



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### METAMIZOLE

#### SUMMARY REPORT (2)

1. Metamizole (synonym: dipyrone) is a pyrazolone non-steroidal anti-inflammatory drug (NSAID). In veterinary medicine it is available as a mono-preparation containing 500 mg/ml metamizole-sodium. There are also combination products containing 500 mg/ml metamizole + 4 mg/ml of an antispasmodic agent such as butylscopolaminium bromide. Metamizole preparations are indicated for use in horses, cattle, swine, and as an adjunct to therapy in many inflammatory conditions of the musculoskeletal and locomotor systems. Metamizole is administered by intravenous or intramuscular injection to horses (20 to 50 mg/kg bw), cattle (20 to 40 mg/kg bw), pigs (15 to 50 mg/kg bw). Administration may be repeated at intervals of 8 hours; maximum duration of treatment was not reported. The Committee were also aware of the availability of oral preparations, but no information concerning dosages was available.

Metamizole is also used in human medicine, the normal oral dose is 500 to 1000 mg per person, 1 to 4 times per day.

Currently, metamizole is included in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Target species	MRLs	Target tissues	Other provisions
Metamizole	4-Methylamino-antipyrin	Bovine, porcine, <i>Equidae</i>	200 µg/kg 200 µg/kg 200 µg/kg 200 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption. Provisional MRLs expire on 1.7.2003.

Additional data were provided in response to the list of questions further to the recommendation of provisional MRLs for metamizole, with regard to use in bovine, porcine and *Equidae* species.

2. In standard pharmacological tests in rats and mice, metamizole was shown to have significant analgesic and antipyretic properties. Metamizole also possessed some antispasmodic activity. The mode of action was similar to that of other non-steroidal anti-inflammatory drugs and involved inhibition of prostaglandin synthesis. In acute studies, metamizole showed no potential for gastric ulceration. No significant effects were observed on cardiovascular or respiratory parameters in anaesthetised cats and dogs (30 to 300 mg/kg bw intravenously). In mice, effects on the central nervous system were observed at oral doses in the range 100 to 2000 mg/kg bw. Four major metabolites of metamizole were evaluated in pharmacological studies in the rat. 4-Aminoantipyrin and 4-methylaminoantipyrin had significant analgesic, antipyretic and anti-inflammatory activity but the other 2 metabolites, 4-acetylaminoantipyrin and 4-formylaminoantipyrin, were inactive.

In the acetic acid writhing test (for analgesic activity), ED<sub>50</sub> values of 90, 104 and 99 mg/kg bw were obtained for metamizole, 4-aminoantipyrin and 4-methylaminoantipyrin, respectively, but 4-acetylaminoantipyrin and 4-formylaminoantipyrin were both inactive. In a test for reversal of yeast-induced motor impairment (a test for anti-inflammatory activity), ED<sub>50</sub> values of 129, 62 and 490 mg/kg bw were obtained for metamizole, 4-aminoantipyrin and 4-methylaminoantipyrin, respectively but 4-acetylaminoantipyrin and 4-formylaminoantipyrin were both inactive. 4-Methylaminoantipyrin was around 50 times more active than metamizole as an inhibitor of cyclooxygenase, 4-aminoantipyrin was less active than metamizole and the other 2 metabolites were almost inactive.

The pharmacological studies in mice were repeated with a dosage schedule covering lower dose levels. In the first study, groups of 6 female Charles River NMRI mice were given oral doses of 0, 1, 5, 10, 100 or 250 mg/kg bw metamizole (as sodium salt). The test substance was dissolved in distilled water. At intervals up to 24 hours after treatment, the mice were evaluated for motor activity, co-ordination, muscle tone, excitability, stereotypic behaviour, body temperature, analgesia, various reflexes and diuresis. At 100 and 250 mg/kg bw, pale skin, depression of respiration, ptosis and sedation were observed. In addition, at 250 mg/kg bw, cyanosis, tremor and increased muscle tone were observed. The NOEL for this study was 10 mg/kg bw.

In another study, groups of 10 female Charles River NMRI mice were given oral doses of 0, 1, 5, 10, 100 or 250 mg/kg bw metamizole (as sodium salt). The test substance was dissolved in distilled water. A statistically significant increase in pentobarbital-induced sleeping time was observed at 250 mg/kg bw.

The pharmacological NOEL from these studies was 10 mg/kg bw.

3. Metamizole was rapidly and almost completely absorbed after oral administration to laboratory animals. Rats were given an oral or intravenous dose of 50 mg metamizole/kg bw. The oral C<sub>max</sub> and t<sub>max</sub> were 23 µg/ml and 2 hours, respectively. Oral bioavailability was calculated to be 80%, based on plasma area under blood-concentration-time curve (AUC) values, and over 90% based on urine excretion data. In Beagle dogs given an oral dose of 50 mg/kg bw, the bioavailability was calculated to be 100% based on plasma AUC values and over 95% based on urine excretion data. The oral C<sub>max</sub> and t<sub>max</sub> were 40 µg/ml and 2 hours, respectively.

