



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

LEVAMISOLE (2)

SUMMARY REPORT

1. Levamisole is a broad spectrum anthelmintic with a long history of use in cattle, sheep, poultry and pigs. When it was initially considered the most severe effect was identified a agranulocytosis in humans. It occurred at relatively low therapeutic doses even when given on non-consecutive days. The mechanism appeared to involve an immune component and no ADI could be identified. Consequently, a provisional MRL of 0.01 mg/kg was recommend at that time, and this expires on 1 January 1995.
2. In vitro biotransformation studies using dog, pig, sheep, cattle and human hepatocytes suggested qualitatively similar metabolic routes in these species. After oral administration to rats, dogs, monkeys and cattle, similar metabolic pathways were identified and these are essentially similar to those in humans. All metabolites noted in cattle were also seen in dogs and rats so that the toxicological potential of residues in beef cattle may be considered to have been evaluated in laboratory species.
3. The induction dose used in these studies was 20 mg/kg bw/day, but in sensitised dogs, challenge with doses of 1.25 mg/kg bw/day and above produced a dose - related incidence of haemolytic anaemia. However, previous studies had demonstrated no haematological effects at a dose of 1.25 mg/kg bw/day for a period of one year.
4. Various immunological parameters were examined in two dog studies, with a view to investigating the mechanism underlying the haematological effects. Sera obtained from dogs which were sensitised to levamisole caused the agglutination of erythrocytes from an untreated dog. The agglutination response was enhanced in the presence of levamisole or some of its metabolites, but only in 3 of 24 animals studied. Erythrocytes isolated from sensitised animals had IgM antibodies and complement on cell surfaces during periods of levamisole-induced haemolytic anaemia. IgG antibodies did not correlate well with anaemia in dogs.
5. Sera from humans, treated with levamisole and showing severe leucopenia or agranulocytosis, caused leucocyte-agglutination or complement-dependent granulocytotoxicity in vitro. The factors responsible for these properties showed a high correlation with haematological toxicity while sera from patients not developing agranulocytosis were not toxic to normal white blood cells. Analysis of sera from a limited number of individuals revealed the presence of IgM but not IgG antibodies. Leucocyte-agglutination was dependent on the presence of levamisole but granulocytotoxicity showed no such reliance.
6. Although the primary target cells in humans and dogs are generally different, there is now evidence supporting an immunological basis for the haematological toxicity in both species. The available evidence implicates the involvement of IgM antibodies and a dependence on complement in the mechanism of cellular destruction. There is also limited evidence that agglutination responses in humans and dogs are mediated through anti-drug antibodies, possibly induced by immunogenic complexes between levamisole and protein, to which the drug is known to bind. The reasons for the differential cell sensitivity in human and dogs are not known. However, the similarities in aetiology and the recent demonstration of leucopenia in dogs, suggests that dogs are a suitable model for the haematological toxicity of levamisole in humans.

7. In establishing the ADI, it was recognised that while continuous dosing of dogs with 1.25 mg/kg bw/day did not result in haemolytic anaemia, it did cause its re-emergence in a number of previously sensitised dogs. Since there is a very small population of humans sensitised to levamisole through therapeutic administration, a safety factor of 200 was applied and an ADI of 0-6 µg/kg bw was established.
8. A new full radiometric residues study in cattle was provided which gave information for the evaluation of the total residues, the non-extractable residues, a marker compound and the setting of suitable MRLs. Urine and faeces were collected at intervals, and tissue samples were collected when the animals were slaughtered at 3, 7, 14 and 21 days after dosing. The total radioactivity in the tissues was determined and the highest concentrations of residues were found in the liver and kidney. The nature of the residues was investigated further in the liver samples by determining the amount of non-extractable residues and identifying and quantifying the major metabolites in the free fraction. Two major metabolites were present in the liver. These were the S-cysteinyl-glycine conjugate and an unidentified metabolite.
9. The concentration of parent drug measured radiometrically was below the limit of determination of the assay. It was measured using the more sensitive GC assay for the unlabelled drug. From this it can be concluded that over a 14 day period the unchanged drug concentration is a small (1.3-3.6%, mean $2.4 \pm 0.7\%$) but a constant proportion of the total residues, other metabolites do not have a linear log ratio with total residues.
10. New studies were provided in which the limit of detection for levamisole is 5 or 10 µg/kg. Several old and new studies using a number of different formulations, dose rates and routes of administration were reported in which the concentrations of levamisole in liver of cattle, sheep, swine and poultry were determined. The residues were <100 µg/kg at 7 days post-dosing and can be >10 µg/kg at 14 days, and <10 µg/kg at 21 days. With cattle doses of >8 mg/kg body weight, most of the values are above 10 µg/kg for at least 14 days and can be above 100 µg/kg at 14 days post-dosing.

The non-extractable residues form about 50% of the total residues and new information estimates above 15% bioavailability.
11. In poultry, residues of parent drug were not measurable in the edible tissues after 1 day withdrawal period at twice the recommended dose. Regarding residues in eggs, using the recommended dose in poultry, the residues in the yolks at 1 day withdrawal were about 800 µg/kg.

There are analytical methods for quantitating the parent drug at a concentration of 10 µg/kg in animal tissues.
12. In establishing the MRLs, the following were taken into account : the ADI; the parent drug as a suitable marker residue; the residues in muscle and fat are equivalent to the parent drug; 50% of the residues in liver are bound and 15% are bioavailable; the residues in kidney are qualitatively similar to those in liver; the residues are similar in cattle, sheep and pigs. As a result, MRLs of 10 µg/kg for muscle, kidney and fat, and 100 µg/kg for liver, of cattle, sheep, pigs and poultry, expressed as parent drug, were established.
13. There is inadequate analytical information to establish an MRL for levamisole in milk.