Aceclofenac: a vulture-toxic drug

Summary of evidence that aceclofenac is toxic to vultures; and that meloxicam, an alternative to aceclofenac, is not toxic to vultures as well as a safe and effective veterinary drug

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SAVE is an international consortium of conservation and research organisations whose mission is to respond to the vulture crisis in Asia by striving to halt vulture population declines and working to minimise their negative impacts on ecological and human health. For further details go to www.save-vultures.org

Aceclofenac is toxic to *Gyps* vultures; however, meloxicam is not toxic to *Gyps* vultures and an effective alternative to aceclofenac for veterinary purposes

Aim

This paper, intended for decision-makers involved in drug licensing, presents evidence of the toxicity of aceclofenac, a non-steroidal anti-inflammatory drug (NSAID), to *Gyps* vultures; and the safety, in both vultures and domesticated animals, and effectiveness, in domesticated animals, of meloxicam, an alternative NSAID.

Executive Summary

1. The NSAID aceclofenac converts into diclofenac in cattle.
2. Wild vultures are exposed to veterinary NSAIDs by feeding on carcasses of domesticated ungulates treated with these drugs shortly before death.
3. The NSAID diclofenac has been shown to be toxic to *Gyps* vultures. Two independent experiments showed that oral doses of diclofenac caused hyperuricemia (i.e., elevated uric acid levels), kidney failure and death in *Gyps* vultures. One of these experiments also showed tissues from domesticated ungulates treated with the recommend dosage of diclofenac prior to death caused hyperuricemia, kidney failure and death in *Gyps* vultures.
4. Aceclofenac has been shown to metabolise into diclofenac in other mammals. A literature review found seven out of seven studies reporting the metabolism of aceclofenac in mammals showed its conversion to diclofenac. However, the list of mammals tested did not include domesticated ungulates likely to be eaten by vultures.
5. An experiment carried out in South Africa showed that cattle *Bos taurus* rapidly metabolise the recommended dose of aceclofenac into diclofenac. Further, the rate of

absorption and elimination of the diclofenac metabolite in cattle plasma was similar to that of pure diclofenac in cattle plasma.

6. In a separate experiment conducted in India, it was shown that water buffalo *Bubalus bubalis* also rapidly metabolise the recommended dose of aceclofenac into diclofenac, showing a similar pharmacokinetic profile to the experiment on South African cattle.
7. Carcasses of cattle treated with aceclofenac shortly before death poses a similar threat to *Gyps* vultures as carcasses of cattle treated with diclofenac shortly before death.
8. The manufacture, importation, retail and use of diclofenac for veterinary purposes are banned in Bangladesh, India, Nepal and Pakistan; however, this is not the case for aceclofenac. Therefore, aceclofenac poses a serious threat to critically endangered *Gyps* vultures in the above-mentioned countries.
9. In contrast to aceclofenac and diclofenac, the NSAID meloxicam has been shown to be non-toxic above the MLE in 50 *Gyps* vultures. Two experiments showed that oral doses of meloxicam above the MLE did not cause hyperuricemia or any other ill effects in African white-backed vultures *Gyps africanus*, Asian white-backed vultures *Gyps bengalensis* and long-billed vultures *Gyps indicus*. These experiments also showed that tissues from cattle and buffalo dosed with meloxicam with some doses at the MLE did not cause hyperuricemia or any other ill effects in *Gyps* vultures. Further, oral doses of meloxicam above the MLE did not cause hyperuricemia or any other ill effects in Egyptian vultures *Neophron percnopterus*, cattle egrets *Bubulcus ibis*, crows *Corvus spp.* and common myna *Acridotheres tristis*, which also scavenge.
10. Meloxicam is manufactured in Bangladesh, India, Nepal and Pakistan. It has been shown to be an effective treatment for pain, inflammation, stress and dysfunction in a variety of veterinary and husbandry situations. In addition, meloxicam has been shown to increase immune response, pregnancy rate, cow productivity and calf productivity. Negative or mixed effects of meloxicam are rare. In contrast, only one experimental study has examined the effect of acelcofenac (in dogs).
11. To conserve critically endangered *Gyps* vultures, veterinary nimesulide and multiple dose vials of human aceclofenac should be banned throughout South Asia. Such a

comprehensive ban would not impact livestock healthcare because meloxicam is a safe, effective and widely available alternative to aceclofenac.

Supporting evidence

We present eight lines of supporting evidence.

1. In Pakistan, searches for dead Asian white-backed vultures *Gyps bengalensis* were undertaken at nesting colonies. Post-mortem examination was performed on 259 vultures of which 219 presented a degree of visceral gout. Among a subset of vultures examined within 24 h of death, 100% of those that were in good condition and without disease tested positive for diclofenac residue, whereas 0% of those with acute disease tested negative for diclofenac. All additional tests for possible causes of kidney failure were negative. In addition, the toxicity of diclofenac to Asian white-backed vultures was examined in a two-phased partially controlled experiment in Pakistan. Phases were: (1) oral-dosing of diclofenac at 0.25-2.5 mg/kg body weight (bw); and (2) feeding diclofenac-contaminated domesticated ungulate tissues at doses of 0.005-1.0 mg/kg vulture bw. In Phase 1, three out of four vultures died. In Phase 2, 13 out of 20 vultures died. All control individuals survived. Elevated uric acid concentration in plasma (hyperuricema) was observed at 24 h in two out of two diclofenac-treated vultures tested. Kidney damage and visceral gout was observed in all diclofenac-treated vultures that died. Further details in pages 9-11.

2. In South Africa, a single-phased controlled experiment examined toxicity of diclofenac to African white-backed vultures *Gyps africanus* and Eurasian griffon vultures *Gyps fulvus*. Vultures were given an oral dose of diclofenac at 0.8 mg/kg body weight (bw). Blood samples were taken for analysis of uric acid and other biochemical components; and all vultures that died and one that survived, but was euthanized, were given full post mortem examinations. All diclofenac-treated individuals died; whereas, all control individuals survived. Elevated uric acid and ALT concentrations in plasma were observed in diclofenac-treated vultures, but not the control individuals. Kidney damage and visceral gout was observed in all diclofenac-treated vultures, but not the control individual that was euthanised. Further details in pages 12-14.

3. Published literature, the PubMed database and package inserts were surveyed for information on the pharmacology and metabolism of aceclofenac. Aceclofenac and diclofenac are structurally similar. Both drugs are arylalkanoic acid derivatives. Seven studies provided evidence that aceclofenac is converted into diclofenac in rats, dogs, monkeys and humans. No study contradicted these findings. Therefore, diclofenac is likely to be the principal metabolite in all mammalian species. The opinions of veterinarians and pharmacists working in Rajasthan, India, on aceclofenac were sought. Aceclofenac was considered a cost- and clinically-effective alternative to diclofenac. The market share of aceclofenac in Rajasthan, India, has increased between 2010 and 2012. Further details in pages 15-16.

4. In South Africa, an experiment was undertaken to examine the pharmacokinetics of aceclofenac and potential diclofenac metabolites in cattle. *Bos taurus* cattle were given the recommended veterinary dose of aceclofenac and their blood was sampled at intervals after treatment. Presence and concentration of aceclofenac and diclofenac metabolites in plasma was determined using liquid chromatography with mass spectrometry. Nearly all of the aceclofenac administered to the cattle was rapidly converted to diclofenac. At 2 h post-treatment, half of the administered aceclofenac had been converted to diclofenac; and, at 12h, four-fifths of the administered aceclofenac had been converted into diclofenac. Diclofenac would be absorbed and eliminated at comparable rates in other tissues (kinetic homogeneity). The time-course of diclofenac concentration in tissues of cattle dosed with aceclofenac was similar to that for cattle dosed with pure diclofenac. Further details in pages 17-19.

5. In India, an experiment was undertaken to examine the pharmacokinetics of aceclofenac in native Water Buffalo *Bubalus bubalis*. Nine buffalo were given the recommended dose of aceclofenac, and their blood sampled at intervals after treatment. After treatment, aceclofenac was barely detectable in the plasma of buffalo, and had been rapidly metabolised into diclofenac, the concentrations of which peaked at 4-8 hours post-treatment, followed by a slower elimination. Further details on pages 20-22.

