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## CVMP assessment report under Article 30(3) of Regulation (EC) No 726/2004

On the risk to vultures and other necrophagous bird populations in the European Union in connection with the use of veterinary medicinal products containing the substance diclofenac

**Procedure no: EMEA/V/A/107**

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# 1. Background information on the procedure

## 1.1. Request for CVMP opinion

On 12 August 2014, the European Commission (EC) presented to the European Medicines Agency a request for an opinion from the Committee for Medicinal Products for Veterinary Use (CVMP), on a scientific matter concerning the risks to vultures and other necrophagous bird populations in the European Union in connection with the use of veterinary medicinal products (VMPs) containing the substance diclofenac, in accordance with Article 30(3) of Regulation (EC) No 726/2004.

## 1.2. Steps taken during the procedure

- During the September 2014 CVMP meeting, the following was agreed:
  - Boris Kolar was appointed rapporteur.
  - Michael Holzhauser-Alberti, Consuelo Rubio Montejano, and Johan Schefferlie were appointed co-rapporteurs.
  - The procedure started on 10 September 2014 CVMP and a timetable was adopted.
- A public consultation was started on 12 September 2014 in order to provide stakeholders with the opportunity to input any information or data that they consider may be helpful to the CVMP in reaching its opinion. The deadline for the provision of information and comments was 10 October 2014.
- The joint rapporteur's and co-rapporteurs' assessment report was circulated to all CVMP members on 3 October 2014.
- During the 7-9 October 2014 CVMP meeting the joint rapporteurs' assessment report was discussed and the Committee agreed to invite the marketing authorisation holder of veterinary medicinal products containing the substance diclofenac (Fatro S.p.A., Fatro Ibérica S.L.), and the bird conservation group BirdLife International to give a presentation to the Committee during the November plenary meeting to address outstanding issues.
- The revised joint rapporteur's and co-rapporteurs' assessment report was circulated to all CVMP members on 30 October 2014.
- During the 4-6 November 2014 CVMP Fatro S.p.A. and Fatro Ibérica S.L. and BirdLife International provided answers to questions of the CVMP.
- On 11 December 2014 the CVMP adopted an opinion in accordance with Article 30(3) of Regulation (EC) No 726/2004.

# 2. Scientific discussion

## 2.1. Introduction

In the European Union diclofenac, a non-steroidal anti-inflammatory substance, has been authorised for veterinary use since 1993 when the product Reuflogin was authorised in Italy for cattle and pigs, and for horses not intended for human consumption. Currently, VMPs containing diclofenac are

authorised in a limited number of Member States: Estonia, Italy and Spain for cattle, pigs and horses, and in the Czech Republic and Latvia for horses only. The marketing authorisation holder for these products is Fatro S.p.A, with the exception of the products in Spain which belong to the affiliated company Fatro Ibérica S.L.

The above mentioned products are indicated for reduction of inflammation and pyrexia in diseases of the respiratory system (e.g. bronchopneumonia), the genitourinary system (e.g. metritis) and mammary gland (e.g. mastitis), and musculoskeletal disorders (e.g. chronic and acute lameness, arthritis, desmitis, tendinitis, myositis).

Following the national authorisation in 2013 of two VMPs containing diclofenac (Dolofenac and Diclovet, respectively) by the Spanish competent authority, conservation organisations, members of the public and politicians wrote to the European Commission expressing their reservations on the risks that these products may represent to vultures and other necrophagous bird populations.

These concerns arose as a result of the decline on the vulture population in South Asia following the use of diclofenac to treat livestock in this region in the 1990s. Vultures were exposed to it by scavenging on livestock carcasses, and consequently died as a result of diclofenac-induced kidney failure.

The dramatic decline in vulture populations, which was estimated to be more than 95%, led in 2006 to the prohibition of the sale of VMPs containing diclofenac in India, Nepal, Pakistan and Bangladesh by the respective governments, while encouraging the development of safer alternatives and the use of substances that are less toxic to necrophagous birds.

The European Commission has requested the CVMP to give its opinion regarding:

- The risk that the use of VMPs authorised in the Union containing the substance diclofenac may represent to vultures and to other necrophagous birds in the Union, taking into account the EU rules on animal by-products;
- If a risk is identified, any actions or mitigation measures that could be implemented to manage effectively the risk.

This opinion is based on the evaluation by the Committee of data from published literature, answers provided by stakeholders during the public consultation including data received from the marketing authorisation holders, information from the presentations that the Committee received from Fatro S.p.A, Fatro Ibérica and BirdLife International, and personal communications.

## **2.2. Assessment approach**

The assessment of the risks to vultures and other necrophagous birds from the use of VMPs containing diclofenac, as a result of ingesting carcasses containing diclofenac residues in feeding stations, or through fallen stock in pastures, is not a standard environmental risk assessment (ERA). Guidance for an assessment of this type of scenario is not included in the CVMP/VICH guidelines on ERA for VMPs (VICH GL6 and VICH GL38 and the CVMP guideline in support of the VICH GLs 6 and 38).

Consequently, given that the CVMP guidelines for the assessment of environmental risks cannot be used for this particular ERA, and in the absence of suitable default parameters, the Committee decided to apply an *ad-hoc* approach by identifying the most suitable species to use as a model organism for the assessment, as well as determining the most adequate inter- and intraspecies extrapolation factors based on expert judgement.

The opinion not only takes into account the responsible use of veterinary medicinal products containing diclofenac in cattle, pigs and horses and the established withdrawal periods in cattle and pigs, but also considers other realistic scenarios of necrophagous birds feeding on carcasses containing diclofenac residues in feeding stations or fallen stock in pastures. Any potential misuse of veterinary medicinal products containing diclofenac has not been considered in the assessment.

Thus, for the assessment of the exposure of necrophagous birds to diclofenac from VMPs containing the substance authorised in the EU and the assessment of the risk arising thereof, the approach taken has been adapted accordingly to allow the development of an adequate and comprehensive opinion as requested by the Commission.

