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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FLUNIXIN

SUMMARY REPORT (1)

1. Flunixin is a non-steroidal anti-inflammatory drug (NSAID), and a non-narcotic analgesic with antipyretic activities. In veterinary medicine, it is used with meglumine as solubilizer as flunixin meglumine. Flunixin meglumine is used in the alleviation of inflammation and pain associated with musculo-skeletal disorders and colic in horses; the control of acute inflammation associated with infectious diseases in cattle and as an aid in the treatment of mastitis metritis agalactia syndrome (MMA) in sows. Flunixin meglumine is available in injectable, granular and paste formulations and can be administered by the intravenous, intramuscular and oral routes in horses at the recommended therapeutic dose of 1.1 mg/kg bw once a day for up to 5 days. A dose of 2.2 mg/kg bw by the intravenous route once a day for up to 3 days is indicated for bovines, whilst 2.2 mg/kg bw intramuscularly (up to 2 injections, 12 hours apart) is recommended for sows affected by MMA syndrome. Flunixin meglumine is also used in veterinary medicine in combination with oxytetracycline for the treatment of bovine pneumonia at a dose of 2 mg/kg bw (once a day for 3 to 5 days by the intravenous or the intramuscular routes).

   The vast majority of studies provided both in the safety file and the residue file were performed with flunixin meglumine.

2. Flunixin demonstrates potent inhibition of the cyclo-oxygenase system involved in the inflammatory pathway. The resultant decrease in production of certain inflammatory mediators accounts for its analgesic, anti-pyretic and anti-inflammatory properties. A series of non-GLP-compliant studies demonstrated that flunixin administered at doses of 0.1, 1.0 and 10 mg/kg bw intravenously to anaesthetised dogs had no significant effects on heart rates or blood pressure. The dose of 10 mg/kg bw had some statistically significant effects on autonomic challenges. Oral doses of 10 and 25 mg flunixin/kg bw had no significant effects on blood pressure, heart rates or electrocardiograms in conscious dogs. Due to the insufficient number of end-points measured in these studies, it was not possible to elucidate a pharmacological NOEL for the dog.

3. Pharmacokinetic studies in the rat show that following the intramuscular administration of a single dose of ^{14}C-flunixin meglumine at a dose of 10 mg/kg bw, only 36.5% and 1.8% of the administered dose was present at the injection site at the 5 minute and 6 hour time points after treatment. More than 90% of the dose (61% in faeces and 29% in urine) was excreted in the first 48 hours after dosing. Approximately 12 to 14% of the dose is excreted unchanged in both urine and faeces. At 24 hours after treatment, the plasma and tissue drug levels were less than 1 µg/g, with the exception of the large intestine and its contents. Following oral dosing of rats with ^{14}C-flunixin meglumine at a dose of 2.5 mg/kg bw, the plasma concentration 2 hours after treatment was 1.0 to 1.5 µg/ml.

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Groups of dogs received flunixin meglumine (non-radiolabelled) at a dose of 2 mg/kg bw by the intravenous, subcutaneous and oral routes. Following oral dosing, peak plasma concentrations were attained at 45 minutes after treatment (mean C_{max}: 4.3 µg/ml). Levels declined to below 0.05 µg/ml by 12 hours after treatment. Following subcutaneous dosing, mean C_{max} of 3.0 µg/ml of flunixin meglumine was reached at 1 hour after treatment. Plasma concentrations were less than 0.029 µg/ml at 18 hours after treatment.

Following intravenous treatment, a mean peak plasma concentration of 10.3 µg/ml was reported at 3 minutes after treatment. At the 12 hours time point after treatment, plasma levels were less than 0.035 µg/ml. From the available data, the plasma bioavailability following oral and subcutaneous administration was 97% and 92%, respectively, as compared to the intravenous route. In a further study, comparing the subcutaneous and oral routes of administration of flunixin meglumine, the elimination half-life of the test compound was 9 to 10 hours for both routes.

^{14}C-Flunixin meglumine was administered as a single intramuscular injection to monkeys at a dose equivalent to 5.0 mg/kg bw of free acid. Absorption from the injection site was rapid and peak plasma concentrations were attained at 24 minutes after treatment. Parent compound was the major component in plasma and urine. Only minor amounts were excreted as hydroxylated metabolites in urine. Faecal excretion accounted for approximately 33 to 37% of administered radioactivity. Urinary excretion accounted for approximately 63 to 68% of administered radioactivity.

4. Following the intramuscular administration of ^{14}C-flunixin meglumine to calves at a dose of 0.25 mg/kg bw, 31% and 43% of the radioactivity was recovered in the urine and faeces respectively. Peak plasma levels of radioactivity occurred 5 to 10 minutes after treatment. Parent compound accounted for more than 90% of plasma radioactivity. Approximately 8% and 21% of the administered dose was excreted as parent compound in urine and faeces respectively. The urine and faeces also contained a large polar fraction and 2 minor metabolites. The distribution half-life was 0.3 hours and the elimination half-life was 10.5 hours in this study. The majority of the recovered drug was eliminated within the first 72 hours.