6. The effect of meloxicam on *Gyps* vultures was examined in a six-phase partially controlled experiment conducted in South Africa and India. Phases were: (1-2) 10 African white-backed vultures *Gyps africanus* were given an oral dose of meloxicam at 0.5-1.0 mg/kg body weight (bw); (3-4) 40 African white-backed vultures *Gyps africanus* were given an oral dose of meloxicam at 2.0 mg/kg bw; (5) six African white-backed vultures *Gyps africanus* were twice fed meloxicam-contaminated cattle tissue and once given an oral dose of meloxicam at >0.01-1.98 and 1.18-2.45 mg/kg bw, respectively; and (6) six Asian white-backed vultures *Gyps bengalensis* and four long-billed vultures *Gyps indicus* were given an oral dose of meloxicam at 0.5-2.0 mg/kg bw. The maximum level of exposure (MLE) of meloxicam to Asian white-backed vulture *Gyps bengalensis* was calculated as 1.83 mg/kg bw. In all Phases, all meloxicam-treated vultures survived with no ill effects. All control vultures survived. Uric acid concentrations in plasma in all meloxicam-treated vultures remained within the normal range. Further details in pages 23-25.

7. The effect of meloxicam on *Gyps* vultures and other common scavenging birds was examined in a three-phase controlled experiment in India. Phases were: (1) six Asian white-backed vultures *Gyps bengalensis* and four Long-billed vultures *Gyps indicus* were given an oral dose of meloxicam at 0.5 or 2.0 mg/kg bw; (2) five Egyptian vultures *Neophron percnopterus*, five cattle egrets *Bubulcus ibis*, five crows *Corvus spp.* and five common myna *Acridotheres tristis* were given an oral dose of meloxicam at 2.0 mg/kg bw; and (3) 19 Asian white-backed vultures *Gyps bengalensis* and two long-billed vultures *Gyps indicus* were fed tissue from buffalo dosed with meloxicam. In all Phases, all meloxicam-treated birds survived with no ill effects. All control birds survived. Uric acid and ALT concentrations in serum in all meloxicam-treated birds was not significantly different to that of control birds. Further details in pages 26-28.

8. A review of studies reporting an effect of aceclofenac and meloxicam in veterinary and husbandry situations found a single study for aceclofenac and 117 studies for meloxicam. The single study examining aceclofenac treatment reported a positive effect. 79% of studies examining meloxicam treatment reported a positive effect. Meloxicam showed positive effects

in cattle, buffalo, horse, goat, sheep and dog; and during castrating, dehorning, general care, handling, infections, limb injuries, reproducing and surgery. In these animals and situations, meloxicam decreased pain, inflammation, stress and dysfunction; increased recovery rate, immune response, pregnancy rate and productivity; and generally resulted in no complications. The proportion of studies examining meloxicam treatment that reported a mixed effect, negative effect, no effect and no conclusion were 9, 3, 7 and 2%, respectively. The studies that reported a negative effect of meloxicam treatment resulted in pregnancy complications, but another eleven studies reported a positive effect of meloxicam treatment on pregnancy rate. Aceclofenac treatment was not examined for pregnancy complications. Further details in pages 29-37.

Specific details

Chemical name: **aceclofenac**

Systematic name: 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic acid.

Formula: $C_{16}H_{13}Cl_2NO_4$

Molar mass: 353.02161 g/mol

Typical formulation: 25 mg/ml injection in 50 ml vials; 500 mg/g bolus.

Example products (manufacturers):

Bangladesh – Flexi (Square)

India – Diclodec (ESPEE formulation), Nicoace (Crescent)

Nepal – Indian products may be imported

Pakistan – Fev-O-nil (S.J. & G. Fazul Ellahie)

Chemical name: **meloxicam**

Systematic name: 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3carboxamide-1,1-dioxide

Formula: $C_{14}H_{13}N_3O_4S_2$

Molar mass: 351.403 g/mol

Typical formulation: 5 or 10 mg/ml injection in 30, 50 or 100 ml vials; and 100 mg bolus

Example products (manufacturers):

Bangladesh – Mel-Vet (ACME), Meximol (Ethical drugs), MP-Vet (Chemist Laboratories)

India – Diclovet-M and Melodic (Umedica), Melonex (Intas), Melosafe and Meloxicon (Safecon), Melovet (Zee), Proxyvet-MP (Health Biotech), Xyclofen (Iskon)

Nepal – Melocam (CTL), Melox (Medivet), Molfen (Qumed)

Pakistan – Camilox and Meloxi-10 (Selmore), Diclostar (Star), Diclosym and Melocsym (Symans), Metrym (Nawan)

1. A carcass survey and toxicity experiment finds diclofenac residue in association with kidney failure and death in the Asian white-backed vulture *Gyps bengalensis*

Published source

Oaks, J. L., Gilbert, M., Virani, Z., Watson, R. T., Meteyer, C. U., Rideout, B. A., Shivaprasad, H. L., Ahmed, S., Chaudhry, M. J. I., Arshad, M., Mahmood, S., Ali, A. and Khan, A. A. (2004). Diclofenac residues as the cause of vulture population declines in Pakistan. *Science* 427: 630633

Background

Between 2000 and 2003, Asian white-backed vultures *Gyps bengalensis* declined by up to 95% in Pakistan. In the same period, diclofenac was the most commonly used veterinary NSAID in Pakistan. Vultures were exposed to diclofenac through the consumption of carcass tissues of domesticated ungulates treated prior to death. This carcass survey and experiment were undertaken to examine the toxicity of diclofenac to Asian white-backed vultures *Gyps bengalensis*.

Action

Searches for dead Asian white-backed vultures *Gyps bengalensis* were undertaken at nesting colonies in Pakistan. Carcasses were given a full post mortem examination. Liquid chromatography with mass spectrometry was used to determine the presence and concentration of diclofenac residue. Additional tests were used to determine the presence of metals, pesticides and viruses that are known to cause kidney failure.

In addition, a three-phased partially controlled experiment was undertaken in Pakistan. Phase 1: two Asian white-backed vultures *Gyps bengalensis* were given an oral dose of diclofenac at 2.5 mg/kg body weight (bw); and another two Asian white-backed vultures were given an oral dose of diclofenac at 0.25 mg kg bw.

Phase 2: twenty Asian white-backed vultures *Gyps bengalensis* were fed domesticated ungulates tissues contaminated with diclofenac at doses of 0.005-1.0 mg/kg vulture bw; and six white-backed vultures *Gyps bengalensis* were fed domesticated ungulates tissues not contaminated with diclofenac. Diclofenac-contaminated tissues were kidney, liver and muscles from buffalo and goat that were given three injections of diclofenac at 2.5 mg/kg bw (the recommended daily dose in Pakistan) over 3 days and slaughtered 4 h after the final dose. Blood samples were taken from one vulture from each treatment group in Phases 1 at 1 and 24 h after dosing. Plasma was analysed for uric acid. All vultures that died and one that survived Phase 2, but was euthanized, were given full post mortem examinations. Kidney samples were analysed for diclofenac residue using liquid chromatography mass spectrometry.

Findings

Between 2000 and 2002, 259 dead Asian white-backed vultures *Gyps bengalensis* were examined. Of these, 219 presented a degree of visceral gout (uric acid deposits on the internal organs); and 42 were examined within 24 h of death. Among the latter subset, 25 out of 25 vultures in otherwise good condition and without a detected disease tested positive for diclofenac residue; and 0 out of 13 vultures with acute disease tested negative for diclofenac. All additional tests for possible causes of kidney failure were negative (number of individual tested was between 13 and 39).

In Phase 1, three out of four vultures died (one vulture dosed with diclofenac at 0.25 mg/kg bw survived). In Phase 2, 13 out of 20 vultures died (seven vultures dosed with diclofenac at 0.005-0.6 mg/kg bw survived). All control individuals survived.

Elevated uric acid concentrations in plasma were observed at 24 h in both diclofenac-treated vultures that were tested (and died). Kidney damage and visceral gout was observed in all diclofenac-treated vultures that died.

Conclusion

Diclofenac is toxic to Asian white-backed vultures *Gyps bengalensis*.

