



## COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

### OXYCLOZANIDE (Extrapolation to all ruminants)

#### SUMMARY REPORT (3)

1. Oxyclozanide (CAS no. 2277-92-1) is a salicylanilide anthelmintic. Oxyclozanide is used for the treatment and control of fascioliasis in cattle and sheep. The substance is currently included in Annex I of Council Regulation (EEC) no. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Oxyclozanide	Oxyclozanide	Bovine	20 µg/kg 20 µg/kg 500 µg/kg 100 µg/kg 10 µg/kg	Muscle Fat Liver Kidney Milk	
		Ovine	20 µg/kg 20 µg/kg 500 µg/kg 100 µg/kg	Muscle Fat Liver Kidney	Not for use in animals producing milk for human consumption

2. In reviewing the availability of endo- and ectoparasiticides for sheep and goats, oxyclozanide was considered for extrapolation from bovine and ovine species to all ruminants. The considerations and criteria leading to the identification of oxyclozanide are described in the Position Paper Regarding Availability of Veterinary Medicines – Extrapolation of MRLs (EMA/CVMP/457/03-FINAL).
3. The scientific justification for this extrapolation was assessed in accordance with the Notes for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMA/CVMP/187/00-FINAL) and on the Establishment of Maximum Residue Limits for Minor Animal Species (EMA/CVMP/153a/97-FINAL).
4. In setting the ADI in the original assessment of oxyclozanide, the data summarised in the paragraphs below were considered.
5. The mechanism of action of oxyclozanide is the uncoupling of oxidative phosphorylation in flukes. No data were available on secondary pharmacodynamic effects.
6. No formal pharmacokinetic studies in laboratory species were available. Plasma concentrations were measured (1 rat per sex per time point) on week 6 and 12 of an oral 90-day rat repeated-dose toxicity study (doses: 0, 0.45, 9 and 44.5 mg/kg bw/day administered in the diet). Maximum plasma concentrations at 6 and 12 weeks were: 1.3 to 3.9 and 1.0 to 3.7 µg/ml (low dose), 5.4 to 7.0 and 8.0 to 15.0 µg/ml (mid dose), 22 to 30 and 43 to 65 µg/ml (high dose).

From limited data collected from the mid dose level, plasma half-lives of 14 and 10 hours were calculated. In a 90-day oral dog study (doses: 0.5, 5 and 25 mg/kg bw/day) maximum plasma levels (1 or 2 animals per sex per dose) at 6 and 12 weeks were: 2.9 to 7.5 and 0.1 to 2.5 µg/ml (low dose), 2.9 to 6.8 and 2.0 to 6.0 µg/ml (mid dose), 8.2 to 23 and 1.7 to 12 µg/ml (high dose). From limited data collected plasma half-lives at 6 and 12 weeks of 16 to 20 hours and 7 hours (5 mg/kg bw) and 5 to 13 and 7 to 9 hours (25 mg/kg bw) were calculated.