### 2.2.1. Species of concern

Populations of birds that display a necrophagous feeding behaviour in the EU have been considered species of concern. The metapopulations of necrophagous birds species (defined as a part of populations of the same species that are spatially separated but interact at some level) that might be potentially affected by diclofenac in Europe are mostly from the *Accipitridae* family, and in particular:

- Vultures: griffon vulture (*Gyps fulvus*), cinereous or black vulture (*Aegypius monachus*), Egyptian vulture (*Neophron percnopterus*), bearded vulture (*Gypaetus barbatus*).
- Eagles: species such as the Spanish imperial eagle (*Aquila adalberti*) and the golden eagle (*Aquila chrysaetos*), although not solely reliant on carrion, are endangered and toxicity of diclofenac could contribute to a decline of their populations.
- Kites: red kite (*Milvus milvus*) and black kite (*Milvus migrans*) are species that, as eagles, do not solely rely on carrion. Nevertheless, toxicity of diclofenac could contribute to a decline of their populations.

Table 1 lists the species considered for the risk assessment of diclofenac to necrophagous birds and also includes their conservation status based on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species. Table 2 lists the estimated metapopulation size of four species of vultures in European countries (Source: Vulture conservation fund-VCF and BirdLife International, public consultation). Dr José Tavares (Director of the Vulture Conservation Foundation) also provided in a personal communication data from 2014 on the numbers of breeding pairs of bearded vultures in the Alps, 26 in total – 3 in Austria, 9 in France, 4 in Italy and 10 in Switzerland.

Besides species from the family of *Accipitridae*, the following species are often seen on the feeding stations and, although they might be affected by diclofenac, have not been considered in this risk assessment given that mammalian carrion is not their main food source: common buzzard (*Buteo buteo*), different species of crows (*Corvus spp.*) and seagulls (*Larus spp.*).

**Table 1:** Necrophagous birds species considered for the risk assessment of VMPs containing diclofenac and their conservation status on the IUCN Red List of Threatened Species.

Species	IUCN global status	Species of European Conservation Concern (SPEC)	European Threat Status
Griffon vulture ( <i>Gyps fulvus</i> )	LC <sup>1</sup>	Non-SPEC <sup>a</sup>	Secure
Black vulture ( <i>Aegypius monachus</i> )	NT <sup>2</sup>	SPEC 1 <sup>b</sup>	Rare
Egyptian vulture ( <i>Neophron percnopterus</i> )	EN <sup>3</sup>	SPEC 3 <sup>c</sup>	Endangered
Bearded vulture ( <i>Gypaetus barbatus</i> )	NT	SPEC 3	Vulnerable
Spanish imperial eagle ( <i>Aquila adalberti</i> )	VU <sup>4</sup>	SPEC 1	Endangered
Golden eagle ( <i>Aquila chrysaetos</i> )	LC	SPEC 3	Rare
Red kite ( <i>Milvus milvus</i> )	NT	SPEC 2 <sup>d</sup>	Declining
Black kite ( <i>Milvus migrans</i> )	LC	SPEC 3	Vulnerable

<sup>1</sup> LC: least concern

<sup>2</sup> NT: near-threatened

<sup>3</sup> EN: endangered

<sup>4</sup> VU: vulnerable

<sup>a</sup> Non-SPEC: Species whose global populations are not concentrated in Europe, but which have a favourable conservation status in Europe

<sup>b</sup> SPEC 1: European species of global conservation concern i.e. classified as critically endangered, endangered vulnerable, near-threatened, or data deficient under IUCN Red List criteria at a global level.

<sup>c</sup> SPEC 3: Species whose global populations are not concentrated in Europe, but have unfavourable conservation status in Europe.

<sup>d</sup> SPEC 2: Species whose global populations are concentrated in Europe and which have an unfavourable conservation status in Europe.

**Table 2:** Estimated metapopulation size of the four vulture species present in Europe

Country	Egyptian vulture	Bearded vulture	Cinereous vulture	Griffon vulture
Albania	8 (2012)	0	0	0 (2012)
Bulgaria	20 (2012)	0	0	67 (2013)
Croatia	0	0	0	141 (2013)
Cyprus	0	0	0	2 (2013)
France	93 (2012)	46 (2012)	28 (2012)	1443 (2012)
Greece	10 (2012)	6-7 (2012)	28 (2012)	270 (2012)
Italy	8 (2012)	0*	0	92 (2012)
Macedonia	25 (2012)	0	0	16 (2012)
Portugal	83-84 (2004)	0	2-3 (2012)	197-361 (2008)
Serbia	0	0	0	130 (2012)
Spain	1300-1500 (2004)	134 (2012)	2068 (2012)	24609-25541 (2008)
Total	1547-1748	196-197	2126-2127	26967-28063

\*Please see text above on numbers of breeding pairs in the Alps in 2014.

### 2.2.2. Approach for the risk assessment

The Oriental white-rumped vulture (*Gyps bengalensis*) has been chosen as the model organism for the risk assessment given that laboratory and field toxicity data are available for this species. The results from the risk assessment performed on the Oriental white-rumped vulture are extrapolated to other species present in the European Union and considered to be at risk in connection with the use of VMPs containing the substance diclofenac.

#### General traits of vultures and other necrophagous bird species in Europe

Most vulture species in the European Union (Table 1) weigh between 6–14 kg, with the Egyptian vulture being the smallest and weighing less than 2.5 kg. Their wingspan can reach up to 3.1 m for the largest vulture (black vulture). Vultures in captivity have been known to live from 37 years (Egyptian vulture) to 55 years (griffon vulture), and they tend to lay one or two eggs during the reproductive season. The onset of reproduction is between the age of 4–8 years. For the eagles of Table 1, their weight ranges between 2.5–6.6 kg depending on the sex and species, they can live up to 57 years in captivity, reproduce at the age of 4–5 years old and lay 1–4 eggs per year.