   ^{14}C-Flunixin meglumine was administered once daily for 2 days to 3 lactating cows and 3 steers by the intravenous route at a dose level of 2.2 mg/kg bw. Approximately 90% of the total administered radioactivity was recovered within 24 hours after the second dose. Five minutes after treatment, mean plasma concentrations were in the range 17.1 to 18.3 µg/ml, declining to values of 0.36 to 0.65 µg/ml at 2 hours after treatment. Levels of total radioactivity in milk were generally low, with mean values of 0.05 to 0.06 µg/ml at the 9 hours time point after treatment.

   Nine lactating cows were given 3 daily doses of flunixin meglumine by the oral, subcutaneous and intravenous routes at a dose of 2.2 mg/kg bw. As compared to the intravenous route, the bioavailability of flunixin meglumine was 53% and 75% by the oral and subcutaneous routes respectively. At the 2 hour time point after intravenous treatment, a second peak plasma concentration occurred at the 3 to 4 hour time point after treatment, possibly indicating reabsorption form the gastrointestinal tract.

   Following a single administration of flunixin meglumine to horses (route of administration not stated) at a dose of 1.1 mg/kg bw, the plasma half-life was determined to be 1.6 hours. In a further study conducted in ponies using the test compound at a dose of 1 mg/kg bw by the oral route (once a day for 5 consecutive days), peak plasma levels were obtained at 1 to 1.5 hours after treatment and were in the range 1.5 to 2.6 µg/ml on each day at that time point.

   ^{14}C-Flunixin meglumine was administered intramuscularly to pigs at a dose of 1.1 mg/kg bw. Approximately 57% of the radioactivity was recovered in urine and 21% in faeces in the 96-hour period after treatment. Peak plasma levels of radioactivity were recorded at 5 to 30 minutes after treatment. The major radioactive component in plasma was parent compound (55 to 83%). Approximately 24% and 10% of the administered dose was excreted as parent compound in urine and faeces respectively. A glusulase hydrolysable conjugate of flunixin meglumine in the urine accounted for a further 23% of the administered dose. Following a single intramuscular injection of 3.3 mg ^{14}C-flunixin meglumine to piglets, a mean of 72% and 18% of total administered radioactivity was recovered in urine and faeces respectively over a period of 16 days. Parent compound was again the major component identified in plasma, urine and faeces.

5. The acute LD_{50} of flunixin meglumine in the mouse was greater than or equal to 170, 306 and 111 mg/kg bw by the oral, intramuscular and intravenous routes; for rats the corresponding values were greater than or equal to 113, 180 and greater than or equal to 90 mg/kg bw. In an acute inhalation toxicity study in the rat, all animals died at an exposure concentration of 0.52 mg/l for 4 hours.

6. A series of repeated dose toxicity studies were conducted in the rat. Groups of 32 rats per treatment group received flunixin orally at doses of 0, 2, 4, 8 and 16 mg/kg bw daily for 6 weeks. Reductions in bodyweight gains were evident at doses higher than or equal to 4 mg/kg bw. Significant reductions in white blood cell counts in males were evident in all treatment groups. Significant pathological changes of the gastrointestinal tract and peritonitis were evident in animals treated at doses higher than or equal to 8 mg/kg bw. The dose of 2 mg/kg bw was retained as a NOEL.

   A 13-week repeated dose oral toxicity study in rats was conducted using doses of 1.5, 3 and 6 mg flunixin meglumine/kg bw. Bodyweight gains were reduced at the highest dose level. Slight decreases in haematocrit and haemoglobin levels as well as increases in white blood cells counts were also recorded at the 6 mg/kg bw dose level. The dose of 3 mg/kg bw can be retained as a NOEL.

   A 1-year GLP compliant repeated dose oral toxicity study was performed in rats at dose levels equivalent to 0, 1, 2 and 6 mg flunixin free acid/kg bw/day. Group sizes consisted of 60 animals (30 males and 30 females) per dose level. Mortality rates of 33% for males and 20% for females were recorded at the highest dose level. Deaths were primarily associated with gastrointestinal ulceration and peritonitis. Statistically significant changes were reported in food intake, body weight, body weight gain and various clinical pathology parameters at the highest dose level. Statistically significant increases in the incidence of faecal occult blood and splenic weights were evident at doses higher than or equal to 2 mg/kg bw/day. *Post mortem* changes reported at doses higher than or equal to 2 mg/kg bw/day included gastro-intestinal tract ulceration, peritonitis, lymphadenopathy, pallor of the body and papillary necrosis of the kidneys. A NOEL of 1 mg/kg bw was retained.