7. One male and one female dog receiving orally 25 mg of <sup>14</sup>C-oxyclozanide/kg bw/day for 7 days excreted 68 to 73% of the dose in faeces and 1.2 to 1.9% in urine as measured from the first dose up to 24 hours after the last dose. Twenty four hours after the last dose, bile contained 1731 to 2307 µg equivalents/g, liver 20.95 to 22.59 µg equivalents/g and kidney 6.517 to 6.675 µg equivalents/g. Total bioavailability was not determined. The residue in liver at 24 hours post dose contained only a minor proportion of parent compound (approximately 2 to 4%), and several unidentified metabolites. Identified components in urine were a sulphone metabolite, two different glucuronides and the parent compound. In faeces the only identifiable component was the parent compound. The same metabolites, except for the sulphone, were identified in sheep urine and faeces. In an *in vitro* experiment, oxyclozanide was hardly or not at all metabolised by dog and sheep liver microsomal suspensions and only slightly metabolised by a bovine liver microsomal suspension.
8. The oral LD<sub>50</sub> in female rats was 3519 mg oxyclozanide/kg bw. Signs of acute toxicity were blood staining of muzzle, pallor, diarrhoea, increased respiration, lethargy, faecal and urine staining of perineum. At autopsy congestion of several organs, signs of liver toxicity (fatty change, accentuation of lobular structure) and dilated fluid filled uterus were found. Other oral LD<sub>50</sub> values in rats, mice and rabbits were in the range of 310 mg/kg bw to more than 2000 mg/kg bw, but could not be assessed because the original reports were not available.
9. In a 3-month oral toxicity study in rats with doses of 0, 0.45, 9 and 44.5 mg oxyclozanide/kg bw/day in the diet, the high dose caused reversible hepatic fat accumulation and reduced liver weights in males, vacuolation of brain cells (medulla) and reduced total plasma protein in females and increased spleen and adrenal weight in both sexes. In a 3-month oral toxicity study in dogs with doses of 0, 0.5, 5 and 25 mg/kg bw/day the high dose caused vacuolation of brain cells in the region of the pons in all animals of both sexes. This phenomenon was also found in some animals of both sexes of a recovery group treated with this dose. The high dose females showed increased alkaline phosphatase. From these data it was concluded that oxyclozanide caused liver and brain toxicity at the highest tested doses. NOELs of 9 and 5 mg/kg bw were derived from the rat and the dog study respectively.
10. Tolerance data in cattle and sheep showed that relatively low single doses (15 mg/kg bw) could already have adverse effects on central nervous system and intestinal function (behavioural depression, diarrhoea, and inappetence). At higher doses the severity of signs of toxicity increased and mortality occurred at 50 mg/kg bw and higher. Presumably these side effects were due to the pharmacodynamic action of the substance.
11. A 2-generation rat reproduction study was conducted with dose levels of 0, 100, 400 and 1200 mg/kg feed (equivalent to varying doses per kg bw; rough estimates: 0, 6, 25 and 75 mg/kg bw/day for males and 0, 9, 35 and 100 mg/kg bw/day for females). At the highest tested dose, bodyweight gain of F0 males was slightly inhibited resulting in a significantly lower body weight at terminal sacrifice. In both F0 and F1 females, the number of animals with brain vacuolisation was increased in the high dose group as compared to the controls. Brain histology was not examined in the low and mid-dose animals. As histopathology was only carried out for the control and high dose group, no NOEL for brain toxicity (i.e. parental toxicity) could be established. At the highest dose tested a slight, possibly treatment related, decrease of F1 litter size was found. No effects on fertility and pre or postnatal development were observed at doses up to about 25 mg/kg bw/day in males and 35 mg/kg bw/day in females. In a one-generation dose-finding study, decreased parental bodyweight and food intake and decreased pup weight and viability were found at doses of 80 to 160 mg oxyclozanide/kg bw/day. Target animals treated with recommended therapeutic doses in several phases of reproduction showed no evidence of foetotoxicity, teratogenicity or effects on fertility. The overall NOEL for reproduction toxicity was 25 mg/kg bw/day.

12. In a rat teratogenicity study with doses of 0, 5, 100 and 200 mg/kg bw/day, no foetotoxicity or teratogenicity was found up to the highest tested dose. Maternal toxicity (soft faeces) was found at the mid and high dose. In a rabbit teratogenicity study with doses of 0, 16, 32 and 64 mg/kg bw/day, teratogenicity (a syndrome characterised by fused cranial bones with associated hydrocephaly, incomplete cranial ossification and vertebral aberrations, and a few cases of umbilical hernia) and foetotoxicity (increased incidence of minor skeletal abnormalities) were found at the highest dose. At the mid and high dose maternal toxicity was found (abnormal faeces, reduced food intake and growth). Based on the rabbit study, an overall NOEL for teratogenicity and foetotoxicity of 32 mg/kg bw/day was derived.
13. Two mutagenicity studies in bacteria with and without metabolic activation (one in *S. typhimurium* only and the other in *S. typhimurium* and *E. coli*) showed no evidence of mutagenicity up to the maximum concentrations tested of 10 to 100 µg/plate. A mouse lymphoma assay with doses up to 62.5 µg/ml with and without metabolic activation was positive at the highest cytotoxic concentration. In 1 out of 3 replications a dose related positive effect was also found at non-cytotoxic doses (30 to 50 µg/ml). A cytogenicity test in cultured human lymphocytes with doses up to 250 µg/ml with and without metabolic activation showed a clastogenic effect of questionable toxicological significance at a cytotoxic concentration of 250 µg/ml (statistically significant but only slightly higher or close to upper limit of historical negative control ranges) in cells of 1 out of 3 donors.  
  
