Analysis of Nine NSAIDs in Ungulate Tissues Available to Critically Endangered Vultures in India

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In 2006, India, Pakistan, and Nepal banned the manufacture of veterinary formulations of the nonsteroidal anti-inflammatory drug (NSAID) diclofenac. This action was taken to halt the unprecedented decline of three *Gyps* vulture species that were being poisoned by diclofenac residues commonly present in carcasses of domestic livestock upon which they scavenged. To assess the affect of this ban and evaluate residue prevalences of other NSAIDs, we present a method to detect diclofenac and eight more NSAIDs by liquid chromatography—mass spectrometry and apply this to 1488 liver samples from carcasses of livestock taken across seven Indian states. Diclofenac was present in 11.1% of samples taken between April and December 2006, and meloxicam (4%), ibuprofen (0.6%), and ketoprofen (0.5%) were also detected. Although meloxicam is safe for a range of avian scavengers, including *Gyps* vultures, data regarding the safety of other NSAIDs is currently limited. If wild *Gyps* on the Indian subcontinent are to survive, diclofenac bans must be completely effective, and NSAIDs that replace it within the veterinary drug market must be of low toxicity toward *Gyps* and other scavenging birds.

Introduction

In 2004, it emerged that the nonsteroidal anti-inflammatory drug (NSAID) diclofenac was causing poisoning and mortality of *Gyps* vultures across the Indian subcontinent (*1, 2*). Residues of this NSAID, widely used to treat pain, fever, and inflammation in livestock, were being ingested by vultures scavenging on carcasses of ungulates that had died soon

after treatment. Residues were detectable in 10.1% of carcasses in India in 2004−2005 (*3*), and the LD$_{50}$ (dose that is lethal to 50% of test subjects) for the Oriental white-backed vulture (*Gyps bengalensis*) was estimated at 98−225 μg kg^{-1} (*1, 4*). These LD$_{50}$ values are an order of magnitude less than, for example, the highly toxic carbamate insecticide aldicarb to red-winged blackbirds (*Agelaius phoeniceus*) and mallard ducks (*Anas platyrhynchos*) and are half that of carbofuran for the same species (*5*). As a class I "highly toxic" compound to *Gyps* vultures (LD$_{50}$ < 1000 μg kg^{-1}) (*6*), diclofenac poisoning has caused resident *Gyps* populations across the Indian subcontinent to collapse over the last 17 years. *G. bengalensis*, long-billed vulture (*G. indicus*), and slender-billed vulture (*G. tenuirostris*) are now listed as Critically Endangered (2000, *G. bengalensis*; 2002, *G. indicus* and *G. tenuirostris*), having previously been listed as Least Concern [International Union for Conservation of Nature (IUCN)] (*7*). In the 1980s, *G. bengalensis* was probably the commonest large bird of prey in the world (*8*), and at the beginning of the 1990s, *Gyps* in India were estimated to number 60 million individuals. Indian populations began to crash in the early to middle 1990s (*9, 10*), with *G. bengalensis* falling 99.9% between 1992 and 2007 at a mean annual decline rate of 43.9% during 2000−2007 (*11*). Similar trends for *G. indicus* and *G. tenuirostris* are reported, and these majestic species now sit on the brink of extinction.

This is the first time that a common veterinary pharmaceutical, thought to be of low toxicity toward numerous species, has had such a widespread, devastating effect at the population level on nontarget wildlife. Modeling suggests diclofenac alone is likely to be the major cause of the observed population collapse (*12, 13*), and two other species, Egyptian vulture (*Neophron percnopterus*, Endangered) and red-headed vulture (*Sarcogyps calvus*, Critically Endangered) are undergoing similar declines in the region due to an as yet unidentified cause (*14*). Also, despite the recognized toxicity of diclofenac toward the *Gyps* genus, its use is now increasing across Africa (*15*). This may endanger four more species, i.e, the cape vulture (*G. coprotheres*, vulnerable), Rueppell's griffon vulture (*G. rueppellii*, Near Threatened), African white-backed vulture (*G. africanus*; Near Threatened), and griffon vulture (*G. fulvus*; Least Concern), whose populations are already under pressure from persecution, hunting, habitat loss, a decrease in wild ungulate food availability, and power line collisions (*7, 16*).

