



## Diclofenac residues in carcasses of domestic ungulates available to vultures in India

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Received 3 November 2006; accepted 15 February 2007

### Abstract

*Gyps* vulture populations across the Indian subcontinent are declining rapidly and evidence indicates that veterinary use of the non-steroidal anti-inflammatory drug (NSAID) diclofenac is the major cause. Exposure of vultures to diclofenac is likely to arise from the consumption of livestock carcasses that have been treated shortly before death, however, detailed information regarding the prevalence and residual levels of diclofenac in carcasses available to vultures in India remains unreported. Here, we present data on diclofenac residues in 1848 liver samples taken from carcasses of dead livestock sampled at 67 sites in 12 states within India, between May 2004 and July 2005. Diclofenac residues were detected in carcasses in all states except Orisa, where only one site was sampled. The overall prevalence of detectable diclofenac ( $> 10 \mu\text{g kg}^{-1}$ ) across all states was 10.1% and varied significantly among states, with up to 22.3% prevalence determined in Bihar. The geometric mean concentration of diclofenac found in samples in which the drug was detected was  $352 \mu\text{g kg}^{-1}$ . The prevalence of carcasses containing diclofenac is similar to that previously proposed to be required to have caused the observed *Gyps* vulture declines in India. On the 11th of May 2006, the Drug Controller General (India) ordered the withdrawal of all licenses granted for the manufacture of diclofenac for veterinary use within India. However, if *Gyps* vultures are to be protected, potentially substantial existing stocks now need to be quickly and effectively removed from the Indian veterinary market.

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**Keywords:** Diclofenac; Conservation; Domestic ungulate; Vulture; NSAID

### 1. Introduction

Since the early 1990s *Gyps* vulture populations have collapsed across the Indian subcontinent at an unprecedented rate (Gilbert et al., 2002; Prakash et al., 2003; Green et al., 2004). Populations of three species (oriental white-backed vulture *Gyps bengalensis*, long-billed vulture *Gyps indicus* and slender-billed vulture *Gyps tenuirostris*) have declined by more than 97% since 1992 (Prakash et al., 2003; Green et al., 2004). All three species are now at high risk of global extinction and listed as Critically Endangered by the IUCN — World Conser-

vation Union (IUCN, 2004), despite the fact that *G. bengalensis* was considered, in the previous decade, to be probably the commonest large raptor in the world (Houston, 1985).

Considerable evidence now indicates that this catastrophic decline has been caused by the non-steroidal anti-inflammatory drug (NSAID) diclofenac, which is widely used to treat pain, fever and inflammation in livestock across the Indian subcontinent (Green et al., 2004; Oaks et al., 2004; Shultz et al., 2004). Vultures ingest diclofenac when scavenging on the carcasses of domestic animals that have been treated with the drug shortly before death. In experiments, captive *G. bengalensis* died soon after feeding on tissues of animals that had received a normal veterinary dose of the drug just before slaughter (Oaks et al., 2004). From these experiments, the median lethal dose

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(LD<sub>50</sub>) of diclofenac for *G. bengalensis* is estimated at only 98–225 µg kg<sup>-1</sup> vulture body weight (bw) (Swan et al., 2006a). Diclofenac is also lethal to *Gyps fulvus* and *Gyps africanus*, and it seems likely that it is toxic at the concentrations typically found in tissues of treated ungulates, to all eight species within the *Gyps* genus (Swan et al., 2006a). Further, simulation modelling by Green et al. (2004) has shown that only 0.13–0.75% of livestock carcasses available to scavenging *Gyps* vultures would need to contain a lethal dose of diclofenac in order to account for vulture declines at the rates observed in India.

Given this information, on the 11th of May 2006 the Drug Controller General of India ordered the withdrawal of all licenses granted across the country to manufacture diclofenac formulations for veterinary use, and required that the marketing of such formulations be phased out within a period of 3 months (Kumar, 2006). Although this is a very positive and a significant step forward in terms of acting to halt the rapid decline of *Gyps* vultures within India, it may, in reality, take a substantial amount of time and effort to effectively remove all existing stocks of the drug from veterinary use. In veterinary medicine, diclofenac is one of the most commonly administered NSAIDs within India. It has been estimated that some 5 million domestic animals were treated with about 12 million doses of the drug each year, across the country (Proffitt and Bagla, 2004), hence substantial stocks are believed to exist.

