



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TOLFENAMIC ACID

SUMMARY REPORT

1. Tolfenamic acid, (N-(2-methyl-3-chlorophenyl)-anthranilic acid, is a non-steroidal anti-inflammatory drug (NSAID) belonging to the fenamate group.
2. The molecule was originally developed over 25 years ago for use in human medicine. More recently, products containing tolfenamic acid have been developed for veterinary use : injectable formulations for use in cattle and pigs and oral paste for horses (not intended for human consumption). In cattle, tolfenamic acid is intended as complementary treatment in respiratory disease and mastitis at a level dose of 2 mg/kg bw/day for two days at 2 days apart administered by intramuscular route. It is also administered by the intravenous route: two injections of 2 mg/kg bw/day for 2 days or a single injection of 4 mg/kg bw. In pigs, tolfenamic acid is administered by the intramuscular route at a single dose of 2 mg/kg bw in case of Metritis Mastitis Agalactia (MMA) syndrome.
3. Tolfenamic acid had a pharmacological profile characteristic of NSAIDs. The two hydroxylated metabolites of tolfenamic acid, the N-(2-hydroxymethyl-3-chloro phenyl)-anthranilic acid and the N-(2-hydroxymethyl-3-chloro-4-hydroxy phenyl) anthranilic acid were much less potent than the parent compound in terms of anti-inflammatory, analgesic and ulcerogenic activity. In animals, pharmacological NOELs can be established from cardiovascular and respiratory effects after intravenous administration of the compound in the dog (1 mg/kg bw) and in the rabbit (5 mg/kg bw). In children, an antipyretic effect was observed after an oral administration of 0.5 mg/kg bw.
4. For rats and the target species, the use of ¹⁴C carboxyl labelled tolfenamic acid allowed the identification of metabolites in excreta and tissues. In all species studied tolfenamic acid was metabolised by hydroxylation and subsequent conjugation of the metabolites.

In rabbits, very little hydroxylation occurred and the drug was excreted almost completely in urine (about 90 %) as glycine and glucuronide conjugates of tolfenamic acid.

In the target species, the major elimination route was urine (for pig, 120 hours after administration, 55% of administered dose and for cattle 46% after 48 hours). Tolfenamic acid was the major component in urine.

In humans, after a single oral administration of 200 mg of tolfenamic acid, 56 % was excreted within 24 hours in urine. Tolfenamic acid and its two hydroxylated metabolites, the N-(2-hydroxymethyl-3-chloro phenyl)-anthranilic acid and the N-(2-hydroxymethyl-3-chloro-4-hydroxy phenyl) anthranilic acid were detected in urine.
5. Tolfenamic acid was of relatively low acute toxicity, with LD₅₀ values in the region of 200-1000 mg/kg bw, depending on the administration route and test species. The two hydroxylated metabolites and the (2-carboxyphenyl)-N-3-chloroanthranilic acid were less toxic than tolfenamic acid.
6. Several repeated dose oral toxicity studies were carried out in rodents (90-day, 6-month, 9-month toxicity studies; doses ranging from 0 to 200 mg/kg bw/day). Although these studies were not always carried out in compliance with GLP, 25 mg/kg bw/day and 10 mg/kg bw/day were the NOELs from the 6- and 9-month toxicity studies.

In the 4-week oral toxicity study carried out in dogs (0, 15, 30, 60 mg/kg bw/day) and in the 6-month oral toxicity study performed in pigs (0, 20, 40, 80 and 160 mg/kg bw/day) no NOELs could be retained.

After oral administration of 0, 1, 4, 16 and 64 mg/kg bw/day for one month in rabbits, 1 mg/kg bw/day was the NOEL.

These studies showed evidence of gastro-intestinal lesions, generally presenting as ulceration and sometimes perforation with resultant peritonitis and deaths.

7. A formal 2-generation reproduction study has not been provided. However, the effects on each reproductive phase were investigated and the studies provide an adequate basis for the assessment of tolfenamic acid on reproduction function;

Segment I in rats treated with 0, 10, 20 and 40 mg/kg bw/day: no effect of tolfenamic acid on fertility of either sex or on progeny: a NOEL of 10 mg/kg bw/day was retained for maternotoxicity and foetotoxicity.

