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

S. CHANDRAMOHAN[a*], KARIKALAN MATHESH[a*], JOHN W. MALLORD[b1], VINNY NAIDOO[c], K. MAHENDRAN[a], MANICKAM KESAVAN[a], GYANENDRA K. GAUR[a], ABHIJIT M. PAWDE[a], NIKITA PRAKASH[d], SACHIN RANADE[d], DEBASISH SAIKIA[d], A. K. SHARMA[a], ROHAN SHRINGARPURE[d], RHYS E. GREEN[e] & VIBHU M. PRAKASH[d].

[a] Centre for Wildlife, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh 243122, India

[b] RSPB Centre for Conservation Science, Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire, SG19 2DL, UK

[c] Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Gauteng 0110, South Africa

[d] Bombay Natural History Society, Hornbill House, Opp Lion Gate, Shaheed Bhagat Singh Road, Fort, Mumbai 400 001, Maharashtra, India

[e] Conservation Science Group, Department of Zoology, University of Cambridge, David Attenborough Building, Cambridge, CB2 3QZ, UK

* Equal contributions by first two authors

[1] Corresponding author: john.mallord@rspb.org.uk

Keywords: pharmacokinetics, liquid chromatography with mass spectrometry, ecotoxicology, non-steroidal anti-inflammatory drugs, NSAID, vulture declines

Abstract

Vulture declines in South Asia were caused by accidental poisoning by the veterinary non-steroidal anti-inflammatory drug (NSAID) diclofenac. Although veterinary use of diclofenac has been banned, other vulture-toxic NSAIDs are legally available, including aceclofenac, which has been shown to metabolise into diclofenac in domestic cattle. We gave nine domestic water buffalo the recommended dose of aceclofenac (2 mg kg^{-1} body weight), collected blood at intervals up to 48 hours, and carried out a pharmacokinetic analysis of aceclofenac and its metabolite diclofenac in plasma. Aceclofenac was rapidly converted to diclofenac, and was barely detectable in plasma at any sampling time. Diclofenac was present within 20 minutes, and peaked 4-8 hours after dosing. Aceclofenac is a prodrug of diclofenac, and behaves similarly in domestic water buffalo as it did in domestic cattle, posing the same risk to vultures. We recommend an immediate ban on the veterinary use of aceclofenac across vulture-range countries.

1. Introduction

The catastrophic population declines of three species of *Gyps* vultures – White-rumped *G. bengalensis*, Indian *G. indicus* and Slender-billed *G. tenuirostris* Vultures – in South Asia from the

mid-1990s onwards, was caused by accidental poisoning by the non-steroidal anti-inflammatory drug (NSAID) diclofenac (Oaks *et al*. 2004, Green *et al*. 2004, 2007). Diclofenac was commonly used to treat injured and dying cattle (domestic cows *Bos taurus* and *B. indicus*) and domestic water buffaloes *Bubalus bubalis* across the Indian subcontinent and remains in illegal use in some areas. Ingestion of the drug by vultures when they fed on the carcasses of animals that had been treated with diclofenac prior to death resulted in kidney failure, visceral gout and death (Oaks *et al*. 2004). Subsequently, the veterinary use of diclofenac was banned by the governments of India, Pakistan, Nepal and Bangladesh (Prakash *et al*. 2012, Sarowar *et al*. 2016). Compliance with the ban was aided by the identification and promotion of meloxicam, an NSAID that is not toxic to vultures (Swan *et al*. 2006, Swarup *et al*. 2006). Tolfenamic acid has recently been identified as another drug that is safe to vultures at doses they are likely to be exposed to in the wild (Chandramohan *et al*. 2022).

