



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### SODIUM 2-METHYL-2-PHENOXY-PROPANOATE

#### SUMMARY REPORT

1. Sodium 2-methyl-2-phenoxy-propanoate is intended for use as injectable 10% aqueous solution in cases of digestive problems, rumen impaction, enterotoxaemia, ketosis, gaseous bloat of the rumen, hepatic failure in cattle (including dairy cows), horses, swine, sheep and goats. The method of administration is by deep intramuscular, intraperitoneal or slow intravenous route at the dosages of 10 mg/kg bw in cattle, horses, swine, sheep and goats.
2. According to an old unpublished report, sodium 2-methyl-2-phenoxy-propanoate has a positive choleretic activity in the rat and a weak choleretic activity in the dog. The primary pharmacodynamic action may be similar to other choleretic chemicals, such as cycloxylic acid and clanobutin, which stimulate secretion of bilirubin and bile acids within the liver as well as enhance the metabolism of cholesterol.

In an inadequate study high dietary exposure levels (equal to or more than 250 mg/kg bw) of sodium 2-methyl-2-phenoxy-propanoate exerted a pronounced stimulating action on liver regenerating activity. Other phenoxy acids shared this action.

Four groups of male CD-1 mice were treated by gavage with a single dose of 0, 125, 250 or 500 mg/kg bw of sodium 2-methyl-2-phenoxy-propanoate to assess effects on intestinal motility. No treatment-related effect was observed in any of the treated group. Four groups of CD-1 mice (6/sex/group) were treated by gavage with a single dose of 0, 125, 250 or 500 mg/kg bw of sodium 2-methyl-2-phenoxy-propanoate to assess variations in hexobarbital-induced sleeping time. A dose-related statistically significant increase of sleeping time was observed in all treated groups, the effect being more marked in females. Three groups of male beagle dogs were dosed by intraduodenal treatment with 0, 65, 200 or 600 mg/kg bw of sodium 2-methyl-2-phenoxy-propanoate to assess effects on cardiovascular and respiratory parameters.

3. Only limited information is available with regard to pharmacokinetics in laboratory animals.

In a satellite study within a oral 90-day rat trial five groups at dosages of 10, 30, 90 and 270 mg/kg bw/day were used to obtain blood samples for proof of absorption on day 1, week 6 and week 10 of treatment.

In a 13-week toxicity study on beagle dogs treated orally with 20, 65 or 200 mg/kg bw, the comparison between AUC values at day 1 and day 91 of treatment indicates that mild accumulation occurred along prolonged treatment. Concurrently,  $C_{max}$  values showed a dose-related increase at the end of treatment, with shorter  $t_{max}$  values. Such changes were more pronounced in mid- and high-dose males.

4. In a single dose intraperitoneal toxicity test carried out in rats the  $LD_{50}$  was 2200 mg/kg bw.

In two limit tests single oral dosages of 2000 mg/kg bw administered to rats and mice did not induce mortality.

5. In an adequate 90-day study five groups of rats were treated by oral gavage with 0, 10, 30, 90 and 270 mg/kg bw of sodium 2-methyl-2-phenoxy-propanoate. Blood glucose levels were significantly increased with a dose-related pattern in males at equal to or more than 30 mg/kg bw. Dose-related increases in kidney and liver weight were observed at equal to or more than 90 mg/kg bw. Compound-related histological liver and kidney lesions were present at 270 mg/kg bw. All the effects were much more pronounced in males. No significant compound-related changes were observed after a 4-week recovery period. The NOEL was 10 mg/kg bw.