To date, very little information has been reported on the actual prevalence and residual concentrations of diclofenac in carcasses available to vultures across India. Taggart et al. (2006) provided the first data of this kind, showing that in liver and kidney tissue samples taken from six livestock carcasses at Pinjore (in Haryana state) and from 15 carcasses at Bikaner (in Rajasthan), 33% and 20% of carcasses respectively contained detectable diclofenac (above 10 µg kg<sup>-1</sup>). Such high local prevalences are likely to be far in excess of those required to cause the observed decline in vulture populations, however the scope of the study and the number of samples taken were limited and as such, firm conclusions could not be drawn. Hence, comprehensive surveys of the residual levels and prevalence of diclofenac in carcasses available to vultures across South Asia are now critical to understand the observed rapid rate of decline, and essential in order to facilitate future assessments regarding the effectiveness of action to remove diclofenac from veterinary use across India.

In this study we report on the prevalence and residual concentrations of diclofenac in liver samples taken from livestock carcasses collected from 12 states in India. We examine differences noted among states, different collection site types, animal species, sex and age class, and begin to consider the impact of the residual levels of diclofenac found in carcasses on remaining vulture populations.

## 2. Methods

### 2.1. Field sampling of livers from domestic ungulate carcasses

Liver samples from Indian cow ( $n=893$ ), water buffalo ( $n=861$ ), sheep ( $n=48$ ), goat ( $n=39$ ), horse ( $n=6$ ) and camel ( $n=1$ ) were collected from 67 sites in 12 states in India between May 2004 and July 2005. Three further states, Assam, Uttaranchal and Meghalaya were also visited (for 20 days in total), but

samples could not be located. Samples were collected from carcasses at carcass dumps managed by local government corporations, co-operatives and private companies/individuals, and from cattle welfare charities and slaughterhouses. Slaughterhouses were included in the survey because, although part of the slaughtered animal is consumed by humans (i.e., the prime cuts), a substantial part (the offal, etc.) is disposed of on carcass dumps and is therefore available to vultures. A small number of carcasses were found in the countryside and alongside roadways, away from such locations, and these were also sampled opportunistically ( $n=4$ ). Sampling sites used were simply those encountered during fieldwork visits, for which it was possible to obtain access and permission to gather samples. Hence, they were not necessarily a representative sample of all locations at which livestock carcasses were available to vultures. It would be difficult to identify the population of such locations from which a random sample of sites could be drawn, however, we did not consciously select sites based on any criteria that were likely to lead to an atypical prevalence of diclofenac-treated animals.

The liver was removed from carcasses by skimmers working on these sites, and three tissue sub-samples of approximately 3–4 g each were then removed by scalpel from three different regions of the liver. These three sub-samples were then bulked together into one watertight 25 ml PP sample container which was then further sealed with tape and labelled appropriately. Batches of ten sample containers were then placed into labelled zip lock bags which were then stored on ice in a portable refrigerator. Subsequently, all samples were transferred to a freezer and stored at  $-20\text{ }^{\circ}\text{C}$  until extraction.

At all sites except one (Ludhiana in the State of Punjab,  $n=61$ ), every carcass that arrived during the sampling visit was sampled. There is therefore no scope for bias with respect to the species, age or condition of the dead animals sampled at 66 of the 67 sites used. At Ludhiana, carcass numbers arriving at the site were  $>50$  per day and it was not possible to sample every carcass. Samples at this site were therefore taken predominantly from young prime, and mature adults.

### 2.2. Diclofenac extraction and measurement

The samples of liver (0.5 g) were weighed into a new glass test tube, 2 ml of HPLC grade acetonitrile (MeCN) was added, and the mixture homogenized for 30 s using an Ultra Turrax IKA T8 handheld homogenizer. The homogenizer was thoroughly stripped down and cleaned in between each sample extraction. The mixtures were then centrifuged at 1000 g for 5 min and the supernatant was filtered using disposable PTFE/PE syringe filter units of 0.45 µm. The filtered extract was then stored in crimp top LC vials at  $-20\text{ }^{\circ}\text{C}$  until analysis.