In May 2006, the Drug Controller General (India) withdrew all licenses to manufacture diclofenac for veterinary use (*17*), and similar action was taken in Nepal and Pakistan. If wild *Gyps* populations are to recover or captive bred birds released successfully these bans must be highly effective. Further, there is currently a lack of data regarding the toxicity of many common NSAIDs toward avian scavengers, and concerns exist that NSAIDs other than diclofenac may pose comparable risks (*18*). It is therefore critical that as diclofenac is phased out, it is replaced with well-studied alternatives known to be of low toxicity. One such alternative, meloxicam, has been clearly identified (*19, 20*) but the risk remains that less well-examined NSAIDs could still emerge (*21*).

Data regarding livestock carcass residues of NSAIDs other than diclofenac have not to date been presented in the literature. Many analytical procedures are available to detect single NSAIDs in differing matrices and to simultaneously analyze multiple NSAIDs in pure mixtures, surface and wastewater, serum, and urine (refs *22−26* represent examples). There are however only two reports to our knowledge describing the analysis of multiple NSAID residues in tissues (*27, 28*). Although these describe method development

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TABLE 1. Suite of Nine NSAIDs Analyzed by Liquid Chromatography Electrospray Ionisation Mass Spectrometry (LC-ESI/MS) in Negative Ion Mode

NSAID	molecular weight	precursor ion	primary fragment ion (fragmentor voltage)	alternative fragment ions
carprofen	273.71	272	228 (200)	193
meloxicam	373.38	350	286 (160)	146, 210
ketoprofen	254.28	253	197 (120)	209
diclofenac	318.13	294	250 (140)	214
naproxen	230.26	229	202 (140)	170, 185
flunixin	491.46	295	251 (180)	231, 279
ibuprofen	206.28	205	193 (120)	161
nimesulide	308.31	307	229 (180)	154, 183
indometacin	357.79	356	312 (140)	139

using spiked tissues, we are also unaware of any report that applies such a method to extensive residue analysis of real tissue samples collected in the field. Here, we give details of a sensitive, relatively simple, LC-ESI/MS (liquid chromatography electrospray ionization single quadrapole mass spectrometry) method that is applied to the analysis of nine NSAIDs simultaneously extracted from liver tissue from >1450 carcasses available to scavengers across seven states in India.

Experimental Section

Field Sampling. Liver samples from Indian cow, buffalo, sheep, goat, and horse were collected from 26 sites in seven Indian states (Figure S1 of the Supporting Information) between April and December 2006 (total $n = 1488$). Samples were from carcasses deposited at dumps managed by government corporations, cooperatives, private companies and individuals, and cattle welfare charities. Locations were typical of sites formerly used by *Gyps* vultures, and all animals had died of natural causes. Sex, species, and condition of animal were recorded as was age, using a classification previously described (*3*). The majority of sites visited were the same as those used in a previous survey (*3*), full details of which (GPS coordinates, city, site name, site type, and carcass intake rates) are provided elsewhere (*29*). After animals were skinned by site staff, the liver was removed and three tissue subsamples (1–2 g each) were taken by scalpel from three liver regions. These subsamples were bulked into one 7 mL polypropylene watertight sample vial that was sealed with parafilm and labeled. Samples were stored in a portable freezer unit (−11 °C) before transfer to permanent laboratory freezers (−20 °C) at the Wildlife Institute of India (WII, Dehradun).