Young adults of a number of vulture species are able to migrate large distances (up to thousands of km), before settling for their adult life. Their main food intake is from scavenging dead animals, however during food shortages they are able to attack and kill their prey, including livestock (Avery and Cummings, 2004)

Feeding behaviour: vultures are very efficient carcass hunters and consumers. Food intake for vultures (based on available data for griffon vultures) is approximately 500–1000 g of meat per meal, however they can eat up to 2000 g in a single feeding event, and store it in a skin pouch to eat it later or bring it to their hatchling. Vultures are able to find a carcass within 30 minutes after the death of the animal (Monsarrat *et al.* 2013). Given that most vulture species feed in groups, a carcass will be consumed (soft parts of the animal, including skin and small bones) by a relatively large number of birds in a short space of time. Indeed, this is one of the reasons why the population decline in the Indian subcontinent was so significant, and it was for instance estimated that <1% of carcasses containing diclofenac residues are able to cause a 99.9% decline in the Oriental white-rumped vulture population (Green *et al.* 2004). However, the estimates from Green *et al.* (2004) were based on the exclusive feeding of vultures from domesticated ungulates in India, and did not account for carcasses of wild ungulates. Hence, given that the latter do not contain diclofenac residues, this was acknowledged by the authors to have led to an overestimated death rate per meal and population decline rate.

### 2.2.3. Determination of the protection goal

The protection goal for the environmental risk assessment of VMPs is not specified in the relevant guidelines on environmental risk assessment or in the legislation (Directive 2001/82/EC). The CVMP/VICH Phase II environmental risk assessment guideline (VICH GL38: Environmental impact assessment for veterinary medicinal products (VMPs) Phase II) states that the protection goal of the environmental risk assessment “is to assess the potential for VMPs to affect non-target species in the environment, including both aquatic and terrestrial species.” In practice, environmental risk assessments address organism-level attributes of a population or community. However, as most of the species considered at risk from VMPs containing diclofenac are included in the IUCN Red List of Threatened Species, the defined level of protection for the current assessment has been established at the individual level (i.e. probability of death of a single individual). This is the common methodology

adopted internationally for assessing environmental risks to endangered species. The reason behind this approach is that for the conservation of endangered species following the reproduction strategy explained below, the protection of a metapopulation is achieved by protecting the population at the individual level.

Vultures and eagles are species whose populations fluctuate at or near the maximum population size that a particular environment can sustain. Species following this reproductive strategy (also called K-selected species) have relatively stable populations and produce low numbers of offspring. They are also characterised by long gestation/incubation periods, slow maturation (and thus extended parental care), and long life spans (up to 50 years for vultures). Endangered K-selected species, such as several vulture species, are particularly vulnerable and conservation plans for these species (included in the IUCN Red List of Threatened Species) need careful planning and are costly. For instance, at least 67 projects under the EU Programme for the Environment and Climate Action (LIFE) have focused on the conservation of vultures to date. Just over the 2008–2012 period, the EU invested 11 million euros in vulture conservation projects (Source: BirdLife International presentation to the CVMP). For instance, the estimated cost to breed a bearded vulture in captivity for reintroduction into the wild has been established at 70–80,000 euros (Frey, 1998). In the last few years, between 9 and 13 birds have been released every year in the three on-going bearded vulture reintroduction projects in the European Union, with yearly costs of about 650,000–900,000 euros (Vulture Conservation Foundation, public consultation).

## **2.3. Critical evaluation**

### **2.3.1. Toxicity of diclofenac to vultures and other birds**

#### **2.3.1.1. Toxicity of diclofenac to vultures**

The mechanism of toxicity to birds from diclofenac is related to the accumulation of uric acid in plasma which eventually leads to kidney failure (Naidoo and Swan, 2009). The differences in sensitivity between species might be related to differences in the half-life of the drug in different species. Indeed, the LD<sub>50</sub><sup>1</sup> values in vultures (as low as 0.225 mg/kg bw for the Oriental white-rumped vulture, Table 3) are 1–2 orders of magnitude lower than in mammals, for which reported LD<sub>50</sub> values range between 53–1500 mg/kg bw (in mice, rat, dog, rabbit and guinea pigs).

Known symptoms of diclofenac intoxication in vultures include lethargy, perch sitting with ruffled feathers, closed eyes and inability to raise the head and neck (dropped head). Within approximately 12 hours the bird enters a catatonic state and becomes highly dehydrated due to the onset of kidney failure. If the bird is stimulated, it attempts to prop its head up but as soon as the stimulus ends, the head drops again (Image 1). Diclofenac intoxication in vultures causes necrosis leading to reduced excretion of uric acid, renal failure and visceral gout, and death within a few days after exposure.

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<sup>1</sup> LD<sub>x</sub> (Lethal Dose x): dose required to kill a specified percentage (x) of a population after a given test duration.



Image 1: A vulture in a catatonic state as a result of diclofenac intoxication (image provided by Kerri Wolter, founder/manager of VulPro, South Africa).

As a result of the large number of deaths of vultures in the Indian subcontinent from feeding on carcasses of animals that had recently been treated with diclofenac, a number of toxicity studies were conducted to investigate diclofenac toxicity. Relevant and peer reviewed studies are summarised below:

#### Toxicity studies of diclofenac to *Gyps* species

- An acute toxicity study with 28 Oriental white-rumped vultures (*Gyps bengalensis*) (including 8 control birds) receiving single oral doses of diclofenac, either by oral administration of 0.25 and 2.5 mg/kg bw or by ingestion of carcass from goats or buffaloes treated with diclofenac (resulting doses ranged from 0.007 to 0.940 mg/kg bw), resulted in an LD<sub>50</sub> of 0.225 mg/kg. No deaths were reported for control birds (Green *et al.* 2007, Swan *et al.* 2006 based on the data from Oaks *et al.* 2004). The calculated LD<sub>10</sub> from the study of Swan *et al.* is 0.074 mg/kg bw, the LD<sub>5</sub> 0.054 mg/kg bw and the LD<sub>1</sub> is 0.030 mg/kg.
- In a study with two African white-backed vultures (*Gyps africanus*) and three griffon vultures (*Gyps fulvus*), all exposed birds died two days after receiving a single dose of 0.800 mg/kg bw, while no deaths were reported for control birds (Swan *et al.* 2006).
- In a similar study as the one by Swan *et al.* 2006, conducted by Naidoo *et al.* (2009) with two Cape griffon vultures (*Gyps coprotheres*), a single dose of 0.800 mg/kg bw caused the death of both birds within 48 hours.

