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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TOLDIMFOS

SUMMARY REPORT

1. Toldimfos sodium is the sodium salt of 4-dimethylamino-2-methyl-phenyl-phosphinous acid, a derivative of phosphoric acid. It is indicated for the treatment and prophylaxis of diseases which arise in connection with parturition and the peri-partum period, developmental and nutritional disorders in young animals, disorders of bone growth and tetany or paresis caused by disorders of calcium, magnesium and phosphorous metabolism. The recommended therapeutic dose is 10 mg/kg bw given by intravenous, intramuscular or subcutaneous injection. Injections are normally repeated to clinical effect, up to a maximum of 10 injections. Toldimfos is indicated for use in the following food producing species: horses, cattle, sheep, pigs and goats.

2. No specific data on the pharmacodynamic action of toldimfos was submitted. The precise mode of action of toldimfos is unknown and it is questionable whether the effect of toldimfos is simply a matter of the substitution of deficient phosphorus. It appears more likely that the effect of toldimfos arises due to multiple stimulation of metabolism within the body. Toldimfos is an aromatic phosphorus compound which falls between phosphorous itself and phosphoric acid in the stages of oxidation.

3. The intramuscular administration of toldimfos to cattle at a dosage level of 10 mg/kg bw led to a peak blood concentration within 10 to 20 minutes after administration. Pharmacokinetic studies in calves showed the mean half life of toldimfos in serum was 1.07 hours, whilst the corresponding value for dairy cows was 1.15 hours. The pharmacokinetic profile corresponded to a one compartment model of distribution. The mean residence time for toldimfos in blood in calves was 3.6 hours, with a value of 3.1 hours being recorded for dairy cows. These studies were not radiolabelled. Urinary excretion studies revealed a rapid elimination of toldimfos following dosing. The major fraction of the administered dose was eliminated within 6 hours, whilst at the 24 hour time-point, only concentrations in the range of the limit of detection (limit of detection: 0.5 µg/kg) were observed. Twelve to twenty four hours following a second injection of toldimfos, similar low concentrations were recorded in urine. Toldimfos was eliminated as parent compound. Elimination of the compound in milk was extremely low, with levels below the limit of quantification of the analytical technique (limit of quantification for milk 0.15 µg/kg) at most time points. Repeated dosing in the pharmacokinetic study did not lead to bio-accumulation of the test compound.

   A further GLP compliant radiolabelled study was performed in which 20 mg ^{14}C-toldimfos/kg bw was administered by intramuscular injection on a single occasion to a total of 12 adult bovines. Toldimfos was rapidly absorbed from the injection site with peak concentrations of 52 to 61 µg equivalents/ml occurring in serum at 10 minutes post administration. The serum half-life was 0.7 to 0.95 hours, with a mean residence time of 1.1 to 1.4 hours. Total clearance from serum was 0.2 to 0.27 l/hour. The volume of distribution in bovines was 0.27 to 0.34 l/kg. Parent compound was the dominant fraction in serum. However, there was a significant unknown metabolite fraction also present in the serum of both sexes. More

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than 95% of the dose was recovered by 48 hours post drug treatment in both sexes. Urine was the principle route of elimination, accounting for over 80% of the recovered radioactivity.

In urine, unchanged toldimfos accounted for more than 90% of the recovered radioactivity. Faecal samples from male animals had both parent compound and a further unknown metabolite present.

4. Urinary excretion studies conducted in the dog indicated that the kidney was the major route of excretion of toldimfos. Male Beagle dogs received an intravenous (n = 2) or an intramuscular (n = 2) injection of 2000 mg of toldimfos per animal. The results demonstrate that following intravenous and intramuscular administration of toldimfos to male Beagle dogs, virtually 100% of the administered dose was excreted within 24 hours as parent compound. This study did not conform to GLP standards.

5. A tissue distribution study was also performed in rats where toldimfos was administered at a dosage rate of 50 mg/kg bw to two treatment groups. A total of 24 rats were used in this study; 12 received toldimfos subcutaneously and 12 received the compound orally. Twenty-four hours later a second injection of toldimfos at the same dosage rate was administered. Twelve rats were sacrificed at both the 24 hour and the 48 hour time-points post first dose. The limit of quantification for the analytical technique in this study was 0.5 mg/kg. Samples of liver, kidney, fat, muscle and injection site were removed for analysis. Examination of the individual data shows that all samples had concentrations below the limit of quantification at all time points, with the exception of one rat who had an injection site value of 2.53 mg/kg at the 24 hour time point. All other rats had injection site concentrations below the limit of quantification. This study was not radiolabelled, but was conducted in accordance with GLP standards.