There are several other NSAIDs which are freely available and legally approved for veterinary use across vulture range states in South Asia (Galligan *et al*. 2020). These include ketoprofen and nimesulide which have both been shown experimentally to be toxic to captive vultures (Naidoo *et al*. 2010, Galligan *et al*. 2022). There is also evidence that carprofen and flunixin are toxic to Gyps vultures (Cuthbert *et al*. 2007, Zorilla *et al*. 2014). The toxicity of several other NSAIDs approved for veterinary use in vulture range states is unknown. As a result of the threat these drugs pose to *Gyps* vultures in South Asia, which are still regarded as Critically Endangered and at risk of global extinction (Birdlife International 2022), conservationists have been recommending that governments should ban veterinary NSAIDs known to be toxic to vultures and to modify procedures for ahead of their approval for veterinary use, to deal with drugs of unknown toxicity (SAVE 2021a). This pressure has only so far been successful for one drug (apart from diclofenac) in Bangladesh, where the government recently banned veterinary use of ketoprofen (SAVE 2021b).

A further NSAID, aceclofenac, has previously been shown to metabolise rapidly into diclofenac when administered to domestic cattle, and thus poses the same threat to vultures that consume contaminated carcasses of domestic cows (Galligan *et al*. 2016). Although the results of this study were conclusive, it was carried out in South Africa on Friesian *Bos taurus*. There was a perceived need to confirm that aceclofenac behaves in the same way when given to an Indian species and under Indian conditions, and would therefore be a threat to vultures in India. Therefore, in this study, we have repeated Galligan *et al*.'s (2016) South African study by giving aceclofenac to nine domestic water buffalo and examining the plasma pharmacokinetics of this drug and its potential metabolites.

2. Material and methods

Approval to carry out experiments on domestic water buffaloes was obtained from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, licence no: F.26-1/2015-16/JD(R), dated 01/03/2017), India. Procedures followed the ARRIVE guidelines (Percie du Sert *et al*. 2020). In India, aceclofenac is mainly available as a bolus formulation, which we purchased locally (Bareilly, Uttar Pradesh). Each bolus contained 300 mg aceclofenac and 1500 mg paracetamol, and was manufactured by Hanuchem Laboratories, Solan, Himachal Pradesh, India (Batch No. T-1752705, Manufacturing date October 2017, Expiry date September 2019).

2.1. Dosing and collection of samples from water buffaloes

Three single dose pharmacokinetic studies were each conducted on three healthy male buffaloes (i.e., total of nine animals). The animals were all older than 12 months of age, and weighed between 210

to 310 kg (mean 255 kg ± 40.0, n = 9). The buffaloes were sourced from the cattle farm at the Division of Livestock Production and Management, Indian Veterinary Research Institute (IVRI), Bareilly, Uttar Pradesh, India. The animals were confirmed to have not been treated with any NSAID in the weeks leading up to the experiment. Two weeks prior to the experiment the buffaloes were housed separately and provided food and water *ad libitum*. During this period the animals received regular veterinary examinations and were declared fit for the study. The experiments were conducted on three dates between 2017 and 2019.

Sampling before dosing with aceclofenac was designated as occurring on day 0. A 10 ml pre-treatment blood sample was drawn into a heparinized tube. The aceclofenac bolus was then administered, using the recommended dose of 2 mg kg^{-1} body weight (b.w.). In the first two experiments, blood samples were drawn 2-, 4-, 8-, 12-, 24- and 48-hours after the bolus was administered. During the third experiment, blood samples were drawn after 20 minutes, 40 minutes, 1-, 2-, 4-, 8-, 12-, 24- and 48-hours. The blood samples were centrifuged at 3000 rpm for 10 minutes. The plasma was transferred to fresh screw cap vials and stored at -80°C.

2.2. Extraction and quantification of aceclofenac and diclofenac

Both aceclofenac and diclofenac were extracted from buffalo plasma samples using a protein precipitation method, and were quantified using liquid chromatography tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI) and multiple reaction monitoring (MRM) in negative ionization mode. The level of detection and quantification was 0.77 ng mL^{-1} for aceclofenac and 5.6 ng mL^{-1} for diclofenac. Methodology was the same for both compounds; in the following description, quantities for measuring concentrations of diclofenac are shown in parentheses.