#### Toxicity studies using field data on *Gyps* species

- In a study by Schultz *et al.* (2004) a number of Oriental white-rumped vultures (*Gyps bengalensis*) and long-billed vultures (*Gyps indicus*) found dead in the field in India with confirmed extensive visceral gout had hepatic and renal concentrations of diclofenac ranging from 0.004 to 0.16 mg/kg. Diclofenac could not be quantified (detection limit of 0.001 to 0.002 mg/kg) in those dead vultures not showing signs of visceral gout or renal failure. These studies provide a strong indication for the link between mortality of vultures in the field and exposure to diclofenac, which was indeed verified by controlled exposure of vulture to diclofenac in other studies (e.g. by Oaks *et al.* 2004).

- In an isolated event a single injured Himalayan vulture (*Gyps himalayensis*), weighing 6.5 kg died two days after being administered 3.8 mg/kg bw diclofenac intramuscularly to treat an injury (Das *et al.* 2011).
- Toxicity concentrations of diclofenac to slender-billed vultures (*Gyps tenuirostris*) and long-billed vultures (*Gyps indicus*) have not been quantified, but the toxicity of the drug to these species is also confirmed by the resulting collapse by more than 95% of the populations in India, Pakistan and Nepal, as reported from other *Gyps* species (e.g. Naidoo and Swan, 2009; Das *et al.* 2011).
- Oaks *et al.* (2004) reported that 85% of a sample of 259 dead Oriental white-rumped vultures (*Gyps bengalensis*) from Pakistan showed grossly apparent urate deposits, characteristic of visceral gout and renal failure. From a subsample of all birds found dead with visceral gout, the only visible histopathological lesion was severe acute tubular necrosis and uric acid crystal formation in the kidneys and other tissues. In all these birds, the renal concentration of diclofenac ranged from 0.051 to 0.643 mg/kg.

#### Toxicity studies to other vulture species

- The Egyptian vulture (*Neophron percnopterus*), and the red-headed vulture (*Sarcogyps calvus*) showed a strong population decline in the period of 2000–2003 (Cuthbert *et al.* 2006) in India. This decline was reported later than that of the *Gyps* populations. This delayed population decrease can be attributed to the fact that *Gyps* species are the first species in the sequential order to scavenge on the carcasses. Thus, only after the population of *Gyps* species collapsed, other species were able to reach and to scavenge the carcass when a significant amount of meat was available, and thus were exposed sufficiently to carcasses containing diclofenac to cause the observed population decline. Shortage of food, persecution and other chemical contaminants were considered unlikely. Given the evidence for diclofenac as cause of the observed decline for the *Gyps* species, exposure to diclofenac is considered as a more likely explanation than disease.
- To examine whether American vultures are equally sensitive to diclofenac as Eurasian vultures, Rattner *et al.* (2008) exposed five Turkey vultures (*Cathartes aura*) to increasing concentrations of diclofenac ranging from 0.08 mg/kg to 2.5 mg/kg bw. After 7 days no deaths were reported, and after 3 weeks vultures were re-dosed with a single oral dose of 2.5 to 25 mg/kg bw. No mortality occurred amongst control and treated animals, and there were no signs of overt toxicity. The results indicate that Turkey vultures are less sensitive to diclofenac than the species from the *Gyps* genus, the Egyptian vulture and the red-headed vulture. The lower sensitivity to diclofenac of this species is also characterised by the lower uric acid levels in the plasma of the exposed organisms.

#### Toxicity of diclofenac to other necrophagous birds

- Two dead steppe eagles (*Aquila nipalensis*) found at a cattle carcass dump in India showed the same histopathological lesions as those observed in the *Gyps* species. Concentrations of diclofenac in the kidneys, estimated by enzyme-linked immunosorbent assay (ELISA) were 0.051 mg/kg. This concentration is similar to the lethal concentrations observed in the field for Oriental white-rumped vulture (*Gyps bengalensis*) and long-billed vultures (*Gyps indicus*) (Sharma *et al.* 2014). This study shows that it is likely that not only species from the genus *Gyps* are very sensitive to diclofenac, but also other necrophagous birds from the family *Accipitridae* might show similar sensitivities.
- No chronic data for toxicity of diclofenac to necrophagous birds or other bird species are available.

### 2.3.1.2. Toxicity of diclofenac to other birds

- Broiler chicks (*Gallus gallus*, 15 days old), pigeons (*Columba livia*, 3 months old), Japanese quail (*Coturnix japonica*, 4 weeks old) and mynah (*Acridotheres tristis*, independent young) were orally exposed to diclofenac at doses of 0 (control), 0.25, 2.5, 10 and 20 mg/kg bw for seven consecutive days. Mortality was observed up to two weeks after administering the last dose. The LD<sub>50</sub> calculated with a log-logistic model from the presented results was 4.1 mg/kg bw for broiler chicks and 15.6 mg/kg bw for pigeons. For these two species a significant reduction in body weight at all doses was also observed. For Japanese quail and mynah toxicity was observed only in organisms exposed to the 2 highest dosages, thus with LD<sub>50</sub> >20 mg/kg bw (Hussain *et al.* 2008).
- Naidoo *et al.* (2007) administered a single intramuscular doses of 0.6 to 10 mg/kg bw of diclofenac to hens (18 weeks old). The LD<sub>50</sub> was 9.8 mg/kg. A concentration of 5 mg/kg bw lead to 33% mortality.
- Reddy *et al.* (2006) administered a single intramuscular dose of 5 mg/kg bw in poultry (6 weeks old) of both sexes. A 40% mortality was recorded for this dose.
- In a study with White Leghorns (6 weeks old) diclofenac was administered at oral doses of 2 and 20 mg/kg bw (Jain *et al.* 2009). The control group and lowest treated group had a 100% survival, whereas only 50% of the treated birds survived in the highest treated group after 12 hours. According to the authors, a 7-day repeated dose exposure would lead to a LD<sub>50</sub> of about a factor of 5 lower than the LD<sub>50</sub> from single dose studies.
- Groups of six pied crows (*Corvus albus*) were dosed by a single oral gavage with 0.8 or 10 mg diclofenac /kg bw. None of the birds died or showed overt signs of toxicity. The no observed adverse effect level (NOAEL) is therefore higher than 10 mg/kg bw diclofenac. The low toxicity is combined with a fast elimination as well as a low direct toxicity to renal and hepatic cells (Naidoo *et al.* 2011).