Two oral mouse micronucleus tests with doses up to 256.5 mg/kg bw and 1200 mg/kg bw and an *in vivo* rat liver unscheduled DNA synthesis test with single oral doses of 0, 632.5 and 2000 mg/kg bw were negative. Since the bacterial tests and three independent *in vivo* mammalian tests were negative, and the toxicological significance of the positive results found in two *in vitro* mammalian tests was doubtful, it was concluded that the overall package of data that oxyclozanide was not mutagenic.
14. No carcinogenicity study was supplied. Since oxyclozanide was not mutagenic or structurally related to a known carcinogen, no carcinogenicity data were needed.
15. No specific data on immunotoxicity were supplied.
16. At levels higher than 1 mg/kg in milk, oxyclozanide might have antimicrobial effects in a test based on *Bacillus stearothermophilus* and at 5 and 8 mg/kg it had clearly inhibiting activity. With a test based on *Streptococcus thermophilus* no activity was found in milk at concentrations up to 128 mg/kg. Because these concentrations were much higher than the maximum residue levels found in milk, the reported activity was not considered of concern.
17. Because oxyclozanide is not used in humans, no information was available on effects in humans.
18. For the calculation of the ADI, an overall NOEL of 5 mg oxyclozanide/kg bw/day was derived from the dog 90-day study. Because of the small difference between the NOEL and the therapeutic dose that elicited side effects in target animals, a safety factor of 200 was used. This resulted in an ADI of 0.03 mg/kg bw, equivalent to 1.8 mg/person.
19. For the extension to include all ruminant species in Annex I the information summarised in the paragraphs below was taken into account.
20. After oral administration of commercial formulations (dose 12.5 mg oxyclozanide/kg bw) in cattle, a plasma  $C_{max}$  of about 14 to 17 mg/l for the parent compound was found at 25 hours. The  $t_{1/2}$  was about 21 hours.
21. After administration of commercial formulations in sheep (dose 12.5 mg oxyclozanide/kg bw) a plasma  $C_{max}$  of parent compound of about 25 to 29 mg/l was reached after a mean  $t_{max}$  of about 22 hours. Area under curve (AUC) values were 880 mg·hour/l for a drench formulation containing only oxyclozanide and 1007 mg·hour/l for a drench formulation containing oxyclozanide in combination with oxfendazole. The  $t_{1/2}$  in sheep was 21.3 hours for a drench containing only oxyclozanide and 26.0 hours for a drench containing also oxfendazole.

A tri-exponential plasma pharmacokinetic model fitted to plasma concentrations in sheep after administration of 15 mg oxyclozanide/kg bw revealed a  $C_{\max}$  of 19.0 mg/l, area under curve of 1224 mg·hour/l,  $t_{1/2\alpha}$  of 7 hours,  $t_{1/2\beta}$  of 14 hours and  $t_{1/2\gamma}$  of 154 hours. A plasma protein binding of 99% was found at concentrations of 10 to 50 mg/l.