In humans, oral bioavailability approaches 100%. An oral dose of 11.7 mg <sup>14</sup>C-metamizole/kg bw, administered to healthy volunteers, produced an AUC of 390 µg·h/ml, whereas an intravenous dose of 9.8 mg/kg bw gave an AUC of 303 µg h/ml. More than 90% of the radioactivity administered by either route was excreted in the urine. Metamizole was extensively metabolised, initially by hydrolysis to 4-methylaminoantipyrin. None of the metabolites were extensively bound to plasma proteins. After intravenous administration to humans, unmetabolised metamizole is rapidly undetectable in plasma. The majority of the administered dose (more than 90%) is excreted in urine as the metabolites 4-acetylaminoantipyrin (approximately 50%), 4-formylaminoantipyrin (approximately 25%), aminoantipyrin (approximately 15%) and methylaminoantipyrin (approximately 10%).

4. Metamizole was of fairly low acute toxicity by the oral and parenteral routes in all the species tested. The acute oral LD<sub>50</sub> values were in the range 3127 to 4800 mg/kg bw in rats and mice and slightly lower in guinea pigs (1000 mg/kg bw). Central nervous system effects such as sedation and convulsions were reported in all species at dosages from 1000 to 4000 mg/kg bw. Four major metabolites of metamizole were evaluated in acute toxicity studies in the rat. 4-Methylaminoantipyrin, 4-formylaminoantipyrin, 4-aminoantipyrin and 4-acetylaminoantipyrin had similar acute toxicity to the parent compound.
5. In rats, 4-week repeated-dose studies were carried out using intravenous dosing (0, 50, 150 or 450 mg/kg bw/day) and subcutaneous dosing (0, 50, 150 or 450 mg/kg bw/day). Reduced bodyweight gain and food consumption, increases in reticulocytes and Heinz bodies were reported in both sexes at 450 mg/kg bw/day by both routes of administration. In females, increases in liver and spleen weights were reported at 450 mg/kg bw/day subcutaneously, and increases in liver and kidney weights at 450 mg/kg bw/day intravenously. In both studies, the NOELs for systemic effects were 150 mg/kg bw/day. Injection site reactions were observed in all treated groups.

In dogs, 4-week repeated-dose studies were carried out using intravenous administration (0, 50, 150 or 450 mg/kg bw/day) and subcutaneous administration (0, 50, 150 or 450 mg/kg bw/day). Adverse effects included weight loss, increased reticulocytes and Heinz bodies, and increased spleen weights. Local injection site reactions were observed in all treated groups and were dose-related in severity. No NOELs could be derived from these studies due to the small group sizes used (2 males and 1 female per dose level).

6. Groups of 25/sex Sprague-Dawley rats were given daily oral doses of 0, 100, 300 or 900 mg/kg bw metamizole/day for 26 weeks. The dose of 900 mg/kg bw/day caused shyness, reduced bodyweight gain and food consumption and increases in reticulocytes and Heinz bodies. Also in the 900 mg/kg bw/day group, spleen weights were significantly increased and haemosiderosis of the spleen was found on microscopic examination. Histopathology was carried out on the control and the 900 mg/kg bw/day groups only and therefore no conclusions could be drawn regarding a NOEL from this study. However, it was not considered necessary to repeat the study because the dog appeared to be the more sensitive species and a lower NOEL would be expected in dogs.

Groups of 3/sex Beagle dogs were given daily oral doses of 0, 30, 100, 300 or 600 mg metamizole/kg bw/day of for 26 weeks. The dose of 600 mg/kg bw/day caused shyness, increased salivation, emesis, reduced bodyweight gain and food consumption. Also at 600 mg/kg bw/day, erythrocyte and haemoglobin counts were significantly reduced and reticulocytes and Heinz bodies were significantly increased and there were increases in serum blood urea nitrogen and bilirubin and a trend towards increased serum aspartate aminotransferase activity. Similar though less severe changes in food consumption and haematology values were observed at 300 mg/kg bw/day. At 100 mg/kg bw/day, emesis and a slight (not statistically-significant) increase in Heinz bodies were the only observed effects. At termination, there were significant dose-related increases in liver, kidney and spleen weights in the 300 and 600 mg/kg bw/day groups. Increased incidence and severity of haemosiderosis in the liver and spleen were observed in the 300 and 600 mg/kg bw/day groups together with periportal infiltration of the liver and cell dense bone marrow. At 100 mg/kg bw/day the only histopathological effect was an increased incidence of slight haemosiderosis of the liver. On re-evaluating the study, the Committee noted that there was no increase in reticulocytes or Heinz bodies at the dose level of 30 mg/kg bw/day and consequently haemosiderosis would not be expected at this dose level. Therefore, although histopathology had not been carried out at 30 mg/kg bw/day, this dose could be considered a NOEL for the study.