Diclofenac levels were determined by LC–ESI/MS (liquid chromatography–electrospray ionisation mass spectrometry) using an Agilent 1100 series instrument (1946D). The instrument was calibrated using 6 standards ranging from 5 to 1000 µg l<sup>-1</sup> in diclofenac concentration, generated using diclofenac sodium salt (Sigma-Aldrich, D6899). The calibration was linear across this range with an  $r^2$  value of  $>0.99$ . Both a blank and a mid-range standard were analysed between every 10 unknown samples to monitor for instrumental drift and/or diclofenac carry-over. Chromatographic separation was achieved on the LC using a Waters Xterra MS C18 column (3.9 mm × 150 mm, 5 µm). Samples and standards (20 µl) were subjected to a binary gradient elution profile using 0.1% acetic acid in water (solution A) and 100% MeCN (solution B). The starting conditions were 75% A/25% B for 0.1 min, followed by a 15 min linear gradient from 75% A/25% B to 5% A/95% B, then by a 5 min column wash step of 5% A/95% B, and a 10 min re-equilibration step with 75% A/25% B. The flow rate was set at 0.7 ml min<sup>-1</sup>, samples were continuously held cool at 4 °C using an autosampler with thermostat, and the column temperature was maintained at 40 °C during analysis. Diclofenac was monitored by the MS at mass/charge ratios of 294 and 296 (the deprotonated and protonated ions) in the negative ion mode, with the capillary voltage set at 3500 V and the fragmentor voltage at 80 V. The instrument was calibrated against both masses and each sample was run through the instrument twice; this allowed 4 values for diclofenac to be gained (at 294 and 296 in two runs), the mean of which was taken to be the concentration in the extract. The limit of quantification (LOQ) for this technique (back calculated to wet tissue concentration) was found to be 10 µg kg<sup>-1</sup> and the limit of detection (LOD) was 4 µg kg<sup>-1</sup>.

### 2.3. Precision of diclofenac measurements

The precision of a single measurement as an estimate of the average diclofenac concentration in a whole liver was estimated by analysing six pieces

Table 1  
Prevalence and detectable residual concentrations of diclofenac in liver samples from domestic ungulate carcasses sampled in 12 states in India

State	Sites ( <i>n</i> )	Samples ( <i>n</i> )	Date sampled	% Prevalence	Residue range ( $\mu\text{g kg}^{-1}$ )	Geometric mean ( $\mu\text{g kg}^{-1}$ )
All states	67	1848	May 2004–Jul 2005	10.1	11–13,723	352
Bihar	1	121	May–Jun 2005	22.3	15–3582	259
Rajasthan	3	310	Jun–Jul 2004	17.1	13–13,723	574
Punjab	7	76	Feb 2005	15.8	34–1879	424
Madhya Pradesh	8	195	Feb–Mar 2005	11.3	14–2156	213
West Bengal	4	94	Apr–May 2005	9.6	63–2038	482
Gujarat	12	65	May–Jun 2004	9.2	86–3183	882
Uttar Pradesh	9	449	Jul–Sep 2004 and Jun–Jul 2005	7.8	11–6524	404
Maharashtra	8	194	Sep 2004 and Mar 2005	5.7	15–4135	240
Jammu and Kashmir	8	77	Jan–Feb 2005	3.9	16–100	41
Jharkhand	2	54	Jun 2005	3.7	105–109	107
Andhra Pradesh	4	161	Apr 2005	3.7	24–269	77
Orisa	1	52	Jun 2005	0.0	n/a	n/a

Geometric mean concentrations were calculated only from those samples with detectable residues. Assam, Uttaranchal and Meghalaya were also surveyed, but no samples were obtained.

of tissue from each of five livers. Two samples were taken from each of the three parts of the same whole liver removed in the field. The standard deviation of the six replicate measurements, expressed as a percentage of their mean, ranged from 11.7 to 41.3% with a mean of  $25\% \pm 5\%$  (standard error).