   A 4-week repeated dose study with flunixin meglumine was conducted by the intramuscular route in the rat. Dose of 1, 2 and 4 mg/kg bw were employed. The haematocrit and haemoglobin values were reduced in some females of the high dose group. The only significant *post mortem* finding was local tissue damage at the injection site. The dose of 2 mg/kg bw was retained as a NOEL with regard to systemic toxicity from this study.

   A 13-week repeated dose study by the intramuscular route was also conducted in rats at doses of 1.5, 3 and 6 mg flunixin meglumine/kg bw. Reduced bodyweight gains were evident at doses higher than or equal to 3 mg/kg bw. Haematocrit and haemoglobin values were reduced at the mid and the high dose levels at *post mortem* examination. Gastrointestinal tract ulcers, adhesions, peritonitis and enlarged mesenteric lymph nodes were present at doses higher than or equal to 3 mg/kg bw. Localised tissue changes at the injection site were recorded in all treatment groups. The dose of 1.5 mg/kg bw can be retained as a NOEL with regard to systemic toxicity from this study.

7. A 9-day repeated dose toxicity study was performed in Beagle dogs at oral doses of 1.1, 3.3 and 5.5 mg flunixin meglumine/kg bw (8 animals per treatment group). Slight decreases in food intake were recorded at the mid and the high dose levels. Effects on haematology were recorded in all treatment groups. Faecal occult blood tests were positive in all treated groups. A NOEL could not be set from this study.

   Two 4-week repeated dose studies using flunixin meglumine by the intramuscular route were performed in dogs at dose of 5, 10 and 20 mg/kg bw of free acid. Marked effects on food intake, bodyweight and haematological parameters were recorded at all dose levels. Bloody vomit and/or faeces and gastrointestinal ulcerations were also noted at all dose levels. No NOELs could be retained from either of these studies.

A 3-month repeated dose study was performed in dogs with flunixin meglumine by oral gavage. The dose levels employed were equivalent to 0, 0.01, 0.05, 0.15, 0.4 and 0.6 mg flunixin free acid/kg bw/day. A total of 10 animals per dose group were included (5 males and 5 females). No compound related changes were observed on survival, clinical condition, body weights, food consumption values or organ weights in any of the test groups. Haematology, biochemistry and urinalysis parameters were unaffected by treatment. Flunixin meglumine administration had no effect on ophthalmic or electrocardiogram examinations. Positive results for faecal occult blood were evident in all test groups (including controls). Food samples were also positive for the presence of occult blood. No treatment-related abnormalities were evident at *post mortem* on either gross pathology or histopathology. A NOEL of 0.6 mg flunixin free acid/kg bw/day can be retained from this GLP compliant study.

8. A 4-week repeated dose toxicity study by the intramuscular route was conducted in monkeys using flunixin meglumine at dose of 3, 10 and 30 mg/kg bw. Treatment had no effect on food intake, bodyweight, haematology/biochemistry or urinalysis. Localised tissue damage at the injection site was recorded in all treatment groups. This was the only significant finding noted at *post mortem*. The NOEL for systemic toxicity was 30 mg/kg bw.

   A 13-week repeated dose oral toxicity study was performed in monkeys using flunixin meglumine at doses of 5, 15, 45 and 60 mg/kg bw of free acid. A slight decrease in bodyweight gain and blood streaked faeces were evident at doses higher than or equal to 45 mg/kg bw. Slight decreases in haemoglobin, haematocrit, albumin and serum protein values were present at doses equal to or greater than 45 mg/kg bw. An increase in activated partial thromboplastin time was recorded at dose levels of equal to or greater than 15 mg/kg bw. The dose level of 5 mg/kg bw can be retained as a NOEL from this study.

   A 13-week repeated dose study by the intramuscular route was performed in monkeys using flunixin meglumine at doses of 5, 15, 45 and 60 mg/kg bw. A compound related decrease in food intake and bodyweight gain as well as reductions in haematocrit, haemoglobin, serum protein and alkaline phosphatase values were recorded at doses higher than or equal to 45 mg/kg bw. A dose related localised irritation at the injection site was noted at doses equal to or greater than 15 mg/kg bw. Bloody stools were occasionally noted in animals treated at all dose levels in the first 5 weeks of the study. No evidence of gastrointestinal tract ulceration was evident on *post mortem* examination. Due to the presence of bloody stools at all dose levels tested, a NOEL could not be retained from this study.

9. Two 21-day repeated dose dermal toxicity studies were performed in rabbits. In the first study flunixin, and in the second study flunixin meglumine, were applied as a cream or a spray formulation to the test animals at doses of 10, 20, 40 and 80 mg/kg bw. Compound related effects recorded in these studies included mortalities, reductions in bodyweight gain and dermal reactions. Histopathological examination of the testes revealed multinucleated giant spermatids in some treated animals. No NOEL could be retained from either of these studies.