Segment II in rats (0, 3.75, 7.5 and 15 mg/kg bw/day) and rabbits (0, 3, 6, 12 and 48 mg/kg bw/day): tolfenamic acid did not induce foetal abnormalities or developmental variations in these species. A NOEL of 3.75 mg/kg bw/day for rats could be retained whereas in rabbits the lowest level dose of 3 mg/kg bw/day induced a significant increase in the placental and foetal weight.

Segment III in rats: a slightly extended gestation period or difficulty in parturition were observed at 40 mg/kg bw but not at dosages equal or lower than 20 mg/kg bw/day. These effects could be attributed to tolfenamic acid treatment.

8. Four *in vitro* tests (Ames Test (2 assays), Rec method test, mouse lymphoma mutation assay, chromosomal aberrations with Chinese ovary cells test) and two *in vivo* test (Micronucleus test in bone marrow, Unscheduled DNA synthesis test in liver) were carried out to investigate mutagenic potential. Although the mouse lymphoma assay gave equivocal results and the chromosomal aberration test was positive at cytotoxic concentrations, all the other mutagenicity tests including *in vivo* tests using two different organ sites were clearly negative and it was concluded that tolfenamic acid is not a genotoxic substance.
9. There was no evidence of carcinogenic potential for tolfenamic acid in either rats (0, 15, 30 or 60 mg/kg bw/day in the diet over a period of 104 weeks) or mice (0, 15, 30 or 60 mg/kg bw administered in the diet for 80 weeks). Both long-term studies showed toxicity typical of that expected for NSAIDs (gut ulceration and renal papillitis). It was not possible to retain NOELs either for renal papillary necrosis in rats or for gut ulceration in mice.
10. The tolerance studies showed that single intravenous administrations of 18 and 20 mg/kg bw induced transient neurologic disorders in cows, but did not lead to the death of animals. A possible relation could be suggested between hepatic parameter modifications and neurologic disorders. As these side effects appeared at doses 9 and 10 times as high as the therapeutic doses, tolfenamic acid had a good general tolerance when it was intravenously administered in cattle. Tolfenamic acid was well tolerated by pigs and cattle when administered by intramuscular route up to level doses corresponding to 4-fold therapeutic doses; however local reactions at the injection sites were reported.
11. Tolfenamic acid is approved for use in human medicine as an anti-inflammatory, analgesic and antipyretic agent. It is used in inflammatory and rheumatic disorders at a dose of 100 to 200 mg given three times daily by the oral route. An epidemiological study of adverse reactions carried out on 16521 patients showed that 3 % of the population treated can have adverse reactions. The most common reaction was gastrointestinal system disorders (1.72 %).

12. Considering the toxicological data set, the NOELs, retained in the 9-month toxicity study and in the segment II toxicity studies were 10 and 3.75 mg/kg bw respectively in rats while in rabbits a NOEL of 1mg/kg bw was obtained in the 4-week toxicity study therefore, it was concluded that rabbits is the most sensitive species. Rabbit data was therefore chosen to calculate the ADI although it was a one-month toxicity study. As the metabolism of tolfenamic acid in this species was different from that of the other laboratory species tested and of humans, a safety factor of 100 was considered sufficient. A toxicological ADI of 0.01 mg/kg bw was established. From the pharmacological data provided, it was not possible to determine a NOEL, an slight antipyretic effect being observed in children after an oral administration of 0.5 mg/kg bw.

13. In pigs, the metabolism and residue kinetics of ¹⁴C-tolfenamic acid were studied after a single intramuscular administration of 2 mg/kg bw (specific activity of the carboxy labelled tolfenamic acid was approximately 2.7 µCi/mg). At one day post administration, the highest levels of total radioactivity were measured in liver (0.45 mg equivalent tolfenamic acid/kg) whereas 0.25, and 0.01 mg equivalent tolfenamic acid were measured in kidney and muscle. The concentrations in fat were below the limit of quantification of the radiometric method. Five days after administration, the radioactivity could only be measured in liver (0.13 mg equivalent tolfenamic acid/kg) and in kidney (0.02 mg equivalent tolfenamic acid/kg).

The concentrations of tolfenamic acid were simultaneously determined by HPLC. One day after dosing, the following concentrations were measured in the different edible tissue: 0.020 mg/kg for muscle, 0.170 mg/kg for liver, 0.050 mg/kg for kidney and 2.16 mg/kg at the injection site. They then declined to attain the limit of quantifications at 120 hours post administration in all edible tissues.

One day after the injection, tolfenamic acid represented 80%, 40% and 20% of the total radioactivity in muscle, liver and kidney.

