The European Agency for the Evaluation of Medicinal Products
*Veterinary Medicines and Inspections*

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# COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

## DICLOFENAC

## SUMMARY REPORT

1. Diclofenac sodium, sodium 2-[(2,6-dichlorophenyl)amino]phenylacetate (CAS No 15307-79-6), belongs to the non-steroidal anti-inflammatory drugs (NSAIDs) and, more specifically, to the phenyl acetic acid derivatives. Diclofenac sodium is intended for treatment in cattle and swine as an anti-inflammatory agent at doses of 2.5 mg/kg bw/day by intramuscular route. The proposed duration of treatment is 1 to 3 days.

   Diclofenac sodium has been used in human medicine for many years for the long-term symptomatic treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and primary nocturnal enuresis. It may also be useful for short-term treatment of acute musculo-skeletal injury and dysmenorrhea. The daily dose varies between 50 and 150 mg/person depending on the route of administration (oral, rectal, intramuscular, intravenous or topical) and on the disease to be treated and may be used up to 12 weeks.

2. As for all non-steroidal anti-inflammatory drugs the pharmacodynamic effects of diclofenac sodium are of anti-inflammatory, analgesic and antipyretic character due to the decrease of the prostaglandin synthesis from arachidonic acid by inhibition of the cyclo-oxygenase activity. It also induces deleterious effects on gastric and intestinal mucosae and an inhibition of platelet aggregation. The pharmacological NOEL for antiphlogistic effects after oral administration in rats was 0.1 mg/kg bw (paw oedema) after a single dose. The dose of 0.1 mg/kg bw/day was a LOEL in the adjuvant arthritis model after repeated administration, while 0.26 mg/kg bw/day represented an effective dose ($ED_{50}$) in this model. The pharmacological NOEL for antipyretic activity of diclofenac sodium administered orally as single dose in rats was 0.1 mg/kg bw. No effect on bleeding time was recorded at the highest dose tested of 0.1 mg/kg bw administered orally in rats. In rabbits the protective dose ($PD_{50}$) of diclofenac inhibiting the mortality induced by intravenous administration of arachidonic acid was 0.05 mg/kg when administered intraperitonally. Constriction of the ductus arteriosus in the foetal rat was demonstrated at single doses of 0.1 mg/kg bw. In humans, a low oral effective dose for the management of pain is 0.4 mg/kg bw every six hours (equal to 1.2 mg/kg bw/day). The major metabolite 4'-hydroxydiclofenac, which shows a comparable acute toxicity as the parent compound, and 3'-hydroxydiclofenac inhibit prostaglandin synthesis only at elevated concentrations [the inhibitory concentration ($IC_{50}$) *in vitro* being 5 to 8 times higher] and display only 1/15 to 1/200 of the activity of diclofenac in various pharmacological animal models whereas the other 4 metabolites are nearly without any effect. An overall pharmacological LOEL of 0.1 mg/kg bw can be established from these studies.

3. Oral doses of a solution containing radiolabelled diclofenac are shown to be rapidly and totally absorbed in the rat, dog, rhesus monkey and in man, with maximal plasma concentrations obtained 10 to 30 minutes after the administration in man. Following oral administration of 50 mg diclofenac sodium in man, as an enteric-coated tablet, absorption was rapid after a lag period of about two hours, and systemic bioavailability was approximately 54%.

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Peak plasma diclofenac levels ranged from 1.4 to 3.0 µg/ml. In human volunteers with single oral doses (75 mg) of tablets resulted in the $t_{max}$ values of 2.01 to 2.08 hours, $C_{max}$ of 0.64 to 0.73 µg/ml and absorption half lives of 22 to 26 minutes.

In Wistar rats, oral administration of diclofenac sodium tablets at 5 mg/kg bw resulted in rapid absorption with absorption half lives of 3.08 to 4.63 min, $t_{max}$ of 6.94 to 8.40 minutes, with $C_{max}$ of 1.77 to 2.12 µg/ml.

Subcutaneous bolus doses of diclofenac administered at 10, 20 and 40 mg/kg to rats were also rapidly absorbed. The three doses showed $C_{max}$ of 4.6, 7.2 and 17.2 µg/ml, with corresponding $t_{max}$ of 0.9, 0.9 and 0.5 ± 0.2 hours, respectively.