Key figure

Table 2 **Results of experimentally feeding meat from diclofenac-treated animals to OWBVs**

Vulture no	Exposure (mg kg^{-1})	Result	Diclofenac residues (μg g^{-1})
11	0.007	Gout	0.38
12	0.025	Survived	ND
8	0.027	Survived	ND
10	0.028	Survived	ND
7	0.029	Survived	ND
9	0.029	Survived	ND
D	0.140	Gout	0.07
F	0.240	Survived	BDL (8 days)
H	0.550	Gout	0.25
B	0.600	Survived	BDL (8 days)
64	0.820	Gout	0.54
65	0.820	Gout	ND
62	0.840	Gout	0.22
63	0.840	Gout	0.12
70	0.860	Gout	0.74
67	0.860	Gout	ND
68	0.860	Gout	ND
66	0.880	Gout	0.26
73	0.910	Gout	0.91
72	0.940	Gout	ND
1	None (control)	Survived	ND
2	None (control)	Survived	ND
3	None (control)	Survived	ND
4	None (control)	Survived	ND
cc1	None (control)	Survived	BDL (8 days)
cc2	None (control)	Survived	BDL (8 days)

Values in parentheses in the last column indicate time after exposure. OWBVs that died and displayed gross characteristics of visceral gout on necropsy are labelled as 'Gout' in the third column. BDL, below detection limit of diclofenac assay (0.005–0.01 μg g^{-1}); ND, analysis not done.

2. A toxicity experiment finds diclofenac causes kidney failure and death in two additional species of *Gyps* vultures

Published source

Swan, G. E., Cuthbert, R. J., Quevedo, M., Green, R. E., Pain, D. J., Bartels, P., Cunningham, A. A., Duncan, N., Meharg, A. A., Oaks, J. L., Parry-Jones, J., Shultz, S., Taggart, M. A., Verdoorn, G., Wolter, K. (2006a). Toxicity of diclofenac to *Gyps* vultures. *Biology Letters* 2: 279-282

Background

Diclofenac is toxic to Asian white-backed vultures *Gyps bengalensis*. This experiment was undertaken to examine toxicity of diclofenac to other species of *Gyps* vulture.

Action

A single-phased controlled experiment was undertaken in South Africa.

Two African white-backed vultures *Gyps africanus* and three Eurasian griffon vultures *Gyps fulvus* were given an oral dose of diclofenac at 0.8 mg/kg body weight (bw); and another two white-backed and three Eurasian griffon vultures *Gyps fulvus* were given an oral dose of sterilized water.

Blood samples were taken from all vultures before and 4, 12 and 24 h after dosing. Plasma was analysed for uric acid and other biochemical components. All vultures that died and one that survived, but was euthanized, were given full post mortem examinations. Liver and kidney samples were analysed for diclofenac residue using liquid chromatography mass spectrometry.

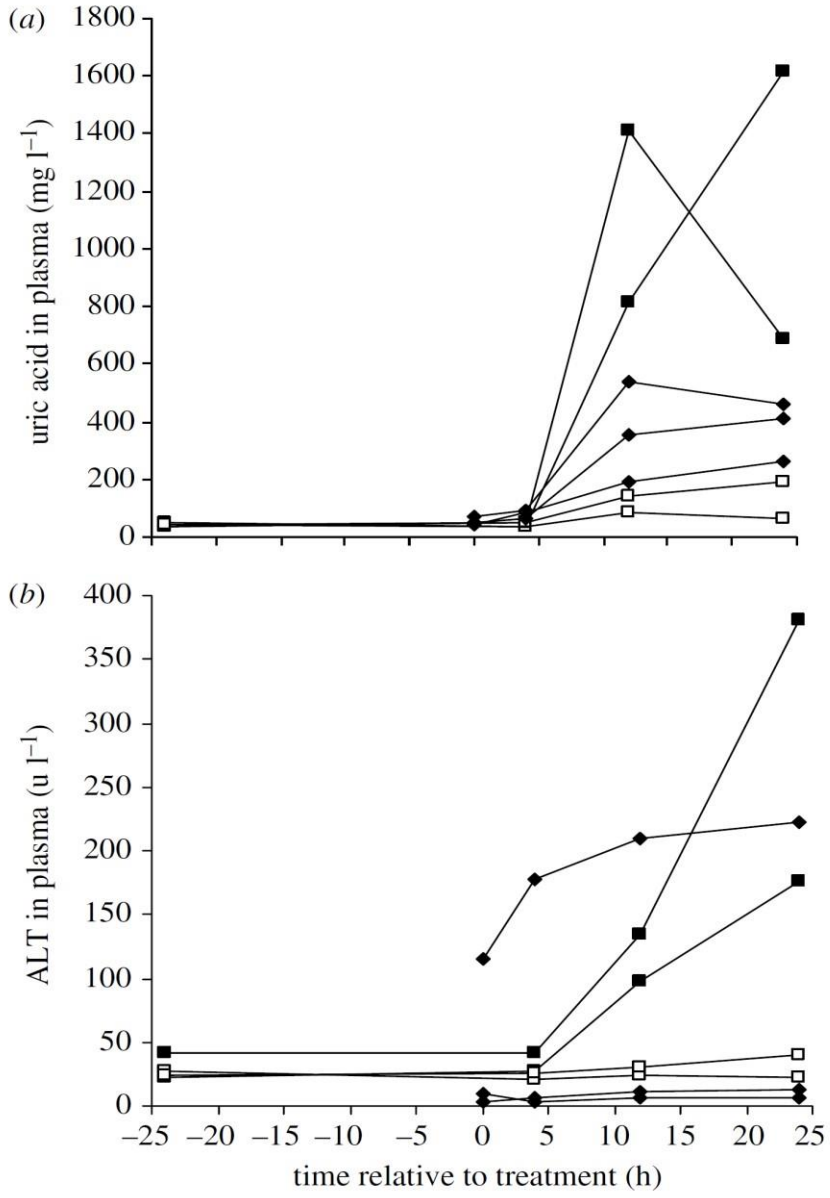
Findings

All diclofenac-treated individuals died; whereas, all control individuals survived. Elevated uric acid and ALT concentrations in plasma were observed at 12 and 24 h in diclofenac-treated vultures, but not the control individuals. Kidney damage and visceral gout was observed in all diclofenac-treated vultures, but not the control individual that was euthanised.

Conclusion

Diclofenac is toxic to *Gyps* vultures in general.

Key figure



(a) Uric acid and (b) ALT concentrations in plasma measured before and after oral treatment of vultures with 08 mg/kg of diclofenac. Lines connect data for the same bird. Results are shown for two diclofenac-dosed (filled squares) and two sham-treated (open squares) G*yps africanus* and for three diclofenac-dosed *Gyps fulvus* (filled diamonds).

3. A review of the literature finds that aceclofenac converts into diclofenac within rats, dogs, monkeys and humans

Published source

Sharma, P. (2012). Aceclofenac as a potential threat to Critically Endangered Vultures in India: a review. *Journal of Raptor Research* 46: 314-318

Background

Diclofenac is toxic to *Gyps* vultures. Like diclofenac, aceclofenac is recommended for use in domesticated ungulates to treat pain and inflammation caused by various diseases and injuries. This review of literature was undertaken to assess the potential risk of aceclofenac to *Gyps* vultures.

Action

Published literature, the PubMed database and package inserts from products available in Rajasthan, India, were surveyed for information on the pharmacology of aceclofenac and its metabolites. In addition, the opinion of veterinarians and pharmacists working in Rajasthan, India, on the cost and clinical effectiveness of aceclofenac was provided.

Findings

Aceclofenac, diclofenac and ketoprofen are structurally similar. These three drugs are arylalkanoic acid derivatives. Meloxicam, which is not toxic to vultures, is an enolic acid derivative.