To prepare stock standards for LC-MS/MS calibration, dimethylsulfoxide (DMSO, 1.0 ml) was added to 1.0 mg of aceclofenac or diclofenac in a 1.5 ml micro centrifuge tube, mixed well and sonicated. Internal standards (IS) were also prepared using deuterated versions of these compounds (aceclofenac D-2 and diclofenac D-4). These IS were used to facilitate correction of data for any variations in instrument sensitivity/response as well as for deviations in expected compound recovery that occurred during the solvent extraction process. For the IS compounds, 1.0 ml of DMSO was added to 5 mg of aceclofenac D-2 (or diclofenac D-4) to create IS stock solutions. Analyte working calibration standards (15.3 to 2,000 ng mL^{-1} for aceclofenac and 112 to 108,000 ng mL^{-1} for diclofenac) and quality control (QC) samples of aceclofenac/diclofenac were also prepared in DMSO. An IS working standard solution (containing both deuterated compounds) was prepared by diluting 0.5 ml of each IS stock solution into 50 ml of acetonitrile (to provide a concentration of 10 µg ml^{-1} for each compound). This solution was mixed well and stored at 2 to 8 °C until used. Calibration standards and QC samples were prepared by spiking 95 µl of blank buffalo plasma with 5 µl of analyte working solution. 100 µL of calibration/QC/study sample was aliquoted into an Eppendorf tube and 10 µL of IS working standard added. All samples were then quenched with 300 µL of acetonitrile, vortexed and centrifuged at 14,000 rpm for 5 min at 4 °C. 200 µL of supernatant was transferred to 1 mL vials for analysis by LC-MS/MS (quantifying aceclofenac and diclofenac, alongside their deuterated equivalents).

Further details of the methods for the extraction and measurement of aceclofenac and diclofenac, along with measured concentrations, can be found in Appendix 1 of the Supplementary Information.

2.3. Pharmacokinetics of aceclofenac and diclofenac in water buffaloes

The relevant pharmacokinetic parameters were calculated for aceclofenac and its metabolite diclofenac by noncompartmental modelling using the package Kinetica 5.1 (Thermo Fisher Scientific, Waltham, MA USA). In addition, diclofenac pharmacokinetics were assessed using a one-compartment model. C_{max} (maximum plasma concentration) taken to be the first peak concentration on the plasma concentration versus time profile, with the corresponding time being the t_{max} (time to maximum concentration). The elimination constant (λ) was determined by least-squares linear regression of the natural logarithm of concentration on time, using results from the last three blood samples. The area under the plasma concentration versus time curve up to the last quantifiable concentration (C_{last}) is referred to as AUC_{last} and was determined using the linear trapezoidal method (AUC_{last} = sum of the area of various trapezoids making up the curve = $\sum 0.5 \times (t_2 - t_1)(C_2 + C_1)$. The area under the moment curve ($AUMC_{last}$) was determined using the same method as for AUC_{last}, except that the concentration portion of the curve was determined as ($t_1 * C_1 + t_2 * C_2$); the mean residence time (MRT)was calculated as $AUCM_{last}$ / AUC_{last}; the area under the curve extrapolated to infinity ($AUC_{inf)}$ was calculated as AUC_{last} + C_{last}/λ) while the terminal half-life ($t_{1/2}$) was calculated as ln(2)/λ. For the one compartment modelling, the elimination constant Beta was determined as the slope of the terminal portion of the plasma concentration versus time profile (following natural logarithmic transformation of the concentration values) using ordinary least-squares linear regression, with the y-intercept being the parameter *B* with the best fit equation representing the extrapolated intravenous curve. To ascertain the rate of formation of diclofenac, the plasma concentration versus time data for diclofenac was fitted to a biexponential equation: Ct = Be^(-β)t –Be^(-αt), where B is the common intercept, Ct the plasma concentration at time t, α the estimated rate constant for absorption and β the rate constant for elimination (Alvinerie *et al*. 1999).

3. Results

Aceclofenac was barely detectable at any sampling time (Fig. 1a), with peak concentrations ranging from just 0.002 – 0.052 μg ml^{-1}. In one buffalo, aceclofenac was not detected at any sampling time. In contrast, diclofenac was detected at the first sampling time (20 minutes) and increased thereafter and peaked at 4-8 hours (mean, 6.22 ± 2.11 h; Table 1). Peak concentrations ranged from 2.22 – 4.94 μg ml^{-1} (3.31 ± 0.93 μg ml^{-1}). Diclofenac was still detectable in all nine animals, albeit at very low levels, after 48 hours (Fig. 1b, Supplementary Information Appendix 2).