10. The tolerance of flunixin meglumine was investigated in the target species. Following intravenous administration to cattle, the recommended therapeutic dose was generally well tolerated except for a low incidence of blood in the faeces (1.9%). Oral and intravenous administration in horses at the recommended therapeutic dose resulted in the presence of occult blood (both routes) and elevations in serum alkaline phosphatase, bilirubin and triglyceride levels (oral route), but was otherwise well tolerated.

    Treatment of puerperal sows at 1 and 3 times the recommended therapeutic dose revealed no significant adverse effects other than an increase in white blood cell counts.

11. No data on a 2-generation reproductive toxicity study were presented.

A 4-month oral study was performed in the rat using doses of 1.5, 3 and 6 mg/kg bw of free acid. Males were dosed from 63 days before mating until the end of the mating period. Females were dosed from 14 days before mating until they were euthanased at day 14 post mating or day 21 *post partum*. Bodyweights were reduced in females at the highest dose level. Mortalities were recorded in parents in the mid and the high dose groups, and was associated with gastrointestinal changes at *post mortem* examination. The length of gestation was increased by an average of 12 hours in all treatment groups when compared to controls. The percentage of pups dying during the lactation period was elevated in all treatment groups. Such deaths resulted from maternal neglect, which was likely related to the toxic effects of the compound on the dams health. No NOEL could be retained from this study.

A second reproductive toxicity study was performed in the rat using the same basic protocol as above, except that flunixin meglumine was administered intramuscularly at doses of 1, 2 and 4 mg/kg bw/day. Treatment had no effect on pregnancy rates, the distribution of foetuses in uterine horns, the number of implantations, litter size and sex distribution. Furthermore, no treatment related effects were recorded for prenatal survival of progeny, bodyweight at birth and postnatal growth rate. The gestation length and post-natal survival rates of progeny were affected, however, at the mid and the high dose levels. Such mortalities were often related to changes in the gastrointestinal tract. The dose of 1 mg/kg bw was retained as a NOEL.

12. Teratogenicity data were available from studies performed in the rat and rabbit. Groups of 25 female Charles River CD rats were given flunixin meglumine at doses of 3, 5 and 7 mg/kg bw/day by oesophageal intubation from day 6 to 15 after mating. Mortalities were recorded in dams especially at the mid and the high dose levels. Gastrointestinal lesions and peritonitis were reported on *post mortem* examination. No adverse effects on reproductive parameters were recorded. The dose of 3 mg/kg bw can be retained as a NOEL for maternotoxicity. Flunixin meglumine was not teratogenic in this study up to the maximum dose tested of 7 mg/kg bw.

    Groups of 25 female rats were administered flunixin meglumine by intramuscular injection at daily dose of 2, 4 and 6 mg/kg bw from day 6 to 15 post mating. There was no evidence of a teratogenic effect in this study up to the highest dose tested i.e. 6 mg/kg bw.

    Groups of 25 female rats were administered flunixin meglumine at dose of 1.5, 3 and 6 mg/kg bw by oesophageal intubation from day 6 to 15 post mating. The highest dose level had significant effects on bodyweight gain and postnatal survival of progeny. Gastrointestinal lesions were evident in dams at the 6 mg/kg bw dose level. The incidence of pups with retarded ossification was significantly increased at the highest dose level. The dose level of 3.0 mg/kg bw can be retained as a NOEL for maternotoxicity and foetotoxicity, whilst there was no evidence of any teratogenic effect in this study up to the maximum dose tested of 6 mg/kg bw.

    Groups of 25 female rats (16 weeks old when mated) were administered flunixin meglumine by the intramuscular route at daily doses of 2, 4 and 6 mg/kg bw from day 14 post mating to day 21 *post partum*. Gastrointestinal changes were reported in all treatment groups and resulted in mortalities at doses higher than or equal to 4 mg/kg bw. The gestation period was prolonged by approximately 18 hours and postnatal survival of pups was reduced at doses higher than or equal to 4 mg/kg bw. Due to the gastrointestinal changes reported at all dose levels, no NOEL could be retained for maternotoxicity. The dose of 2 mg/kg bw was retained as a NOEL for foetotoxicity. Flunixin meglumine was not teratogenic in this study up to the maximum dose tested of 6 mg/kg bw.

    Flunixin meglumine was administered orally to pregnant New Zealand White rabbits at doses of 3, 9 and 15 mg/kg bw from day 6 to 18 post mating. Treatment had no significant effect on various reproductive parameters. Mortalities were reported at the highest dose level. Skeletal anomalies/variations were seen at maternotoxic doses. The dose of 9 mg/kg bw was retained as a NOEL for maternotoxicity. Flunixin meglumine was not teratogenic in this study up to the maximum dose level tested.