### 3. Statistical analysis

#### 3.1. Factors affecting prevalence

We tested for differences in the proportion of samples with detectable diclofenac among states, types of sampling site, ungulate species, sex class and age using multiple logistic regression models. Whether or not each sample had detectable diclofenac it was treated as a binary dependent variable with

binomial error and logit link function. Independent variables (all of which were factors) were state (12 categories, see Table 1), type of sampling site (the five categories listed in Table 2 and samples in the countryside), species (the four species in Table 2), sex (3 categories: male, female, unsexed) and age (the four categories in Table 2). Data for horse and camel were excluded from this analysis because of the small sample size ( $n=6$  and 1 respectively).

Minimum adequate models (MAMs) were selected by stepwise backward elimination. We used likelihood-ratio tests and deleted from the model variables with the least significant  $p$  value at each step until only variables with  $p < 0.05$  were retained. Because of the large possible number of interaction terms we did not include them all in the starting model. Instead, we chose to

Table 2  
Prevalence and concentration of detectable residual diclofenac in liver samples of domestic ungulate carcasses in 12 states in India, by species, sex, type of death, type of site and age

Factor	Category	Samples	% Prevalence	Residue range ( $\mu\text{g kg}^{-1}$ )	Residue geometric mean ( $\mu\text{g kg}^{-1}$ )
Species	Indian cow	893	14.7	13–13,723	394
	Buffalo	861	6.0	11–6524	284
	Sheep	48	0.0	n/a	n/a
	Goat	39	2.3	n/a	53
	Horse	6	33.3	24–793	138
	Camel	1	0.0	n/a	n/a
Sex	Female	1138	13.5	14–5074	347
	Male	707	4.5	11–13,723	375
Cause of death	Natural death	1569	11.5	13–13,723	372
	Slaughtered	279	2.2	11–269	66
Site type	Private dump	437	12.6	15–3582	276
	Corporation dump	1068	11.1	13–13,723	412
	Co-operative dump	28	10.7	459–1976	816
	Cow charity dump	32	9.4	86–3183	687
	Slaughterhouse	279	2.2	11–269	66
Age	Old adult (>5 years old)	439	17.1	15–4248	254
	Young prime adult (2–5 years old)	488	14.1	14–5074	324
	Immature (6 months to 2 years old)	180	3.9	11–4135	397
	Infant (<6 months old)	741	4.7	13–13,723	811

Geometric mean concentrations were calculated only from those samples with detectable residues.

Three samples were of unknown sex and four were collected opportunistically by roadsides or in fields and are excluded from the respective sections of this table.

include the main effects and the two- and three-way interactions among species, sex and age because interactions among these variables seemed to us the most plausible on biological grounds. We first tested the significance of all the interaction terms together. We then tested the three-way interaction, then all two-way interactions and then the main effects.

### 3.2. Factors affecting residual diclofenac concentration

We analysed the diclofenac concentrations using ordinary least squares multiple linear regression of log-transformed concentrations. We included all samples with detectable diclofenac. The independent variables and model selection procedure were the same as for the logistic regression analysis of prevalence described above, except that *F* tests were used to assess significance.

## 4. Results

### 4.1. Prevalence of diclofenac in liver from carcasses of domestic ungulates

Of the 1848 carcass samples, 10.1% were found to contain diclofenac concentrations above the LOQ of  $10 \mu\text{g kg}^{-1}$  (Table 1). By state, the prevalence detected varied from 3.7% in Andhra Pradesh and Jharkhand up to 22.3% in Bihar (Fig. 1). Diclofenac was not detected in any of the 52 samples taken at one site in the state of Orisa, however, this was a slaughterhouse site which may be more likely to process younger, healthy animals in less need of drug/diclofenac treatment prior to death.