6. Two acute toxicity studies were performed with toldimfos in mice with conflicting results. In the first study the acute LD_{50} following subcutaneous administration of toldimfos was 164 mg/kg bw; following intramuscular administration, the corresponding value was 132 mg/kg bw whilst the value for the intravenous route was 4000 mg/kg bw. A later acute toxicity study in mice revealed an acute LD_{50} of 8000 mg/kg bw following subcutaneous administration and 6000 mg/kg bw following intravenous administration. As both studies are over 30 years old, no raw data was available to evaluate these two studies. These studies did not meet GLP standards. Acute toxicity studies by the intravenous route in the rabbit revealed that dosage levels between 5000 and 6000 mg/kg bw led to death. Observed signs of toxicity included increases in heart rates and respiratory rates and increased voiding of urine. Transient paralysis was also a feature.

7. Repeated dose toxicity studies were performed in the rat. In the first study, 10 rats received 20 mg toldimfos/kg bw subcutaneously, 6 days a week for a period of 4 weeks. This dose level caused parenchymal degeneration and fatty degeneration of the liver in some rats. Three weeks after cessation of drug administration, no abnormalities were recorded in the liver. This study did not correspond to GLP standards. More recently a 91 day repeated dose subcutaneous toxicity study was performed in the rat. The dosage levels used in this study were 10, 20 and 50 mg/kg bw. A control group was also incorporated. No abnormal clinical signs nor changes in behaviour were recorded in this study. Bodyweight and growth curves were similar for treated and control animals. Food intake was slightly increased in the highest dosage group. Minor changes were recorded on haematology, but such changes were transient and reversible with time. The white blood cell count was markedly decreased at the 10 mg and 50 mg/kg bw dosage levels following drug withdrawal. On biochemical evaluation, a significant increase in alamine transaminase values was recorded in the highest dosage group, particularly in males. Glucose and aspartate transaminase values were also significantly increased in the highest dosage group females. Statistically significant alterations in organ weights (both relative and absolute) were recorded at all dosage levels. Nephrocalcinosis was recorded at post mortem in 50% of females treated at the 50 mg/kg dosage level. As nephrocalcinosis is commonly recognised in rats as a background problem (particularly in female laboratory sewer rats), the significance of such a finding is doubtful. Due to the changes seen on haematology and *post mortem* examination, even at the lowest dosage level, it was not possible to set a NOEL for this study.

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A further GLP compliant study was performed in which groups of 10 male and 10 female rats were fed toldimfos in the diet at dosage levels of 0, 140, 1400 and 14000 mg/kg feed/day for 90 days. No abnormal behaviour nor mortalities were recorded during this study. Body weight and food consumption were unaffected by treatment. Haematological examination at study termination was unremarkable, but serum biochemistry revealed statistically significant increases in bilirubin concentrations in both sexes at the highest dose level. Statistically significant increases were recorded in the concentrations of alanine transaminase and aspartate transaminase in either one or both sexes at intermediate and high dose levels. Blood glucose levels were significantly increased in males of all dosage groups. However, the mild increase in blood glucose concentrations was not considered to be of toxicological significance. Absolute and relative lung weights were statistically significantly decreased in mid and high dose males. Decreased absolute ovarian weights were observed in high dose females, whilst increased relative pituitary weights were observed in mid dose females. Histopathology of affected tissues was unremarkable. A dosage level of 140 mg/kg feed/day (equal to 10 and 11mg/kg bw/day in males and females respectively) was retained as a NOEL from this study.