A second oral teratogenicity study was performed in rabbits at dose of 3, 9 and 15 mg/kg bw from day 6 to 18 post mating. Bodyweight and bodyweight gains were reduced at the mid and the high dose levels. A dose-related increase in resorptions was evident at doses equal to or greater than 9 mg/kg bw. The NOEL for maternotoxicity was 3 mg/kg bw, whilst flunixin meglumine was not teratogenic in this study up to the maximum dose level tested.

Flunixin meglumine was administered intramuscularly to pregnant New Zealand White rabbits at doses of 3, 6 and 12 mg/kg bw from day 6 to 18 post mating. Treatment with the test compound had no significant effect on various reproductive parameters. There was no evidence of a teratogenic effect in this study.

13. A series of 21 separate mutagenicity studies were undertaken with flunixin, flunixin meglumine or meglumine alone. In a battery of *Escherichia coli* DNA polymerase I deficient assays, flunixin meglumine was positive in 2 out of 3 such assays at a concentration of 300 mg/ml, flunixin gave rise to equivocal results at the 0.06 mg/ml concentration level and meglumine alone was negative.

    Flunixin meglumine and meglumine were negative in the *Salmonella*/mammalian microsome mutagenicity assay (*Salmonella. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 investigated). Flunixin meglumine was positive in the *Saccharomyces cerevisiae* mitotic gene conversion assay at test concentrations of 8 and 24 mg/ml. In a second such study, a similar positive result was obtained at test concentrations of flunixin meglumine of 5 to 25 mg/ml. Flunixin alone was tested in the same assay at concentrations ranging from 0.081 to 8.1 mg/ml. Flunixin gave a positive result in this assay at test concentrations greater than 0.27 mg/ml. When meglumine alone was investigated in this assay, an initial positive result was obtained at the 100 mg/ml concentration level. A subsequent test at this and a higher concentration were negative, however, and overall it is thought that meglumine should be considered negative in this assay.

    Flunixin meglumine was tested for chromosomal aberrations in Chinese hamster ovary (CHO) cells *in vitro*. Cell cultures were exposed to the test compound for 6 hours in the presence and 24 hours in the absence of metabolic activation. The test concentrations ranged from 25 to 400 µg/ml. A significant positive response was obtained at 200 and 400 µg/ml in the presence and 100 µg/ml in the absence of metabolic activation (higher concentrations were highly toxic to cells in the absence of metabolic activation).

    Flunixin meglumine was tested in a unscheduled DNA synthesis (UDS) assay using rat primary hepatocyte cultures. Test concentrations ranged from 8 to 1000 µg/ml. Flunixin meglumine was negative in this assay.

    Flunixin meglumine was investigated in two separate mouse lymphoma forward mutation assays. In the first assay, flunixin meglumine was tested at concentrations ranging between 15.6 and 300 µg/ml in the presence of metabolic activation. This assay was positive, primarily at the higher test concentrations. The second assay again demonstrated a positive result at test concentrations up to 500 µg/ml, in the presence of metabolic activation.

    Flunixin meglumine was negative in the mouse micronucleus test at dose levels of 40 and 80 mg/kg bw intraperitoneally once daily for 2 days (animals sacrificed 6 hours after the last dose). Flunixin was negative in the same assay at dose levels of 100 and 150 mg/kg bw once daily for 2 days. Meglumine was investigated in two separate micronucleus assays. In the first assay, meglumine was administered intraperitoneally at dose levels of 500 and 1000 mg/kg bw once daily for 2 days. Both test concentrations were positive. In the second study, test concentrations of meglumine ranged between 300 and 700 mg/kg bw (administered intraperitoneally). Meglumine was negative in this second study.

    An overall assessment of the above data indicates that flunixin was mutagenic *in vitro*, but this was not confirmed in the *in vivo* mutagenicity studies.

14. A 2-year carcinogenicity study was conducted in rats with flunixin meglumine (free acid) being administered orally at doses of 2, 4 and 8 mg/kg bw. Treatment groups consisted of 60 male and 60 female animals per dose level. The test compound was administered intramuscularly for the first 4 weeks of this study at the lower doses of 1, 2 and 4 mg/kg bw. A significant increase in mortality rates was evident at the high dose level. A decrease in bodyweight gains was also recorded at the high dose level. A dose-dependent increase in gastrointestinal tract lesions (ulcers, perforations, peritonitis and oedematous regional lymph nodes) was reported in this study. There was no increase in the incidence of tumours in treated rats as compared to controls in this study.