7. Pharmacovigilance data indicated that the incidence of adverse reactions in the target species was very low. It was estimated that more than 1.5 million doses had been sold for use in cattle but not a single adverse reaction had been reported. An estimated 4.3 million treatments had been sold for use in horses and a total of 8 adverse reactions had been reported; 4 of these were local reactions at the injection site, there were two cases of generalised reactions with unknown outcome, one case of penile prolapse and one death with no other symptoms reported.
8. In a reproductive toxicity study, Sprague-Dawley rats were given daily oral doses of 0, 100, 250 or 625 mg/kg bw/day. Treatment of the F0 males commenced 10 weeks prior to mating. The F0 females were treated for 2 weeks prior to mating and treatment continued throughout pregnancy and lactation. On day 20 of gestation 50% of the dams were laparotomised; the remaining dams were allowed to litter and rear the offspring to weaning. The dose of 625 mg/kg bw/day was toxic to the dams and sires and caused 6 deaths associated with gastric haemorrhage, reduced bodyweight gain and food consumption and enlarged spleens. One dam given 250 mg/kg bw/day also died. In the 625 mg/kg bw/day group, there were significant reductions in the numbers of pups born per dam and in pup survival up to day 4 of lactation. Pup survival was also reduced at 250 mg/kg bw/day. After weaning, selected males and females from the F1 generation were mated; these rats remained untreated throughout mating and gestation; no significant effects were observed. The NOEL was 100 mg/kg bw/day. No adverse effects on litter or foetal parameters were reported in dams sacrificed on day 20.

9. Groups of 30 male and 30 female Wistar rats were fed diets containing 0, 1000, 3000 or 9000 mg/kg feed, equivalent to approximately 0, 100, 300 and 900 mg metamizole/kg bw/day. Treatment started 60 days before mating, and treatment of the females continued throughout pregnancy and lactation. Half the females were killed on day 21 of gestation and the uterine contents examined. The remaining females were allowed to litter down naturally and rear the offspring to weaning. Some of the offspring (15 rats/sex/dose) were mated and allowed to rear their young. Parental bodyweight gain was significantly reduced in the 3000 and 9000 mg/kg feed groups. In the 9000 mg/kg feed group, the number of implantations was significantly reduced. There was no evidence of teratogenicity, though only 12 or 13 pregnant dams per dose were available for Caesarean section (OECD recommends at least 20/dose). The percentage of foetuses with less than 6 sternebrae ossified was slightly increased in the 9000 mg/kg feed group. Pup survival and bodyweight gain were adversely affected in the 3000 and 9000 mg/kg feed groups. The overall NOEL for the study was 1000 mg/kg feed, equivalent to 100 mg/kg bw/day.

In a poorly conducted and reported teratology study, groups of 20 pregnant Sprague-Dawley rats were given daily oral doses of 0, 100, 400 or 800 mg/kg bw/day of metamizole from days 6 to 15 of gestation. Maternal toxicity (shyness, unkempt fur, reduced bodyweight gain) was observed at 400 and 800 mg/kg bw/day. The dose of 800 mg/kg bw/day also resulted in an increased incidence of resorptions with corresponding reductions in the total number of foetuses and the numbers of foetuses per dam. Also at 800 mg/kg bw/day, mean foetal weight was reduced and there were 4 dead foetuses and 4 runts. There was no evidence of teratogenicity, with no malformation found in any group. Details of skeletal variations were not given. A subsequent statement by the author of the study report claimed that examination of the foetuses revealed some variations, including delayed ossification of the skeleton, but that these occurred with similar frequency in the treated and control groups and were not attributable to treatment. Therefore, the NOEL for foetotoxicity was 400 mg/kg bw/day.

In a poorly designed and reported teratology study, groups of 10 pregnant New Zealand White rabbits were given daily oral doses of 0, 25, 100 or 400 mg/kg bw/day of metamizole from days 6 to 18 of gestation. Maternal toxicity (shyness, inappetance, reduced bodyweight gain) was observed at 400 mg/kg bw/day. The doses of 100 and 400 mg/kg bw/day also resulted in an increased incidence of resorptions with corresponding reductions in the total number of foetuses and the numbers of foetuses per dam. Also at 400 mg/kg bw/day, mean foetal weight was reduced. There was no evidence of teratogenicity with no malformation found in any group. Details of skeletal variations were not given. A subsequent statement by the author of the study report claimed that examination of the foetuses revealed some variations, including delayed ossification of the skeleton, but that these occurred with similar frequency in the treated and control groups and were not attributable to treatment. Therefore, the NOEL for foetotoxicity was 25 mg/kg bw/day.