As well as varying among states, the prevalence of diclofenac also varied according to species, sex, age, and type of collection site (Table 2). Diclofenac was detected more frequently in Indian cow than in water buffalo, prevalence was lower still in goats, and no diclofenac was detected in sheep. One-third of a small sample of horses ( $n=6$ ) had detectable levels of the drug in their livers. Combining all species,



Fig. 1. Prevalence of diclofenac in livers of domestic ungulate carcasses from India by state. Assam, Uttaranchal and Meghalaya were surveyed but samples were unavailable. States shown in white were not surveyed. Inset map shows the number of samples collected per state and the number of sites from which they were collected in brackets.

diclofenac prevalence was higher in females than in males, and higher in older animals. The logistic regression analysis (see Methods) indicated that the main effects of state, type of site, species, sex and age were all statistically significant (likelihood-ratio tests;  $\chi^2_{(11)}=37.05$ ,  $p < 0.005$ ;  $\chi^2_{(5)}=26.92$ ,  $p < 0.005$ ;  $\chi^2_{(3)}=9.98$ ,  $p < 0.025$ ;  $\chi^2_{(2)}=6.99$ ,  $p < 0.05$ ;  $\chi^2_{(3)}=61.94$ ,  $p < 0.005$  respectively). Inspection of Table 2 indicates that prevalence was similar for all types of carcass dumps and lower for slaughterhouses. A revised classification of types of site into slaughterhouses and all other types did not result in a significantly poorer fit of the model ( $\chi^2_{(4)}=1.82$ ,  $p > 0.2$ ) and the effect of the simplified classification of site type was also highly significant ( $\chi^2_{(1)}=25.14$ ,  $p < 0.0005$ ). Regardless of how site type was classified, the interaction terms among species, sex and age class were not significant together ( $p > 0.30$ ) or separately ( $p > 0.20$ ).

#### 4.2. Residual levels of diclofenac in liver from carcasses of domestic ungulates

The range of residual diclofenac concentrations detected was 11–13,723  $\mu\text{g kg}^{-1}$ , with a geometric mean of 352  $\mu\text{g kg}^{-1}$ . The final model identified in the multiple least squares linear regression analysis described above included significant effects of state ( $F_{10,182}=1.94$ ,  $p < 0.05$ ) and age class ( $F_{3,175}=2.87$ ,  $p < 0.05$ ). None of the other main effects was statistically significant when added to this model (site type  $F_{4,168}=1.00$ ,  $p > 0.25$ ; species  $F_{3,169}=0.85$ ,  $p > 0.25$ ; sex  $F_{1,171}=1.79$ ,  $p > 0.10$ ), although when site type was simplified to slaughterhouse and all other site types, there was a near significant tendency for concentrations to be lower in carcasses from slaughterhouses ( $F_{1,171}=3.54$ ,  $p=0.08$ ). None of the interactions tested was statistically significant. The estimated geometric mean concentrations from the fitted model declined progressively with age in a similar way to the observed values in Table 2.

## 5. Discussion

The results presented here show that the food sources upon which *Gyps* vultures rely in India are heavily contaminated with residues of diclofenac. It is clear that diclofenac is used widely in India because we have found diclofenac in all but one of the twelve states sampled. In that state, Orisa, we sampled at only one site, and this was a slaughterhouse. Diclofenac prevalence at slaughterhouses in other states (2.6%) was much lower than at other types of sites (11.4%), hence it seems likely that diclofenac contamination would also have been found in Orisa if more sites, including carcass dumps, had been sampled.

Diclofenac prevalence was higher in cows than in water buffaloes and other species, higher in female than male animals, higher at carcass dumps than at slaughterhouses and increased with advancing age. The logistic regression model indicated that these were all significant separate effects and not attributable to correlations among the explanatory variables. These differences might be as expected, as older animals are perhaps more likely to need treatment for injuries and diseases, and since slaughtered animals may be those specifically reared to provide meat and may be healthier than the average. Veterinarians also report that NSAIDs (including diclofenac) are commonly administered in combination with antibiotics to treat mastitis in lactating female cows and water buffalo. More surprisingly, in view of the low prevalence of diclofenac in young animals, the residual con-

centration of diclofenac was higher in young than in old animals. This may be because a standard quantity of the drug is being administered per animal, rather than a dose dependent on the animal's weight, as is recommended.

Diclofenac is rapidly metabolized in most mammals (EMEA, 2004) including the two most prevalent domestic ungulate species in India (Indian cow and goat), (Green et al., 2006; Taggart et al., 2006). Diclofenac tissue residues in both species were found to be undetectable ( $< 10 \mu\text{g kg}^{-1}$ ) 6 days after a normal veterinary dose. Hence, livestock would presumably have to die within 6 days of treatment to contain detectable residues, and within hours to 1 or 2 days for their tissues to contain sufficiently high concentrations of diclofenac to pose a risk to scavenging vultures.