NSAID Extraction. Subsamples of liver (0.5 g) were weighed to ±0.001 g into new borosilicate test tubes, and 2 mL of HPLC grade acetonitrile added. The mixture was homogenized for 45 s using an Ultra-Turrax IKA-T8 handheld homogenizer. The homogenizer was thoroughly stripped down and cleaned between extractions. Homogenates were centrifuged at 1000 g for 5 min, and the supernatant was filtered with disposable 0.45 μm PTFE/PE syringe filter units directly into crimptop 2 mL LC vials that were capped and stored at −20 °C until analysis. This extraction is similar to that utilized elsewhere (*1, 27*); however, we did not undertake sample cleanup using solid phase extraction (SPE) cartridges as trials suggested this was unnecessary for these NSAIDs (as in ref *28*, albeit for different NSAIDs).

Target NSAIDs Determined. Nine NSAIDs (Table 1) were determined simultaneously by LC-ESI/MS using an Agilent 1100-LC coupled with a 1946D-ESI/MS. Molecular weights in Table 1 are for the standards used. Precursor and primary fragment ion values are for deprotonated ions, and all precursors were monitored at a fragmentor voltage of 80 V. Alternative fragment ion values listed were not monitored

here but are derived from studies that also targeted one or more of these compounds (*24, 25, 27, 28, 30, 31*).

All standards were from Sigma-Aldrich and were Vetranal carprofen (product number 33975), meloxicam sodium salt hydrate (M3935), Vetranal ketoprofen (34016), diclofenac sodium salt (D6899), naproxen (N8280), flunixin meglumine (F0429), ibuprofen (I4883), nimesulide (N1016), and indometacin (I8280). These NSAIDs met at least one of the following criteria: (a) They were licensed or available within the Indian veterinary market (authors unpublished market surveys and ref *32*). (b) They were highlighted as an NSAID previously used to treat avian species and had caused mortality (*18*). (c) They had potential to enter the Indian market in the future (*21, 33*). (d) During method development, they were found suitable for inclusion in a multiresidue technique. In addition to these nine NSAIDs, criteria highlighted three other compounds that were also initially investigated, i.e., dipyrone (analgin, D8890), salicylic acid (S5922), and phenylbutazone (P8386). As standards, these were successfully analyzed and good calibrations gained; however, they were determined unsuitable for use here. In spikes, we observed poor recovery, compound instability, and/or potential for significant interferences from natural compounds common in liver extracts, which would ultimately have led to high limits of quantification (LOQs). Phenylbutazone was particularly unstable (as noted in ref *34*), even as a standard in the short term. Modification of procedures to include SPE cleanup may facilitate inclusion of one or more of these three compounds, but further work is required to validate these additions.

Stock standards (1000 mg l^{-1}) were made for all nine drugs (Table 1) in 50:50 acetonitrile:Milli-Q water and mixed working standards generated by serial dilution from 1000–8.3 μg l^{-1} in acetonitrile. Working solutions were stored at −20 °C in 2 mL liquid chromatography (LC) vials, and stocks were stored at −4 °C. Stocks and working standards were periodically cross analyzed against fresh solutions over a period in excess of three months, and no significant recovery degradation was observed.

Liquid Chromatography−Mass Spectrometry. Chromatographic separation was optimized using an Agilent Zorbax-300SB-C18 column (4.6 mm × 150 mm, 5 μm), with a matching 12.5 mm guard column. Flow rate, column temperature, and elution conditions were adjusted and found to be optimal at 0.75 mL min^{-1}, 40 °C, and with a binary gradient elution profile using 0.1% acetic acid in water (A) and 100% acetonitrile (B). 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, a 5 min wash step of 5% A/95% B, and a 10 min re-equilibration with 75% A/25% B. Twenty microliters of sample/standard were injected, and samples/standards were held cool at 4 °C using an autosampler thermostat.

For MS, working standards were initially analyzed in positive and negative ion scan mode. For these compounds, negative ion monitoring was optimum. During analysis of tissue extracts, the instrument was calibrated with six mixed standards (from 8.3–500 μg l^{-1}) for all NSAIDs. Each compound was monitored in single ion monitoring (SIM) mode, and calibrations were all linear across this range (r^2 of >0.99). Blank and midrange standards were analyzed between every 10 unknown samples to monitor instrumental drift and/or NSAID carry over. Carry over was at no point observed, and nominal drift was compensated for using a rolling correction where required. An internal standard was not used as a suitably well-studied and characterized compound has not been identified for this specific purpose.