### 2.3.1.3. Field data in Europe

In the public consultation the Italian Veterinarian Association (Federazione Nazionale Ordini Veterinari Italiani, FNOVI) reported that two vultures kept and fed in captivity were found dead with visceral gout (Zucca *et al.* 2003), but no additional *post mortem* investigations on the cause of death were conducted, thus it can not be concluded whether the cause of death was intoxication by diclofenac or any other substance.

### 2.3.1.4. Conclusion on toxicity

The field and experimental data confirm the high susceptibility of all *Gyps* species to diclofenac leading to renal failure and death as a result of increased uric acid concentrations in plasma, after exposure to low concentrations of diclofenac.

Studies on other organisms show that this substance can be toxic to other necrophagous bird species from the *Accipitridae* family, such as the Egyptian vulture (*Neophron percnopterus*), the red-headed vulture (*Sarcogyps calvus*) and the steppe eagle (*Aquila nipalensis*). Thus, it is important to consider that although data are missing for other species, those in the same family might also be at risk (for instance the golden eagle (*Aquila chrysaetos*)). On the contrary, other species of vultures appear to be rather insensitive, such as the New World vultures.

An overview of the derived LD<sub>50</sub> values with equivalent concentrations in food is presented in Table 3. The LD<sub>50</sub> values show that some species of vultures are highly sensitive to diclofenac with the lowest reported LD<sub>50</sub> value of 0.225 mg/kg bw for *Gyps bengalensis*, however similar doses (0.25 mg/kg bw/day) in other bird species did not lead to lethal effects but caused weight loss to exposed organisms (broiler chicks and juvenile pigeons).

**Table 3.** LD<sub>50</sub> values of diclofenac for different species of birds

Species name	Scientific name	LD <sub>50</sub> [mg/kg bw]	LC <sub>50</sub> [mg/kg feed]
Oriental white-rumped vulture	<i>Gyps bengalensis</i>	0.225	1.0
Griffon vulture	<i>Gyps fulvus</i>	<0.80	
African white-backed vulture	<i>Gyps africanus</i>	<0.80	
Cape Griffon vulture	<i>Gyps coprotheres</i>	<0.80	
Turkey vulture	<i>Cathartes aura</i>	>25	
Pied crow	<i>Corvus alba</i>	>10	
Chicken	<i>Gallus gallus</i>	4.1	32.8
Pigeon	<i>Columba livia domestica</i>	15.6	
Japanese quail	<i>Coturnix japonica</i>	>20	
Mynah	<i>Achridotheres tristis</i>	>20	

The only available LD<sub>50</sub> for necrophagous birds (derived for the Oriental white-rumped vulture) is 0.225 mg/kg bw, for a single exposure, which equals 1.045 mg of diclofenac/bird applying the average weight of 4.75 kg (Green *et al.* 2007) for the Oriental white-rumped vulture. With a meat consumption of 1.023 kg per meal (Green *et al.* 2007), the diclofenac residue concentration in food at the LD<sub>50</sub>, i.e. the LC<sub>50</sub> is 1.044 mg/kg diclofenac residues in food.

Using the LD<sub>10</sub> of 0.074 mg/kg bw calculated from the same study by Green *et al.* (2007), the derived LC<sub>10</sub> is 0.343 mg/kg food (concentration in food at which 10% of the exposed animals would die). The LD<sub>50</sub> and the LD<sub>10</sub> were derived from the (modelled) dose-response relationship based on the acute oral toxicity study in Oriental with-rumped vultures in which the mortality was measured at different dose levels.

Similarly, for 1% of the animals, the lethal concentration LC<sub>1</sub> is calculated to 138 µg/kg (from the estimated LD<sub>1</sub> is 0.030 mg/kg), which is about a factor of 10 below the LC<sub>50</sub>.

To protect all avian species in an ecosystem, normally the following assessment factors are considered, based on a 5-day laboratory exposure on avian species (Technical Guidance Document on Risk Assessment, Part II):

**Table 4.** Applicability of assessment factors to current risk assessment

Factor	Explanation	Is it applicable to the risk assessment of diclofenac to necrophagous birds?
3	To extrapolate from caloric differences between laboratory food vs. food ingested in the field.	No, the risk assessment for vultures is based on the dose of diclofenac taken up with the total amount of ingested food, and not in the type of diet.

Factor	Explanation	Is it applicable to the risk assessment of diclofenac to necrophagous birds?
10	To extrapolate from acute LC <sub>50</sub> to the concentration that will cause low/non-significant mortality	No, as the dose-response curve and the EC <sub>10</sub> <sup>2</sup> from the study by Swan <i>et al.</i> 2006 can be used.
10	To extrapolate from acute no effect levels to chronic no effect levels	No, chronic exposure of vultures to low concentrations of diclofenac has not been considered a realistic scenario for the assessment, as feeding with contaminated meat is viewed as a seldom event.
10	To extrapolate the data from the most sensitive species to other species	Yes, there are no comprehensive studies on other necrophagous birds, and data from other species from the family <i>Accipitridae</i> , to which most European scavengers belong, show signs of high sensitivity to diclofenac
10	To extrapolate the data to potentially more sensitive species life-stages	Yes, often no data on nestlings are available or other more vulnerable life stages.

Based on the protection goal determined for this assessment, i.e. on the protection of individuals and not at population/community level, to ensure the safety of all potentially exposed scavengers an assessment factor of 100 (10 to account for species to species variability x 10 to account for variability in species life stages) on the lowest LC<sub>10</sub> should be applied. This approach is in accordance with current risk assessment practices in the European Union.

Consequently, the maximum concentration of residues of diclofenac in tissue to ensure the safety of vultures would result in a value of 3 µg/kg in tissue (LC<sub>10</sub> 0.343 mg/kg/100).

### 2.3.2. Diclofenac residues in food-producing animals

For treatment, the approved dosage of VMPs containing diclofenac in cattle is 2.3 mg of diclofenac per kg bw for 1–3 days. In the case of acute lameness a dose of 1.15 mg of diclofenac per kg bw for 3 days may be used. The dosing regimen in pigs is 2.3 mg of diclofenac per kg bw for 3 days, and in horses 2.3 mg diclofenac per kg bw for 3–5 days.