10. Several *in vitro* assays for gene mutation were carried out using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, and Escherichia coli WP2uvrA. All the studies which were adequately described gave negative results. Positive results were reported in a number of published studies but there were no details of the methods used and full details of the results were not given; these studies are therefore difficult to interpret. Positive results were claimed in published *in vitro* cytogenetics assays in human lymphocytes and in sister chromatid exchange assays in Chinese hamster cells but little or no information was provided concerning the test methods or the purity of the substances tested and full details of the results were not provided; the results were therefore difficult to interpret. Oral doses of 0, 25, 200 or 1600 mg/kg bw were administered to NMRI mice in an *in vivo* micronucleus test; a negative result was obtained but there was no positive control group and no evidence of bone marrow toxicity. A second *in vivo* micronucleus test was reported in the literature but gave no details beyond a statement that metamizole was positive. Published studies were also reported on the mutagenic potential of nitrosation products of metamizole in the *in vitro* assay for gene mutation in bacteria (Ames test); 2 studies claimed positive results but the third, which was the most adequately reported, claimed negative results.

Two new mutagenicity assays were carried out. Both studies were well-conducted and were in compliance with Good Laboratory Practice and relevant OECD guidelines. An *in vitro* assay to investigate gene mutations at the HPRT locus in Chinese hamster V79 cells gave negative results in both the absence and presence of metabolic activation. In an *in vivo* micronucleus test, male and female NMRI mice were given 2 oral doses of 0, 200, 650 or 2000 mg/kg bw, 24-hours apart. The dose levels were selected on the basis of results in a preliminary toxicity study. The mice were killed 24 hours after the second dose and 2000 polychromatic erythrocytes were scored for each animal; there were no significant increases in micronucleated polychromatic erythrocytes in any treated group but a statistically-significant increase was observed in a positive control group given an oral dose of 50 mg/kg bw cyclophosphamide. Overall, it was concluded that metamizole was not genotoxic.

11. Three carcinogenicity studies were carried out in mice. In the first study, groups of approximately 50/sex B6C3F1 mice were given metamizole in the drinking water for 78 weeks and were then given untreated water for a further 10-weeks. The doses used were 0, 0.125% and 0.5% (males) and 0, 0.25% and 1.0% (females); doses as mg/kg bw could not be calculated from the data available. Mortality was significantly increased in females given 1.0% and liver weights were significantly increased in the survivors at termination. The incidence of hepatic adenomas was significantly increased in females given 1.0% (82% *versus* 4% in controls) and in both male treated groups (30 and 50% *versus* 11% in controls). Liver necrosis was found in more than 50% of the mice given 1.0% and hyperplastic nodules were found in around 40%. These lesions were not observed in other dose groups or controls. Tumours developed earlier in metamizole-treated mice compared with the controls, and the multiplicity of liver tumours was also increased. In this study, tumour incidence was increased in groups in which there was also evidence of hepatotoxicity. The NOEL for hepatotoxicity in females was 0.25% in females but no NOEL was established for males.

In a second study, 3 strains of mice (B6C3F1, Balb/c and ICR) were given drinking water containing metamizole for 18 months followed by a further 2 months untreated water. Only one dose level (0.75%, approximately 30 to 37 mg/kg bw/day in B6C3F1 animals) was used and group sizes were lower than specified in the OECD guidelines. There were clear increases in the incidence and multiplicity of liver tumours in all 3 strains. However there was no statistically significant increase in the incidence of malignant tumours.

The third study was carried out in groups of 50/sex/dose NMRI mice which were fed diets containing 0, 400, 1000 or 2500 mg metamizole/kg feed for 104 weeks (males: 59, 141 and 375 mg/kg bw/day, females: 72, 177 and 442 mg/kg bw/day). Liver weights were increased in the mice given 2500 mg/kg feed but there were no corresponding histopathological changes. No evidence of carcinogenicity was found in this study. It was concluded that the liver tumours found in some strains of mice were probably due to a non-genotoxic mechanism involving hepatotoxicity.

According to a published report, groups of 50/sex/dose Wistar rats were fed diets containing 0, 1000 or 3000 mg/kg feed metamizole for 24 months (48 and 145 mg/kg bw/day in males and 62 and 193 mg/kg bw/day in females). At termination, there were significant increases in absolute and relative thyroid and pituitary weights in the 3000 mg/kg feed group but no corresponding pathological findings. There was no evidence of carcinogenicity. A second carcinogenicity study was also carried out in Fischer rats with metamizole administered in the drinking water at concentrations of 0, 0.0625% or 0.125%. No details of the study design, methods employed or details of the results were provided. The claimed negative result for this study cannot be substantiated.

12. No antimicrobial activity was detected in milk samples taken from cows for up to 3 days following intravenous treatment with metamizole at twice the indicated therapeutic dose.

13. Metamizole is used in human medicine as an oral analgesic. The normal dose is 500 to 1000 mg/person, one to 4 times per day. The reported adverse reactions include allergic skin reactions and cross-sensitivity with aspirin. The most severe adverse reaction to metamizole is a reversible but potentially fatal agranulocytosis. It was suggested that the metamizole-induced agranulocytosis in humans was an immunologically mediated response and that stimulation of lymphocytes was a prerequisite; therefore, data from a lymphocyte transformation study in patients sensitised to the pyrazolones could be used to determine the optimum concentration for the effect. However, no data were provided to support this hypothesis and a NOEL for lymphocyte stimulation had not been established.