Studies in the rat using radiolabelled diclofenac indicate that apart from the liver, bile, and kidneys, relatively high concentrations also occur in blood, followed by heart and lung. The highest levels of radioactivity in individual organs and tissues occur mostly one minute after intravenous administration, indicating extremely rapid distribution and uptake are processes in rats, dogs, rhesus monkeys and man. Distribution of diclofenac into plasma (more than or equal to 0.064 µg/ml), synovial fluid more than or equal to 0.118 µg/ml) and synovial tissue more than or equal to 0.130 µg/g) has also been observed following topical administration 4-times daily of 2.5 g of a gel formulation containing 1.16% (w/w) diclofenac ammonium on the hands of arthritic patients. The volume of distribution of diclofenac is small in man (0.15 to 0.21 l/kg after two single doses of 25 mg enteric-coated diclofenac sodium tablets). This is explained by extensive binding (99.7%) to plasma proteins.

Biotransformation, but also the route of excretion seems to be species-specific.

Diclofenac sodium is extensively metabolised in humans via direct conjugation and hydroxylation with subsequent conjugation. Besides parent drug, the following compounds have been identified in human urine: 3'-hydroxydiclofenac, 4'-hydroxydiclofenac, 5-hydroxy-diclofenac, and 4',5-dihydroxydiclofenac. In human and baboon plasma, another metabolite, 3'hydroxy-4'-methoxydiclofenac, has been identified. In contrast to the rat, rhesus monkey, baboon and man, which excrete mainly hydroxylated metabolites, the dog does not oxidise diclofenac. Dog urine contained a relatively stable taurine conjugate of diclofenac, and in the bile an ester glucuronide was excreted. The unstable ester glucuronide is hydrolysed in the dog duodenum, releasing diclofenac, which undergoes enterohepatic circulation. In the rat, 4'-hydroxydiclofenac together with 5-hydroxydiclofenac are the major metabolites in urine.

From studies with radiolabelled $^{14}$C-diclofenac in animal species (1 mg/kg bw intramuscularly) and man (50 mg/person orally), it is seen that elimination via urine is important accounting for 39% of the administered dose in rats, 35 to 40% in dogs, 74% in the baboon, 71% in the Rhesus monkey and 64 to 71% in man. Elimination in bile accounted for 74% in rats and about 90% in dog, of which 40 and 89%, respectively where found to be unchanged compounds. The sum of excretion in urine and bile in rats and dogs is more than 100%, indicating enterohepatic circulation.

A bioavailability study was performed in 8 lactating cows receiving 2.5 mg diclofenac/kg intramuscularly and intravenously. The $C_{max}$ were 167.15 and 4.6 µg/ml by intravenous and intramuscular routes, respectively. The $t_{max}$ following intramuscular administration was 3.4 hours. Half lives of elimination were 5.9 and 11.3 hours for intravenous and intramuscular routes, respectively. Similar areas under the curve (AUC) (68 923 and 69 759 ng.h/ml for intravenous and intramuscular administration routes, respectively) were obtained showing an absolute bioavailability of 100% for the intramuscular route.

Eight young bovines (140 to 280 kg) of both sexes were treated daily for 6 days with 2.5 mg diclofenac/kg into the neck muscles. The resulting ratio of diclofenac to 4'-hydroxydiclofenac and 5-hydroxydiclofenac metabolites was consistently above 20 and the ratio diclofenac to total residues in plasma was always above 0.9. The plasma concentrations of the other 3 metabolites (3'-hydroxydiclofenac, 4',5-dihydroxydiclofenac and 3'-hydroxy-4'-metoxydiclofenac) were always below the limit of quantification (10 ng/ml).

Sixteen pigs of both sexes with mean body weight of 28 kg received 6 consecutive intramuscular injection of 2.5 mg diclofenac/kg in the neck once a day. Blood was sampled up to 12 hours after the last drug administration. $C_{max}$ was 4667 ng/ml at 0.5 hour. Estimated half-life of elimination was 3.4 hours. Kinetics similar to that of parent compound were observed for two metabolites, i.e. 4'-hydroxydiclofenac and 5-hydroxydiclofenac, with $C_{max}$ of about 350 and 180 ng/ml and $t_{max}$ around 0.5 to 1 hours and 1 to 2 hours, respectively. The other metabolites had concentrations always below the limit of quantification (10 ng/ml, HPLC method with coulometric detection). The bioavailability in swine after intramuscular administration has not been investigated.