To estimate the amounts of diclofenac that necrophagous birds can be exposed to by feeding on contaminated carcasses from treated animals, the residue concentrations in the organs and tissues of these animals must be known, as well as the feeding behaviour of the birds. Residue studies were provided by the marketing authorisation holder using the commercial product at the maximum dose (2.3 mg/kg) and duration (3 days) in pigs and cattle. These studies are relevant for the risk assessment. Other residue depletion studies as summarised in the MRL Summary Report on diclofenac were performed with a different formulation and therefore the results are less representative for the field situation and consequently not taken into account in the risk assessment.

Typically, in residue studies, the target food-producing animals are treated with the product at the maximum dose and duration in accordance with the product label. At different time points after

<sup>2</sup> EC<sub>x</sub> (Exposure concentration x): exposure to a chemical that is required to kill a specified percentage (x) of a population after a given test duration

treatment, groups of animals are slaughtered and edible tissues are analysed for the presence of residues.

### 2.3.2.1. Diclofenac residues in cattle

A residue study in cattle was carried out using the commercial formulation; animals were slaughtered at 8, 10 and 12 days after the final injection. From this study it becomes clear that at any time after treatment, the highest residue concentrations of diclofenac are found in the sites (muscle tissue) where the injections were given. The first slaughter time point was 8 days after treatment, at which residue concentrations in the injection sites were up to approximately 500 µg/kg. The residues in the injection sites declined rapidly to less than 3 µg/kg at 10 days after treatment. The residues in other tissues were much lower and declined to undetectable levels at 12 days after treatment, except for fat. In fat, diclofenac appeared to deplete very slowly, although the residue levels were generally below 1 µg/kg. In Table 5, the residue concentrations in all tissues are given.

**Table 5:** Residues of diclofenac (µg/kg) in cattle tissues after three intramuscular injections

Group (Slaughter time point)	Animal identification	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Muscle (µg/kg)	Last Injection site Core sample (µg/kg)	Last Injection site Surrounding sample (µg/kg)
<b>Group 1</b> (8 days)	092	5.5	<LOQ	1.28	5.9	467.3	2.7
	782	<LOQ	<LOD	2.72	<LOD	9.6	<LOQ
	790	5.1	5.3	1.49	12.0	42.1	5.8
	823	7.4	10.9	2.71	<LOQ	453.6	43.5
<b>Group 2</b> (10 days)	176	<LOQ	<LOD	0.71	<LOD	2.6	<LOQ
	657	<LOQ	<LOD	0.76	<LOD	<LOQ	<LOQ
	770	2.7	<LOD	0.64	<LOD	2.9	<LOQ
	817	3.3	<LOD	1.02	<LOQ	3.3	<LOQ
<b>Group 3</b> (12 days)	080	<LOD	<LOD	0.62	<LOD	<LOQ	<LOQ
	180	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ
	791	<LOD	<LOD	0.64	<LOD	3.0	<LOD
	793	<LOD	<LOD	0.77	<LOD	<LOQ	<LOQ

As mentioned above, the worst-case residue concentrations of diclofenac are found in the injection sites. These sites will be eaten by necrophagous birds. It is unfortunate that there are no residue data of injection site muscle tissue between day 0 and day 8. To overcome this problem for the risk assessment, the Committee estimated the depletion of the diclofenac concentration in injection sites using the amount administered, 500 mg per injection site (bw of the animals was around 200 kg), and the maximum amount of residues at 8 days (approximately 500 µg per injection site), and using a mono-exponential depletion model. The following residue concentrations were estimated:

**Table 6:** Estimated amounts of diclofenac residues (mg) present in the injection sites of treated cattle

Time after injection (days)	Amount at injection site (mg)
0	500
1	210
2	88
3	37
4	16

Time after injection (days)	Amount at injection site (mg)
5	6.5
6	2.7
7	1.1
8	0.5

It should be noted that the animals in this study weighed approximately 200 kg, and because the dose is (linearly) adjusted to bodyweight, heavier animals receive greater amounts of diclofenac in the injection site. Moreover, the target animals are treated on three consecutive days, therefore three injection sites per carcass will be available for consumption. In addition, injection sites may overlap, which could result in higher local residue concentrations.

Information on residue concentrations in injection sites related to the situation in South-East Asia was not available. However, Green *et al.* (2004) showed that liver samples taken from cattle carcasses in India were quite high: between 1 and 100 mg/kg in approximately 5% of the samples. Figure 1 depicts the distribution of residue findings in cattle liver.

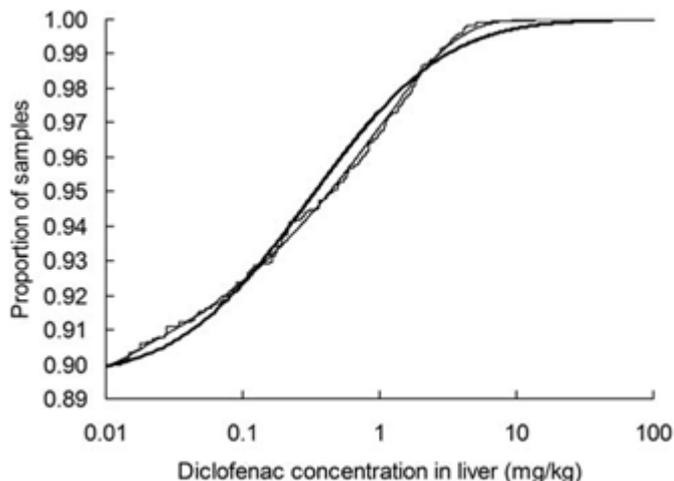


Figure 1. Distribution of residue concentrations of diclofenac (mg/kg) found in cattle carcasses in India (Green *et al.*, 2004)

This small percentage of very high residue concentrations might reflect the residue status of animals treated very shortly before dying. It is quite difficult to compare these data to the data of the product, as it is not possible to estimate the concentrations in the liver between days 0 and 8. However, in a radiolabel study from the MRL Summary report it seems that the diclofenac residue concentrations in liver were around 0.6 mg/kg at day 3. Therefore it appears that the data from Green *et al.* do not contradict the results from controlled residue studies.