No conclusions can be drawn regarding a possible NOEL for the induction of agranulocytosis. Further human epidemiology data were subsequently provided, including data from the International Agranulocytosis and Aplastic Anaemia Study, IAAAS (known colloquially as the "Boston" study), a population-based case-control study. The study was carried out in 8 regions; the excess risk attributable to metamizole use in 2 regions was estimated to be 0.6 cases per million treated patients; for 4 other regions, there was no discernable increase in risk. The Committee noted that the risk in this study was much lower than that observed for the induction of aplastic anaemia following therapeutic treatment with chloramphenicol (1 case per 20 to 40 000 treated patients).

Additional data were subsequently provided concerning 7 human cases of agranulocytosis, which had been reported to the Swedish pharmacovigilance scheme since the reintroduction of metamizole in 1995, leading to an incidence of one case per 1700 treated patients, and which had led to the decision to suspend the use of the substance as a human medicinal product in that country. Two of the cases had cancer and several had been treated with other drugs which had also been implicated in agranulocytosis; consequently not all of the cases could be definitely attributed to it metamizole-induced agranulocytosis. A number of reports on the incidence of agranulocytosis in other countries were also provided and gave reassurance. There were apparent differences in the frequency of agranulocytosis associated with metamizole across countries, the causes of which were unknown. The CVMP noted that the Swedish cases involved repeated daily administration of 500 to 6000 mg per person per day and concluded that the overall risk of agranulocytosis to humans from the ingestion of residues from treated animals was negligible.

14. A toxicological ADI of 0.15 mg/kg bw was established by applying a safety factor of 200 to the NOEL of 30 mg/kg bw/day in the 26-week repeated-dose study in dogs; the safety factor of 200 was justified by the lack of histopathology at this dose level. Although a lower NOEL (25 mg/kg bw/day) was established for foetotoxicity in a rabbit teratology study, applying a safety factor of 100 would lead to a higher ADI (0.25 mg/kg bw).

A lower pharmacological ADI of 0.01 mg/kg bw was established by applying a safety factor of 1000 to the pharmacological NOEL of 10 mg/kg bw in the mouse. It was noted that the lowest oral therapeutic dose level used in humans was 500 mg/day, corresponding to the slightly lower dose of approximately 8 mg/kg bw. Although a pharmacological NOEL in humans had not been established, it was considered that the safety factor of 1000 which had been used in the calculation of the pharmacological ADI would afford a sufficient margin of safety.

15. Metamizole is degraded rapidly both *in vitro* and *in vivo* to 4-methylaminoantipyrin. 4-Methylaminoantipyrin is the only primary metabolite, it is metabolised further to form 4-formyl-aminoantipyrin, aminoantipyrin and 4-acetylaminoantipyrin. 4-methylaminoantipyrin and its 3 major secondary metabolites account for approximately 77% of the total residue in human serum 2 to 8 hours after administration of <sup>14</sup>C-metamizole. In horses, pigs and cows, 4-acetylaminoantipyrin accounted for less than 5% of the total area under the curve, 4-formylaminoantipyrin accounted for 5 to 7%, aminoantipyrin accounted for 6 to 21% and 4-methylaminoantipyrin accounted for 74 to 89%. From the similarity in the volume of distribution values for metamizole (0.7 l/kg) and 4-methylaminoantipyrin (0.61 l/kg), and the distribution of metabolites detected in humans treated with <sup>14</sup>C-metamizole, it was assumed that the metabolite pattern of tissues is similar to that of plasma. Where the time dependent concentrations of metabolites were measured, 4-methylaminoantipyrin accounted for 42 to 97 % of the metabolites in horse plasma, 33 to 83% in pigs plasma, 69 to 96% in cows plasma.

In milk, after therapeutic administration of cattle with metamizole, 4-methylaminoantipyrin was detectable only in the first milking (1000 µg/l), 4-formylaminoantipyrin was detected in the first 3 milkings (440, 170, 110 µg/l), aminoantipyrin was found only in the first milking (110 µg/l) and 4-acetylaminoantipyrin was not found in any milk samples.