NSAIDs were detected and quantified at precursor ion values (Table 1) with the fragmentor voltage at 80 V. NSAIDs detected were reconfirmed as primary fragment ions in a

TABLE 2. Prevalence (%) of Diclofenac and Other NSAIDs Detected in Liver Samples from Ungulate Carcasses Sampled in Seven States in India in 2006[a]

state	DIC	MEL	IBU	KET	DIC + MEL	DIC + IBU	DIC + IBU + KET
all States	11.1 (165)	4.0 (60)	0.6 (9)	0.5 (8)	0.9 (13)	0.5 (7)	0.3 (5)
Jammu and Kashmir	3.6 (4)	11.6 (13)	0.9 (1)	–	–	0.9 (1)	–
Gujarat	4.1 (9)	–	–	–	–	–	–
Maharashtra	11.2 (27)	4.6 (11)	–	0.4 (1)	0.8 (2)	–	–
Punjab	12.3 (28)	7.5 (17)	–	–	2.2 (5)	–	–
Madhya Pradesh	12.7 (30)	0.8 (2)	0.4 (1)	–	0.4 (1)	0.4 (1)	–
Rajasthan	14.5 (49)	4.7 (16)	1.5 (5)	2.1 (7)	1.5 (5)	1.5 (5)	1.5 (5)
West Bengal	16.4 (18)	0.9 (1)	1.8 (2)	–	–	–	–

[a] Abbreviaitons are DIC (diclofenac), MEL (meloxicam), IBU (ibuprofen), and KET (ketoprofen). Number of samples containing residues (*n* values) are given in brackets.

second run at an optimized fragmentor voltage, while monitoring solely for the NSAID of interest (to maximize sensitivity toward the fragment, Table 1). The optimized fragmentor voltages were determined using trials on pure standards, while adjusting the fragmentor voltage between 70 and 200 V (Figure S2 of the Supporting Information shows relative ion abundances with changing fragmentor voltages for four NSAIDs, as examples, and matching scan spectra). Capillary voltage used was 3500 V throughout. LOQs (wet liver tissue concentrations) are given in Table S1 of the Supporting Informaiton, with spike recoveries for fortified bovine liver, avian plasma (crow, *Corvus* sp.), and pure extracted standards.

Results and Discussion

Analytical Technique. The technique described permits simultaneous analysis of nine common NSAIDs in animal tissue and plasma. Recoveries from spiked liver or plasma were >70% and >76%, respectively, in all cases (Table S1 of the Supporting Information). Recoveries and LOQs for meloxicam and to a lesser extent flunixin were particularly good in both matrices. We previously described a similar extraction and chromatographic method based on ref *1* that has been used in several studies regarding diclofenac (*3, 4, 29, 35*), meloxicam (*20*), and ketoprofen (authors unpublished data) individually. Full validation against an enzyme-linked immunosorbant assay (ELISA) method for diclofenac has also been reported (*36*); however, the ability to screen residues of nine NSAIDs simultaneously is obviously advantageous.

Figure S3 of the Supporting Information shows the total ion count (TIC) and SIM data for these NSAIDs. The SIM data were collected from a mixed spike into bovine liver and show that compounds eluted free of significant interferences at these target masses. Total run time was 30 min, with the nine NSAIDs eluting between 8.7 and 11.2 min. Possible structures of the primary fragments monitored (Table 1) are also shown, all of which are plausible, except perhaps for meloxicam. Although this primary fragment has been used elsewhere (*27*) and is certainly correct, its structure is perhaps less immediately obvious.