#### 2.3.2.2. Diclofenac residues in pigs

A residue study in 12 pigs, slaughtered at 3, 7 and 9 days after the last injection with the commercial formulation, showed that the residue concentrations were highest in the injection sites: up to approximately 900 µg/kg at day 3. The residue concentrations were much lower in other tissues: up to approximately 170 µg/kg in liver and kidney, 17 µg/kg in fat and skin, and 13 µg/kg in (non-injection

site) muscle. At 9 days after treatment, diclofenac was undetectable in all tissues including the injection sites. The residue concentrations in all tissues are given in Table 7 below.

**Table 7:** Residues of diclofenac ( $\mu\text{g}/\text{kg}$ ) in pig tissues after three intramuscular injections

<i>Groups (Slaughter time point)</i>	<i>Animal identification</i>	<i>Liver (<math>\mu\text{g}/\text{kg}</math>)</i>	<i>Kidney (<math>\mu\text{g}/\text{kg}</math>)</i>	<i>Fat+skin (<math>\mu\text{g}/\text{kg}</math>)</i>	<i>Muscle (<math>\mu\text{g}/\text{kg}</math>)</i>	<i>Injection site Core sample (<math>\mu\text{g}/\text{kg}</math>)</i>	<i>Injection site Surrounding sample (<math>\mu\text{g}/\text{kg}</math>)</i>
<i>Group 1</i> <i>3 days</i>	5	63.5	57.8	11.94	6.0	457.3	13.5
	6	98.1	141.8	17.01	7.1	687.6	3.7
	15	171.0	171.8	13.53	14.2	891.7	18.3
	16	65.4	59.2	11.98	5.9	932.4	12.9
<i>Group 2</i> <i>7 days</i>	1	2.6	<LOQ	1.88	<LOQ	5.4	4.7
	2	14.4	7.4	<LOD	<LOQ	3.2	<LOQ
	4	7.1	11.5	3.22	<LOQ	3.9	3.3
	7	4.1	8.3	0.63	<LOQ	3.5	<LOQ
<i>Group 3</i> <i>9 days</i>	11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	12	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD
	13	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	14	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD

It should be noted that up to 150 mg diclofenac was administered per injection site (bodyweight up to 60 kg). Because the dose is expressed as mg/kg bw, heavier animals will receive greater amounts of diclofenac in the injection site. Moreover, the target animals are treated on three consecutive days, therefore three injection sites per carcass may be available for consumption. In addition, injection sites may overlap, which could result in higher local residue concentrations.

It was noted that there are no residue data between treatment and three days after treatment, however it appears to be unrealistic that animal carcasses will be fed to vultures immediately after treatment. Thus, the Committee considered that a 3-days period between treatment and exposure to birds would represent a worst-case scenario.

### **2.3.2.3. Diclofenac residues in other species**

No residue depletion data were available in other food-producing species treated with the authorised veterinary products. During the stakeholder presentations at the CVMP, Fatro S.p.A and Fatro Ibérica S.L reported that they have no knowledge of the products being used under the cascade. BirdLife International stated that they have verbal reports from veterinarians indicating that this product is used in goats and sheep. The extent of the use was not known, and no detailed information is available regarding this statement.

Diclofenac is authorised for horses not intended for human consumption (3-5 day treatment). As such, no MRLs or withdrawal periods have been established for this species and no residue data are available. As non-food producing horses are generally categorised as farm animals, their carcasses might be taken to feeding stations.

### **2.3.2.4. Conclusion on residues**

From the data available it is clear that the worst-case residue concentrations are found in the injection sites of cattle and pigs, and that the amount present in injection sites is correlated to the bodyweight

of the animals. Because cattle are the heaviest animals that are treated with the authorised injectable product, the data from cattle can be considered as worst-case exposure data.

In Europe, it is not very likely that animals will be treated with diclofenac immediately before they die (See 2.3.3.2 Fallen stock) and hence, it is very unlikely that animals would be available for consumption by birds immediately after being treated. The Committee considered that a 3-day period between treatment and exposure to birds would represent a worst-case scenario.

The reasonable worst-case amount of diclofenac in the injection sites available for consumption by birds would be approximately 37 mg in cattle and 0.9 mg in pigs.

At 10 days after treatment of cattle, the residue concentrations in all tissues were below 3 µg/kg.

At 9 days after treatment of pigs, the residue concentrations in all tissues were no longer detectable.

The residue data in cattle carcasses found in the Indian subcontinent do not contradict those found in the residue studies above.

No data were available in other food-producing species or in horses not intended for human consumption.

### **2.3.3. Considerations on exposure routes of vultures and other necrophagous birds to diclofenac residues**

Necrophagous birds fill an important niche in European and Mediterranean ecosystems. By feeding on dead animals necrophagous birds are placed at the top of the food chain. For centuries, they have played an important role in extensive farming by sanitation of pasture sites and preventing the spread of disease. Disposing of dead animals in the carcass dumps has been the traditional way of removing dead animals from households and farms in the Mediterranean and in the Balkan countries, countries inhabited also by necrophagous birds and scavenging carnivores (bears, wolves, jackals). The main purpose of carcass dumps is that it is an inexpensive and easy way for removing dead animals, as well as to feeding and hence preventing large carnivores (mainly bears) from attacking economically important livestock such as sheep, goats and other animals on the pasture.

In 2002, and after the outbreak of Bovine Spongiform Encephalopathy (BSE), Regulation (EC) No 1774/2002 (repealed by Regulation (EC) No 1069/2009 and its implementing Regulation (EC) 142/2011) set new rules concerning the disposal of animal by-products not intended for human consumption, and also defining special feeding purposes of animal by-products, including those for necrophagous birds. This regulation set strict rules for the disposal of animal carcasses which before then could be left in fields or taken to dumps and entered the vultures food chain. With the new restrictions implemented by Regulation (EC) No 1774/2002 the strict removal of carcasses from rural areas led to a very severe impact on necrophagous birds populations, given that their food sources became considerably reduced. The lack of available carcasses in fields led to not only large numbers of starving birds, but also changes in the birds' behaviour.

