The European Agency for the Evaluation of Medicinal Products *Veterinary Medicines Evaluation Unit* 

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# **COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS**

# DOXYCYCLINE HYCLATE

## **SUMMARY REPORT (1)**

- 1. Doxycycline is a semisynthetic tetracycline derivative. As hyclate salt doxycycline is presented as injectable solution (intramuscular, intravenous), water soluble or lactodispersable powders, and (for dogs and cats) tablets and capsules. Doxycycline hyclate is indicated in cattle, pigs, poultry, turkeys, dogs and cats for the treatment of infections due to bacteria sensitive to doxycycline. Doxycycline is not for use in lactating cattle and layers.
- 2. Doxycycline belongs to the group of tetracycline antibiotics. All tetracyclines have a broad spectrum of activity which includes gram-positive and gram-negative bacteria, chlamydias, rickettsias, mycoplasmas and spirochaetes. Doxycycline tends to be more active against some of these species than other tetracyclines. It is primarily a bacteriostatic antibiotic and has its main mechanism of action on inhibition of protein synthesis. The potency of doxycycline is not less than 880 IU/mg.
- 3. Doxycycline is processed separately from oxytetracycline, chlortetracycline and tetracycline. For these three tetracyclines, which were also evaluated by JECFA, the Committee for Veterinary Medicinal Products established a final ADI (0-3 µg/kg bw, based on effects on the human gut flora) and final MRLs in all food producing species for the marker residue defined as "sum of parent drug and its 4-epimer" (kidney 600 µg/kg, liver 300 µg/kg, muscle and milk 100 µg/kg and eggs 200 µg/kg; Commission Regulation (EC) No 281/96).
- 4. Data have been provided on the antimicrobial activity of doxycycline on the human intestinal flora in comparison with the activity of oxytetracycline, on the residue distribution of doxycycline in pigs and poultry after oral administration, and on routine analytical methods for the determination of doxycycline residues in tissues for cattle, pigs and poultry. Data on the toxicology and pharmacology of doxycycline, and on residue depletion in other target animals than pigs and poultry have been extracted from dossiers from national marketing authorisations and from handbooks.
- 5. After oral administration doxycycline is rapidly and well absorbed from the gastrointestinal tract. Doxycycline has a longer half-life (15-22 h) and is more lipid-soluble than other tetracyclines. Following absorption through various routes of administration, doxycycline is widely distributed in the body with highest levels in kidney and liver, and in bone and dentine. Doxycycline may be metabolised up to 40%, and is largely excreted in faeces (via bile and intestinal secretion), mostly in a microbiologically inactive form.
- 6. Doxycycline is of low acute oral toxicity. From several repeated dose and chronic toxicity studies with rats, hamsters, mini-pigs, dogs and monkeys, it appears that sensitivity for the hepatoxic effects of doxycycline is idiosyncratic in dogs (NOEL 25 mg/kg bw in a 1-month study, although the hepatic changes did not progress upon continuation of the drug for 1 year, and the changes were reversible after drug withdrawal). There is no evidence of reproductive or developmental toxicity and there is no evidence of genotoxic potential. It can be concluded that the toxicological profile of doxycycline is roughly comparable to that of oxytetracycline, chlortetracycline and tetracycline.

- 7. The microbiological activity of doxycycline compared to that of oxytetracycline was determined in *in vitro* MIC-studies with human enteric isolates. From these data it can be concluded that the tested human enteric microorganisms have a comparable or slightly higher susceptibility for doxycycline than for oxytetracycline. These antimicrobial data provide the most appropriate endpoint for the safety evaluation of doxycycline. In view of the similarity in antimicrobial activity against human enteric microorganisms, the microbiological ADI of 0-3  $\mu$ g/kg bw for oxytetracycline (and chlortetracycline and tetracycline) can be adopted for doxycycline.
- 8. From residue data with pigs, poultry and cattle after oral administration and with cattle after intravenous administration it can be concluded that the residue distribution of doxycycline in these food-producing animals is roughly comparable to that of oxytetracycline. Highest residues are found in kidney, followed by liver, skin and muscle. Given the polarity of doxycycline, it is not detectable in fat to any great extent.
- 9. For the complete recovery of doxycycline the 4-epimer of doxycycline has to be determined. The 4-epimer of doxycycline occurs in samples and is built during sample preparation. The 4-epimer is in equilibrium with the parent compound. Therefore, the marker residue is the sum of the parent drug and its 4-epimer.
- 10. For the determination of doxycycline in tissues of cattle, pig and poultry HPLC-methods are available. However, none of the methods is completely validated, mainly since the 4-epimer of doxycycline is not taken into account. No information is available whether the 4-epimer is separated under the analytical conditions described. The specificity of the analytical methods is not demonstrated for doxycycline and its 4-epimer, particularly with respect to the possible interference of the other tetracyclines and their 4-epimers.
- 11. As the residue distribution of doxycycline in cattle, pigs and poultry is roughly comparable to that of oxytetracycline, tetracycline and chlortetracycline, the MRLs that have been established for oxytetracycline, tetracycline and chlortetracycline can be adopted for doxycycline, with the exception of the MRLs for eggs and milk, since doxycycline is not indicated for use in lactating cattle and layers. In addition, an MRL of 300  $\mu$ g/kg can be established for skin/fat of pigs and poultry.

### **Conclusions and recommendation**

Having considered that :

- a toxicological ADI has been set at 0-0.003 mg/kg bw;

- the physico-chemical analytical method available is not fully validated for the determination of doxycycline and 4-epimer in tissues of cattle, pigs and poultry,

the Committee recommends the inclusion of doxycycline in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically	Marker residue	Animal	MRLs	Target	Other
active substance(s)		species		tissues	provisions
Doxycycline	Sum of parent	Porcine,	600 µg/kg	Kidney	Provisional
	drug and its	poultry	300 µg/kg	Liver	MRLs expire
	4-epimer		300 µg/kg	Skin/fat	on
	_		100 µg/kg	Muscle	01.01.1998
		Bovine	600 µg/kg	Kidney	
			300 µg/kg	Liver	
			100 µg/kg	Muscle	

### LIST OF QUESTIONS

- 1. For the determination of doxycycline in tissues of cattle, pig and poultry the HPLC-methods proposed are fairly well described but not completely validated. In order to fully validate these methods in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community the followings points should be addressed :
  - the 4-epimer of doxycycline should be taken into account, and information should be given whether the 4-epimer is separated under the analytical conditions described;
  - the specificity of the analytical methods should be demonstrated for doxycycline and its 4-epimer, particularly with respect to the possible interference of the other tetracyclines and their 4-epimers;
  - if the detector-response in the HPLC-method is the same for doxycycline and its 4-epimer, the limit of quantification can be established as the sum of doxycycline and its 4-epimer. If the detector-response is not the same, the HPLC-method should also be validated for the 4-epimer, and the limit of quantification has to be established for doxycycline and for its 4-epimer.

In both cases, the sum of the limit of quantification for doxycycline and the limit of quantification for its 4-epimer should be well enough below the provisional MRLs for the different tissues.

In all cases the methods should be presented in an internationally recognized format (e.g. ISO 78/2).