NSAID Residues Detected. A previous survey of 1848 carcasses from 67 sites in 12 Indian states undertaken between May 2004 and July 2005 (*3*) showed 10.1% (0.0–22.3% by state) of liver samples had detectable diclofenac residues (geometric mean = 352 μg kg^{-1}, range = 11–13723 μg kg^{-1}). Here, we have detected a highly comparable prevalence of 11.1% (318 μg kg^{-1}, 11–10088 μg kg^{-1}) across seven states, which varied from 4.1 to 16.4% by state. The prevalence from the 2004–2005 survey, excluding slaughter houses (not sampled here) was 11.4% and for the same seven states was 11.5%. The prevalence and concentrations of diclofenac found previously (2004–2005 survey) have been estimated to be more than sufficient to account for the observed rate

of *Gyps* vulture population decline, without the need to invoke any other causes (*13*). Because the prevalence and concentrations of diclofenac found here are so similar, it is clear that the level of diclofenac contamination remains incompatible with the recovery of vulture populations in India. The factors (sex, age, site type, species, and location) affecting the prevalence and concentrations of diclofenac in carcasses (2004–2005 survey) have been analyzed and discussed in detail previously (*3, 29*). As such, further analysis for this survey, of these factors, is not presented; however, for comparison, a detailed breakdown of the diclofenac (and meloxicam) data is given in Figure S1 and Tables S2 and S3 of the Supporting Information.

In terms of NSAIDs other than diclofenac, no comparable data currently exists. However, our results show that meloxicam is the second most common NSAID residue encountered (Table 2 and Figure S1 and Table S3 of the Supporting Information), being present in 4% of samples at levels between 11–1647 μg kg^{-1} (mean = 262 μg kg^{-1}). For vulture conservation, this is encouraging because meloxicam would seem to be well-established in at least some states within the Indian market and is already defined as being of low toxicity toward *Gyps* (*19*) and a broader range of avian scavengers (*18, 20*). Its use appears to be greater than diclofenac in Jammu and Kashmir and is also quite high (7.5% prevalence) in the Punjab, two states within the NW region of India (Figure S1 of the Supporting Information). Interestingly, these states were also the last to be surveyed (November–December 2006), 6–7 months after the ban on the manufacture of diclofenac was announced. Although further temparo–spatial data is obviously still required, this may indicate that the ban was beginning to have an effect in at least some Indian states by this point, and that meloxicam was also effectively replacing diclofenac as the NSAID of preference in these areas.

Ibuprofen was the third (0.6%, mean = 54 μg kg^{-1}, range = 14–681 μg kg^{-1}) and ketoprofen (0.5%, 403 μg kg^{-1}, 155–5,603 μg kg^{-1}) the fourth most common NSAID detected (Table 2), and there is currently little information regarding the safety of these drugs toward *Gyps*. Ketoprofen administered alone at therapeutic doses was not noted to cause mortality in avian species in a previous survey (*n* = 20 birds, 13 species, including 3 *Gyps* sp.) (*18*). In contrast, however, it apparently caused mortality in eiders (*Somateria fischeri, S. spectrabilis*) (*37*), and when given in combination with carprofen (which caused mortality alone), a *G. africanus* was noted to have died (*18*). Results from the same survey showed ibuprofen may also have caused mortality in one cinereous vulture (*Aegypius monachus*); however, more extensive data remains unavailable. Our results also indicate that the use of various NSAID combinations, including diclofenac, meloxicam, ibuprofen, and ketoprofen, is not uncommon. Or, that such drugs are being administered sequentially and separately, but because of variations in half-life of elimination,