22. Four lactating cows which received a single oral dose of 15 mg  $^{14}\text{C}$ -oxyclozanide/kg bw showed a mean plasma peak concentration of 26  $\mu\text{g}$  equivalents/ml at 24 hours after administration. Elimination half-life was about 24 hours. During five days after administration 84% of the dose was excreted in faeces, 7.5% in urine and less than 0.07% in milk. The highest individual concentration of  $^{14}\text{C}$ -residues in milk from these animals was 0.153 mg/kg, found at the second milking after administration, the corresponding concentration of parent compound was 12.5  $\mu\text{g}$  equivalents/l. In most samples no parent compound could be detected. Twenty-four hours after single oral treatment of nine heifers with 13.3 to 14.6 mg/kg bw of  $^{14}\text{C}$ -oxyclozanide a peak plasma concentration of 38  $\mu\text{g}$  equivalents/ml was found. The elimination half-life of total radioactivity and parent compound in whole blood was 26 and 15 hours. Less than 10% of the dose was recovered over a period of 3 days in urine (4% in the first 24 hours). Total concentration of radiolabelled compound in bile at 1, 7 and 14 days post dose was 600 to 1624  $\mu\text{g}$  equivalents/g, 2.63 to 5.19  $\mu\text{g}$  equivalents/g and 0.32 to 0.68  $\mu\text{g}$  equivalents/g. Evidence was found that most of the urinary elimination product consisted of two or three different O-glucuronides.
23. A radiolabel study was conducted in sheep with a single dose of 15 mg  $^{14}\text{C}$ -oxyclozanide/kg bw formulated as a commercial drench. The highest plasma radioactivity of 10 to 20  $\mu\text{g}$  equivalents/ml was found at 8 hours after treatment (2 animals/time point; time points examined: 2, 4, 8 and 24 hours). Eight days after treatment, plasma concentrations decreased to 0.12 to 0.15  $\mu\text{g}$  equivalents/ml. Within 14 days after treatment, 67 to 75% of the ingested radioactivity was recovered in faeces and 11 to 22% in urine. Most of the radioactivity was excreted during the first two days after administration. The mean concentrations in the bile of animals slaughtered after withdrawal periods of 4, 7, 14 and 21 days (4 animals/time point) were 4.79, 0.11, 0.08 and 0.17  $\mu\text{g}$  equivalents/g. Excretion product in sheep faeces consisted predominantly of parent compound. In sheep urine, 2 glucuronide conjugates besides oxyclozanide were identified. Other possible metabolites in faeces and urine accounted for only small proportions (less than 10%) of the total radioactivity recovered in these excreta. *In vitro* sheep liver microsomes hardly metabolised or did not metabolise oxyclozanide. Tissue residues were examined producing radio-HPLC profiles of residue extracts of livers of animals slaughtered at 4, 7, 14 and 21 days and of kidneys, muscle and fat tissue of animals slaughtered at 4 days after administration using two different solvent systems (one to recover parent compound, the other to resolve a large polar area found with the first system). Several peaks and diffuse areas of radioactivity were found. No attempt was done to identify the possible metabolites present in these fractions. With these procedures, the proportion of material co-chromatographing with parent compound in liver extract decreased from about 18 to 21% on day 4 to about 2 to 4% on days 7, 14 and 21. The proportions of this material in kidney, muscle and fat tissue extracts on day 4 were 3 to 8%, 18 to 22% and 24 to 44%, respectively.
24. After single oral doses of 13.3 to 14.6 mg  $^{14}\text{C}$ -oxyclozanide/kg bw in heifers, (three heifers per slaughter time), total residue concentrations at withdrawal times of 1, 7 and 14 days were 21600 to 27600, 4700 to 8200 and 1700 to 4100  $\mu\text{g}$  equivalents/kg in liver; 8500 to 9500, lower than 300 and lower than 300  $\mu\text{g}$  equivalents/kg in kidney; and 1300 to 1700, lower than 100, 150 to lower than 100  $\mu\text{g}$  equivalents/kg in muscle, respectively. In fat tissue, residues were not reliably measured, but concentrations were possibly slightly higher than those in muscle tissue. At 24 hours the ratio parent compound/total  $^{14}\text{C}$ -residues was in the range 45 to 61% for liver, 50 to 60% for kidney, 68 to 89% for muscle. A proportion of 110% was reported in one fat sample, while no proportion could be determined for the remaining fat samples. Only the ratio for liver could be determined at 7 and 14 days (residue concentrations in remaining tissues were too low), this was 1.1% on day 7 (all 3 animals) and 1.1 to 1.5% on day 14 (2 animals; in the third animal no ratio could be determined).