4. The oral $LD_{50}$ values in mice, rats, dogs, rabbits and guinea pigs ranged between 95 to 1300 mg/kg bw, 53 to 1500 mg/kg bw, 59 mg/kg bw, 157 mg/kg bw and 1250 mg/kg bw, respectively.

   Adult male ICR mice (CD-1 strain) were administered a single intraperitoneal injection diclofenac sodium at 32.5, 65 and 104 mg/kg bw. Signs of toxicity, manifest as hypoactivity were observed in mice of the highest dose group at 24 and 48 hours. No deaths were reported in this study.

   In other studies an oral $LD_{50}$ for diclofenac in rats has been determined as 226 and 240 mg/kg, females and males, respectively. The hydroxylated derivatives of diclofenac were of similar or lower acute toxicity.

5. In rats, SPF Fisher CDF (F-344)/CrlBR strain, diclofenac sodium was administered orally at doses of 0.5, 2.5 and 5.0 mg/kg bw for 91 days. Significant decreases of absolute and relative liver and epididymis weight were recorded in males treated with 5.0 mg/kg bw. No adverse and toxic effects were observed at 0.5 and 2.5 mg/kg bw. A NOEL was determined as 2.5 mg/kg bw.

   Diclofenac sodium was administered orally (capsules) at 0.3 or 1 mg/kg bw/day to 2 male and 2 female Beagle dogs per group for four-weeks. At the lowest dose cortical tubular dilatation was observed in the kidneys of most animals. In addition, females showed urothelial hyperplasia in the renal papillae. The high dosed animals showed severe effects at gastro-intestinal, kidney and spleen sites accompanied by diarrhoea, anaemia, protein loss and kidney dysfunction. Chronic inflammation of the livers of treated males and females was seen, in females associated with bile duct proliferation. One high dosed male had to be sacrificed in extremis. No oral toxicological NOEL could be established in this study.

   Four male and four female Beagle dogs per group were administered diclofenac sodium (0.03, 0.1 and 0.3 mg/kg bw/day) by oral gavage for 13 weeks. No mortality, or post-dose observations were noted during the study. No treatment-related changes were seen in body weight, food and water consumption, physical examination, ophthalmoscopy, electrocardiography, blood pressure, haematology, clinical chemistry, urinalysis, faecal analysis, organ weights, macroscopic and microscopic evaluations. No significant adverse effects were seen in any dose group The NOEL is 0.3 mg/kg in this study.

   Diclofenac sodium was administered to Beagle dogs daily by intramuscular administration at doses of 0, 0.1, 0.5 and 1.0 mg/kg bw for 90 days (4 males and 4 females per group). Satellite animals from the control and high dose groups were subject to a 28-day recovery period. One animal died in the high dose group on day 87. The group dosed 1.0 mg/kg bw showed a lower body weight gain, reduced food consumption, enhanced incidence of scours, often with blood admixture, reduction of haemoglobin, and haematocrit values and red blood cell counts in males and females, increases in white blood cells and a significant elevation of platelet and segmented neutrophil in males and in both sexes a reduction of serum albumin and total protein content.

   Aspartate aminotransferase activity was also enhanced and the excretory function of the kidneys was reduced transiently. Medium and high dosed females showed a shortened prothrombin time, a significant increase in urobilinogen values in urine as well as an enhancement of protein values was demonstrated. Gross necropsy showed an increased dose-dependent incidence of focal erosions, haemorrhage and enteritis in the jejunum and ileum in groups treated with 0.5 and 1.0 mg/kg bw. Haemorrhage or haematomas were also observed at the site of administration.

Microscopic examination showed atrophy of the epithelium in the small intestine villi, focal enteritis, focal erosions, desquamation and hyperaemia in male and female animals of the groups administered 1.0 mg/kg bw, and sporadically, with a lower intensity in the group treated with 0.5 mg diclofenac/kg bw. All lesions were reversible in animals allowed to recover. A parenteral NOEL was set at 0.1 mg/kg bw for this study.