TABLE 3. Concentrations (LC$_{50}$) of Diclofenac in Liver, Muscle, and Whole Carcass (Edible Parts)[a]

	feeding interval (meal size)	LC$_{50}$ (in μg kg^{-1}, using LD$_{50}$ of 98 μg kg^{-1})[b]	LC$_{50}$ (in μg kg^{-1}, using LD$_{50}$ of 225 μg kg^{-1})[b]
liver	daily (0.341 kg)	>1369 (2.29%)	>3143 (0.54%)
	every 2nd day (0.682 kg)	>685 (3.90%)	>1572 (1.95%)
	every 3rd day (1.023 kg)	>456 (4.91%)	>1048 (3.02%)
	every 4th day (1.364 kg)	>342 (5.78%)	>786 (3.70%)
whole carcass (edible parts)	daily (0.341 kg)	>3603 (0.34%)	>8272 (0.07%)
	every 2nd day (0.682 kg)	>1802 (1.61%)	>4136 (0.27%)
	every 3rd day (1.023 kg)	>1201 (2.69%)	>2757 (0.74%)
	every 4th day (1.364 kg)	>901 (3.36%)	>2068 (1.21%)
muscle	daily (0.341 kg)	>8054 (0.07%)	>18491 (0.00%)
	every 2nd day (0.682 kg)	>4027 (0.27%)	>9245 (0.07%)
	every 3rd day (1.023 kg)	>2685 (0.81%)	>6163 (0.20%)
	every 4th day (1.364 kg)	>2013 (1.28%)	>4623 (0.27%)

[a] Concentrations required to exceed two reported LD$_{50}$ values for *G. bengalensis*, depending on the feeding interval (meal size). [b] Values in parentheses give the percentage of carcasses found to exceed these concentrations across seven states in India in 2006.

multiple residues remain detectable within the same sample. Again, information regarding toxicity of combinations of these compounds to avian scavengers is completely lacking, and it is notable that in 0.9% of samples we detected drugs known to be safe and highly toxic (i.e, meloxicam and diclofenac, respectively).

Analyses also indicated suspected residues of naproxen in four samples (two from Gujarat, one from West Bengal, and one from Madhya Pradesh), indometacin in five (Madhya Pradesh), and flunixin in one (West Bengal). However, concentrations were very low and could not be conclusively confirmed with MS fragmentation. We did not encounter residues of carprofen or nimesulide. Previous work (*18*) has reported a 13% mortality for carprofen ($n = 40$, mortality in 5 individuals from 3 species) and 30% mortality for flunixin ($n = 24$, mortality in 7 individuals from 5 species) in a range of birds (raptors, storks, cranes, and owls). The toxicity of flunixin toward birds has also been highlighted elsewhere (*38, 39*). As such, these two compounds have been noted to be of particular concern (*18*). However, neither drug is thought to be available for veterinary use in South Asia at present (*18*), and our results seem to confirm this.

Phenylbutazone, aspirin, and analgin are also marketed in India for veterinary treatment (*32*). Limited data (*18*) suggest aspirin may be safe toward certain avian scavengers ($n = 3$ individuals from 3 species), but phenylbutazone may have caused mortality of one lappet-faced vulture (*Torgus tracheliotus*). We know of no information regarding the avian toxicity of analgin. In order to fully address the issue of NSAID residue availability and toxicity to vultures in India, it would be necessary to carry out further research on these compounds. The methods used here were not suitable for these drugs, and alternative analytical approaches should be developed or suitable existing techniques applied.

Prevalence and Levels of Diclofenac in Relation to Toxicity toward *Gyps*. The LD$_{50}$ for diclofenac toward *G. bengalensis* has been calculated at between 98 and 225 μg kg^{-1} (*4*) (depending on the toxicity data used from ref *1*), using oral dose experimental data and results from feeding trials where vultures were fed meat from slaughtered animals that had been given diclofenac within a few hours of death. Feeding experiments have also shown 100% mortality in vultures fed meat containing 6400 μg kg^{-1} (*1*), a level exceeded in this data set in 0.20% of carcasses (liver tissue). Also, a prevalence of between 0.13% and 0.75% of carcasses containing a lethal dose of diclofenac would be sufficient to account for the declines seen in vulture numbers in India over the last 17 years (*40*). Using information on the mean mass of *G. bengalensis* (4.75 kg) (*41*) and the amount of food needed per day to sustain these vultures (0.341 kg) (*19, 41*)