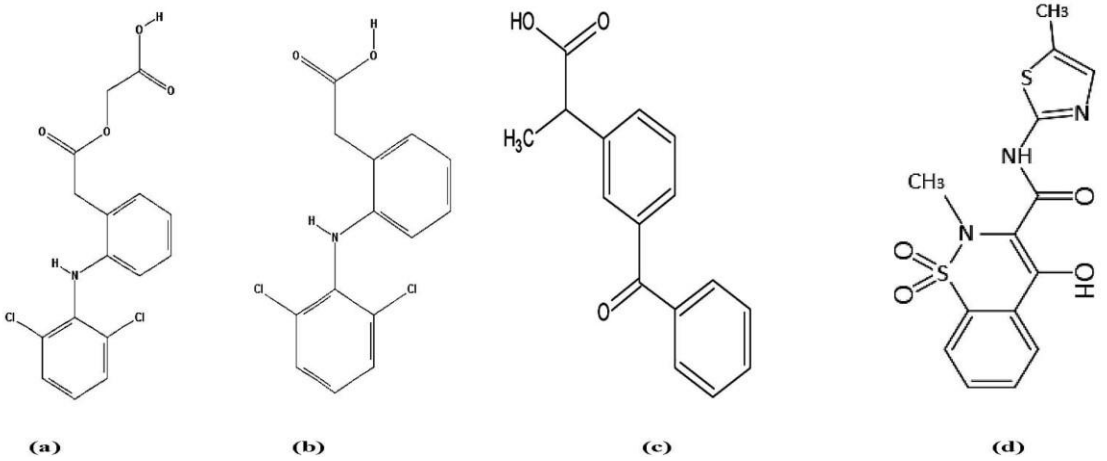
Seven studies provide evidence that aceclofenac is converted into diclofenac and 4'-hydroxydiclofenac in rats, dogs, monkeys and humans. These are the only mammalian species in which the pharmacology of aceclofenac has been examined so far. No study contradicts these findings.

Aceclofenac formulations are relatively cheap and concentrated (thereby requiring less injections) compared to diclofenac. Aceclofenac is considered a cost- and clinically-effective alternative to diclofenac among veterinarians and pharmacists in Rajasthan, India. The market share of aceclofenac in Rajasthan, India, has increased between 2010 and 2012.

Conclusion

Diclofenac is a possible metabolite of aceclofenac in domesticated ungulates.

Key figure



Chemical structure of (a) aceclofenac, (b) diclofenac, (c) ketoprofen and (d) meloxicam.

4. An experiment finds cattle metabolise aceclofenac almost entirely into diclofenac

Published source

Galligan, T. H., Taggart, M. A., Cuthbert, R. J., Svobodova, D., Chipangura, J., Alderson, D., Parkash, V. M. and Naidoo, V. (2016). Metabolism of aceclofenac in cattle to vulture-killing diclofenac. *Conservation Biology* DOI: 10.1111/cobi.12711

Background

Diclofenac is toxic to *Gyps* vultures. Aceclofenac is an analogue of diclofenac. Like diclofenac, aceclofenac is recommended for use in domesticated ungulates to treat pain and inflammation caused by various diseases and injuries. Aceclofenac was known to convert into diclofenac in a number of mammal species, but not cattle or other domesticated ungulates. This experiment was undertaken to examine the pharmacokinetics of aceclofenac and potential diclofenac metabolites in cattle.

Action

In South Africa, six mature *Bos taurus* cattle were given the recommended veterinary dose of aceclofenac (2 mg/kg body weight). The aceclofenac product used was a bolus formulation, manufactured and purchased in India, and administered orally. The recommended dose was that of India. Blood (plasma) was sampled 12 times between 0 and 12 h. In the United Kingdom, plasma samples were examined for the presence and concentration of aceclofenac, diclofenac and 4'-hydroxy-diclofenac using liquid chromatography with mass spectrometry. The pharmacokinetic profiles of aceclofenac, diclofenac and 4'-hydroxy-diclofenac in plasma were modelled and the parameters of each drug compared.

Findings

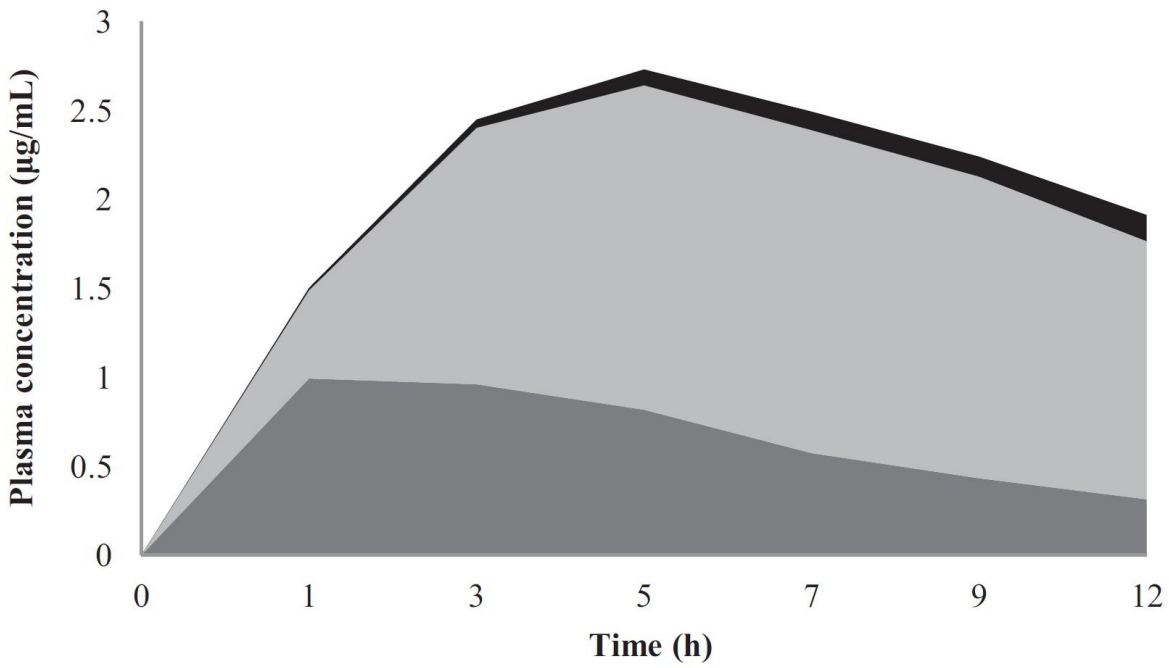
Nearly all aceclofenac administered to the cattle was rapidly converted to diclofenac. Diclofenac was first detected at 0.25 h post-administration and at every subsequent sampling time. At 2 h, half of the administered aceclofenac had converted to diclofenac; and, at 12h, four-fifths of the administered aceclofenac had been converted into diclofenac. 4'-hydroxydiclofenac was a minor metabolite.

Pharmacokinetic profiles in plasma are considered proportional throughout the body (kinetic homogeneity); therefore, the diclofenac metabolite of aceclofenac would be absorbed and eliminated at comparable rates in other tissues. The profile of diclofenac in cattle dosed with aceclofenac was similar to that of cattle dosed with pure diclofenac; therefore, carcasses of cattle treated with aceclofenac prior to death are as dangerous to *Gyps* vultures as those treated with pure diclofenac prior to death.

Conclusion

Cattle metabolise aceclofenac into diclofenac.

Key figure



Additive geometric mean plasma concentration over time for aceclofenac (dark grey), diclofenac metabolite of aceclofenac (light grey) and 4'-hydroxy-diclofenac metabolite of aceclofenac (black) in 6 female *Bos taurus* cattle.

5. An experiment conducted in India on water buffalo *Bubalus bubalis* confirms that aceclofenac is rapidly metabolised into diclofenac.

Published source

Chandramohan, S., Mathesh, K., Mallord, J.W., Naidoo, V., Mahendran, K., Kesavan, M., Gaur, G.K., Pawde, A.M., Prakash, N., Ranade, S., Saikia, D., Sharma, A.K., Shringarpure, R., Green, R.E. & Prakash, V.M. 2022. Metabolism of aceclofenac to diclofenac in the domestic water buffalo *Bubalus bubalis* confirms it as a threat to Critically Endangered *Gyps* vultures in South Asia. *Environmental Toxicology and Pharmacology*, **96,** 103984

Background

Diclofenac is toxic to *Gyps* vultures. Aceclofenac, another NSAID recommended for the treatment of pain and inflammation in domesticated ungulates, has been shown to metabolise into diclofenac in several mammal species, including domesticated cattle in South Africa. To confirm that the drug behaves the same way in an Indian species of domesticated ungulate and would thus be available to vultures feeding on contaminated carcasses, this experiment was undertaken to examine the pharmacokinetics of aceclofenac and its diclofenac metabolite in Indian Water Buffalo *Bubalus bubalis*.

