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

EMEA/MRL/626/99-FINAL
July 1999

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

PROPETAMPHOS

SUMMARY REPORT

1. Propetamphos is an organophosphorus compound. Technical grade propetamphos consists of 4 isomers, 2 geometric (*cis* and *trans)* isomers and 2 optical (S- and R-)isomers. The *cis* form predominates as the active ingredient, usually exceeding 90% w/w of the technical material. From analogy with the chiral separation of propetamphos-oxon, *cis*-propetamphos is a racemate of equal proportions of the S- and R-enantiomers. The *trans*-form is generally present at less than 0.1% in the technical grade material.

   In veterinary medicine, propetamphos is applied topically to sheep, including lactating ewes. It is used as an ectoparasiticide for the control of sheep scab, blowfly strike, ticks, keds and lice. Sheep dip formulations may contain 5.6 to 40% propetamphos and are intended for dilution with water to provide a bath solution of approximately 0.03% w/v propetamphos. The establishment of MRLs was only requested for non-lactating sheep.

   Propetamphos is not used on crops and has not been reviewed by Joint FAO/WHO Meeting on Pesticide Residues (JMPR).

2. Pharmacokinetic studies were carried out in rats following oral administration of ^{14}C-labelled propetamphos following single (0.5, 0.6, 6, 16 or 18 mg/kg bw) and multiple (6.4 mg/kg bw/day for 8 days or 18 mg/kg bw/day for 15 days) doses. In all cases propetamphos was rapidly and almost completely absorbed. Peak serum concentrations were achieved within 1 hour of treatment and were proportional to the dose administered. Residues of radiolabelled material were widely distributed throughout the body tissues. ^{14}C-Labelled residues were highest in the lungs, fat, liver and skin. Excretion was mostly *via* exhaled air, as carbon dioxide, with lesser amounts present in urine. The half-life of elimination was approximately 25 hours. At least 7 metabolites were found in urine but no unmetabolised propetamphos. There was no evidence of bioaccumulation. The metabolites in urine were characterised but the nature of the residues in tissues was not studied.

3. The acute toxicity of technical propetamphos was variable, depending on strain, sex and age of the test animals, and the solvent vehicle. The oral LD_{50} values ranged from 59.5 mg/kg bw (female Wistar rat) to 119 mg/kg bw (male Charles River CD rat). It was less toxic via the percutaneous route. The acute dermal LD_{50} was 486 mg/kg bw in New Zealand White rabbits after undiluted application under an occlusive dressing and greater than 2260 mg/kg bw in female Wistar rats, following application in polyethylene glycol 200. Technical grade propetamphos was not a skin or eye irritant in the rabbit.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Switchboard (44-20-7) 418 8400 Fax (44-20-7) 418 8447
E-mail: mail@emea.eudra.org http://www.eudra.org/emea.html

4. Groups of Sprague-Dawley rats were fed diets containing 0, 2, 4 or 8 mg propetamphos/kg feed (86% pure) for 13 weeks. This pre-GLP study was poorly carried out, without analyses of the test diets, and dietary concentrations were not adjusted as the rats grew; consequently they received proportionally more of the test substance at the beginning. The only substance-related effects were dose-related reductions in plasma and erythrocyte cholinesterase activities. However,the reductions were not always statistically significant and rarely exceeded 20% of the control values. Consequently they merely reflected exposure rather than a true toxicological effect. There was no reduction in brain cholinesterase activity. The high dose level of 8 mg/kg feed, equivalent to 0.60 and 0.70 mg/kg bw for males and females respectively, was established as NOEL.

5. Groups of Beagle dogs (4 animals per sex and dose) were fed diets containing 0, 4, 20 or 100 mg propetamphos/kg feed for 52 weeks. The study was well conducted, in accordance with GLP requirements. One male given 100 mg/kg feed was euthanased in a collapsed condition. In the 100 mg/kg feed group, the dogs had diarrhoea, reduced food consumption, increased liver enzymes and liver weights. Focal hepatic necrosis was observed in 2 dogs given 100 mg/kg feed. Liver weights were also increased in the 20 mg/kg feed group, but there were no corresponding pathological changes. Plasma, erythrocyte and brain cholinesterase activities were significantly reduced in the 100 mg/kg feed group. Significant reductions were also observed in plasma and erythrocyte cholinesterase activities in the 20 mg/kg feed group. The NOEL/NOAEL was 4 mg/kg feed, equivalent to 0.13 and 0.14 mg/kg bw/day for males and females, respectively.