for feeding intervals of 1−4 days, it is possible to tabulate (Table 3) the estimated concentration (LC$_{50}$) in various tissues required to exceed the LD$_{50}$ values noted. Concentrations in Table 3 are calculated for liver, muscle (because this accounts for >50% of the edible tissue of a carcass) and then for the average concentration for the edible parts of the whole carcass (*13*). Calculations assume the concentration in muscle, and for the whole carcass are 17% and 38% of that detected in liver, respectively (*3, 13*). As discussed previously (*13*), in relation to 2004−2005 survey data, residues detected here remain at levels which would in many scenarios, give rise to *Gyps* receiving doses of diclofenac in excess of the LD$_{50}$ values available. As also noted previously (*3*), the residues detected here are sometimes so high (Tables S2 and S3 of the Supporting Information) that it appears livestock are commonly given doses well beyond those recommended in India. The recommended livestock treatment regime for diclofenac in India was three daily doses of 1000 μg kg^{-1} body weight. In the European cow (*Bos taurus*), 3 h after being given daily doses of 2500 μg kg^{-1} for six days, mean liver residues were 2874 μg kg^{-1} (*42*) and disposition in Indian cow is very similar (*35*). Yet, levels in liver detected here are >2874 μg kg^{-1} in 0.67% of samples tested and up to 3.5 times higher than this. Because vultures live and feed in large colonies, one carcass of an animal dosed well above previously recommended levels (that died within hours of treatment) would decimate that colony. Just as aspirin, paracetamol, and ibuprofen are extremely common in human medicine, so diclofenac has become extremely common in treating livestock in India. Residue prevalences of 11% may seem high, but livestock are extremely valuable resources in this region for the provision of milk and as working animals. If livestock are ailing, broad spectrum antibiotics, steroids, and NSAIDs such as diclofenac are relatively inexpensive and widely available in markets without the need to consult or use a qualified veterinarian. While diclofenac is not a curative drug, acting only to relieve pain, fever, and inflammation, it may temporarily relieve the signs of underlying sickness and thus be widely perceived as effective.

Data presented here are for samples taken over a nine month period, which commenced a few weeks before the Drug Controller General (India) announced a manufacturing ban on diclofenac. The Drug Controller stated that licensed wholesale manufacturing and marketing of diclofenac for veterinary use should cease within three months of May 11, 2006 (*17*). Hence, this survey is not strictly a post ban survey but should be viewed as a baseline (with 2004−2005 data) (*3*) against which future reductions in diclofenac and increases in the use of other NSAIDs can be measured. Large stocks of diclofenac exist within the Indian veterinary market

and, importantly, inexpensive injectable human formulations also remain widely available, which may well be used on animals. Illegal manufacture, distribution, black market trade, and/or importation from neighboring countries may also occur. In order to address such issues, the 2006 restrictions were recently strengthened (July 2008) to include a critical ban on the actual sale of diclofenac for veterinary purposes (*43*).

Beyond meloxicam, the safety of a wide variety of available NSAIDs and other common pharmaceutical groups (steroids, antibiotics, etc) toward avian and other nontarget scavengers is currently very poorly characterized. Recently, diclofenac dosing trials on turkey vultures (*Cathartes aura*) have shown it to be nontoxic in this particular species (*44*), and there is still significant debate as to how and why diclofenac is so highly toxic toward *Gyps* (*45, 46*) yet seems safe in at least one species of New World vulture. Further, concerns in relation to the long-term effects of antibiotic residues on vultures have also been raised recently (*47*). Although, the high toxicity of diclofenac toward *Gyps* demonstrates an unusual and perhaps extreme case of nontarget effects in wildlife, it also clearly supports calls (*48−50*) for a much increased level of environmental pharmacovigilance globally.

Acknowledgments

Supporting Information Available

Further breakdowns regarding the prevalences and concentrations of diclofenac and meloxicam detected and further details regarding the LC-ESI/MS methods used. This information is available free of charge via the Internet at http://pubs.acs.org.

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