8. A GLP-compliant reproductive toxicity study was conducted in SPF Wistar rats, where toldimfos was administered subcutaneously at dosage levels of 10, 20 and 50 mg/kg bw. Parental and F1 generation females were treated from 14 days prior to mating until the end of lactation. Male rats were treated from the time of weaning until the end of mating in both generations. Each treatment group consisted of 26 female and 13 male rats. A decrease in male fertility was recorded in all F_1 parental generation groups (both treated and untreated). The percentage of females mated in the parental P generation varied between 77 and 96%. Due to the decrease in male fertility recorded in all F_1 parental generation groups, the percentage of females mated in the F_1 generation varied between 56% and 73%. This effect was not dose dependent. The following parameters were not adversely affected by treatment in the period up to weaning; the mean number of days required for mating, the percentage of gravid females, gestation period, mean litter size, sex distribution of pups, live birth index, developmental stage of offspring and the bodyweight of pups and growth rate of such offspring. At the highest dosage level tested (50 mg/kg bw), there was a 10% loss of whole litters surviving in both the F_1 and F_2 generations. A total of 45 pups were lost up to the time of weaning in the F_1 generation versus 27 in the control group. A significant incidence of pup mortality in the F_2 generation occurred in all treated groups. The incidence was highest in the 10 mg/kg treated group (the incidence for pup mortality in the F_2 generation was 36% for the 10 mg/kg dosage group, whilst the corresponding figure for the control group was 10.5%). The major time for pup mortality was 2 to 7 days post partum. Such mortality seemed to be associated with an increased incidence of cannibalism by dams. Maternal cannibalism occurred in approximately 25% of control litters and 53% of litters treated at 10 mg/kg bw. The increase in pup mortality in the 10 mg/kg bw treatment group was primarily attributable to two dams that ate almost their entire litters. The increased incidences of cannibalism and pup mortality were not dose dependant. Histopathological examination of parent generations revealed no abnormalities in the reproductive system nor in the pituitary gland which could be treatment related. Although a NOEL value could not be retained from this study, this fact should not be considered of pivotal importance in the overall safety assessment of toldimfos, as the route of administration in this study was subcutaneous, not oral.

9. The study on reproductive toxicity also investigated any teratogenic effects of toldimfos. Gross malformations were recorded in two F_1 pups of the control group and in one F_1 pup of the 50 mg/kg dosage group. Four F_2 pups in litters of a 10 and a 20 mg/kg treated mother demonstrated aplasia of the ureters, anus and kidneys. No malformations were recorded in F_2 pups from dams treated at the highest dosage level. Overall, it would appear that malformations were sporadic and not dosage dependent.

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10. A total of 3 studies were performed to investigate the mutagenic potential of toldimfos. The *Salmonella* microsomal assay was conducted using two strains of *Salmonella typhimurium* (TA98 and TA100). Studies were performed both in the presence and absence of metabolic activation. The dosage levels of toldimfos investigated were as follows : 140 mg, 105 mg, 70 mg, 35 mg, 17.5 mg and 7 mg per plate. The results recorded in this study were negative for both strains TA98 and TA100, both in the presence and absence of metabolic activation. A cytogenetic analysis study was undertaken whereby toldimfos was injected intraperitoneally to Balb C mice at dosage levels of 120, 300 and 600 mg/kg bw. Five male and 5 female mice were incorporated into each group. The animals were sacrificed 24 hours following drug administration and smears of bone marrow cells were prepared for cytogenetic analysis. Fifty metaphases were analysed for each test animal. This study demonstrated no *in vivo* mutagenic effects of toldimfos on mammalian cells. A further study was undertaken to investigate whether biochemical markers of mutagenicity were induced by toldimfos. Toldimfos was dissolved in dimethyl-sulfoxide and injected intraperitoneally at dosage levels of 0.1 to 100 mg/kg bw to 10-week old male C57 B1/6 mice. Measurement of microsomal protein fractions demonstrated that toldimfos did not induce certain bioactivating or detoxifying enzymes. All studies were performed to GLP standards. The available evidence indicates no mutagenic hazard for toldimfos.

11. No carcinogenicity data was available. In line with the negative results obtained in the mutagenicity studies, and in the absence of any structural alerts, carcinogenicity studies were not considered necessary.

12. Toldimfos has not been subjected to any specific tests for immunotoxicity, but the findings of the repeated dose toxicity tests do not indicate that toldimfos is likely to be immunotoxic.

13. No information on the microbiological properties of residues was available. As no such effects are expected for toldimfos, specific studies were not considered necessary.

14. Toldimfos has been historically used in human medicine since 1920. However, it is not currently indicated for human use. In humans, the product was used as an injectable or an oral preparation for states of debility, following major operations and infectious diseases, the treatment of chronic stress and as treatment for physical and mental overexertion. The recommended dosage rate was 10 to 20 mg of toldimfos per person per day by subcutaneous administration or 300 mg of toldimfos per person per day by oral administration. Toldimfos was well tolerated after both parenteral and oral administration. Although a significant amount of human data was available, the quality of such data was poor, making meaningful interpretation difficult.

15. A toxicological ADI of 0.1 mg/kg bw (i.e. 6 mg/person for a 60 kg person) was established based on the NOEL of 10 mg/kg bw/day from the 90-day rat study, and applying a safety factor of 100.