The total exposure of diclofenac (AUC_{inf}) was 62.07 μg.h ml^{-1}, the half-life of elimination ($t_{1/2}$) was 6.59 h (± 0.85 h) and the mean residence time (MRT) was 12.4 h (± 1.49 h). Other pharmacokinetic parameters are shown in Table 1.

4. Discussion

Our results confirm that treating domestic water buffalo in India with the recommended dose of aceclofenac in bolus form results in its rapid conversion to diclofenac, to a similar extent to that shown previously for Friesian domestic cattle in South Africa (Galligan *et al*. 2016). This finding is relevant to vulture conservation because diclofenac prevalence has been found to be high in liver samples taken from carcasses of both domestic cattle and domestic water buffaloes in India (Taggart *et al.* 2007). Diclofenac was detectable within 20 minutes after dosing of buffalo with aceclofenac, with concentrations peaking after around 6 hours. It was still detectable after 48 hours in all birds. In

contrast, aceclofenac was only detected at very low concentrations in all animals throughout. In fact, in one animal, concentrations of aceclofenac were below the level of quantification/detection at all sampling times. The rapid conversion of aceclofenac to diclofenac is consistent with the former being a prodrug of the latter, and confirms that the treatment of livestock with aceclofenac will have the same catastrophic effect on *Gyps* vultures feeding on contaminated carcasses as would the use of diclofenac.

The barely detectable aceclofenac in the plasma of buffalo was in contrast to aceclofenac administered to domestic cattle at the same dose and route (Galligan *et al*. 2016). In cattle, aceclofenac was detectable for a considerable time after dosing. In rats, oral bioavailability of aceclofenac of 15% was described (Noh *et al*. 2015). In humans (Hinz *et al*. 2003) and dogs (Liu *et al*. 1997) aceclofenac was detectable in plasma at high concentrations. This would indicate that the aceclofenac had an almost complete presystemic elimination in buffalo. While this is the first instance of a near complete presystemic elimination being recorded for aceclofenac, this is not a completely unexpected phenomenon and has been described for other orally administered medication such as lidocaine (Boyes *et al*. 1971) and morphine (Brunks & Delle 1974, Bock *et al*. 1978, Säwe *et al*. 1981) in humans and rats (Iwamoto & Klaassen 1977). At this point, while the actual metabolic pathways for aceclofenac metabolism is not elucidated in ruminants, we can speculate on the reason for the rapid conversion to diclofenac. In humans, both diclofenac and aceclofenac are metabolised by the hepatic CYP2C9 enzyme (Hinz *et al*. 2003). Considering that the half-life of diclofenac administered orally in our study is similar to that of intravenously-administered diclofenac in cattle (EMA 2003), it is unlikely that hepatic metabolism would explain the rapid conversion of aceclofenac to diclofenac. This suggests that a degree of extra-hepatic metabolism is occurring, which seems likely to occur in the rumen because aceclofenac is known to undergo rapid ester cleavage by anaerobic bacteria in wastewater, (Pérez & Barceló 2008).