6. No long-term repeated dose toxicity study has been performed.

7. Diclofenac tolerance was investigated in cattle and pigs administered 2.5 mg/kg bw/day for six days. No clinical signs and adverse effects on biochemical parameters, attributable to treatment, were observed in the target species. Adverse effects on haematological parameters (decrease in haematocrit) were observed in cattle, but not in pigs. Histopathology of injected muscle showed polymorphous inflammatory infiltration alone and with necrotic spots in few samples (10 out of 96 in cattle and 15 out of 95 in pigs) of the injected sites. No drug related changes were observed in kidney and liver, other organs not being investigated. No information on frequency and severity of gastro-intestinal lesions were included in the studies.

8. A one-generation reproductive toxicity study in rats established adverse effects after repeated oral administration of diclofenac sodium at 0.5, 2.5 and 5.0 mg/kg bw. In females receiving diclofenac sodium at 2.5 and 5.0 mg/kg bw/day adverse effects on body weight, food consumption, mortality, percentage fertility, percentage of mated parental females, litter size and number, live birth index, adverse effects in the absolute and relative weights in liver, spleen and uterus, number of corpora lutea, pathological and histopathological effects were observed. A depression of parental body weight and a reduction in the percentage of mated females was still observed at 0.5 mg/kg bw. A NOEL could not be determined for this study.

   In a two-generation reproductive toxicity study rats were administered diclofenac sodium orally at 0.25, 1.25 and 2.5 mg/kg bw/day. Adverse and toxic effects were reported at 2.5 mg/kg bw/day for food consumption, percentage of mated parental $F_1$ females, litter size in $F_1$ and $F_2$ generations, sex distribution and a reduction of epididymides weight in parental $F_1$ males. Mild adverse and toxic effects, manifest as a reduction in litter size in the $F_1$ generation pups, and in the relative weights of epididymides in parental males at 1.25 and 2.5 mg/kg bw were observed. The NOEL for fertility was 1.25 mg/kg bw. The NOEL for parental toxicity and neonatal toxicity was 0.25 mg/kg bw this study.

   A single oral dose of 0.1 mg/kg bw to rat dams on day 21 of pregnancy caused a constriction of the ductus arteriosus in the offspring.

   Enlargement of the portal area and bile duct proliferation were seen in 4 week old offspring of Wistar females treated from the $5^{th}$ to the $20^{th}$ day of pregnancy with intramuscular doses of 1 mg diclofenac/kg bw per day. Additionally, all diclofenac-treated pups had diffuse sinusoidal dilatations, granular and vacuolar degeneration in parenchymal cells and pycnosis in the nuclei of hepatocytes.

   Repeated daily oral administration of diclofenac sodium (1 and 2 mg/kg bw) for 65 days to male rats, produced a significant decrease in testicular weight, epididymal sperm cell concentration, progressive motility percentage and number of seminiferous tubules containing spermatozoa, significant increase in total number of sperm abnormalities and a significant decrease in serum testosterone concentration. Histopathological examination of testes and accessory glands of treated rats showed degenerative changes in some seminiferous tubules of the testes.

   In the rabbit, an intragastric dose of 10 mg/kg bw given one hour after administration of human chorionic gonadotrophin to induce ovulation, decreased ovulation to 23%; at 20 mg/kg bw ovulation was interrupted.

   Daily subcutaneous administration of diclofenac sodium (2 mg/kg bw/day) to male dogs for 42 days revealed a significant increase in the number of spermatids in every biopsy until the $28^{th}$ day which was then sustained for the remainder of the study.

   An overall LOEL of 0.1 mg/kg bw for foetal effects has been established.

9. Teratogenicity studies carried out in mice *in vivo* as well *in vitro* showed a teratogenic potential of diclofenac. *In vivo* tests were carried out in 15 animals with a total of 114 embryos. From these 82% were normal whereas 12% showed cleft palate and 6% resorbed embryos at an intramuscular dose of 4 mg/kg bw on day 13.5 of pregnancy. *In vitro* tests were carried out on homotypic pairs (53) of cultured palate processes taken from embryos on days 13.5 and 14.5 and cultured in a dose of 50 µg. The explants of day 13.5 showed 89% unfused pairs whereas this number was 11% for the 14.5 days explants.