It seems likely, given the liver levels found here, that a significant proportion of these animals were indeed given a final dose of diclofenac less than 48 h before death. Taggart et al. (2006) have shown that for Indian cow (*Bos indicus*) given one dose of 1000  $\mu\text{g kg}^{-1}$  bw, liver diclofenac residues dropped to just 41  $\mu\text{g kg}^{-1}$  ( $n=2$ ) after 46 h. Likewise, the EMEA (2004) has shown that even when given 6 daily 2500  $\mu\text{g kg}^{-1}$  bw doses, in European cow (*Bos taurus*) liver residues fall to 426  $\mu\text{g kg}^{-1}$  after only 24 h, (in India, a course of three daily doses of 1000  $\mu\text{g kg}^{-1}$  bw is commonly recommended for diclofenac, although this depends upon the nature and severity of the condition being treated). The results presented here show residues are  $> 41 \mu\text{g kg}^{-1}$  and  $> 426 \mu\text{g kg}^{-1}$  in 8.8% and 5.1% of all carcasses tested and in 87% and 51% of all those found with residual diclofenac  $> \text{LOQ}$ . Further, the maximum diclofenac residue reported here is 13,723  $\mu\text{g kg}^{-1}$  in liver. This suggests that not only did this animal (a male, infant cow from Rajasthan) receive a diclofenac dose within only a few hours of death, but that the dose was far in excess of that which is commonly recommended in India. This level is almost five times the mean level (2874  $\mu\text{g kg}^{-1}$ ;  $n=4$ ) detected just 3 h post-treatment in liver of *B. taurus* when given 6 daily doses of 2500  $\mu\text{g diclofenac kg}^{-1}$  bw (EMEA, 2004). This raises the question of whether diclofenac is occasionally being administered either by those who are unqualified (farmers, etc.), or by veterinary practitioners who are disregarding the recommended diclofenac dosage for India.

Recent estimates suggest that there are 503 million domestic and feral ungulates in India (Prakash et al., 2005), including 185 million Indian cows, 98 million water buffaloes, 124 million goats and 61 million sheep (NDDDB, 2006). Assuming that 10 to 20% of these animals die per year, of which only a small proportion are consumed by humans, around 50–100 million animals per year would be expected to die in India and potentially be available to vultures. The Indian pharmaceutical industry estimates that about 5 million animals are treated with diclofenac per year (Proffitt and Bagla, 2004). Diclofenac is detectable in liver samples from cattle given the standard veterinary dose for about 6 days after the last dose (Green et al., 2006; Taggart et al., 2006). Cattle given the standard course of three daily injections would therefore be expected to have detectable diclofenac in the liver for 8 days after the first injection. Hence, if ungulates were treated at random times with respect to the time of death, we would expect that  $(8 \times 5) / (365 \times 503) = 0.02\%$  of carcasses would

contain detectable diclofenac. Our observed value of 10.1% prevalence is more than two orders of magnitude larger than this. However, if all animals treated with diclofenac were given their first injection less than 8 days before death, the expected proportion of animals with detectable diclofenac in the liver would be between  $5 / 100 = 5\%$  and  $5 / 50 = 10\%$ , which is much closer to the observed level. Even so, it seems very unlikely that all, or even the majority, of diclofenac is administered to dying animals, so it may be that much more diclofenac is used than the industry has estimated. Further research on the number of doses of diclofenac administered to livestock and their provenance would be useful in ensuring that the veterinary use of the drug in India is reduced swiftly.