While very little aceclofenac was detected, complete profiles for diclofenac were obtained. The poor bioavailability of aceclofenac and the presence of diclofenac as early as the first time point, indicates that aceclofenac is rapidly and efficiently metabolised to diclofenac in buffalo, possibly while it is in the rumen of the alimentary canal. When the rate of formation was determined, the half-life of formation was rapid at less than 2 hours (Alpha-HL, Table 1). The half-life of elimination ($t_{1/2}$) was 6.59 hours which was similar to domestic cattle (Galligan *et al*. 2016). However, the extent of absorption (i.e., total exposure of animals to the drug, AUC_{inf}) was 50% higher in buffalo (62.07 ug ml^{-1}*h), in comparison to that previously determined for cattle (48.34 ug ml^{-1}*h) (Galligan *et al*. 2016). To further understand the risks posed to vultures, the extent of absorption obtained in this study was compared to the pharmacokinetics of diclofenac following intravenous administration in buffalo at the concentration of 1 mg kg^{-1} (Kumar *et al*. 2003). In that study, an area under the curve of 11 ug ml^{-1}*h was calculated, which was six-fold lower than in this study. The significance of this finding needs to be considered in relationship to the initial toxicity studies undertaken by Oaks *et al*. (2004), where a dose of 2.5 mg kg^{-1} administered to domestic water buffalo was sufficient to cause the death of vultures fed on its tissues. While Oaks *et al*. did not determine the pharmacokinetics of diclofenac in vultures, a 2.5-fold increase in AUC based on an assumption of linear pharmacokinetics, will still be substantially lower than the AUC obtained in this study; therefore, the amount of diclofenac detectable in this study is higher than that previously reported to be toxic to vultures in food. It should also be noted that all the animals showed linear depletion of the diclofenac metabolite, which would further support this extrapolation as being valid i.e., there were no metabolic constraints present in buffalo.

The ban on the manufacture and use of diclofenac for veterinary purposes has had a positive impact on vulture populations in South Asia (Chaudhry *et al*. 2012, Prakash *et al*. 2017, Galligan *et al*. 2019). Despite this, the illegal use of human formulations of the drug remain stubbornly high in India (Galligan *et al*. 2020), and still poses a significant threat to vulture populations. However, sales of aceclofenac to treat injured cattle are increasing in some areas of India (BNHS, unpublished data), so given the results of this and other studies, there is an increasing risk of vultures being poisoned by diclofenac derived from aceclofenac. There is now sufficient evidence for governments in vulture-range states in South Asia to act, as they did for diclofenac, and immediately ban the manufacture, distribution, sale and use of bolus and injectable formulations of aceclofenac in doses suitable for large animals (i.e., vials > 3 ml) (Galligan *et al*. 2016). Failure to act will threaten the progress that has been made supporting the partial recovery of vulture populations across South Asia.

Acknowledgements

We are grateful to BNHS and the Director, ICAR-IVRI for their general support. We are also grateful to the Haryana Forest Department for allowing us to carry out work at the Vulture Conservation Breeding Centre at Pinjore. Extraction and quantification of aceclofenac and diclofenac in plasma samples was carried out by Eurofins Advinus Ltd., Bengalaru, India. Funding was provided by the Haryana Forest Development Corporation. The funders played no role in the design of the study, collection, analysis and interpretation of data, writing of the manuscript nor in the decision to submit the article for publication.

References

Alvinerie, M., Sutra, J.F., Galtier, P. and Mage, C. 1999. Pharmacokinetics of eprinomectin in plasma and milk following topical administration to lactating dairy cattle. *Research in Veterinary Science*, **67**, 229-232.

BirdLife International, 2022. *IUCN Red List for birds*. Downloaded from http://www.birdlife. org on 09/03/2022.

Bock, K. W., Brunner, G., Hoensch, H., Huber, E. & Joshing, D. 1978. Determination of microsomal UDP-glucuronyltransferase in needle-biopsy specimens of human liver. *Eur. J. clin. Pharmac*., **14,** 367-373.

Boyes, R.N., Scott, D.B., Jebson, P,J., Godman, M.J. & Julian, D.G.. 1971. Pharmacokinetics of lidocaine in man. *Clin. Pharmacol. Ther*. **12,** 105-116.

Brunk, S. F. & Delle, M. 1974. Morphine metabolism in man. *Clin. Pharmac. Ther*. **16,** 51-57.