16. In horses (doses not given), the elimination half-life of metamizole residues was reported to be 4 to 5 hours. In another study, horses were given a single intravenous dose of a combination product containing butylscopolaminium bromide and metamizole at a dose rate corresponding to 25 mg/kg bw metamizole. Blood samples were collected at intervals from 0.5 hours up to 72 hours after treatment. Maximum plasma concentrations of 4-methylaminoantipyrin (mean 26 µg/ml, range 20.0 to 32.9 µg/ml) were found in the samples taken 0.5 hours after dosing. Mean maximum plasma concentrations of 0.88 µg/ml of 4-aminoantipyrin were found in plasma samples taken 1 hour after treatment. Mean maximum plasma concentrations of 0.62 µg/ml 4-formylaminoantipyrin were found in plasma samples taken 8 hours after treatment. Mean AUC values of 133, 24 and 23 µg.h/ml were calculated for 4-methylaminoantipyrin, 4-aminoantipyrin and 4-formylaminoantipyrin respectively. 4-Acetylaminoantipyrin was detected only in plasma samples taken at one time point.
17. In pigs, 4 hours after intravenously treatment with 200 mg metamizole/kg bw the urinary aminoantipyrin concentrations were greater than twice that of 4-methylaminoantipyrin (the proposed marker residue). From the results of urine elimination studies, it was evident that the relative concentrations of the four main metabolites of metamizole (4-methylaminoantipyrin, aminoantipyrin, 4-formylaminoantipyrin and 4-acetylaminoantipyrin) continually changed with time after dosing. 4-Methylaminoantipyrin was the metabolite accounting for the greatest fraction of the total metabolite content of plasma from metamizole-treated pigs being twice the concentration of aminoantipyrin. In urine samples the reverse was true. Aminoantipyrin was present in the major fraction at concentrations more than twice that of 4-methylaminoantipyrin. When pigs were dosed intramuscularly with 50 mg metamizole/kg bw, plasma 4-methylaminoantipyrin concentrations fell from approximately 38 000 µg/l, 0.25 hours after treatment to less than 100 µg/l, 79 hours after treatment. In another study pigs were given a single intravenous dose of a combination product containing butylscopolaminium bromide and metamizole at a dose rate corresponding to 96.2 mg metamizole/kg bw. Blood samples were collected at intervals from 0.25 hours up to 72 hours after treatment. Maximum plasma concentrations of 4-methylaminoantipyrin (mean 205 µg/ml) were found in the samples taken 0.25 hours after treatment. Mean maximum plasma concentrations of 4-aminoantipyrin, 4-formylaminoantipyrin and 4-acetylaminoantipyrin were 9.2 µg/ml, 2.3 µg/ml and 0.61 µg/ml respectively and were found in samples taken 8, 8 and 2 hours after treatment, respectively. Mean AUC values of 944, 221, 75 and 52 µg.h/ml were calculated for 4-methylaminoantipyrin, 4-aminoantipyrin, 4-formylaminoantipyrin and 4-acetylaminoantipyrin respectively. Mean concentrations of 4-methylaminoantipyrin, 4-aminoantipyrin, 4-formylaminoantipyrin and 4-acetylaminoantipyrin in urine samples taken 4 to 8 hours after treatment were 966, 119, 68 and 1.9 µg/ml respectively.
18. When cows dosed intramuscularly with 50 mg metamizole/kg bw, the plasma and urine 4-methylaminoantipyrin concentrations were more than twice those of aminoantipyrin at all time points (0 to 72 hours). In another study in which dairy cows were given a single intravenous dose of a combination product containing butylscopolaminium bromide and metamizole at a dose rate corresponding to 22.6 mg metamizole/kg bw, blood samples were taken from 0.25 to 72 hours after treatment. Maximum plasma concentrations of 4-methylaminoantipyrin (mean 303 µg/ml) were found in the samples taken 0.5 hours after treatment. Mean maximum plasma concentrations of 4-aminoantipyrin and 4-formylaminoantipyrin were 0.86 µg/ml and 0.20 µg/ml, respectively. and were found in plasma samples taken 0.25 and 8 hours after treatment. Concentrations of 4-acetylaminoantipyrin were below the limit of quantification. Mean AUC values of 420, 24 and 68 µg.h/ml were calculated for 4-methylaminoantipyrin, 4-aminoantipyrin and 4-formylaminoantipyrin, respectively. Concentrations of 4-methylaminoantipyrin, 4-aminoantipyrin and 4-formylaminoantipyrin in urine samples taken 4 hours after treatment were 1510, 90 and 16 µg/ml, respectively. Residues of 4-acetylaminoantipyrin were undetectable in urine until 48 hours after treatment when low residues were found (mean 0.19 µg/ml).

19. In pigs, intramuscular doses of 96 to 214 mg metamizole/kg bw resulted in the following concentrations of 4-methylaminoantipyrin at the injection site: 9 days after treatment, 480 µg/kg were detected in one animal, while in three others residue concentrations were below the limit of quantification; 12 days after treatment, 2 out of 5 animals had residue concentrations of 1840 µg/kg and 940 µg/kg respectively, while the remainder were below the limit of quantification; 15 days after treatment 1 out of 4 animals had residue concentrations of 120 µg/kg while the remainder were below the limit of quantification (100 µg/kg).

In another study, pigs were given a single intramuscular dose of a combination product containing butylscopolaminium bromide and metamizole. The dose corresponded to 105.9 mg/kg bw metamizole. The pigs were slaughtered (4 or 5 per time point) at 9, 12, 15 or 20 days after dosing. Samples of liver, kidney, muscle and the injection site were taken and analysed using HPLC with UV detection. Residues of 4-methylaminoantipyrin at the injection site were in the range from below the limit of quantification to 780 µg/kg, 9 days after treatment and in the range from below the limit of quantification to 2100 µg/kg at 12 days after treatment. Residues of 4-methylaminoantipyrin were also found in one sample of kidney taken 20 days after treatment (160 µg/kg). Residues in all other tissues were below the limit of quantification (100 µg/kg).