14. Two radiometric depletion studies were carried out in cattle according to two different therapeutic regimen.

In the first study, the intravenous administration was followed by an intramuscular administration 24 hours later of ¹⁴C-tolfenamic acid at a rate of 4 mg/kg bw.

At the 7-day withdrawal period, the total residues of tolfenamic acid in fat were 1.6 mg/kg, i.e. 10-fold lower then those measured in muscle (20 mg/kg).

At the 14-day withdrawal period, the mean radioactivity levels ranged from 60 to 90 mg equivalent tolfenamic acid/kg in the edible tissues except for the injection site (85700 mg equivalent tolfenamic acid/kg)

In the second study, the animals received two intramuscular administrations of ¹⁴C-tolfenamic acid at a level dose of 2 mg/kg bw at 48 hour apart. At one day post dosing, the total radioactivity levels were in the magnitude of 1400 mg equivalent tolfenamic acid for liver, 1000 mg equivalent tolfenamic acid/kg for kidney, 500 mg equivalent tolfenamic acid/kg for muscle and 305000 mg equivalent tolfenamic acid/kg for the injection site. At four days after the end of the treatment, the amounts of radioactivity were below 100 mg/kg in all edible tissues. The residues at the injection site were still high (62420 ± 72000 mg equivalent tolfenamic acid/kg).

After treatment by beta-glucuronidase, nearly all the radioactivity could be extracted from tissue samples. Tolfenamic residues are therefore not irreversibly bound to the tissues.

The concentrations of tolfenamic acid were simultaneously determined by HPLC. Four days after dosing, the following concentrations were measured in the different edible tissues: 20 mg/kg for muscle, 100 mg/kg for liver, 30 mg/kg for kidney and 39000 mg/kg for the injection site respectively.

At 24 hours post dosing, tolfenamic acid represents 80%, 70% and 30% of the total radioactivity in muscle, liver and kidney.

15. Twenty-four hours after intravenous and intramuscular administrations of 4 mg/kg bw, these two administrations being separated by 24 hours, radioactivity could be measured in milk (47 mg

equivalent tolafenamic acid/kg). The radioactivity then declined rapidly to reach levels close to the limit of quantification 10 ng equivalent tolafenamic acid/kg within three days.

In a second study carried out at the therapeutic regimen intravenous administration followed by intramuscular administration of ¹⁴C-tolafenamic acid, the concentrations of tolafenamic acid were below 20 ng/kg in the milk samples collected 24 hours after the last injection.

The parent compound represents 30 % of the total radioactivity measured in milk.

16. A validated HPLC routine analytical method was proposed. The limits of quantification were 20 ng/kg for pig edible tissues and cattle muscle and milk and 50 ng/kg for bovine liver and kidney. The limit of detection was 10 ng/kg except for bovine kidney and porcine muscle (15 ng/kg).

Conclusions and recommendation

Having considered that :

- Σ the toxicological ADI is 0.010 mg/kg bw (i.e. 600 µg per person);
- Σ tolafenamic acid is the marker residue;
- Σ in pigs, at one day post treatment, the tissue distribution observed showed that the highest concentrations of tolafenamic acid were measured in liver (170 ng/kg) whereas the levels were close to 50 ng/kg for kidney and 20 ng/kg for muscle. The ratio of parental compound to total residues is known for the edible tissues: 0.8 for muscle, 0.4 for liver and 0.20 for kidney;
- Σ in cattle, at 4 days post treatment, the tissue distribution observed showed that the highest concentrations were measured in liver (100 ng/kg) whereas the levels were close to 30 ng/kg for kidney and 20 ng/kg for muscle. The ratio of parental compound to total residues is known for the edible tissues : 0.6 for muscle, 0.7 for liver, 0.30 for kidney and 0.3 for milk.

The Committee recommends the inclusion of tolafenamic into 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 tissues	Other provisions
Tolafenamic acid	Tolafenamic acid	Bovine	50 µg/kg 400 µg/kg 100 µg/kg 50 µg/kg	Muscle Liver Kidney Milk	
		Porcine	50 µg/kg 400 µg/kg 100 µg/kg	Muscle Liver Kidney	

As the concentrations of tolafenamic acid in bovine and porcine fat were much lower than those measured in muscle no MRLs were allocated for this edible tissue.

Based on these MRL values, the daily intake will represent about 60% of the toxicological ADI.