Action

Nine Indian Water Buffalo *Bubalus bubalis* were given the recommended dose (2 mg kg-1 body weight) of a locally-sourced (N India) bolus formulation of aceclofenac, administered orally. Blood (plasma) was sampled at 0 (pre-treatment), 20 min, 40 min, 1-, 2-, 4-, 8-, 12-, 24- and 48-hours post-treatment. Both aceclofenac and diclofenac were extracted from plasma samples using a protein precipitation method and quantified using liquid chromatography tandem mass spectrometry (LC-MS/MS). The pharmacokinetic profiles of aceclofenac and diclofenac in plasma were modelled and the parameters of each drug compared.

Findings

Aceclofenac was barely detectable at any sampling time (see Fig. 1a below). In contrast, diclofenac was detected after 20 mins, and increased thereafter, peaking at 4-8 hours after treatment. Diclofenac was still detectable in all nine animals, albeit at very low levels, after 48 hours (Fig, 1b).

Conclusion

Aceclofenac is rapidly metabolised to diclofenac in Water Buffalo. Buffalo carcasses treated with aceclofenac prior to death will be as dangerous to *Gyps* vultures as those treated with pure diclofenac. Thus, the consequences to vulture populations will be just as catastrophic as the diclofenac-induced declines in the mid-1990s to early 2000s.

Key figure

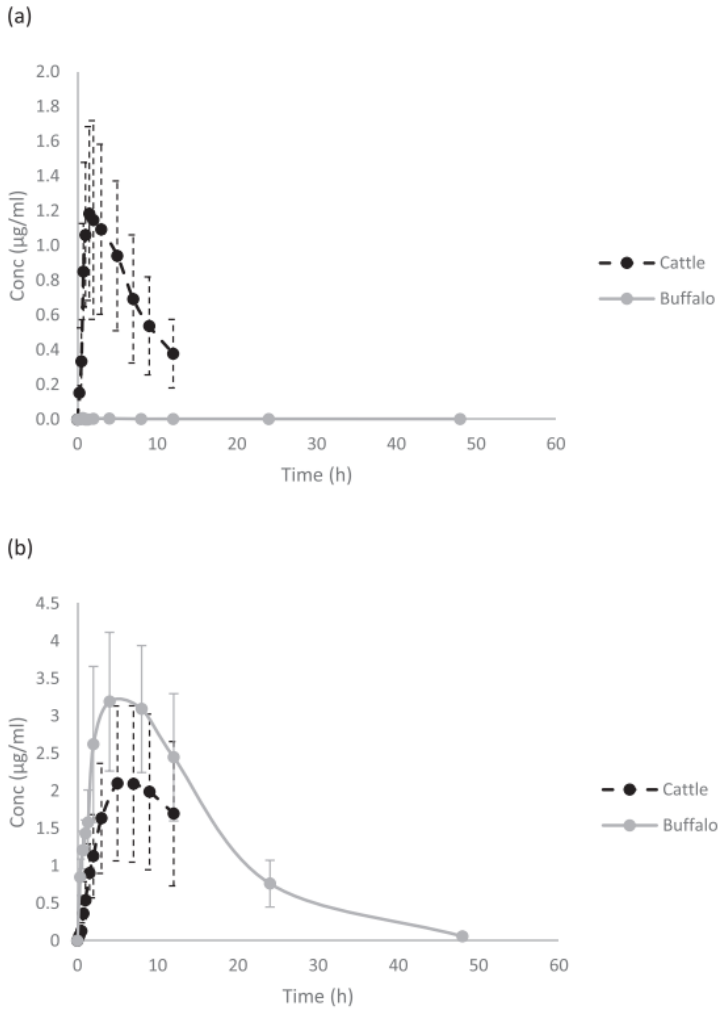


Fig. 1. Change in plasma concentrations over time of (a) aceclofenac and (b) diclofenac metabolite of aceclofenac in nine male domestic water buffaloes *Bubalus bubalis* and domestic cattle *Bos taurus*. Note different scales on *y*-axes. Data for domestic cattle are rom Galligan et al. (2016).

6. An experiment finds doses above the MLE of meloxicam to Asian whitebacked vultures *Gyps bengalensis* does not cause kidney failure, death or elevation of blood uric acid levels in African and Asian *Gyps* vultures

Source

Swan, G., Naidoo, V., Cuthbert, R., Green, R. E., Pain, D. J., Swarup, D., Prakash, V., Taggart M. A., Bekker, L., Das, D., Diekmann, J., Diekmann, M., Killian, E., Meharg, A., Patra, R. C., Saini, M. and Wolter, K., (2006b). Removing the threat of diclofenac to critically endangered Asian vultures. *PLoS Biology* DOI: 10.1371/journal.pbio.0040066.

Background

A previous questionnaire survey on the use of NSAIDs in vultures, found no mortality in 39 cases of meloxicam treatment involving six species of *Gyps* vultures. This experiment was undertaken to further examine the toxicity of meloxicam to *Gyps* vultures.

Action

The effect of meloxicam on *Gyps* vultures was examined in a six-phase partially controlled experiment in South Africa and India.

African and Asian *Gyps* vultures were used. The African white-backed vulture *Gyps africanus* and the Asian white-backed vulture *Gyps bengalensis* are of similar size (4-7 and 3.5-7.5 kg, respectively).

One injectable meloxicam product, manufactured and purchased in India, was used. Double the recommended dose for cattle in India was used to reflect frequent overdosing behaviour in India.

Phases 1-2: 10 African white-backed vultures *Gyps africanus* were given an oral dose of meloxicam at 0.5-1.0 mg/kg body weight (bw); and six African white-backed vultures *Gyps africanus* were given sterilised water.

Phases 3-4: 40 African white-backed vultures *Gyps africanus* (including 21 wild vultures) were given an oral dose of meloxicam at 2.0 mg/kg bw; and three captive and four wild African white-backed vultures *Gyps africanus* were given sterilised water.

Phase 5: six African white-backed vultures *Gyps africanus* were twice fed tissue from cattle dosed with meloxicam and once given an oral dose of meloxicam at >0.01-1.98 and 1.18-2.45 mg/kg bw, respectively. Meloxicam-contaminated cattle tissues were muscle and liver from three cattle given daily injections of meloxicam at 1 mg/kg bw for five days and slaughtered 8 h after the final dose.

Phase 6: six Asian white-backed vultures *Gyps bengalensis* and four long-billed vultures *Gyps indicus* were given an oral dose of meloxicam at 0.5-2.0 mg/kg bw; and two Asian white-backed vultures *Gyps bengalensis* and three long-billed vultures *Gyps indicus* were given sterilised water.

Blood samples were taken from all vultures in Phases 1-5 before the experiment and at up to six time points up to 168 h after dosing. Serum was analysed for uric acid and three other biochemical components.

The maximum level of exposure of meloxicam to Asian white-backed vultures *Gyps bengalensis* was calculated using the average body weight (4.75 kg), estimated large meal weight (1.02 kg) and highest meloxicam residue concentration in cattle liver tissue (8.54 mg/kg bw); and was equal to 1.83 mg/kg bw.

Findings

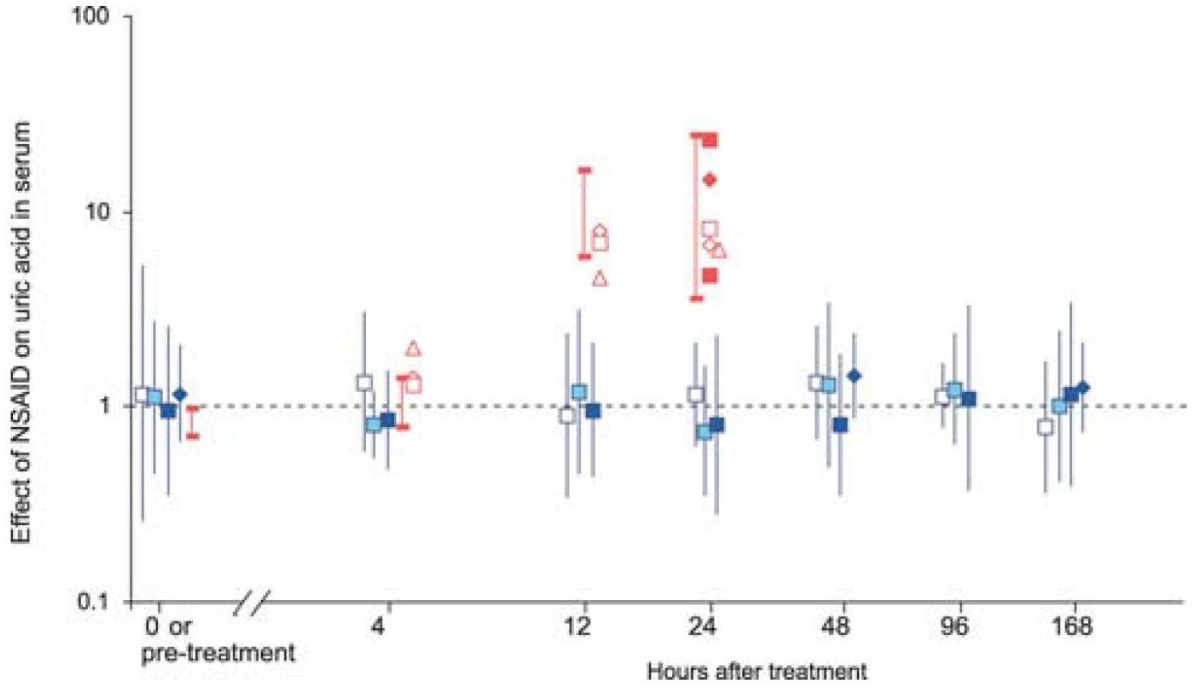
In all Phases, all meloxicam-treated vultures survived with no ill effects. All control vultures survived.