   Pregnant rats were given daily oral (gavage) administration of diclofenac sodium at 2.5, 5.0 and 10 mg/kg bw/day, from gestation days 5 to 15. At 10 mg/kg bw/day, significant decreases in body weight and food consumption in dams was observed. Furthermore, mortality of 4 animals occurred, in which haemorrhagic gastroenteritis and necroses of the mucous membranes, affecting the submucosa was observed. These lesions resulted in inflammatory processes within the abdominal cavity. Inflammatory processes, accompanied by an increased formation of fibrous tissues and cellular infiltration, were found in capsules of the liver, spleen, adrenal gland and kidneys and peritoneum. Haemometra was also diagnosed. Visceral and skeletal examination of foetuses revealed no pathological alterations or malformations attributable to dosing with diclofenac sodium at any dosing level. No embryotoxic and teratogenic effects of diclofenac sodium were observed. A NOEL for maternotoxicity was determined as 5 mg/kg bw/day.

   Diclofenac sodium was administered orally at doses of 2.5, 5 and 10 mg/kg bw/day from day 6 to 18 of pregnancy in rabbits. A significant decrease in feed consumption was noted in dams receiving diclofenac sodium at 2.5, 5 and 10 mg/kg bw/day was accompanied by a significant suppression of body weight gain from gestation days 10 to 28 in the 5 and 10 mg/kg bw/day dose groups only. Females dosed at 5 and 10 mg/kg bw/day, demonstrated an increase in the number of abortions (two abortions per 14 pregnant females), a significant increase in early, late and total resorptions and a significant reduction in number of live foetuses. The pre-, post- and total number of implantation deaths was increased after administration of diclofenac sodium at 5 and 10 mg/kg body weight to rabbit dams. A dose related increase in post-implantation and total implantation deaths was observed. No teratogenicity was observed in this study. The NOEL for foetotoxicity was 2.5 mg/kg. No NOEL could be established for maternotoxicity.

   A prolongation of the duration of gestation was observed after intramuscular administration of diclofenac at the dose of 1 mg/kg daily from the 5$^{th}$ to 20$^{th}$ day of pregnancy in Wistar rats. In the two-generation reproduction study, a slight effect on the prolongation of the duration of gestation was observed at 2.5 mg/kg bw but not at 1.25 mg/kg bw.

10. *In vitro* mutagenicity studies on diclofenac included reverse mutation in the *Salmonella typhimurium* assay and a chromosome aberration assay with human lymphocytes, both performed with an without metabolic activation. No gene mutations were observed. At concentrations that were not cytotoxic, diclofenac did not induce chromosomal aberrations. *In vivo*, the micronucleus assay in mouse bone marrow was negative. It can be concluded that diclofenac is not mutagenic.

11. No carcinogenicity studies are available for diclofenac, however in view of lack of a genotoxic potential such studies appear not necessary. There is experience from long-term treatment with diclofenac in human therapy and the diclofenac molecule is not considered to be related to those that pose a carcinogenicity hazard.

12. Diclofenac can act as a negative chemokinetic agent, for migration of both stimulated and unstimulated polymorphonuclear neutrophils. At concentrations below 100 µg/ml in a gel diclofenac reduced in a dose-dependent manner, the directed locomotion of polymorphonuclear neutrophils induced by a gradient of C5a-activated serum, peptide N-formyl-methionyl-leucyl-phenylalanine or *Klebsiella pneumoniae* culture supernatant. Diclofenac also inhibited the random locomotion of unstimulated polymorphonuclear neutrophils, as well as the polymorphonuclear neutrophil chemokinetic activity induced by various amounts of N-formyl-methionyl-leucyl-phenylalanine or activated serum.

    Other effects include a reversible decrease in the number of megakaryotic cells as shown in sheep, toxicity on hepatocytes, gastrointestinal lesions in dogs. No neurotoxicity studies have been performed as no potential for neurotoxicity has been observed in toxicological studies.

13. Diclofenac has demonstrable antimicrobial activity against pathogenic bacterial species. The following bacteria were inhibited by diclofenac at 50 to 100 µg/ml level: *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Staphylococcus aureus*, The lowest reported. $MIC_{50}$ against *Brucella* species is 16 µg/ml. The antimicrobial activity against bacteria of the human gut flora is weak. The most sensitive strains were *Fusobacterium necrophorum* and *Fusobacterium nucleatum* with MICs of 64 µg/ml. A microbiological ADI of 47 µg/kg was established.