In addition, depletion and composition of residues were studied in two groups of four lactating dairy cattle which received a single oral dose of 9 to 10 mg  $^{14}\text{C}$ -oxyclozanide and were sacrificed 4 and 7 days later. Total residue concentrations at 4 days were 2961 to 7989  $\mu\text{g}$  equivalents/kg (mean value 6035) in liver, 223 to 989  $\mu\text{g}$  equivalents/kg (mean value 631) in kidney, 20 to 152  $\mu\text{g}$  equivalents/kg (mean value 84) in muscle and 98 to 316  $\mu\text{g}$  equivalents/kg (mean value 213) in fat. At 7 days total residue concentrations were 3426 to 6972  $\mu\text{g}$  equivalents/kg (mean value 5198), 88 to 203  $\mu\text{g}$  equivalents/kg (mean value 159), lower than 13 to 24  $\mu\text{g}$  equivalents/kg and lower than 46 to 163  $\mu\text{g}$  equivalents/kg in liver, kidney, muscle and fat, respectively. At 4 days, marker residue concentrations as measured by LC-MS (limit of detection 2  $\mu\text{g}/\text{kg}$  and limit of quantification 5  $\mu\text{g}/\text{kg}$ ) in liver, kidney, muscle and fat were 139 to 569  $\mu\text{g}/\text{kg}$  (mean value 400), 44 to 164  $\mu\text{g}/\text{kg}$  (mean value 97), lower than 2 to 49  $\mu\text{g}/\text{kg}$  and 5 to 57  $\mu\text{g}/\text{kg}$  (mean value 33), respectively. At 7 days concentrations were 2 to 10  $\mu\text{g}/\text{kg}$ , lower than 2 to 6  $\mu\text{g}/\text{kg}$ , lower than 2 to 8  $\mu\text{g}/\text{kg}$  and lower than 2  $\mu\text{g}/\text{kg}$  in liver, kidney, muscle and fat, respectively. At 4 days the mean marker to total residue ratios for liver, kidney, muscle and fat were 6, 17, 31 and 14%. At 7 days marker percentages could not be reliably established due to low concentration of marker residue in all tissues and of total residues in muscle and fat. Radio-HPLC chromatograms showed that the parent compound was extensively metabolised and constituted only a small proportion of the total residue.

Metabolites were not identified. Glucuronidase treatment of liver samples hardly changed the radio-chromatogram. Maximum concentrations of total residues in milk were found at the third and fourth milking after administration and were in the range 30 to 80  $\mu\text{g}$  equivalents/kg. Marker residue concentration at the third milking was lower than limit of detection (2  $\mu\text{g}/\text{kg}$ ) in five out of eight animals and 3 to 5  $\mu\text{g}/\text{kg}$  (i.e. equal to or less than the limit of quantification) in the remaining three cows. Marker residue percentages at this milking were lower than 2.5 to 9% (but not reliable because marker residue was below the limit of quantification in most samples).