In a 13-week GLP compliant study four groups of beagle dogs were dosed orally by gavage with 0, 20, 65 or 200 mg/kg bw/day of sodium 2-methyl-2-phenoxy-propanoate. A dose-related incidence of diarrhoea was observed in all treated animals at all doses. Slightly lower weight gain was present in the groups treated with 65 and 200 mg/kg bw. A dose-related reduction of serum cholesterol and bilirubin levels was present in all dosed groups. At doses equal to and higher than 65 mg/kg bw reduced red blood cells, haematocrit and total haemoglobin were observed. Reduced spleen weight was present in females from all treated groups; testicular weight was reduced in males at 65 and 200 mg/kg bw. Compound-related histopathological alterations were present only at 200 mg/kg bw, including liver chronic inflammation and bile duct proliferation. In all treated groups a parafollicular thyroid hyperplasia was present at higher incidence than in controls. Since slight effects were present even at the lowest dose level tested, 20 mg/kg bw, no NOEL was retained.

6. In a pharmacokinetic study on calves the blood levels of creatine kinase enzyme were determined as indicator of tissue damage in 5 calves after a single intramuscular treatment with 10 mg/kg bw of sodium 2-methyl-2-phenoxy-propanoate. Creatine kinase levels rose from 90 minutes to 12 hours post-treatment whereas at 24 hours post-treatment the levels were at the pre-dosing values.

No data were provided on tolerance in the other target species.

7. In an adequate one-generation gavage study on rats, no effects on fertility, litter size, morphological development or growth were elicited even at the highest dose level tested, 270 mg/kg bw/day. Marginal effects were observed at 270 mg/kg bw, including reduced maternal *post partum* weight gain, reduced testicular weight without alteration of sperm parameters, and slight delay of startle response in pups. The NOEL was 52 mg/kg bw.
8. In an adequate embryotoxicity/foetotoxicity study, pregnant rats were treated orally with 0, 30, 135 and 600 mg/kg bw from days 6 to 17 of pregnancy. No maternal toxicity was observed. No increase of malformation was observed. However, a slight, but dose-related increase of kidney (dilated renal pelvis) and ureter (kinky and/or dilated) anomalies was observed in foetuses at all dose levels, including 30 mg/kg bw. No NOEL could therefore be retained for foetotoxicity in rats.

In an adequate embryotoxicity/foetotoxicity study, pregnant rabbits were treated orally with 0, 50, 230 and 1000 mg/kg bw/day from day 6 to 18 of pregnancy. Maternal toxicity, including significantly reduced weight gain up to the end of the study, was observed at high and intermediate dose levels. Embryotoxicity (increased postimplantation loss, supernumerary ribs) was present only at the highest dose level. The NOEL was 50 mg/kg bw for maternal toxicity and 230 mg/kg bw for embryotoxicity/foetotoxicity.

9. The potential for genotoxic effects of sodium 2-methyl-2-phenoxy-propanoate was assayed in the following adequate tests: reverse mutation in *Salmonella typhimurium* with and without metabolic activation; gene mutation in L5178YTK<sup>+/-</sup> mouse lymphoma cells with and without metabolic activation; chromosome aberrations in human lymphocyte cultured *in vitro* with and without metabolic activation; micronucleus test in CD-1 mice by oral gavage at dose levels of 750 and 1500 mg/kg bw. All tests gave negative results.
10. No carcinogenicity tests were performed. Since the compound has no potential for genotoxicity, there is no need to provide such study.

11. No studies on microbiological activity were provided. There are no indications that sodium 2-methyl 2-phenoxy-propanoate is likely to exert any significant antimicrobial activity.
12. No specific studies on immunotoxicity were provided.

No alterations of parameters or organs relevant to immune function were detected in the 90-day study on rats. In the 90-day study on dogs, a reduced spleen weight was observed in females even at the lower dose level tested of 20 mg/kg bw; no histopathological alterations or changes in the myeloid cells of bone marrow were present.

13. No data are available concerning possible effects on humans.
14. An ADI of 0.1 mg/kg bw (i.e. 6 mg per person) can be determined on the basis of the NOEL of 10 mg/kg bw in the 90-day rat study. A safety factor of 100 is utilised to determine the ADI since the study was of adequate quality and no persistent effects were observed.
15. In a study performed according to GLP, five weaned calves were treated by intramuscular route with 0.1 ml/kg bw of the finished product, a 10% solution of sodium 2-methyl-2-phenoxy-propanoate, i.e. a dose of 10 mg/kg bw of the active ingredient. An HPLC assay was carried out on plasma for determination of concentrations of the active ingredient (limit of quantification: 0.2 µg/ml). The blood peak for sodium 2-methyl-2-phenoxy-propanoate appears rapidly (30 to 45 minutes) and the half-life is short (90 to 130 minutes).

