken production, for example, where poultry production in Europe is essentially pyramidal with a clear segregation of production stages (*e.g.* grand-parents, parental flocks and broiler flocks with no direct contact between flocks). Between-flocks transmission is limited in poultry production through a strict all in/all out production with thorough cleaning and disinfection procedure between broiler flocks. Thus, resistance selection, development, and dissemination over time are limited through the above mentioned practices in poultry production. Food rabbit production is confined traditionally to one and the same farm with the different life stages of rabbit present and in relatively close contact to each other, constituting a closed, dynamic and structured animal population. In this type of population, resistance is selected, developed, and disseminated continuously over time.

Therefore, it is expected that HIPRALONA ENRO-S and its generics would lead to increased selection pressures over time after repeated use in a given rabbit farm.

Current situation in EU

At present, EU food rabbit production is concentrated in Mediterranean Countries such as Italy, Spain and France which together account for 76.4% of total production. Although the total rabbit meat production is minor compared to other food animal species, by no means does this lessen the importance of rabbits to the food animal industry and thus rabbits deserve the same full consideration as all other food animals species in terms of risk.

HIPRALONA ENRO-S is authorized and commercialised in Spain. Oral enrofloxacin for use in rabbits is authorised in other European countries. Some data exist: the sensitivity of enrofloxacin to *E. coli* isolated in rabbits is high in France (90%, RESAPATH 2009) whereas it is less sensitive in Member States using enrofloxacin-containing products authorised for rabbits (*e.g.* in Italy with respectively 78% in Camarda 2004, 62% in Pisoni 2007, 56% in Grilli 2009). The same pattern is observed for *Staphylococcus aureus* (89% sensitive in France versus 70% in Italy). However, in the latter publications there is insufficient information on methodology, and in particular on sample selection and interpretation criteria, to ascertain whether data are comparable or not.

Orally administered fluoroquinolone are currently not authorized in all EU countries. For example, France is one of the countries where the products are not authorized for use in food rabbits. Pasteurellosis in rabbits is treated with authorized first line antibiotics: sulfonamides, oxytetracycline, and tilmicosin. Flumequine, a quinolone, is authorized for oral use in rabbits in France, but its use is sporadic. Flumequine use is limited by a relatively narrow spectrum (in comparison to fluoroquinolones). In France, the use of oral enrofloxacin-containing products under the cascade encompasses 12% of maternity batches and 1% of growing rabbit batches (ANSES, 2011). Flumequine use entails only 1% of batches both in maternity and in growing rabbits (ANSES, 2011). Moreover, RESAPATH shows that most bacteria resistant to flumequine are still sensitive to enrofloxacin.

Enrofloxacin in rabbits: Increased activity, but no demonstration of added clinical benefit in this indication

There is a consensus that non-wild type pasteurella have good susceptibility towards enrofloxacin, but also first line antibiotics. For instance, RESAPATH (2009 data) shows that the susceptibility of *Pasteurella multocida* to first line antibiotics used to treat pasteurellosis is very good, varying from 93% (for sulfonamides + trimethoprim), to 97 and 98% (for oxytetracycline and tilmicosin). The extra activity of enrofloxacin (up to 100%) is considered to be minimal when compared to other antibiotics and is not expected to be of any great clinical benefit.

Limits of the existing clinical study: In the provided clinical trial in rabbits, HIPRALONA ENRO-S was demonstrated to be non-inferior to a sulfonamide product. The efficacy of oral enrofloxacin in the treatment of respiratory disease due to pasteurella is therefore considered to be established; but any added clinical benefit from oral enrofloxacin treatment is not shown.

Specific incentives and risks linked to pharmaceutical form

Oral enrofloxacins are incentives for the use in rabbit farms because:

- The oral route of administration will facilitate the herd-level use of this antimicrobial when compared to injectable products. Indeed, treatment in water is easier for the farmer in terms of workload.
- The authorized product will have a withdrawal period of two days which is far shorter than the withdrawal period applicable for off-label use of poultry oral enrofloxacin products in countries where the product is not licensed for use in rabbits. This will add an extra incentive for product use, especially in the finishing stages where veterinary drug use normally decreases. Moreover, rabbits treated in the finishing stage carry a higher chance of arriving at the slaughterhouse with resistant microflora.

Oral enrofloxacin represents a high risk of selection of resistance because:

- It is acknowledged that the first risk posed by oral administration is immediate exposure to the intestinal flora. This risk may be amplified in this coprophagic species.
- Oral administration means mass treatment. Indeed, the treatment might be administered in drinking water to an entire batch of animals because, in most cases, only one drinking water system is available for each batch. Both diseased and healthy rabbits will be exposed, both as a treatment and prevention of spread of disease.
- With administration in drinking water, there is variability of uptake among individual rabbits, especially sick rabbits; hence, each individual animal will not always receive the exact recommended dose. In severe cases of respiratory disease, it is probable that the water intake is reduced for the majority of rabbits in the batch. Here, underdosing with a concentration-dependent antibiotic will lead to higher selection pressure of resistance.

Potential emergence of fluoroquinolone-resistant pasteurella

A report provided by an applicant during the referral procedure (Badiola, 2011) has presented a study on *Pasteurella multocida* isolated from clinical samples from 30 Spanish rabbit farms in 2006 (13 isolates), 2007 (1 isolate) and 2011 (16 isolates). Most of the MICs obtained in that study are above the range expected for wild-type isolates. This observation could either be explained by methodological factors, or reflect a true emergence of decreased susceptibility. Alternatively, the distribution indicates that isolates with decreased susceptibility have emerged, although only one isolate was classified as clinically resistant.

It is noted that the risk characterization is incomplete. Relevant studies are not available that allow to quantify and rule out the risk of antibiotic resistance in zoonotic bacteria like *Salmonella* spp and *Staphylococcus aureus*.

Benefit:risk

The CVMP opinion providing the basis for decision which would allow the use of the product everywhere in the EU cannot be supported.

The clinical benefit as demonstrated is not considered to outweigh the risks. Enrofloxacin is a critically important antibiotic and should be truly reserved to last resort treatment indications. HIPRALONA ENRO-S and its generics are and will be used for mass treatment, not only of sick rabbits, but also healthy ones.

The consequences of any antibiotic use on the level of resistance of pathogenic bacteria are widely acknowledged. In the case of HIPRALONA ENRO-S and its generics, increased use would take place where early warning on *Pasteurella multocida* can already be documented. On commensal and zoonotic bacteria, the consequence on the level of resistance is also assumed to be present, though it currently is not quantified.

Furthermore, it is noted that CVMP has relied upon a comparative risk analysis whereby rabbit production is seen as minor when compared to poultry production. Such comparisons are flawed for the basis of an additional risk.

Consequently, the benefit:risk for extension of the product into countries where it is not yet used is considered as unfavourable.

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