6. Groups of New Zealand White rabbits were treated topically with 0 (water), 0 (corn oil), 0.5, 2.5 or 5 mg/kg bw/day of propetamphos, 6 hours per day, 5 days per week for 3 weeks. Dermal irritation was dose-related in severity and was observed in all treated groups and the corn oil controls. However, histopathological examination showed no significant differences between the treated and control groups. Plasma and erythrocyte cholinesterase activities were reduced in all treated groups. Brain cholinesterase activities were not measured. Due to the inconsistencies in the study report no conclusion could be drawn regarding a NOEL.

7. In a 2-generation reproduction study, groups of male and female Wistar rats were fed diets containing 0, 4, 30 or 75 mg propetamphos/kg feed. Concentrations of 30 and 75 mg/kg feed caused parental toxicity (overt signs of toxicity at 75 mg/kg feed, dose-related reductions in body weight gain and in plasma, erythrocyte and brain cholinesterase activity). There was evidence of treatment-related infertility in F1 males given 75 mg/kg feed. This dose was also toxic to offspring causing reduced litter size, pup viability and body weight gain and significantly reduced plasma, erythrocyte and brain cholinesterase activities. The NOEL/NOAEL was 4 mg/kg feed, equivalent to 0.3 to 0.5 mg/kg bw/day.

8. Daily oral doses of 0, 1.5, 3 and 6 mg/kg bw/day were administered to pregnant Wistar rats on days 6 to 15 of gestation. Overt signs of toxicity were observed in the dams given 3.0 and 6.0 mg/kg bw, and maternal body weight gain was significantly reduced at 6 mg/kg bw. There was no evidence of teratogenicity or foetotoxicity at any dose level.

   Daily oral doses of 0, 1, 4 or 8 mg/kg bw/day were administered to pregnant New Zealand White rabbits from days 6 to 18 of gestation. Severe maternal toxicity (1 death, diarrhoea, weight loss) were observed at 8 mg/kg bw. The incidence of resorptions was significantly increased at 8 mg/kg bw. However, there was no evidence of teratogenicity at any dose level.

9. Propetamphos was not mutagenic in an *in vitro* bacterial assay for gene mutation using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with and without metabolic activation. Propetamphos gave negative results in an *in vitro* assay for mitotic chromosome non-disjunction, and an *in vitro* assay for gene mutation in *Saccharomyces cerevisiae* with and without metabolic activation. An *in vitro* assay for gene mutation in mammalian cells in mouse lymphoma L5178Y cells (HPRT locus), with and without metabolic activation, an *in vitro* unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes and an *in vitro* sister chromatid exchange assay in Chinese hamster ovary (CHO) cells with and without metabolic activation also gave negative results.

An *in vivo* micronucleus test in mice given oral doses of 0.0009 or 0.009 ml propetamphos/kg bw and an *in vivo* cytogenetics assay in bone marrow of rats given an intraperitoneal dose of 58 mg/kg bw were also negative. It was concluded that propetamphos was not genotoxic.

10. In a well-conducted combined chronic toxicity/carcinogenicity study, groups of 50 CD-1 mice per sex were fed diets calculated to provide 0 (control I), 0 (control II), 1, 6 or 21 mg/kg bw/day propetamphos for up to 93 weeks. Satellite groups of 10 mice per sex, including an extra group given 0.05 mg/kg bw, were kept for assessment of haematology and clinical chemistry values and for interim necropsy. There were dose-related reductions in plasma, erythrocyte and brain cholinesterase activities. At 1 mg/kg bw, there were statistically significant reductions in plasma, erythrocyte and brain cholinesterase activity in males and statistically significant reductions in plasma cholinesterase activity in females. At 6 and 20 mg/kg bw all three cholinesterases were significantly reduced in both sexes. The NOAEL, based on inhibition of plasma and erythrocyte esterase was 0.05 mg/kg bw/day. There was no evidence of carcinogenicity.