Uric acid concentrations in serum in all meloxicam-treated vultures remained within a 95% range calculated from measurements from these vultures before treatment and control vultures.

Conclusion

Meloxicam is not toxic to *Gyps* vultures at the maximum level of exposure.

Key figure



The effect of adminstration of meloxicam and diclofenac by [oral dose] on uric acid in serum of vultures. Blue symbols show the ratio of the geometric mean (with 95% confidence interval) serum concentration of uric acid for a group of *Gyps* vultures treated with meloxicam to that for a control group sampled at the same time. Each group of blue symbols show the effects of increased doses of meloxicam, from left to right. Red symbols and vertical lines show the equivalent ratio but for vultures given diclofenac. See article for more detail.

7. An experiment finds doses above the MLE of meloxicam to Asian whitebacked vultures *Gyps bengalensis* does not cause kidney failure, death or elevation of blood uric acid levels in *Gyps* vultures and other common scavenging birds native to South Asia

Source

Swarup, D., Patra, R. C., Prakash, V., Cuthbert, R., Das, D., Avarti, P., Pain, D. J., Green, R. E., Sharma, A.K., Saini, M. Das, D. and Taggart M. A. (2007). Safety of meloxicam to critically endangered *Gyps* vultures and other scavenging birds of India. *Animal Conservation* 10: 192-198.

Background

Meloxicam is not toxic to *Gyps* vultures. This experiment was undertaken to further examine the toxicity of meloxicam to *Gyps* vultures and other common scavenging birds of South Asia.

Action

The effect of meloxicam on *Gyps* vultures and other common scavenging birds was examined in a three-phase controlled experiment in India.

One injectable meloxicam product, manufactured and purchased in India, was used. Double the recommended dose for buffalo in India was used to reflect frequent overdosing behaviour in India.

Phases 1: six Asian white-backed vultures *Gyps bengalensis* and four Long-billed vultures *Gyps indicus* were given an oral dose of meloxicam at 0.5 or 2.0 mg/kg body weight (bw); and two Asian white-backed vultures *Gyps bengalensis* and two Long-billed vultures *Gyps indicus* were given sterilised water.

Phases 2: five Egyptian vultures *Neophron percnopterus*, five cattle egrets *Bubulcus ibis*, five crows *Corvus spp.* and five common myna *Acridotheres tristis* were given an oral dose of meloxicam at 2.0 mg/kg bw; and four Egyptian vultures *Neophron percnopterus*, four cattle

egrets *Bubulcus ibis*, five crows *Corvus spp.* and five common myna *Acridotheres tristis* were given sterilised water.

Phase 3: 19 Asian white-backed vultures *Gyps bengalensis* and two long-billed vultures *Gyps indicus* were fed tissue from buffalo dosed with meloxicam; and five Asian white-backed vultures *Gyps bengalensis* were fed tissue from goats not dosed with meloxicam.

Meloxicamcontaminated buffalo tissues were muscle and liver representing doses of 0.3-2.1 mg/kg bw from two buffalo given daily injections of meloxicam at 1 mg/kg bw for five days and slaughtered 8 h after the final dose.

Blood samples were taken before dosing and 48h after dosing from all birds in Phases 1 and 2 (except common myna *Acridotheres tristis*); and only 48h after dosing from birds in Phase 3. Serum was analysed for uric acid and other biochemical components. All birds were observed for seven days for symptoms of toxicity.

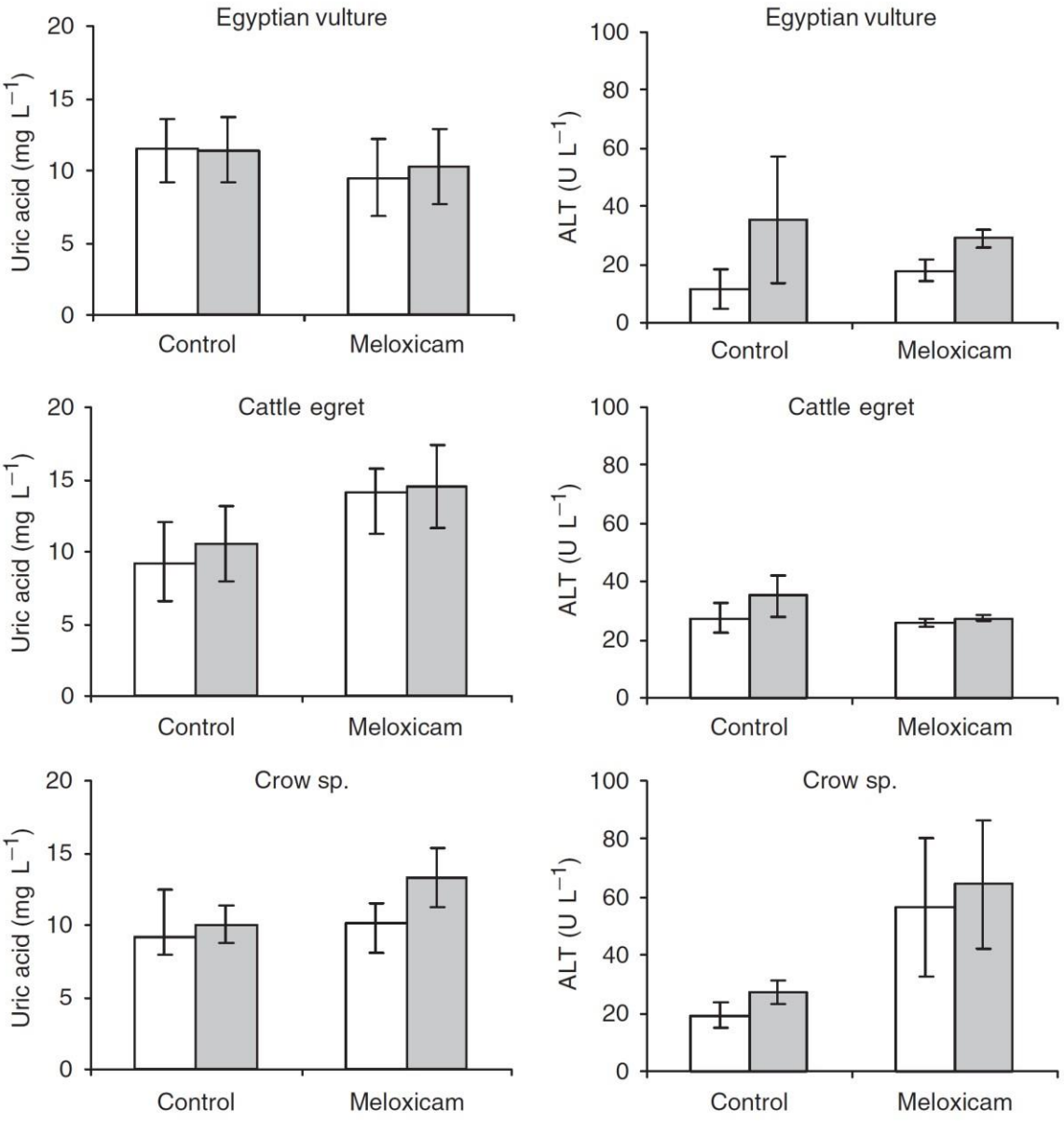
Findings

In all Phases, all meloxicam-treated birds survived with no ill effects. All control birds survived. Uric acid and ALT concentrations in serum in all meloxicam-treated birds was significantly different to that of control birds.