Green et al. (2004) presented a vulture population model which showed that just 0.13–0.75% of carcasses available to vultures would need to contain a lethal level of diclofenac to cause the recently observed rates of population decline. These proportions are much lower than the prevalence of detectable diclofenac reported in our survey, but the lethal level to vultures varies, and is much higher than the detectable level. Swan et al. (2006a) recently estimated the  $LD_{50}$  for *G. bengalensis* as between 98 and 225  $\mu\text{g kg}^{-1}$ . A free-living wild *G. bengalensis* requires around 0.34 kg of animal tissue per day (Swan et al., 2006b), and weighs about 4.75 kg (Del Hoyo et al., 1994). However, wild vultures do not usually feed every day, and are thought to frequently eat enough to sustain them for 3 days, i.e., 1.02 kg (Swan et al. 2006b). Using these estimates, in order for the tissue eaten (in this case, liver) to contain enough diclofenac to exceed the  $LD_{50}$  range given, residual levels would need to be  $>1369$  and  $>3143 \mu\text{g kg}^{-1}$  if a vulture fed every day, and  $>456$  and  $>1048 \mu\text{g kg}^{-1}$  if it fed every 3 days. Such residual levels are exceeded within the data presented here in 2.6–0.8% and 5.0–3.2% of cases respectively. Hence, the prevalence of carcasses found to be available to *Gyps* in India that contained a potentially lethal dose of residual diclofenac in their livers is in excess of even the highest estimate of 0.75% suggested by Green et al. (2004). However, it is important to note that vultures may more typically consume a variety of tissues, some of which (such as kidney) may contain higher levels of diclofenac than the liver, and some of which may contain somewhat less (such as muscle; see Green et al., 2006; Taggart et al., 2006). Even given this, the lower estimate of prevalence required according to Green et al. (2004) is only 0.13%, nearly six times less than (and 16% of) the lowest 0.8% prevalence noted above in relation to liver concentrations. Data from Taggart et al. (2006) suggests that the level of diclofenac in muscle tissue of Indian cow will also tend to be approximately six times less than (and 17% of) that in the liver after drug administration. Much more detailed and comprehensive modelling is ongoing (Green et al., in preparation), but it certainly seems given the data reported here, that the prevalence of carcasses containing sufficient levels of diclofenac is broadly consistent with that previously proposed to be required to have caused the observed *Gyps* vulture declines in India.

Further comprehensive surveys of this nature in coming years are now needed in order to determine whether the recent ban on the manufacture and marketing of diclofenac in India has been

effective. Likewise such surveys should also look to provide information on other NSAIDs detected in ungulate carcasses, and monitor whether meloxicam, identified as being non-toxic to *Gyps* vultures and other scavenging avian species, and suggested to be a viable safe alternative to diclofenac (Swan et al., 2006b; Swarup et al., in press), is becoming increasingly present in such carcasses.

Unfortunately, there now remains a significant risk that diclofenac use in India will be replaced by alternative drugs other than meloxicam, which are not yet known to be safe to vultures and other scavenging species, and which have not undergone rigorous testing in such species. For example, Reddy et al. (2006) recently compared the toxicity of nimesulide with that of diclofenac in chickens, and whilst identifying high levels of mortality (40%) when using diclofenac, showed no mortality in those birds receiving nimesulide. Reddy et al. (2006) then went on to incorrectly conclude from this limited work, that given a lack of evidence to the contrary, (i.e., a range of robust experimental data using a variety of scavenging avian species, including vultures), that nimesulide should be considered ‘completely safe for birds’.

It now also seems that two further vulture species within India (Egyptian vulture *Neophron percnopterus* and red-headed vulture *Sarcogyps calvus*) are undergoing very rapid population declines due to an unidentified cause (Cuthbert et al., 2006), and Cuthbert et al. (in press) have recently highlighted a range of other NSAIDs (including carprofen and flunixin) which may also be potentially toxic to scavenging bird species. Current understanding of NSAID toxicity in bird species is extremely low, and safety trials on these and a much wider range of veterinary pharmaceuticals have unfortunately, to date, completely failed to address the impact of such compounds on both avian and other scavenging species alike.

### Acknowledgements

The authors gratefully acknowledge funding from the UK Government’s Darwin Initiative for the Survival of Species and the Royal Society for the Protection of Birds; and support from the Wildlife Institute of India (WII), the Bombay Natural History Society (BNHS) and the University of Aberdeen (UK). For their assistance in the collection of tissues in the field we would also like to thank Mr Anant Khot and Mr Kamal Kumar Kashyap from BNHS, and for his assistance in sample extraction we gratefully acknowledge Mr Rakesh Sundriyal (WII). Thanks are also given to all the skinners who co-operated and contributed immensely in terms of providing tissue samples from the livestock carcasses.

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