    A 2-year carcinogenicity study was performed orally in mice whereby flunixin meglumine was administered in the diet at doses of 0.6, 2.0 and 6.0 mg/kg bw. Treatment groups consisted of 60 male and 60 female animals per dose level. An increase in mortality rate was evident at dose levels higher than or equal to 2 mg/kg bw. A reduction in bodyweight gain was reported at the highest dose level in females. Gastrointestinal lesions, pelvic cavity lesions (adhesions, abscesses) and splenomegaly were reported at doses higher than or equal to 2 mg/kg bw. There was no increase in tumour incidence in this study in treated mice as compared to controls. Due to the fact that insufficient data was presented from this study in the areas of haematology, serum biochemistry and histopathology, it was not possible to use such information for the purposes of a chronic toxicity study.

    The data demonstrate that flunixin meglumine is not carcinogenic.

15. Flunixin, flunixin meglumine and meglumine showed poor activity against bacteria and fungi (MIC ranging between 32 to 256 µg/ml). These values are greatly in excess of the plasma concentrations following treatment of animals.

16. Flunixin meglumine is not used in human medicine. No information on experience in humans was provided.

17. Based on the NOEL value of 0.6 mg flunixin free acid/kg bw/day from the 90-day repeated dose gavage study in the dog, and applying a safety factor of 100, a toxicological ADI of 0.006 mg/kg bw (i.e. 360 µg/60 person) was established.

18. In a non-radiometric residue depletion study performed in horses, 3 male and 3 female animals were sacrificed 10 days following the oral administration of flunixin meglumine at a dose of 1 mg/kg bw daily for 5 consecutive days. Flunixin was assayed in this study. The limit of detection of the analytical method (HPLC) was 50 µg/kg. No residues could be detected above the limit of detection in all tissues at the single sacrifice time point, with the exception of kidney where values ranged between 100 to 291 µg/kg. The single, late, time-point of sacrifice was a deficiency in this study.

    Three groups of 3 horses per time point were treated with flunixin meglumine by the intramuscular route at a dose level of 1.1 mg/kg once daily for 5 days. Flunixin concentrations were assayed by an HPLC method, with a limit of detection of 50 µg/kg. Two days after the last dose, highest levels of flunixin were detected at the injection site (range of 49000 to 60000 µg/kg). By 7 days after the last dose, all tissue residue levels were less than 100 µg/kg. The number of animals used per slaughter time point did not meet the requirements of Volume VI.

    Flunixin meglumine was administered orally via stomach tube to 3 groups of horses (3 animals/group) at a dose of 1.1 mg flunixin/kg bw once daily for 5 consecutive days. Animals were sacrificed at 2, 7 and 14 days after treatment. A single horse served as a control. Residues of flunixin in edible tissues were assayed by HPLC, with a limit of quantification of 50 µg/kg. All samples were negative at all time points employed in this study.

    Due to the lack of measurable residues in both oral studies and in the absence of data following the intravenous use of the substance, no conclusion could be reached on the marker residue in this species.

19. ^{14}C-Flunixin meglumine was administered on a single occasion intramuscularly to piglets (4 animals per time point) at a dose of 3.3 mg/kg bw. Total residue concentrations declined in liver from a value of 139 to 10 µg equivalents/kg between days 3 and 16 respectively. The mean total residues at the injection site declined from 7249 to 8 µg equivalents/kg between days 3 and 16 respectively. Mean concentrations of total residues in muscle were 18.0, 6.6 and 3.2 µg equivalents/kg at 3, 7 and 16 days respectively, whilst the corresponding values for kidney were 116, 24 and 6.2 µg equivalents/kg at the same time points. Only one single dose was administered in this study.

    Following the intramuscular administration of ^{14}C-flunixin meglumine to 3 piglets at a dose of 1 mg/kg bw, the mean total residue concentrations as measured by liquid scintillation counting were below 10 µg/kg for all edible tissues at 13 days after treatment. The highest values were recorded in liver (5 to 8 µg/kg) and at the injection site (3 to 6 µg/kg). The insufficient number of animals and slaughter time points, as well as the use of a sub-optimal dose were identified as deficiencies in this study.

    In a repeated dose residue depletion study in pigs, a total of 12 animals were treated at 2 mg/kg bw daily for 5 days by the intramuscular route. Flunixin was assayed by a HPLC method (limit of detection: 25 µg/kg for liver and muscle; 50 µg/kg for fat and injection site and 75 µg/kg for kidney). Animals were sacrificed on days 1, 2, 14 and 21 after treatment. By 2 days after treatment, mean liver and kidney residues were below the limit of detection. Residues of flunixin persisted longest in fat and injection site (range of 60 to 358 and 123 to 690 µg/kg respectively at 2 days after treatment). Mean residue concentrations at day 14 were below the limit of detection for muscle; the values for fat and the injection site were less than 78 and 83 µg/kg respectively at this time point. Mean residue concentrations were below the limit of detection for fat and injection site muscle by day 21 after treatment. The dose level used was slightly below the recommended therapeutic dose.