Conclusion

Meloxicam is not toxic to *Gyps* vultures and other common scavenging birds at the maximum level of exposure.

Key figure



Concentration of uric acid and ALT in serum of Egyptian vultures *Neophron percnopterus*, cattle egrets *Bubulcus ibis* and crows *Corvus spp.* given an oral dose of meloxicam at 2.0 mg/kg bw (filled columns) or sterilised water (open columns).

8. A review of the literature finds that meloxicam is a safe and effective treatment for domesticated animals

Source

Galligan, T. H., et al. Vulture-safe meloxicam is a safe and effective treatment for a variety of ailments in domesticated animals (unpublished)

Background

Meloxicam is not toxic to vultures. Therefore, meloxicam is a vulture-safe alternative to aceclofenac. This review of literature was undertaken to assess the safety and effectiveness of meloxicam as an alternative to aceclofenac for the treatment of domesticated animals.

Action

The PubMed literature database (online and open access) was surveyed for *in vivo* experimental and clinical studies reporting the effect of aceclofenac and meloxicam in cattle, buffalo, camel, horse, goat, sheep and dogs.

Findings

Among 118 studies: 1 reported an effect of aceclofenac treatment; and 117 reported an effect of meloxicam treatment. No study reported an effect of both aceclofenac and meloxicam treatment.

All studies were grouped into broad veterinary and husbandry situations for treatment (e.g. infection, surgery, reproducing); broad objectives of treatment (e.g. decrease pain, increase reproductive rate, no complications); and categorised as having a: positive effect; negative effect; mixed effect (partially positive and negative); no effect; or no conclusion.

A single study examining aceclofenac treatment in dogs reported a positive effect (decreased pain and inflammation).

A positive effect was reported in 79% of studies examining meloxicam treatment. The proportion of studies examining meloxicam treatment that reported a mixed effect, negative effect, no effect and no conclusion were 9, 3, 7 and 2%, respectively.

Meloxicam showed positive effects: in cattle, buffalo, horse, goat, sheep and dog; and during castrating, dehorning, general care, handling, infections, limb injuries, reproducing and surgery. In these situations, meloxicam decreased pain, inflammation, stress and dysfunction; increased recovery rate, immune response, pregnancy rate and productivity; and generally resulted in no complications.

Overall, three studies reported negative effects of meloxicam treatment resulting in pregnancy complications (one each in cattle, horse and sheep). However, eleven studies reported positive effects of meloxicam treatment resulting in no pregnancy complications (nine in cattle and two in sheep). Further, no study examined the effect of aceclofenac on pregnancy.

Conclusion

Meloxicam is as safe as and probably more effective than aceclofenac in a variety of veterinary and husbandry situations in a variety of animals.

Key figure

One-hundred and eighteen studies reported an effect of either meloxicam, ketoprofen or meloxicam and ketoprofen treatment in domesticated animals. Data provided are: NSAID(s) examined; reference for the study; study animal; veterinary or husbandry situation; objective of treatment; total sample size (n); and effect reported.

NSAID	Study	Animal	Situation	Objective	Method	n	Effect
Aceclofenac	Grau et al. 1991	Dog	Limb injury	Decrease pain and inflammation	controlled	8	Positive
Meloxicam	Roberts et al. 2016	Cattle	Castration	Decrease pain	controlled	83	Mixed
Meloxicam	Roberts et al. 2015	Cattle	Castration	Decrease pain	controlled	30	Positive
Meloxicam	Olson et al. 2016	Cattle	Castration	Decrease pain	controlled	60	Positive
Meloxicam	Coetzee et al. 2012a	Cattle	Castration	Decrease pain	controlled	258	Positive
Meloxicam	Brown et al. 2015	Cattle	Castration	Decrease pain and increase calf productivity	controlled	62	Positive
Meloxicam	Repenning et al. 2013	Cattle	Castration	Decrease pain and stress	controlled	157	None
Meloxicam	Allen et al. 2013	Cattle	Dehorning	Decrease pain	controlled	30	Positive
Meloxicam	Stewart et al. 2009	Cattle	Dehorning	Decrease pain	controlled	46	Positive
Meloxicam	Clapp et al. 2015	Cattle	Dehorning	Decrease pain	not controlled	25	Mixed
Meloxicam	Theurer et al. 2012	Cattle	Dehorning	Decrease pain	not controlled	12	Positive
Meloxicam	Coetzee et al. 2012b	Cattle	Dehorning	Decrease pain and increase calf productivity	controlled	12	Positive
Meloxicam	Heinrich et al. 2010	Cattle	Dehorning	Decrease pain and stress	controlled	60	Positive
Meloxicam	Heinrich et al. 2009	Cattle	Dehorning	Decrease stress	not controlled	60	Positive
Meloxicam	Bates et al. 2015	Cattle	Dehorning	Increase calf productivity	controlled	202	Positive
Meloxicam	Roy et al. 2015	Cattle	General	Decrease inflammation	controlled	12	Positive

Meloxicam	Carpenter et al. 2016	Cattle	General	Increase cow productivity and longevity	controlled	.	Positive
Meloxicam	Amiridis et al. 2009	Cattle	General	Increase pregnancy rate	controlled	402	Positive
Meloxicam	Cooke et al. 2016	Cattle	Handling	Decrease stress	controlled	943	None
Meloxicam	Guarnieri et al. 2014	Cattle	Handling	Decrease stress	controlled	84	Positive
Meloxicam	Van Engen et al. 2016	Cattle	Handling	Decrease stress	controlled	87	Positive
Meloxicam	Van Engen et al. 2014	Cattle	Handling	Decrease stress	not controlled	97	Positive
Meloxicam	Maslanka et al. 2011	Cattle	Immunisation	Avoid dysfunction	controlled	10	None
Meloxicam	Rodrigues et al. 2015	Cattle	Immunisation	Avoid dysfunction	controlled	21	None
Meloxicam	Bednarek et al. 2002	Cattle	Infection	Decrease inflammation	not controlled	.	Positive
Meloxicam	Konigsson et al. 2002	Cattle	Infection	Decrease inflammation	not controlled	4	Positive
Meloxicam	Bednarek et al. 2003a	Cattle	Infection	Improve recovery	not controlled	30	Positive
Meloxicam	Todd et al. 2010	Cattle	Infection	Increase calf productivity	controlled	62	Positive
Meloxicam	Bednarek et al. 2003b	Cattle	Infection	Increase immune response	controlled	18	Positive
Meloxicam	Bednarek et al. 2005	Cattle	Infection	Increase immune response	not controlled	30	Mixed
Meloxicam	Coetzee et al. 2014	Cattle	Limb injury	Decrease pain	controlled	18	Positive
Meloxicam	Offinger et al. 2013	Cattle	Limb injury	Decrease pain	controlled	19	Positive
Meloxicam	Barrier et al. 2014	Cattle	Reproduction	Decrease pain	controlled	110	Positive
Meloxicam	Fitzpatrick et al. 2013	Cattle	Reproduction	Decrease pain	controlled	24	Positive
Meloxicam	Newby et al. 2013b	Cattle	Reproduction	Decrease pain and stress	controlled	103	Positive
Meloxicam	Mainau et al. 2015	Cattle	Reproduction	Decrease pain and stress	not controlled	60	Mixed
Meloxicam	McDougall et al. 2009	Cattle	Reproduction	Improve recovery	not controlled	727	Positive
Meloxicam	Lopes et al. 2015	Cattle	Reproduction	Increase pregnancy rate	controlled	85	Positive