20. In cattle, intramuscular doses of 40 mg metamizole/kg bw resulted in the following concentrations of 4-methylaminoantipyrin at the injection site: 9 days after treatment, 130 µg/kg in one animal, while in three others, residue concentrations were below the limit of quantification; 15 days after treatment, 500 µg/kg in one animal, while in the 3 others residue concentrations were below the limit of quantification. In another study, 11 cows and one bull were given a single intramuscular dose of a combination product containing butylscopolaminium bromide and metamizole. The dose administered to the cows corresponded to 37.5 mg/kg bw metamizole and that given to the bull corresponded to 27.8 mg/kg bw. The cattle were slaughtered (4 per time point) at 9, 15 or 20 days after treatment. Samples of liver, kidney, muscle and the injection site were taken and analysed using HPLC with UV detection. Residues of 4-methylaminoantipyrin were found in the injection sites taken from the 3 cows slaughtered 9 days after treatment (100, 300 and 2200 µg/kg). In all other tissues, residues of 4-methylaminoantipyrin were below the limit of quantification (100 µg/kg).

In a GLP-compliant study, calves were given six intramuscular injections of a combination product containing butylscopolaminium bromide and metamizole. The calves were slaughtered (4 per time point) at 24 hours, 18 days or 28 days after the last dose. Samples of liver, kidney, muscle, fat and the injection site were taken and analysed using HPLC with UV detection. Residues of 4-methylaminoantipyrin at the sixth injection site were in the range 200 to 640 µg/kg at 24 hours; though higher residues (up to 2470 µg/kg were found at some earlier injection sites). At 18 days, residues at all the injection sites were in the range below the limit of quantification to 420 µg/kg. Residues in all samples of muscle were below the limit of quantification at all time points (100 µg/kg). At 24 hours after treatment mean residues in liver and kidney and fat were 160 µg/kg, 377 µg/kg, respectively. At 24 hours, residues in fat were in the range below the limit of quantification to 180 µg/kg. At subsequent time points residues in liver, kidney, muscle and fat were below the limit of quantification (100 µg/kg).

21. Three dairy cows were given a single intravenous administration of a combination product containing butylscopolaminium bromide and metamizole at a dose rate corresponding to 22.6 mg/kg bw metamizole and milk samples were taken 7 hours after treatment. Residues in milk were determined using HPLC with UV detection. Mean residues of 4-methylaminoantipyrine in milk were 420 µg/l. Residues of 4-aminoantipyrin and 4-formylaminoantipyrin were in the range below the limit of quantification to 150 µg/kg and below the limit of quantification to 180 µg/kg respectively. In all samples, residues of 4-acetylaminoantipyrin were below the limit of quantification (100 µg/kg).

22. Horses were given a single intravenous dose of a combination product containing butylscopolaminium bromide and metamizole at a dose rate corresponding to 25 mg/kg bw metamizole. The horses were slaughtered (3 per time point) at 6, 9 or 12 days after treatment. Samples of liver, kidney and muscle were taken and analysed using HPLC with UV detection. Residues of 4-methylaminoantipyrin were found in 1 (out of 3) kidney samples (300 µg/kg), 2 (out of 3) muscle samples (700, 3430 µg/kg) and 2 (out of 3) liver samples (240, 750 µg/kg) taken 6 days after treatment. In all other tissues, residues of 4-methylaminoantipyrin were below the limit of quantification (100 µg/kg).

Three groups of horses were administered a single intravenous injection of 25 mg metamizole/kg bodyweight as sodium metamizole in a veterinary medicinal product. Groups of four animals (2 per sex) were slaughtered after 24, 48 and 96 hours. 4-Methylaminoantipyrin was identified as the marker residue. The 4-methylaminoantipyrin concentrations were above 100 µg/kg in liver, kidney and fat 24 hours after treatment, but depleted rapidly and were below 100 µg/kg in all tissues 48 and 96 hours after treatment. The limits of quantification for 4-methylaminoantipyrin for each of the tissues were 50 µg/kg for muscle and kidney, and 25 µg/kg for liver and fat.