No data on the pharmacokinetics in other target species were provided.

16. No radiolabel studies in target species were provided.
17. Three female pigs, housed in metabolic cages, received a single intramuscular injection of 0.1 ml/kg bw of the finished product (10 mg/kg bw as active ingredient). The urine and faeces of each animal were collected from pre-dose to 96 hours after dosing, at 12 hour intervals and analysed for the presence of sodium 2-methyl-2-phenoxy-propanoate by HPLC (limit of quantification: 2500 µg/kg). Urinary and faecal excretion of sodium 2-methyl-2-phenoxy-propanoate was detected in the 24 hours following treatment. Of the administered dose 39.8% and 2.2% were recovered in the urine and faeces respectively. However, the remaining more than 50% of the dose were not accounted for.

Another study was performed to identify possible metabolites of sodium 2-methyl-2-phenoxy-propanoate in urine and faeces samples of 3 swine treated with a single intramuscular injection of 0.1 ml/kg bw of the finished product (10 mg/kg bw as active ingredient). As swine was reported as a species leading to phase II conjugated metabolites, an investigation of the urine was carried by liquid chromatography coupled to mass spectrometry (limit of quantification: 2500 µg/l). Two phase II metabolites were identified in urine. These metabolites are the glycine conjugate and the glucurono conjugate. A specific hydrolysis of the former and a chemical analysis of the latter were performed for the quantification of the metabolites in the faeces and urine samples from the above reported study. All procedures were fully validated and performed in compliance with GLP regulations. For both metabolites assays were conducted on samples of interest: pre-dose, 0 to 12, 12 to 24 and 24 to 36 hours. The total balance including parent drug and its two metabolites reached 78.3%.

Three healthy Italian Friesian cows, housed in metabolic cages, received a single intramuscular injection of 0.1 ml/kg bw/day of the finished product (10 mg/kg bw as active ingredient). The urine and faeces of each animal were collected from pre-dose to 96 hours after dosing, at 12 hour intervals and analysed for the presence of sodium 2-methyl-2-phenoxy-propanoate by HPLC (limit of quantification: 2500 µg/kg).

The maximum elimination of sodium 2-methyl-2-phenoxy-propanoate occurred in the urine in the 12 hours post-dose (mean value: 970 000 µg/kg). The mean urine recovery, expressed as a percentage of the administered dose, was 87.8%, with individual values between 78.3 and 100.7%.

The mean faeces recovery, expressed as a percentage of the administered dose, was 14.9%, with individual values between 14 and 16.2%.

A LC-MS-MS method was used for the measurement of sodium 2-methyl-2-phenoxy-propanoate in bovine and swine urine and faeces (limit of quantification: 2500 µg/kg; limit of detection: 1250 µg/kg).

18. In a GLP study five groups, each of 4 swine, were treated with a dose of the finished product of 0.1 ml/kg bw (i.e. 10 mg/kg bw as active ingredient) by intramuscular route for 3 consecutive days. The residue concentrations were determined by HPLC assay method (limit of quantification: 100 µg/kg for muscle, liver and kidney; 200 µg/kg for fat and skin, limit of detection: 14 µg/kg for muscle, 7 µg/kg for liver, 3 µg/kg for kidney and 200 µg/kg for fat and skin).

By day 2, no detectable residues were found in the muscle (including injection site), liver, fat or skin. As regards to the kidney, 3 samples of 4 were negative. Only one sample was positive (160 µg/kg). After 4 days all kidney samples were free of detectable residues.