Meloxicam	Aguiar et al. 2013	Cattle	Reproduction	Increase pregnancy rate	controlled	207	Positive
Meloxicam	Rajkumar et al. 2010	Cattle	Reproduction	Increase pregnancy rate	not controlled	.	Positive
Meloxicam	Erdem et al. 2010	Cattle	Reproduction	No complications	controlled	87	Negative
Meloxicam	Hirsch et al. 2009	Cattle	Reproduction	No complications	controlled	41	Positive
Meloxicam	Newby et al. 2014	Cattle	Reproduction	No complications	controlled	.	Positive
Meloxicam	Beausoleil et al. 2012	Cattle	Surgery	Decrease pain	controlled	18	None
Meloxicam	Blois et al. 2010	Dog	General	Avoid dysfunction	controlled	10	Positive
Meloxicam	Wooten et al. 2009	Dog	General	Avoid dysfunction	not controlled	8	Positive
Meloxicam	Punke et al. 2008	Dog	General	Avoid dysfunction	not controlled	8	Positive
Meloxicam	Gilmour et al. 2012	Dog	General	Decrease inflammation	controlled	15	None
Meloxicam	Gilmour et al. 2009	Dog	General	Decrease inflammation	not controlled	38	Positive
Meloxicam	Hare et al. 2013	Dog	General	No complications	controlled	40	Mixed
Meloxicam	Bostrom et al. 2006	Dog	General	No complications	controlled	8	Positive
Meloxicam	Boston et al. 2003	Dog	General	No complications	controlled	20	Positive
Meloxicam	Narita et al. 2006	Dog	General	No complications	not controlled	15	Positive
Meloxicam	Fusellier et al. 2008	Dog	General	No complications	not controlled	10	Positive
Meloxicam	Borges et al. 2013	Dog	General	No complications	not controlled	24	Positive
Meloxicam	Luna et al. 2007	Dog	General	No complications	not controlled	36	Positive
Meloxicam	Viking et al. 2002	Dog	Infection	Decrease pain	controlled	12	Positive
Meloxicam	Brainard et al. 2007	Dog	Limb injury	Avoid dysfunction	not controlled	8	Positive
Meloxicam	Cross et al. 1997	Dog	Limb injury	Decrease inflammation	not controlled	12	Positive

Meloxicam	Van Bree et al. 1994	Dog	Limb injury	Decrease inflammation and no complications	controlled	4	Positive
Meloxicam	Jones et al. 2002	Dog	Limb injury	Decrease pain	controlled	12	Positive
Meloxicam	Moreau et al. 2003	Dog	Limb injury	Decrease pain	controlled	71	Positive
Meloxicam	Matyas et al. 2013	Dog	Limb injury	Decrease pain	controlled	11	Positive
Meloxicam	Borer et al. 2003	Dog	Limb injury	Decrease pain	controlled	60	Positive
Meloxicam	Cozzi and Spensley	Dog	Limb injury	Decrease pain and inflammation	controlled	280	Positive
Meloxicam	Schmid et al. 2010	Dog	Limb injury	Decrease pain and inflammation	controlled	.	Positive
Meloxicam	Walton et al. 2014	Dog	Limb injury	Decrease pain and inflammation	controlled	111	Positive
Meloxicam	Peterson and Keefe 2004	Dog	Limb injury	Decrease pain and no complications	controlled	217	Positive
Meloxicam	Nell et al. 2002	Dog	Limb injury	Decrease pain and no complications	controlled	214	Positive
Meloxicam	Doig et al. 2000	Dog	Limb injury	Decrease pain and no complications	controlled	40	Positive
Meloxicam	Sauve et al. 2003	Dog	Limb injury	No complications	controlled	46	Positive
Meloxicam	Mullins et al. 2012	Dog	Surgery	Avoid dysfunction	not controlled	.	Mixed
Meloxicam	Budsberg et al. 2002	Dog	Surgery	Decrease pain	controlled	40	Positive
Meloxicam	Zanuzzo et al. 2015	Dog	Surgery	Decrease pain	controlled	40	Positive
Meloxicam	Teixeira et al. 2013	Dog	Surgery	Decrease pain	controlled	27	Positive
Meloxicam	Tsai et al. 2013	Dog	Surgery	Decrease pain	controlled	27	Positive
Meloxicam	Teixeira et al. 2013	Dog	Surgery	Decrease pain	controlled	24	Positive

Meloxicam	Leece et al. 2005	Dog	Surgery	Decrease pain	controlled	43	Positive
Meloxicam	Fowler et al. 2003	Dog	Surgery	Decrease pain	controlled	20	Positive
Meloxicam	Caulkett et al. 2003	Dog	Surgery	Decrease pain	controlled	15	Positive
Meloxicam	Moak et al. 2011	Dog	Surgery	Decrease pain	not controlled	25	None
Meloxicam	Gruet et al. 2011	Dog	Surgery	Decrease pain and inflammation	controlled	146	Positive
Meloxicam	Lafuente at al. 2005	Dog	Surgery	Decrease pain and inflammation	controlled	16	Positive
Meloxicam	Yilmaz et al. 2014	Dog	Surgery	Decrease stress	not controlled	24	Positive
Meloxicam	Nakagawa et al. 2007	Dog	Surgery	Increase recovery and no complications	controlled	5	Positive
Meloxicam	Kum et al. 2013	Dog	Surgery	No complications	controlled	18	Positive
Meloxicam	Kazakos et al. 2005	Dog	Surgery	No complications	controlled	38	Positive
Meloxicam	Fresno et al. 2005	Dog	Surgery	No complications	not controlled	20	Positive
Meloxicam	Laredo et al. 2004	Dog	Surgery	No complications	not controlled	32	Positive
Meloxicam	Mathews et al. 2001	Dog	Surgery	Decrease pain and no complications	controlled	36	Positive
Meloxicam	Gruet et al. 2013	Dog	Surgery	Decrease pain and inflammation	not controlled	174	Positive
Meloxicam	Khan and McLean 2012	Dog, cat	General	No complications	controlled	22206	Mixed
Meloxicam	Regan et al. 2016	Donkey	Limb injury	Decrease pain and inflammation	controlled	40	Mixed
Meloxicam	Ingvast-Larsson et al. 2011	Goat	Dehorning	Decrease pain	not controlled	11	None
Meloxicam	van der Heide et al. 2008	Goat	Limb injury	No complications	controlled	18	Positive

Meloxicam	Little et al. 2007	Horse	General	Avoid dysfunction	controlled	18	Positive
Meloxicam	Beretta et al. 2005	Horse	General	Decrease pain and inflammation	controlled	.	Positive
Meloxicam	Raidal et al. 2014	Horse	General	No complications	controlled	9	Mixed
Meloxicam	Nobel et al. 2012	Horse	General	No complications	controlled	33	Positive
Meloxicam	D'Arcy-Moskwa et al. 2012	Horse	General	No complications	controlled	25	Positive
Meloxicam	Raidal et al. 2013	Horse	General	No complications	controlled	10	Positive
Meloxicam	Vander Werf et al. 2012	Horse	General	No complications	not controlled	7	Positive
Meloxicam	Brideau et al. 2001	Horse, Dog	General	Decrease inflammation	controlled	30, 48	Positive
Meloxicam	de Walter et al. 2016	Horse	Limb injury	Decrease inflammation	controlled	24	No conclusion
Meloxicam	de Grauw et al. 2009	Horse	Limb injury	Decrease pain and inflammation	controlled	6	Positive
Meloxicam	Lima et al. 2015	Horse	Reproduction	Increase pregnancy rate	controlled	11	Negative
Meloxicam	Naylor et al. 2014	Horse	Surgery	Decrease pain	controlled	60	Mixed
Meloxicam	Walliser et al. 2015	Horse	Surgery	Decrease pain and inflammation	controlled	66	Positive
Meloxicam	Paull et al. 2012	Sheep	Castration	Decrease pain	controlled	48	Positive
Meloxicam	Small et al. 2014	Sheep	Castration	Decrease pain	controlled	60	Positive
Meloxicam	Colditz et al. 2011	Sheep	Limb injury	Decrease pain and inflammation	controlled	.	Positive
Meloxicam	McKeown et al. 2000	Sheep	Reproduction	Increase pregnancy rate	controlled	.	Positive
Meloxicam	Dorniak et al. 2011	Sheep	Reproduction	No complications	controlled	.	Negative
Meloxicam	Rac et al. 2007	Sheep	Reproduction	No complications	controlled	.	Positive

Meloxicam	Barrett et al. 2016	Cattle	Surgery	Decrease pain	controlled	24	No conclusion
Meloxicam	Newby et al. 2013	Cattle	Surgery	Decrease pain	not controlled	10	Positive
Meloxicam	Deneuche et al. 2004	Dog	General	Decrease pain and no complications	controlled	60	Positive
Meloxicam	Forsyth et al. 1998	Dog	General	No complications	not controlled	24	Positive