Cuthbert, R., Parry-Jones, J., Green, R.E. & Pain, D.J. 2007. NSAIDs and scavenging birds: potential impacts beyond Asia's critically endangered vultures. *Biol. Lett*. **3,** 90-93

Chandramohan, S., Mallord, J.W., Mathesh, K., Sharma, A.K., Mahendran, K., Kesavan, M., Gupta, R., Chutia, K., Pawde, A., Prakash, N.V., Ravichandran, P., Saikia, D., Shringarpure, R., Timung, A., Galligan, T.H., Green, R.E. & Prakash, V.M. 2022. Experimental safety testing shows that the NSAID tolfenamic acid is not toxic to *Gyps* vultures in India at concentrations likely to be encountered in cattle carcases. *Science of the Total Environment*, **809,** 152088

Galligan, T.H., Green, R.E., Wolter, K., Taggart, M.A., Duncan, N., Mallord, J.W., Alderson, D, Li, L. & Naidoo, V. 2022. The non-steroidal anti-inflammatory drug nimesulide kills *Gyps* vultures at concentrations found in the muscle of treated cattle. *Science of the Total Environment*, **807,** 150788

Galligan, T.H., Mallord, J.W., Prakash, V.M., Bhusal, K.P., Alam, A.B.M.S., Anthony, F.M., Dave, R., Dube, A., Shastri, K., Kumar, Y., Prakash, N., Ranade, S., Shringarpure, R., Chapagain, D., Chaudhary, I.P., Joshi, A.K., Paudel, K., Kabir, T., Ahmed, S., Azmiri, K.Z., Cuthbert, R.J., Bowden, C.G.R., Green, R.E., 2020. Trends in the availability of the vulture-toxic drug, diclofenac, and other NSAIDs in South Asia, as revealed by covert pharmacy surveys. *Bird Conserv. Int*. https://doi.org/10.1017/S0959270920000477

Galligan, T.H., Taggart, M.A., Cuthbert, R.J., Svobodova, D., Chipangura, J., Alderson, D., Prakash, V.M., Naidoo, V., 2016. Metabolism of aceclofenac in cattle to vulture-killing diclofenac. *Conserv. Biol*. **30,** 1122–1127.

Green, R.E., Newton, I., Shultz, S., Cunningham, A.A., Gilbert, M., Pain, D.J. & Prakash, V.M. 2004. Diclofenac poisoning as a cause of vulture population declines across the Indian subcontinent. *J. App. Ecol*. **41,** 793–800.

Green, R.E., Taggart, M.A., Senacha, K.R., Raghavan, B., Pain, D.J., Jhala, Y., Cuthbert, R.J., 2007. Rate of decline of the oriental white-backed vulture population in India estimated from a survey of diclofenac residues in carcasses of ungulates. *PLoS ONE* **2(8),** e686. https://doi.org/10.1371/journal.pone.0000686.

Hinz, B., Auge, D., Rau, T., Rietbrock, S., Brune, K. and Werner, U., 2003. Simultaneous determination of aceclofenac and three of its metabolites in human plasma by high-performance liquid chromatography. *Biomedical Chromatography*, ***17*(4),** 268-275.

Iwamoto, K. & Klaassen, C. (1977). The first pass effect of morphine in rats. *J. Pharmac. Exp. Ther*. **200,** 236-244.

Kumar, N., Singh, S.D. and Jayachandran, C., 2003. Pharmacokinetic study of diclofenac and its interaction with enrofloxacin in buffalo calves. *Journal of Veterinary Science*, ***4*(2),** 155-159.

Liu, X.Q., Chen, X.J., Zhao, L.H. and Peng, J.H., 1997. High performance liquid chromatographic assay for aceclofenac in plasma and its pharmacokinetics in dogs. *Yao xue xue bao = Acta Pharmaceutica Sinica*, ***32*(7),** 546-548.

Naidoo, V., Venter, L., Wolter, K., Taggart, M., Cuthbert, R., 2010. The toxicokinetics of ketoprofen in *Gyps coprotheres*: toxicity due to zero-order metabolism. *Arch. Toxicol*. **84,** 761–766.

Noh, K., Shin, B.S., Kwon, K.I., Yun, H.Y., Kim, E., Jeong, T.C. and Kang, W., 2015. Absolute bioavailability and metabolism of aceclofenac in rats. *Archives of pharmacal research*, ***38*(1),** 68-72.