    In a combined chronic toxicity and carcinogenicity study, groups of Sprague-Dawley rats were fed diets containing 0, 6, 12 or 120 mg propetamphos/kg feed for up to 2 years. The study was poorly conducted during the late 1970s and was not in accordance with GLP. Survival was poor and surviving males were killed during week 91 due to the high mortality. Most deaths including those in the controls were attributed to chronic nephropathy. Brain cholinesterase activity was significantly reduced in the rats given 120 mg/kg feed and plasma and erythrocyte cholinesterase activities were reduced at 12 and 120 mg/kg feed. The NOAEL, based on cholinesterase inhibition, was 6 mg/kg feed, equivalent to 0.376 and 0.412 mg/kg bw/day in males and females, respectively. Although there was no apparent increase in the incidence of any tumour type, the study was inadequate for the assessment of carcinogenicity due to the poor survival.

    Taking into account the negative mutagenicity results and the lack of carcinogenicity in an adequately-conducted study in the mouse, it was concluded that propetamphos was not carcinogenic.

11. Neurotoxicity was studied in *in vivo* and *ex vivo* studies in hens and rats. In an acute delayed neurotoxicity study in hens, a single oral dose of 180 mg/kg bw did not cause clinical ataxia. There was evidence of axonal degeneration in the brains and spinal cords of propetamphos-treated and negative control groups and a moderate reduction of brain neuropathy target esterase activity in the propetamphos group. However, these effects were marginal, compared to the marked axonal degeneration and reduced neuropathy target esterase activity observed in the positive control group.

    Ex vivo investigations were conducted in both hens and rats. Single oral doses of technical propetamphos of 200 mg/kg bw given to adult female hens or 40 mg/kg bw given to adult male rats (with atropine protection), caused statistically significant reductions in brain acetylcholinesterase activity lasting up to 72 hours. There were no significant effects on brain or spinal neuropathy target esterase in either species. In a range finding study in the rat, a single protected dose of 120 mg/kg bw (an unprotected lethal dose), caused a statistically significant, but biologically not significant decrease of brain neuropathy target esterase of 15%. This indicated that biologically significant efffects on neuropathy target esterase were only likely at *supra*-lethal doses.

    The effects of chirally resolved enantiomers and racemic propetamphos-oxon on antiesterase activity were investigated *in vitro* using rat brain homogenates. Both the oxon enantiomers and the racemate were potent inhibitors of acetylcholinesterase activity at concentrations of 1.563 to 6.25 µmol/l, with rate inhibition constants (k_a) of 13 262 and 10 527 for the enantiomers, and 11 553 for the racemate. Activity against neuropathy target esterase was very weak, with mean k_a values of 2.96 and 5.62 for the enantiomers and 9.70 for the racemate at concentrations greater than 5000 µmol/l. The rate constant ratios for the neuropathy target esterase and acetylcholinesterase are considered to correlate with a compound's *in vivo* activity. Those with a ratio of less than 0.25 are generally not neurotoxic. The ratios for propetamphos oxon enantiomers and racemate were 0.00022 and 0.00053, and 0.00084, respectively.

No data was available for the *trans*-enantiomers and other impurities in the technical grade material, but due to the low concentrations of these compounds such data were considered unecessary. It was also noted that the structure of the major desisopropyl metabolite gave no indication of concern, and no evidence to neurotoxicity was observed in rats exposed to propetamphos in the diet for up to two years. Overall, the available data indicated that propetamphos would not induce delayed neuropathy.

12. Propetamphos was not a sensitiser when tested in the guinea pig using the Buehler technique.

13. Published reports of alleged cases of human poisoning with propetamphos were provided. From the information provided it was not possible to determine whether the cases were attributable to exposure to propetamphos and there was no indication of the likely extent of exposure. Cholinesterase measurements from workers dipping sheep and working in a manufacturing plant were also provided. Again there was no information concerning the likely extent of exposure and no base-line cholinesterase measurements. No conclusions could be drawn regarding a possible NOAEL for cholinesterase inhibition in humans.

14. NOAELs for cholinesterase inhibition were determined in several studies in 3 different species. The lowest NOAEL was 0.05 mg/kg bw/day which was established in the chronic toxicity and carcinogenicity study in the mouse, based on the inhibition of brain, plasma and erythrocyte cholinesterase activity.