25. A single dose of 15 mg  $^{14}\text{C}$ -oxyclozanide/kg bw formulated as a commercial drench was administered to sheep. Four animals per time point were slaughtered at 4, 7, 14 and 21 days after administration. On day 4, the mean concentrations of radioactivity in liver, kidney, muscle and fat were 4377, 337, 54 and 74  $\mu\text{g}$  equivalents/kg, respectively. In liver and kidney they were 2125 and 89  $\mu\text{g}$  equivalents/kg on day 7; 1538 and 53  $\mu\text{g}$  equivalents/kg on day 14 and 1221 and 60  $\mu\text{g}$  equivalents/kg on day 21, respectively. The concentrations in muscle were in the range of lower than 14 to 17  $\mu\text{g}$  equivalents/kg on days 7 and 14 and lower than 14  $\mu\text{g}$  equivalents/kg on day 21. In fat concentrations on days 7, 14 and 21 were in the range of lower than 14 to 31, lower than 14 to 16 and lower than 14  $\mu\text{g}$  equivalents/kg, respectively. The concentrations of parent compound (i.e. the marker residue) were determined by HPLC-MS (liver and kidney) or HPLC-UV (muscle and fat) methods. At 4 days they were 315 to 1611  $\mu\text{g}/\text{kg}$  (mean value 775) in liver, lower than 33 to 102  $\mu\text{g}/\text{kg}$  in kidney, lower than 25 to 25  $\mu\text{g}/\text{kg}$  in muscle and lower than 25 to 39  $\mu\text{g}/\text{kg}$  in fat tissue. At 7 days withdrawal, oxyclozanide was only detectable in one out of four liver samples (concentration 39  $\mu\text{g}/\text{kg}$ ) and was below the limit of quantification of the analytical method (50  $\mu\text{g}/\text{kg}$ ) on days 14 and 21 in liver and on day 7 in the other tissues. Ratio of marker to total residue was 10 to 33% (mean 18%) for liver at 4 days withdrawal and 23 to 26% for kidney (only 2 animals with residue concentrations above the limit of quantification). Marker residue concentrations were too low (lower than the limit of quantification) for calculation of meaningful ratios in kidney of the remaining two animals and in muscle and fat of all four animals.
26. In two residue depletion studies, with calves treated orally with 10 and 15 mg oxyclozanide/kg (commercial formulations) in animals slaughtered after a withdrawal period of 7 days, livers contained slightly higher concentrations of parent compound than kidney, muscle and fat tissue; however, the concentrations were generally below the limit of quantification (50  $\mu\text{g}/\text{kg}$ ) of the method (HPLC-UV). Only in three calves (out of 4 examined) treated with 15 mg/kg bw and slaughtered at 7 days, concentrations of 51 to 71  $\mu\text{g}/\text{kg}$  (slightly above the limit of quantification) were found in liver. At this time point no significant quantity of glucuronide was found in liver. In pooled livers of two ruminating calves dosed with 10 mg/kg of oxyclozanide and killed two days later, after deglucuronidation the concentration of oxyclozanide increased from 1730 to 2220  $\mu\text{g}/\text{kg}$ , indicating the presence of glucuronides.

27. Tissue residue studies with commercial formulations in sheep (single oral doses of 15 mg/kg bw) showed that 7 and 21 days after treatment, concentrations in edible tissues of the parent compound (and at 7 days: its glucuronide) were below the limit of quantification of the HPLC-UV method (50 µg/kg).
28. In four available milk residue studies with doses of 5.4 to 12.6 mg oxyclozanide/kg bw in cattle, only concentrations of parent compound below the limit of quantification (50 µg/kg) were found in milk. No significant quantities of glucuronide were found in milk.
29. Ruminant species such as bovine, ovine and caprine share a similar gastro-intestinal physiology. The available pharmacokinetic and residues depletion data do not indicate any significant variability between cattle and sheep, therefore, it was considered that other ruminants were unlikely to show any significant differences in these parameters. The existing tissue MRLs for bovine and ovine species are identical and so it was considered appropriate to recommend the extension of the MRLs so that the same tissue MRL values would apply to all ruminants, the MRL for bovine milk was also recommended to be extended to all ruminants.
30. Reverse phase HPLC-MS methods were validated for the routine measurement of residues in bovine and ovine tissues and bovine milk. They were described in ISO 78/2 format. The reported limits of quantification were 250 µg/kg for bovine and ovine liver, 50 µg/kg for bovine and ovine kidney, 10 µg/kg for bovine and ovine muscle and fat, and 5 µg/kg for bovine milk. This method should be applicable to other ruminant species and therefore from this aspect extrapolation to the tissues and milk of other ruminants is possible.

## Conclusions and recommendation

Having considered that:

- a toxicological ADI of 0.03 mg/kg bw (i.e. 1800 µg/person) for oxyclozanide was previously established,
- MRLs were established in bovine and ovine species; these MRLs are identical,
- an analytical method for the monitoring of residues in tissues and milk of all ruminant species was available;

the Committee for Medicinal Products for Veterinary Use recommends the modification of the current entry for oxyclozanide for bovine and ovine species in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table;

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Oxyclozanide	Oxyclozanide	All ruminants	20 µg/kg 20 µg/kg 500 µg/kg 100 µg/kg 10 µg/kg	Muscle Fat Liver Kidney Milk	

Based on these MRLs, consumer intake of residues from ruminant tissues and milk would represent about 100% of the ADI.