Three groups, each of two male and two female cattle, were treated by daily intramuscular administration with 0.1 ml/kg bw/day of the finished product (10 mg/kg bw/day as active ingredient) for 3 consecutive days. Both kidneys, the liver, a sample of an untreated muscle and the muscle relative to the injection sites were taken from each animal. The analyses for the residue depletion profile of sodium 2-methyl-2-phenoxy-propanoate were performed by the HPLC method (limit of quantification: 100 µg/kg). Concentration of sodium 2-methyl-2-phenoxy-propanoate in liver, kidneys and muscle rapidly decreased within 1 day of the last treatment ( $52 \pm 37$  µg/kg and  $25 \pm 29$  µg/kg for the first and second day respectively in liver;  $193 \pm 30$  µg/kg and  $102 \pm 34$  µg/kg in kidney;  $18 \pm 11$  µg/kg and below 100 µg/kg in muscle); after two days fat, liver and muscle values were below the limit of quantification of the analytical method.

One group of eight lactating dairy cattle (four selected as high milk yielding cattle at an early stage of lactation and four as low milk yielding cattle at a late stage of lactation) were treated with daily intramuscular administration of 0.1 ml/kg bw/day of the finished product (10 mg/kg bw/day as active ingredient) for 3 consecutive days. Samples of milk (approximately 100 ml each) were collected 12, 24, 36, 48, 60 and 72 hours after the last administration. Milk samples were a representative mix of all 4 quarters. The analyses were performed by the HPLC method (limit of quantification: 100 µg/kg).

Mean values of residues of [sodium 2-methyl-2-phenoxy-propanoate](#) in milk samples of cattle treated with the finished product at a dose of 10 mg/kg bw/day by intramuscular route were in the range of 118 to 148 µg/kg after 12 hours and below the limit of detection after 24 hours.

No residue depletion studies for sheep, goats and horses have been provided.

19. An HPLC method was set up for swine and cattle tissues and milk utilising two extraction procedures: one with ethyl acetate for muscle, liver and kidney and the other with n-hexane for fat and skin. The limit of quantification of the method was 100 µg/kg, except for swine fat where the value was 200 µg/kg. The limits of detection in bovine tissues were as follows: 12 µg/kg for muscle, 13 µg/kg for liver and fat, 24 µg/kg for kidney and 14 µg/kg for milk, while the values for porcine tissues were 14 µg/kg for muscle, 210 µg/kg for skin and fat, 7 µg/kg for liver and 3 µg/kg for kidney.

## Conclusions and recommendation

Having considered that:

- [sodium 2-methyl-2-phenoxy-propanoate](#) is used only for treatment of individual animals,
- the animals are unlikely to be sent to slaughter immediately after treatment,
- a toxicological ADI of 0.1 mg/kg bw (6 mg/person) has been established,
- pharmacokinetics in cattle indicate that a rapid and complete excretion occurs as unchanged parent compound, and pharmacokinetics in swine indicate that a rapid and nearly complete excretion occurs as unchanged parent compound and two metabolites (glycine conjugate and glucurono conjugate),
- residue depletion studies show that residues below the limit of quantification (100 µg/kg) are detected in all edible tissues 2 days after the end of treatment in cattle,
- therefore, a worst-case (residues equal to limit of quantification) calculation in cattle will lead to a theoretical maximum daily intake of 0.20 mg one day after the end of treatment, i.e. less than 5% of the ADI,
- residue depletion studies in other target species (sheep, goat, horse) have not been provided;

the Committee concludes that there is no need to establish an MRL for [sodium 2-methyl-2-phenoxy-propanoate](#) for cattle and pigs and, taking into account the CVMP Note for Guidance on the Establishment of MRLs for Minor Animal Species (EMEA/CVMP/153a/97-FINAL), for goats and horses, whereas no recommendation can be proposed for sheep, and recommends its inclusion in Annex II to Council Regulation (EEC) No 2377/90 in accordance with the following table:

| Pharmacologically active substance(s)                | Animal species                    | Other provisions |
|------------------------------------------------------|-----------------------------------|------------------|
| <a href="#">Sodium 2-methyl-2-phenoxy-propanoate</a> | Bovine, porcine, caprine, equidae |                  |