    Based on this overall NOAEL, and using a safety factor of 100, an ADI of 0.0005 mg/kg bw (i.e. 0.030 mg/person) was established.

15. Groups of 3 castrated male lambs were dipped in a commercial formulation at the indicated rate and residues of propetamphos were determined 7, 14 and 21 days later using gas chromatography with electron capture detection (GC-ECD). The limit of detection was 1 µg/kg for all tissues. The residues in tissues were very variable. Residues of 400 µg/kg in muscle and 220 µg/kg in kidney were found in 1 lamb killed 14 days after treatment but residues in all other muscle and kidney samples were below the limit of detection. Residues were highest in fat and were in the range 330 to 3570 µg/kg at 7 days after treatment but below the limit of detection, 21 days after treatment. Residues were detectable in only 1 sample (out of 3) of liver taken 7 days after treatment (310 µg/kg) and 1 (out of 3) taken 14 days after treatment (620 µg/kg).

16. In another study, groups of 4 Suffolk cross sheep (both sexes) were dipped in a commercial formulation at the intended dose rate and slaughtered 7, 10, 14, 28 and 35 days later. Residues of propetamphos were determined using GC-ECD; the limits of detection and quantification were 10 µg/kg and 25 µg/kg, respectively, for all tissues. Residues in all samples of muscle, liver and kidney were below the limit of detection at all time points. The residues in subcutaneous fat were variable and in the range from less than 10 to 244 µg/kg, 7 days after treatment, less than 10 to less than 25 µg/kg at 10 days after treatment, and from less than 10 to 280 µg/kg, 14 days after treatment. Residues in all fat samples taken 28 and 35 days after treatment were below the limit of detection.

17. In a GLP-compliant study, twenty-four sheep (6 groups of 4 animals each) were plunge dipped in to 320 mg propetamphos/litre then sacrificed 1, 3, 7, 10, 14 and 21 days after treatment. The concentrations of propetamphos in muscle and fat were determined by an in-house gas chromatography with electron capture detection (GC-ECD) method (limit of detection: 10 µg/kg and limit of quantification: 25 µg/kg). The concentrations of propetamphos in muscle were less than 10 µg/kg in all muscle samples. In fat, propetamphos was detected at concentrations between the limits of detection and quantification in 1 of the 4 samples collected on days 7 and 10 after treatment. Because no raw data were provided and the protocol and supporting data for the analytical method were not included in the study report, no firm conclusions could be drawn.

18. In a pilot residue depletion study, 2 sheep were topically dosed with 200 mg ^{14}C-propetamphos in a shaved area (100 × 200 mm) along the backbone, then killed 1 or 2 days after treatment. The total residue concentrations of propetamphos in tissues were determined by combustion followed by liquid scintillation counting (LSC) and/or extraction into acetone followed by LSC. The metabolites in urine and faeces were determined by radiometric thin layer chromatography (radio-TLC) and the isolated desisopropyl propetamphos-fraction quantified by GC-ECD. The highest concentrations of total residue were found in kidney (529 and 443 µg equivalents/kg on days 1 and 2 after dosing respectively) of which desisopropyl propetamphos accounted for approximately 24%. The total residue concentrations in liver, muscle and fat were 559, 137 and 141 µg equivalents/kg at day 1 and 317, 63 and 27 µg equivalents/kg at 2 days after treatment. The concentrations of desisopropyl propetamphos in liver, muscle and fat accounted for 1.1, 6.6 and 7.1% of the total residue 1 day after treatment and 1.9, 6.3 and 48.1% of the total residue 2 days after treatment, respectively. The parent compound represented 34% of the total residues in fat in the sheep killed 1 day after dosing but only a minor fraction of the total residues detected in other tissues, the majority (i.e. greater than 80%) being unidentified polar and non-extractable components.