14. In humans diclofenac is well tolerated. The most frequently reported adverse effects are gastrointestinal effects such as vomiting and diarrhoea, indigestion, nausea, constipation and flatulence. Central nervous system effects are the second most frequent adverse reactions associated with diclofenac therapy: headache, dizziness, vertigo, insomnia, drowsiness, agitation, depression, irritability and anxiety. The other main effects are elevation in liver function tests and skin rash and pruritis. Hypersensitivity has been occasionally observed. Premature closure of the ductus arteriosus in the fetus with persistent pulmonary hypertension in the newborn has been reported, when the drug was given to women near term.

15. An overall pharmacological-toxicological ADI of 0.5 µg/kg bw (30 µg per person) can be established from the overall pharmacological and the toxicological LOEL of 0.1 mg/kg bw, applying a safety factor of 200.

16. In 4 pigs $^{14}$C-diclofenac sodium was administered by the intramuscular route, at a target dose rate of 2.5 mg/kg bw/day for 3 consecutive days. Following the first intramuscular administration of $^{14}$C-diclofenac sodium, absorption of radioactivity into the systemic circulation was rapid, with the peak mean concentration ($C_{max}$ equals 7450 µg equivalents/kg) observed at 1 hour following administration of the first dose. Low levels of radioactivity (302 µg equivalents/kg) remained in the plasma at 24 hours after treatment. Following administration of the final administration (dose 3), concentrations of radioactivity present in the plasma increased gradually, with the highest concentrations ($C_{max}$ equals 7063 µg equivalents/kg) observed at 1 hour after the last administration. By 48 hours after the last administration, mean concentrations of radioactivity had fallen to lowest levels of 299 µg equivalents/kg. The major route of elimination was via urine, which accounted for a mean of 51 % of the administered dose. Excretion in faeces accounted for a mean of 10 %. Diclofenac, 5-hydroxy diclofenac and 4'-hydroxy diclofenac were observed in urine and faeces.

    In cattle $^{14}$C-diclofenac sodium was administered by the intramuscular route, at a target dose rate of 2.5 mg/kg per day for 3 consecutive days to 16 calves. Absorption of radioactivity into the systemic circulation was rapid, with the peak mean concentration ($C_{max}$ equals 7009 µg equivalents/kg) observed at 2 hours following the first administration. Low levels of radioactivity (1252 µg equivalents/kg) remained in the plasma at 24 hours after treatment. Following administration of the final administration (dose 3), concentrations of radioactivity present in the plasma increased gradually, with highest concentrations ($C_{max}$ equals 9006 µg equivalents/kg) observed at 2 hours after the last administration. By 48 hour after the last administration, mean concentrations of radioactivity had fallen to lowest levels of 411 µg equivalents/kg. The major route of elimination was via urine, which accounted for a mean of 61% and 80% in male and female cattle, respectively. Diclofenac, 4'-5-dihydroxy diclofenac, 5-hydroxy diclofenac 4'-hydroxy diclofenac and 3'-hydroxy diclofenac were observed in urine. Excretion in faeces accounted for a mean of 29% and 16% in male and female cattle, respectively. Diclofenac, 5-hydroxy diclofenac and 4'-hydroxy diclofenac were observed in faeces.

17. Radiometric studies were performed in pigs and cattle.

    In pigs $^{14}$C-diclofenac sodium was administered by the intramuscular route, at a target dose rate of 2.5 mg/kg bw/day for 3 consecutive days to 16 animals (8 males and 8 females). The animals were sacrificed at 3, 7, 14 and 17 days following the administration of the last administration. Mean concentrations of total radioactive residues in edible tissues at each of the sacrifice times were 911, 553, 317 and 130 µg equivalents/kg in liver ; 661, 137, 92 and 79 µg equivalents/kg in kidney ; 70, 30, 15 and 11 µg equivalents/kg in skin+fat ; 11, 6, 4 and 0 µg equivalents/kg in muscle.

At the same time points mean concentrations of total radioactive residues at injection site were 3744, 1380, 440 and 56 µg equivalents/kg. Analysis for tissues for diclofenac at 3 days showed average ratios of 0.035 µg equivalents/kg in liver and 0.12 µg equivalents/kg in kidney. At 7 days after treatment, diclofenac was not detectable in liver and kidney (less than 5µg/kg). Total residue in muscle, fat and skin with adhering fat contained levels of radioactivity which were too low to allow radio chromatographic analysis.