Oaks, J.L., Gilbert, M., Virani, M.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., Khan, A.A., 2004. Diclofenac residues as the cause of vulture population decline in Pakistan. *Nature*, **427,** 630–633.

Percie du Sert, N., Ahluwalia, A., Alam, S., Avey, M.Y., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Hurst, V., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T. and Würbel, H. 2020. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.* **18(7),** e3000411. https://doi.org/10.1371/journal.pbio.3000411

Pérez, S. & Barceló, D. 2008. First evidence for occurrence of hydroxylated human metabolites of diclofenac and aceclofenac in wastewater using QqLIT-MS and QqTOF-MS. *Anal. Chem*. **21,** 8135-8145.

Prakash, V., Bishwakarma, M.C., Chaudhary, A., Cuthbert, R., Dave, R., Kulkarni, M., Kumar, S., Paudel, K., Ranade, S., Shringarpure, R., Green, R.E., 2012. The population decline of Gyps vultures in India and Nepal has slowed since veterinary use of diclofenac was banned. *PLoS ONE*, **7,** e49118.

Sarowar, A.B.M.S., Ahmed, S., Rahman, S., 2016. *Vultures and Vulture Safe Zones of Bangladesh.* IUCN International Union for the Conservation of Nature, Bangladesh Country Office, Dhaka, Bangladesh.

SAVE 2021a. A Blueprint for Recovery of Asia's Globally Threatened Vultures. Pp29. Accessed from https://save-vultures.org/wp-content/uploads/2021/04/SAVE-Blueprint-2021.pdf on 27/4/22

SAVE 2021b. Bangladesh First Country to Declare National Ketoprofen Ban. Posted 18 February 2021. Accessed from https://save-vultures.org/2021/02/bangladesh-first-country-to-declare-national-ketoprofen-ban on 27/4/22

Säwe, J., Dahlstrom, B., Paalzow, L. & Rane, A. 1981. Morphine kinetics in cancer patients. *Clin. Pharmac. Ther*. **30,** 629-635.

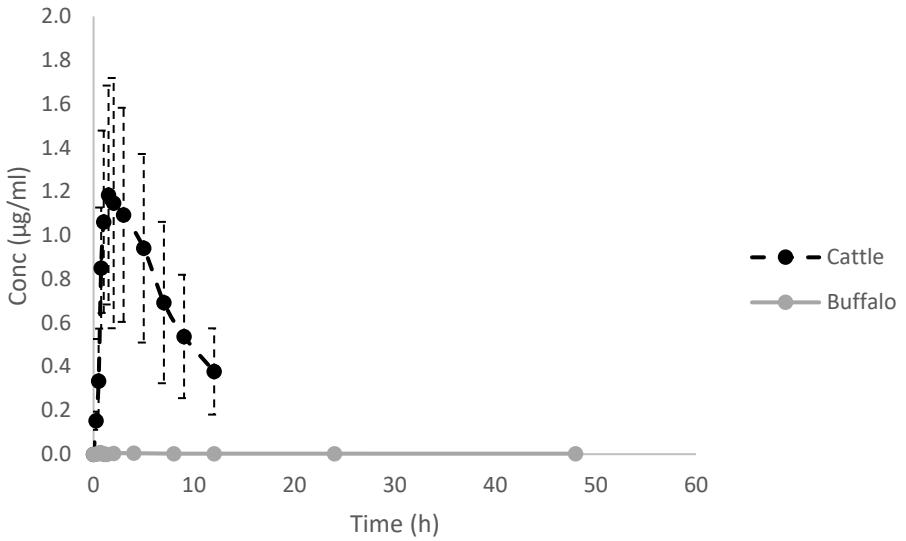
Swan, G., Naidoo, V., Cuthbert, R., Green, R.E., Pain, D.J., Swarup, D., Prakash, V., Taggart, M., Bekker, L., Das, D., Diekmann, J., Diekmann, M., Killian, E., Meharg, A., Patra, R.C., Saini, M., Wolter, K., 2006. Removing the threat of diclofenac to critically endangered Asian vultures. *PLoS Biol*. **4(3),** e66.