    In a follow-up pivotal study, sheep were topically dosed with 200 mg ^{14}C-propetamphos, as before, then 4 animals killed on days 1, 3 and 7 after treatment. The total residue concentrations of propetamphos in tissues were determined by LSC and the desisopropyl propetamphos concentration was determined by GC-ECD. Residues of unmetabolised propetamphos were not measured in this study. The total residue concentrations in kidney, liver, muscle and fat were: 513, 174, 35 and 17 µg equivalents/kg, 1 day after treatment, 441, 364, 75 and 72 µg equivalents/kg, 3 days after treatment and 376, 348, 65 and 50 µg/kg, 7 days after treatment. The desisopropyl propetamphos concentrations in kidney were: 207, 56 and 41.2 µg/kg on days 1, 3 and 7 after treatment, respectively. The concentrations of desisopropyl propetamphos in liver, muscle and fat were less than their respective limits of quantification (102, 20 and 20 µg/kg, respectively) on days 1, 3 and 7 after treatment.

19. Two milk residues depletion studies were carried out in lactating ewes. In the first study used 6 sheep, were plunge dipped in a commercial formulation at the indicated rate. Thirty hours post dipping, residues of propetamphos were undetectable in the milk from 2 sheep; residues in milk from the remaining 4 sheep ranged from 340 to 1370 µg/kg. Forty four hours after dipping, residues were detectable in milk from only 1 sheep (40 µg/kg). In the second study 3 crossbred ewes were dipped in a commercial preparation at the recommended rate. Seventeen hours post-dipping, residues of propetamphos in the milk ranged from 140 to 710 µg/kg and were undetectable 66 hours post-dipping. In the second study, oxytocin was administered to "let-down" the milk and detergent was used to clean the udders; it was not clear whether these substances would interfere with the analysis. There was no information concerning the composition of the residues in milk and no justification for the choice of marker residue.

20. The proposed routine analytical method was based on liquid-liquid extraction and GC-ECD and appeared to have a limit of quantification of 25 µg/kg for propetamphos for all sheep tissues and limits of quantification for desisopropyl-propetamphos of 102, 20, 20 and 20 µg/kg for liver (fresh samples) kidney, muscle and fat respectively. However, the method has not been validated for propetamphos in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community.

Conclusions and recommendation

Having considered that:

- an ADI of 0.0005 mg/kg bw (i.e. 0.03 mg/person) was established for propetamphos,
- from the available data a suitable marker residue and its concentration relative to that of total residues in sheep tissues could not be determined,
- the marker residue was considered to be the sum of the residues of propetamphos and desisopropyl-propetamphos,
- the marker residue represents approximately 2%, 24%, 8% and 60% of the total residues in liver, kidney, muscle and fat, within 24 to 48 hours of treatment,
- residues of propetamphos in muscle and liver were usually below the limit of detection of the analytical method; in the pivotal study residues of desisopropyl-propetamphos were in all cases below the limit of quantification; consequently it was considered unnecessary to set MRLs for these tissues,
- there was no information concerning the composition of the residues in milk and no justification for the choice of a marker residue, consequently no MRLs could be proposed for milk,
- an analytical method for the simultaneous determination of residues of propetamphos and desisopropyl-propetamphos in tissues was available but the method was not fully validated,

the Committee for Veterinary Medicinal Products recommends the inclusion of propetamphos in Annex III 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
Propetamphos	Sum of the residues of propetamphos and desisopropyl-propetamphos	Ovine	90 µg/kg 90 µg/kg	Fat Kidney	Not for use in animals from which milk is produced for human consumption. Provisional MRLs expire on 1.1.2002

Based on these MRLs, the maximum theoretical daily intake of total residues would represent approximately 88% of the ADI.

Before the Committee can consider the inclusion of propetamphos in Annex I, the applicant should provide answers to the following list of questions:

LIST OF QUESTIONS

1. In the original residues depletion studies using unlabelled propetamphos and application by dipping, significant residues of unmetabolised propetamphos were found in some tissues, particularly fat. In the new pilot study using radio-labelled propetamphos, residues of propetamphos were very low and the metabolite desisopropyl-propetamphos accounted for a significant proportion of the residues in most tissues. In the follow-up pivotal study, significant amounts of desisopropyl-propetamphos were found only in kidney. The Applicant should clarify these inconsistent results and confirm the ratio of marker to total residues at later time points.

2. The proposed routine analytical method should be fully validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community and re-presented in a suitable international format (e.g. ISO 78/2).