    A GLP compliant residue depletion study was performed in pigs in which ^{14}C-flunixin meglumine was administered intramuscularly at a dose of 2.4 mg ^{14}C-flunixin free acid/kg bw once a day for 3 consecutive days. A total of 4 animals per group were employed and the pigs were sacrificed at 1, 4, 7, 10 and 13 days post last dose. Total residue concentrations were measured by liquid scintillation counting with an limit of quantification of 5, 6, 3 and 3 µg equivalents/kg for liver, kidney, muscle and skin + fat respectively. Besides the injection site, liver and kidney contained the highest total residue concentrations. Mean total residue concentrations assayed in liver were 745, 243, 112, 53 and 5 µg equivalents/kg at the 1, 4, 7, 10 and 13 day time points respectively. The corresponding values for kidney were 552, 115, 42, 25 and 23 µg equivalents/kg at the same respective time points. Mean total residue concentrations detected in muscle were much lower and averaged 19, 7, 3, 1 and 2 µg equivalents/kg at the indicated time points, whilst the corresponding values for skin + fat were 28, 12, 8, 5 and 5 µg equivalents/kg. The mean concentrations of total residues in injection site muscle declined from an initial value of 10200 at day 1, to 128, 68, 147 and 6 µg equivalents/kg at the 4, 7, 10 and 13 day time points respectively. The corresponding values for injection site skin were 5390 at day 1 and 54, 145, 80 and 26 µg equivalents/kg at days 4, 7, 10 and 14 respectively. Various residue fractions were identified in porcine tissues by HPLC analysis. Flunixin and an unknown metabolite were the dominant fractions. Flunixin was identified as the marker residue, and ratios of marker to total residues of 0.35, 0.07, 0.25 and 0.1 were established for liver, kidney, muscle and skin + fat, respectively. These ratios were primarily based on data derived from the 4-day sacrifice time point.

20. A total of 4 cattle (2 male and 2 female) were injected intravenously with ^{14}C-flunixin meglumine (free acid) at a dose of 3.3 mg/kg bw, once a day for 3 consecutive days. The animals were sacrificed 12 hours following the final dose. Total residue concentrations were measured by liquid scintillation counting with a limit of detection of 7 µg equivalents/kg. The mean total residue concentrations recorded were 3800, 2500, 60 and 10 µg equivalents/kg in liver, kidney, fat and muscle, respectively. Only one slaughter time-point was analysed.

^{14}C-Flunixin meglumine was administered intravenously to 4 groups of 3 cattle each on 3 consecutive days at a dose of 2.2 mg/kg bw. The mean total residue concentrations of in liver and kidney were 1700 and 1100 µg equivalents/kg respectively at the 12 hour time-point post last dose. By 5 days post last dose, the respective concentrations in liver and kidney had declined to values of 120 and 80 µg equivalents/kg. The limit of detection of the analytical method was 8 µg equivalents/kg and values for muscle and fat were below this limit of detection within 1 day post last dose. The number of animals per slaughter time point was not in line with the requirements of Volume VI.

Flunixin meglumine was administered daily for 3 consecutive days by a single intravenous injection to Hereford cattle at a dose of 2.2 mg/kg bw (5 groups of 5 animals each). Flunixin levels were assayed in liver by HPLC and GC-MS. The mean concentration of flunixin in liver was 389, 53, and 13 µg/kg at the 12 hour, 1 day and 2 day time points post last dose. The limit of quantification of the analytical method was 8 µg/kg; no residues above the limit of quantification were present in liver 3 days after the last dose. Analysis was not performed on residue levels in other edible tissues in this study.

^{14}C-Flunixin was administered intravenously to cattle once a day for 3 consecutive days at a dose of 3.6 mg/kg bw (3 animals per time group). Mean total residue concentrations of in liver declined from 1950, 520, 440 to 390 µg equivalents/kg at the 1, 2, 3 and 4 day time-points post last dose respectively. In the case of kidney, mean total residue concentrations of 1420, 470, 370 and 250 µg equivalents/kg were detected at the corresponding time points. Total residue concentrations in fat were assayed by liquid scintillation counting, whilst radiocombustion analysis was used to assay muscle samples. The mean concentrations of total residues determined in muscle and fat 1 day post last dose were 30 and 58 µg equivalents/kg, respectively. At 2 days post last dose, the mean total residue concentrations had declined to 14 and 26 µg equivalents/kg in muscle and fat, respectively. The number of animals per slaughter time point did not meet the requirements of Volume VI. Flunixin was identified as the marker residue in all edible tissues of bovines using LC-MS/MS analytical technique, with an limit of quantification of 0.3, 0.2, 0.6 and 0.4 µg equivalents/kg for liver, kidney, muscle and fat respectively. The ratio of marker to total residues at the day 2 time point post last dose was 0.3, 0.1, 0.3 and 0.25 for liver, kidney, muscle and fat, respectively.