Diclofenac was not identifiable at the injection site. Ethanol extracts of liver, kidney, injection sites, fat and skin with adhering fat collected 3 and 7 days after the final administration of $^{14}$C-diclofenac sodium contained 10.4 to 38.1% total radioactive residues, 23.5 to 54.1% total radioactive residues, 43.6 to 74.0% total radioactive residues,, 15.7 to 37.4% total radioactive residues and 25.4 to 40.1% total radioactive residues, respectively. Unknown metabolites were significantly present in liver, kidney and injection site. The generally low overall extraction efficiency data indicate that a non-extractable residue was formed through interaction of diclofenac and/or its metabolites with tissue matrix components of liver and kidney. Extensive investigations of incurred tissue residues using rigorous extraction procedures (enzymes and 6 N HCL) showed that 50% and 20% of the total residues in liver and kidney were practically irreversibly bound.

In cattle $^{14}$C-diclofenac sodium was administered by the intramuscular route, at a target dose rate of 2.5 mg/kg bw/day for 3 consecutive days to 16 calves (8 males and 8 females). The animals were sacrificed at 3, 7, 14 and 17 days following the administration of the last dose. Mean concentrations of total radioactive residues in edible tissues at each of the sacrifice times were 623, 1040, 150 and 251 µg equivalents/kg in liver ; 324, 145, 117 and 194 µg equivalents/kg in kidney ; 40, 81, 15 and 7 µg equivalents/kg in fat ; 14, 4, 1 µg equivalents/kg and below the limit of detection in muscle. At the same time points mean concentrations of total radioactive residues at injection site were 6363, 234, 849 and 947 µg equivalents/kg. Analysis for tissues for diclofenac at 3 days showed average ratios of 0.11 in liver and 0.20 in kidney. At 7 days post dose, the ratio in liver was 0.09 and diclofenac was not detectable in kidney (less than 5µg/kg). Total residues in muscle and fat (excepted for one animal) contained levels of radioactivity which were too low to allow radio chromatographic analysis. At the injection site the ratio was 0.43 at 3 days and 0.18 at 7 days. Ethanol extraction of liver, kidney, injection sites and fat collected 3 and 7 days after the final dose administration of $^{14}$C-diclofenac recovered 20.7 to 29.0% total radioactive residues, 24.9 to 48.6% total radioactive residues, 51.9 to 94.4% total radioactive residues and 35.3 to 82.0% total radioactive residues, respectively. Unknown metabolites were significantly present in liver, kidney and injection site. The generally low overall extraction efficiency data indicate that a non-extractable residue was formed through interaction of diclofenac and/or its metabolites with tissue matrix components of liver and kidney. Extensive investigations of incurred residues using rigorous extraction procedures (enzymes and 6 N HCL) showed that 40% and 30% of the total residues in liver and kidney were practically irreversibly bound.

No radiolabelled studies investigating total residues in cow's milk were available.

18. Non-radiometric depletion studies were carried out in cattle, lactating cows and pigs.

Sixteen pigs of both sexes (8 males and 8 females) with a mean body weight of 28 kg, received 6 consecutive intramuscular injection of 2.5 mg/kg bw diclofenac in the neck once a day. Groups of 2 males and 2 females were slaughtered at 3, 12, 24 and 168 hours after the last administration. Diclofenac in tissues and plasma and hydroxy metabolites in plasma (4' and 5 hydroxy diclofenac, respectively) were assayed using HPLC with coulometric detection. Mean diclofenac concentrations in edible tissues at 3, 12 and 24 hours were 3746, 576 and 75 µg/kg in liver; 2622, 463 and 53 µg/kg in kidney; 430, 29 and 246 µg/kg in skin+fat and 189, 16 and 7 µg/kg in muscle. At the same time points mean concentrations at injection site were 451, 31 and 70 µg/kg. At 168 hours after treatment diclofenac was at the limit of quantification of 5 µg/kg with the exception of two injection site samples in two pigs (51 and 176 µg/kg). Hydroxy metabolite concentrations were below the limit of quantification already 12 hours after the last administration.