23. It has been demonstrated that metamizole is rapidly metabolised *in vitro* and *in vivo* and is not suitable as the marker residue. Of the metamizole metabolites, only 2 were shown to retain pharmacological activity: 4-methylaminoantipyrine and 4-aminoantipyrine. Of these two metabolites the 4-methylaminoantipyrine is the most lipophilic metabolite and it has been shown to be the most prevalent metabolite in the radiometric studies and the pharmacokinetic studies and is therefore the choice as the marker residue.
24. Twelve Large White pigs were treated with a single intramuscular administration of C<sup>14</sup> labelled metamizole at the recommended dose level of 50 mg/kg. Animals were less than a year old and weighed between 16 and 24 kg on arrival. Groups of four animals (2 per sex) were slaughtered after 24, 48 and 96 hours. Metamizole was rapidly metabolised and both metamizole and the marker metabolite, 4-methylaminoantipyrin were excreted rapidly. 4-Methylaminoantipyrin accounted for the following percentages of the total residues at 24 hours: 47% in muscle, 47% in injection site, 9% in liver, 15% in kidney and 27% in fat but 7% in skin + fat. At 48 hours the 4-methylaminoantipyrin concentrations were below the limit of quantification of 25 µg/kg in most of the samples. All 4-methylaminoantipyrin concentrations were below the limit of quantification at 96 hours. Therefore the ratio of marker to total residue can only be calculated after 24 hours.

Twelve Limousin cross-strain cattle were treated with a single intramuscular administration of C<sup>14</sup> labelled metamizole at a dose level of 50 mg/kg. The animals used for this investigation were ruminating calves between 90 and 148 kg on arrival, thus the results are considered valid also for adult cattle. Groups of four animals (2 per sex) were slaughtered after 24, 48 and 96 hours. Again, metamizole was rapidly metabolised and both metamizole and the marker metabolite 4-methylaminoantipyrin were excreted rapidly, but somewhat slower than in the pig. 4-Methylaminoantipyrin accounted for the following percentages of the total residues at 24 hours: 50% in muscle, 20% in injection site, 22% in liver, 69% in kidney and 41% in fat. At 48 hours the 4-methylaminoantipyrin concentrations were below the limit of quantification of 25 µg/kg in the muscle and fat samples (except 1 fat sample with 25.2 µg/kg). All 4-methylaminoantipyrin concentrations were below the limit of quantification at 96 hours. Therefore the ratio of marker to total residue can only be calculated after 24 hours.

Four Friesian-Holstein strain dairy cows were administered a single intramuscular injection of <sup>14</sup>C-labelled metamizole at the recommended dose level of 40 mg/kg. Milk samples were taken twice daily until 7 days following administration. Total residues and 4-methylaminoantipyrin depleted rapidly in milk with the maximum levels of radioactivity found less than 2 hours after treatment, when 4-methylaminoantipyrin accounted for 75% of the total residue. This had fallen to 22% after the third milking, which was approximately 26 hours after administration. All the levels of radioactive residues had fallen below the limit of detection at the fourth milking, except for those in 1 cow and were all below the limit of detection at the fifth milking.

25. An analytical method for the determination of the proposed marker residue in tissues of the target species was based on HPLC with mass spectrometry detection. The method has been fully validated and was presented in an internationally recognised format (ISO 78/2). For all bovine edible tissues, and bovine milk the limit of quantification was 25 µg/kg. For porcine liver, kidney and muscle the limit of quantification was also 25 µg/kg, but for fat + skin it was 100 µg/kg. Although this value is the same as the proposed MRL, it was considered acceptable for this tissue because fat + skin would not be the determining tissue when setting withdrawal periods. The analytical method was also tested for horse tissues in accordance with CVMP Note for Guidance on the establishment of maximum residue limits for Minor Species (EMEA/CVMP/153a/97-FINAL) and considered applicable.

### Conclusions and recommendation

Having considered that:

- an ADI of 0.01 mg/kg bw was established,
- the major 4-methylaminoantipyrin was identified as marker residue,
- the marker residue represents the following percentages of the total residues at 24 hours after treatment in porcine edible tissues: 47% in muscle, 9% in liver, 15% in kidney and 5% in skin + fat,
- the marker residue represents the following percentages of the total residues at 24 hours after treatment in bovine edible tissues: 50% in muscle, 32% in liver, 69% in kidney and 21% in fat and 27% for bovine milk,
- in order to accommodate bovine milk, the provisional MRL values needed to be reduced to 100 µg/kg for porcine and bovine edible tissues.
- 4-methylaminoantipyrin was also identified as the marker residue in horses and therefore similar MRL values can be established in this animal species in accordance with the CVMP Note for Guidance on the establishment of maximum residue limits for Minor Species (EMEA/CVMP/153a/97-FINAL),
- a fully validated analytical method for monitoring residues in bovine and porcine tissues including bovine milk is available; the method is also applicable to horse tissues;

the Committee for Veterinary Medicinal Products recommends the inclusion of metamizole in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Target species	MRLs	Target tissues	Other provisions
Metamizole	4-Methylaminoantipyrin	Bovine	100 µg/kg 100 µg/kg 100 µg/kg 100 µg/kg 50 µg/kg	Muscle Fat Liver Kidney Milk	
		Porcine	100 µg/kg 100 µg/kg 100 µg/kg 100 µg/kg	Muscle Fat +skin Liver Kidney	
		<i>Equidae</i>	100 µg/kg 100 µg/kg 100 µg/kg 100 µg/kg	Muscle Fat Liver Kidney	

Based on these MRL values, the daily maximum intake of marker residue will represent 98% of the ADI.