While flunixin is licensed in several EU Member States for intramuscular as well as intravenous administration, for up to a maximum of 5 days of treatment, the residue depletion studies provided relate only to the use of flunixin by the intravenous route for a maximum of 3 consecutive days.

21. ^{14}C-Flunixin meglumine was administered intravenously to lactating cows (number of animals not stated) at a dose of 2.2 mg/kg bw for 3 consecutive days. HPLC with a radioactive flow monitor and UV detection was the analytical technique employed. The predominant radioactive peak obtained was the metabolite 5-hydroxy-flunixin. No peaks were associated with flunixin.

^{14}C-Flunixin meglumine was administered once a day for 2 days by intravenous injection to 3 lactating dairy cows at a dose of 2.2 mg/kg bw. Measured total residue concentrations in milk were low and ranged from 40 to 90 µg equivalents/kg up to 33 hours post first dose (mean values of 50 and 60 µg equivalents/kg for the 9 and 33 hour samples, respectively). The number of animals employed in this study was low and did not meet the requirements of Volume VI.

^{14}C-Flunixin meglumine was administered intravenously to 8 lactating cows at a dose of 2.2 mg flunixin free acid/kg bw once daily for 3 consecutive days. Radioactivity in milk was assayed using liquid scintillation counting with a limit of quantification of 0.1 µg equivalents/kg.

At the 12-hour time point post last dose, the total residue concentration present ranged between 26 and 142 µg equivalents/kg, with a mean value of 66 µg equivalents/kg. At the 24 hour time point post last dose, the values obtained lay in the range of 1 to 5 µg equivalents/kg, with the exception of 1 animal that had a value of 32 µg equivalents/kg. A similar low range of values of 3-12 µg equivalents/kg was recorded at the 36 hour time point, again with the exception of the same single animal reported previously (value of 67 µg equivalents/kg). Analysis of milk samples by HPLC indicated that the 2 major fractions present were 5-hydroxy flunixin and parent compound. The dominant fraction present at the 12 hour time point post last dose was the 5-hydroxy metabolite. The limit of quantification of the HPLC assay for 5-hydroxy flunixin was 1 µg equivalents/kg. The ratio of 5-hydroxy flunixin to total radioactive residues was 0.46, 0.17 and 0.22 at the 12, 24 and 36 hour time points post last dose, respectively. The corresponding values for the ratio of parent compound to total radioactive residues were 0.18, 0.20 and 0.22 at the same time points as before. The metabolite 5-hydroxy flunixin was chosen as the marker residue as it was the dominant fraction at early time points, and the results of previous studies had indicated that the parent compound flunixin was not always detectable in milk.

22. The proposed routine analytical method presented was based on liquid chromatography with MS/MS detection (LC - MS/MS). The method was designed to monitor residues of flunixin in porcine and bovine tissues, and residues of 5-hydroxy flunixin in bovine milk. The limit of quantification of the analytical technique for all edible porcine tissues, bovine tissues and bovine milk was 1 µg/kg. The method was validated in accordance with the requirements of Volume VI and was presented in the ISO 78/2 format.

    No method was available for monitoring residues of flunixin in the edible tissues of equines.

Conclusions and recommendation

Having considered that:

- a toxicological ADI of 6 μg/kg bw (i.e. 360 μg/person) was established for flunixin,
- flunixin was identified as the marker residue in bovine and porcine tissues, whilst 5-hydroxy flunixin was identified as the marker residue in bovine milk,
- the ratios of marker to total residues were 0.3. 0.1, 0.3 and 0.25 in liver, kidney, muscle and fat of bovine (2 days after treatment),
- the ratio of marker to total residues was 0.4 in bovine milk (12 hours after treatment),
- the ratios of marker to total residues were 0.35, 0.07, 0.25 and 0.1 in liver, kidney, muscle and skin/fat of porcine (4 days after treatment),
- validated routine analytical methods are available for monitoring residues of flunixin and
- 5-hydroxy flunixin in edible tissues and milk respectively,
- due to deficits in the data relating to the depletion of residues in edible tissues of equines, and in the absence of an analytical technique for the monitoring of such residues, no recommendation could be made for this species;

the Committee recommends the inclusion of flunixin 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 tissue	Other provisions
Flunixin	Flunixin	Bovine	20 μg/kg 30 μg/kg 300 μg/kg 100 μg/kg	Muscle Fat Liver Kidney	
	5-Hydroxy flunixin	Bovine	40 μg/kg	Milk	
	Flunixin	Porcine	50 μg/kg 10 μg/kg 200 μg/kg 30 μg/kg	Muscle Skin + fat Liver Kidney	

Based on the above MRL values, the daily intake will represent approximately 90% of the ADI.