Sixteen young bovines (140 to 280 kg) of both sexes (8 males and 8 females) were treated daily for 6 days with 2.5 mg diclofenac/kg bw into the neck muscles. Groups of four animals were slaughtered 3, 12, 24 and 144 hours after the last administration. Diclofenac in tissues and plasma and hydroxy metabolites in plasma (4' and 5 hydroxy diclofenac, respectively) were assayed using HPLC with coulometric detection. Mean diclofenac concentrations in edible tissues at 3, 12, 24 and 144 hours were 2874, 1232, 426 and 27 µg/kg in liver; 3244, 1511, 423 and 21 µg/kg in kidney; 1270, 504, 100 and 9 µg/kg in fat, 470, 172, 347 and less than 5 µg/kg in muscle. At the same time points mean concentrations at injection site were 1586, 2947, 1090 and 33 µg/kg. Only traces of hydroxy metabolites could be detected (up to 25 µg/l).

Eight dairy cows were treated intramuscularly with 2.5 mg diclofenac/kg bw once a day for 6 days. The tissue diclofenac concentrations 96 hours after the last administration, were 82 µg/kg in liver, 88 µg/kg in kidney, 10 µg/kg in muscle, 358 µg/kg at injection site and 55 µg/kg in fat. At 176 hours, the values decreased to 25 µg/kg in liver, 23 µg/kg in kidney, 5 µg/kg in muscle, 148 µg/kg at injection site and 10 µg/kg in fat.

19. Only non-radiometric studies were performed in lactating cows.

    Eight dairy cows were treated intramuscularly with 2.5 mg diclofenac/kg once a day for 6 days. A total of 8 consecutive samples taken twice a day starting at the afternoon of the last day of treatment were taken. Diclofenac concentrations above the limit of quantification (5 µg/l) were found only in 12 out of 64 samples, i.e. less than 20%. Concentrations ranged between 5.1 and 17.2 µg/l, with only 3 exceeding values, i.e. 40.5 µg/l ($4^{th}$ milking) and 35 µg/l and 38 µg/l (last milking). In an additional study, in 12 dairy cows the diclofenac levels in all samples analysed were below the limit of quantification (5 µg/l), approximately 12 hours after the end of the treatment.

20. A validated routine analytical method based on HPLC MS/MS for the determination of diclofenac in edible tissues of pigs and cattle is available. The limit of quantification was 0.5 µg/kg for all edible tissues of pigs. In cattle the limit of quantification was 1 µg/kg for liver and kidney and 0.5 µg/kg for muscle and fat. The limit of detection was 0.2 µg/kg for liver, kidney and muscle and 0.25 µg/kg for skin+fat of pigs. For edible tissues of cattle the limit of detection was 0.8 µg/kg for liver and kidney and 0.25 µg/kg for muscle and fat.

    For milk a routine analytical method based on HPLC MS/MS was developed with an limit of quantification of 5 µg/kg and an limit of detection of approximately 1 µg/kg.

**Conclusions and recommendation**

Having considered that:

- an ADI of 0.5 µg/kg bw (i.e. 30 µg/person) was established for diclofenac,
- diclofenac was retained as the marker residue in porcine and bovine tissues,
- the average marker to total residues ratios tissues 3 days after intramuscular administration in porcine and bovine tissues were 0.035 and 0.11 in liver and 0.12 and 0.20 in kidney, respectively; data were not available for muscle and fat or skin+fat and so a conservative ratio of 0.1 for these tissues has been retained;
- 50% and 20% of the total residues in liver and kidney were irreversibly bound in pig tissues; 40% and 30% of the total residues in liver and kidney were irreversibly bound in bovine tissues;
- for harmonisation purposes similar MRLs should be recommended for both species
- there were no radiometric residues data for milk,
- validated routine analytical methods for monitoring residues of diclofenac in porcine and bovine tissues are available;

the Committee for Veterinary Medicinal Products recommends the inclusion of diclofenac 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 |
|---|---|---|---|---|---|
| Diclofenac | Diclofenac | Bovine | 5 µg/kg<br>1 µg/kg<br>5 µg/kg<br>10 µg/kg | Muscle<br>Fat<br>Liver<br>Kidney | Not for use in animals from which milk is produced for human consumption |
| | | Porcine | 5 µg/kg<br>1 µg/kg<br>5 µg/kg<br>10 µg/kg | Muscle<br>Skin+fat<br>Liver<br>Kidney | |

Based on the MRLs for pigs, the theoretical maximum daily intake was calculated to be 86% of the ADI.