16. A tissue residue study was performed with the use of toldimfos in calves. A total of 8 calves were slaughtered at 2 separate time points (4 animals per time point). Toldimfos was administered by intramuscular injection at a dose rate of 10 mg/kg bw on two occasions, 24 hours apart. Four calves were sacrificed 24 hours following the second injection, with the remaining 4 being slaughtered at the 48 hour time point following the second injection. Samples of liver, kidney, muscle, fat and injection sites were removed for analysis. The parent compound was assayed by an HPLC technique with a limit of quantification of 500 µg/kg. This study, however, was not radiolabelled. No residues above the limit of quantification were detected in any tissue samples at either of the 2 slaughtering time points. The highest residue values detected were recorded in liver and fat. Following the second administration of toldimfos, a slight increase in some tissue concentrations was detected, but all levels remained below the limit of quantification. Residues did not appear to persist at the injection site, with no residues being detected at this site in all four calves at the 48 hour time point post second injection. The study was performed to GLP standards. No information was available on tissue residue levels in other indicated species.

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A GLP-compliant radiolabelled residue depletion study was conducted in adult cattle. Groups of 4 animals each were sacrificed at 8, 24 and 48 hours following a single intramuscular injection of ^{14}C-toldimfos at 20 mg/kg bw. Total radioactivity was measured by liquid scintillation counting, with an limit of quantification of approximately 100 µg equivalents/kg of tissue for all matrices except fat, where the limit of quantification was approximately 300 µg equivalents/kg. Highest mean concentrations of total residues were recorded at the injection site and in kidney, although variations according to sex were apparent. The mean concentrations of total residues at the injection site in males were 8200, 1900 and 2300 µg equivalents/kg at the 8, 24 and 48 hour time points, respectively. The corresponding values for the female animals were 17 100, 2200 and 1300 µg equivalents/kg at the same respective time points. Mean total residue concentrations in kidney were 8000 and 2400 µg equivalents/kg in males and females, respectively, at the 8-hour time point post treatment. Thereafter, mean values declined to similar concentrations in both sexes, and were below 250 µg equivalents/kg at the 24 and 48-hour time points. Mean total residue concentrations in liver and fat were below 700 µg equivalents/kg in both sexes at the 8-hour time point, with values declining to below 300 µg equivalents/kg by 24 hours. Muscle, other than injection site, had the lowest mean total residue concentrations, with values below 100 µg equivalents/kg at all time points assayed.

Samples from the above studies were also assayed for concentrations of toldimfos, using a HPLC assay technique with an limit of quantification of 500 µg/kg. Quantifiable residues were only detected in kidney at the 8 hour time point, with mean concentrations of 6000 and 1400 µg/kg recorded for males and females respectively at this time point. Kidney tissue from later time points, and all samples of muscle (other than injection site), fat and liver had residue concentrations below the limit of quantification. Measurable residues of toldimfos were only detectable at the injection site at the 8 hour time point post dosing. Individual values recorded ranged between 1000 and 9000 µg/kg.

17. Residues of toldimfos in milk were analysed as part of a pharmacokinetic experiment involving 2 dairy cows. Milk samples were collected from 2 cows who received two intramuscular injections of toldimfos at the recommended therapeutic dosage, 24 hours apart. The limit of detection and the limit of quantification for the analytical method was 150 µg/kg. Residues of toldimfos were assayed at 12 hour intervals over a period of 2 days and were below 200 µg/kg at all time points in milk from both dairy cows. Twenty-four hours following the second dose of toldimfos, residue levels were below the limit of quantification in both animals. Although the analytical techniques were performed to GLP standards, the number of animals incorporated in this study was totally inadequate for meaningful interpretation. No radiolabel was attached to the toldimfos administered in this experiment.

    The analytical technique utilised for residues of toldimfos in tissues and body fluids was based on HPLC. A similar method was used for all experiments; only the elution procedure was changed depending on the sample. The reference substance utilised was toldimfos.

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Conclusions and recommendation

Having considered the criteria laid down by the Committee for Veterinary Medicinal Products for the inclusion of substances into Annex II of Council Regulation (EEC) No 2377/90, and in particular that:

- A toxicological ADI of 0.1 mg/kg bw (i.e. 6 mg/day for a 60 kg human) was established,
- the substance is used infrequently in a small number of individual animals,
- the pharmacokinetic data from bovines and laboratory animal species indicate that toldimfos is rapidly eliminated,
- at 24 hours after treatment, the amount of residues likely to be ingested by consumers represents only a fraction (less than 25%) of the toxicological ADI;

the Committee for Veterinary Medicinal Products concluded that there is no need to establish MRLs for toldimfos and recommends its inclusion into Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Toldimfos	All food producing species	

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