Swarup, D., Patra, R.C., Prakash, V., Cuthbert, R., Das, D., Avari, P., Pain, D.J., Green, R.E., Sharma, A.K., Saini, M., Das, D., Taggart, M., 2007. Safety of meloxicam to critically endangered *Gyps* vultures and other scavenging birds in India. *Anim. Conserv*. **10,** 192–198.

Taggart, M.A., Senacha, K.R., Green, R.E., Jhala, Y.V., Raghavan, B., Rahmani, A.R., Cuthbert, R., Pain, D.J. & Meharg, A.A. 2007. Diclofenac residues in carcasses of domestic ungulates available to vultures in India. *Environ. Int.* **33,** 759-765. doi:10.1016/j.envint.2007.02.010

Zorilla, I., Martinez, R., Taggart, M.A. & Richards, N. 2014.Suspected flunixin poisoning of a wild Eurasian Griffon Vulture from Spain. *Conserv. Biol*. **29,** 587-592.

(a)



(b)

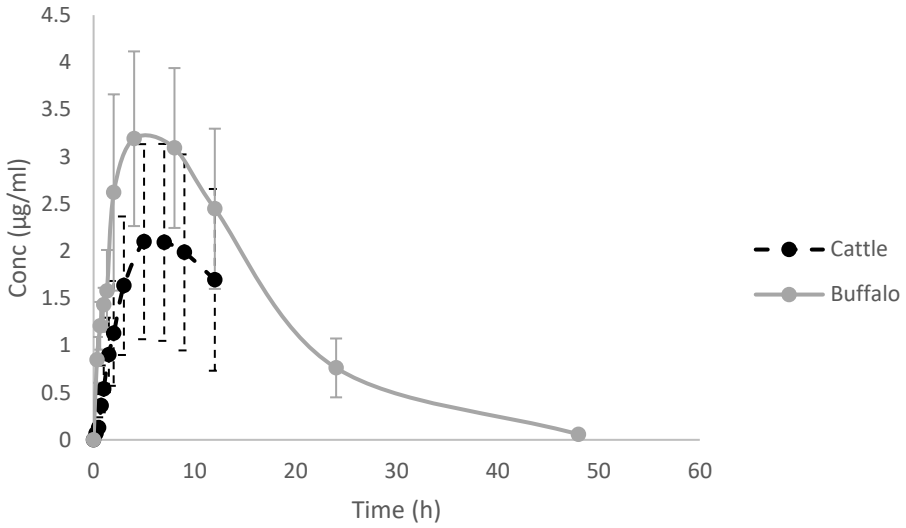


Figure 1. Change in plasma concentrations over time of (a) aceclofenac and (b) diclofenac metabolite of aceclofenac in nine male domestic water buffaloes *Bubalus bubalis* and domestic cattle *Bos taurus*. Note different scales on *y*-axes. Data for domestic cattle are rom Galligan *et al*. (2016)

Table 1: Mean pivotal pharmacokinetic parameters with standard deviations in parenthesis

Parameter	Units	Aceclofenac	Diclofenac
C_{max}	µg ml^{-1}	0.012 (0.017)	3.31 (0.93)
t_{max}	h	1.87 (1.1)	6.22 (2.11)
AUC_{last}	µg ml^{-1}*h	0.13 (0.18)	61.47 (18.82)
AUC_{inf}	µg ml^{-1}*h	0.87 (1.51)	62.07 (18.98)
λ	h^{-1}	0.05 (0.04)	0.11 (0.01)
$AUMC_{last}$	µg ml^{-1}*h^2	3.2 (4.41)	745.43 (254.97)
$t_{1/2}$	h	77.93 (129.32)	6.59 (0.85)
MRT	h	113.06 (186.05)	12.4 (1.49)
AUC_{extra}	%	57.5 (18.25)	0.94 (0.56)
Alpha	h^{-1}		0.42 (0.16)
Alpha-HL	h		1.84 (0.58)
B	µg ml^{-1}		5.37 (1.70)
Beta	h^{-1}		0.09 (0.01)
Beta-HL	h		7.89 (0.70)