As a result of the impact of Regulation (EC) 1774/2002 to vulture and other wildlife populations, and to facilitate the resolution of this problem, the European Commission adopted Decisions 322/2003 and 830/2005, which lay down derogation conditions to allow feeding of the endangered necrophagous bird populations in Portugal, Spain, France, Italy and Greece. These Decisions are implemented in Spain through Royal Decree No 664/2007 of 25 May 2007, which defines the health and safety conditions required of necrophagous bird feeders. However, the application of the measures authorised in the Decisions cited above and expressed in the Royal Decree can only very partially replace the enormous

quantity of food lost through the compulsory removal from fields of livestock carcasses (Council of the European Union, 2007).

Implementing Regulation (EC) No 142/2011 is specifying special feeding rules, general requirements and species of necrophagous birds in each Member State, the feeding of certain species in feeding stations, as well as wild animals outside the feeding stations.

In the EU two main potential routes of exposure of necrophagous birds to diclofenac used in VMPs have been reported by stakeholders from replies to the public consultation on this issue. These routes are:

- Exposure at feeding stations
  - from animal by-products from slaughterhouses,
  - from animals that have died from natural causes taken to feeding stations by farmers.
- Exposure through fallen stock.

### **2.3.3.1. Exposure at carcass dumps/feeding stations**

#### From animal by-products from slaughterhouses

Since 2002, and as a result of bovine spongiform encephalopathy (BSE), carcass dumps are not permitted in EU Member States, with the exception of bird feeding stations and stations for feeding of large carnivores. Bird feeding stations must be registered and supervised, and all carcasses used in the feeding stations have to have the adequate documentation stating the history of treatment of the animal and allowing for traceability to the farm. If a slaughter house does not have the correct documentation for an animal, this animal or its by-products cannot be taken to a feeding station. Nevertheless, it should be noted that currently diclofenac is not considered a substance of risk, thus control of diclofenac residues or any other special measures are not in place.

#### From animals that have died from natural causes taken to feeding stations by farmers

Stakeholders have reported that these feeding stations provide livestock owners responsible for these sites an easy and inexpensive way to dispose of their carcasses. However, many of these stations are poorly managed (IUCN Vulture Specialist Group, public consultation).

It is also important to consider that although carcass dumps are not allowed in the EU, a number of stakeholders mention that these still exist, as they represent a traditional and, more importantly, a cheap alternative of disposing of dead animals (Vulture Conservation Foundation – VCF, public consultation).

#### Examples of feeding stations in EU Member States

##### **Italy, the northern Adriatic and southern Alps**

Italy has the following feeding stations which use (mainly) domesticated animals (Source: Federazione Nazionale Ordini Veterinari Italiani-FNOVI, public consultation)

- Reserve Cornino Friuli for griffon vulture and golden eagle (other meat source: pig by-products)
- Velino Regional Park Sirente CFS Abruzzo for griffon vulture and golden eagle
- Pollino National Park for griffon vulture and golden eagle
- Regional Park Nebrodis Sicily for griffon vulture

- National Park Gran Sasso Laga, there is a feeding station with veterinary supervision (other meat source: mutton and goat)
- Montio Sibillini National Park is about to set up a feeding station.

Additionally, the Natural Park of the Belluno Dolomites is working on setting up a new station.

The station in Cornino (Riserva Naturale Regionale Lago di Cornino) receives up to 200 bird visits. Moreover, metapopulations from neighbouring countries (e.g. from Croatia) can fly more than 200 km and thus feed from this station, as well. The feeding station is mostly visited by griffon vultures from the Italian and Kvarner (Croatia) populations (Figure 2). However, bearded vultures from Italian, Austrian and probably Swiss metapopulations can also be seen on the site. Other regular visitors are cinereous vulture, Egyptian vulture, golden eagle, red kite, black kite, ravens, crows and seagulls. In the year 2013, 46.6 tons of meat were used in this station (from which 60–70% were pig carcasses from local farms), the rest was game collected from car collisions (Figure 3). In this station sheep and goat carcasses represent a minor source of meat used.

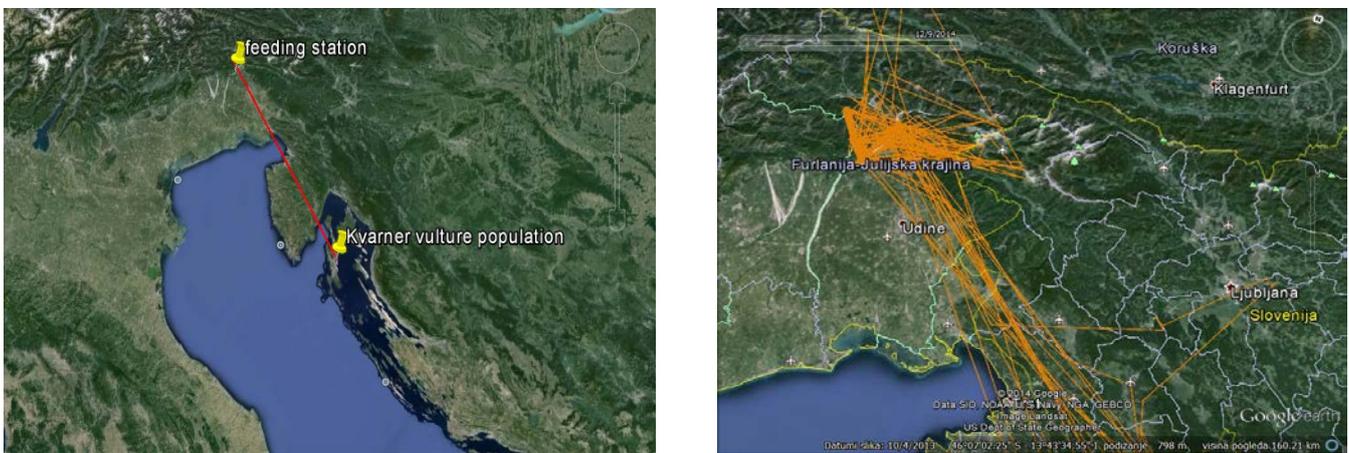


Figure 2: The Kvarner population is regularly flying over 200 km to search for carrion at the feeding station in Cornino. Tracks of satellite tracking of birds (Sušić, 2014, unpublished data).

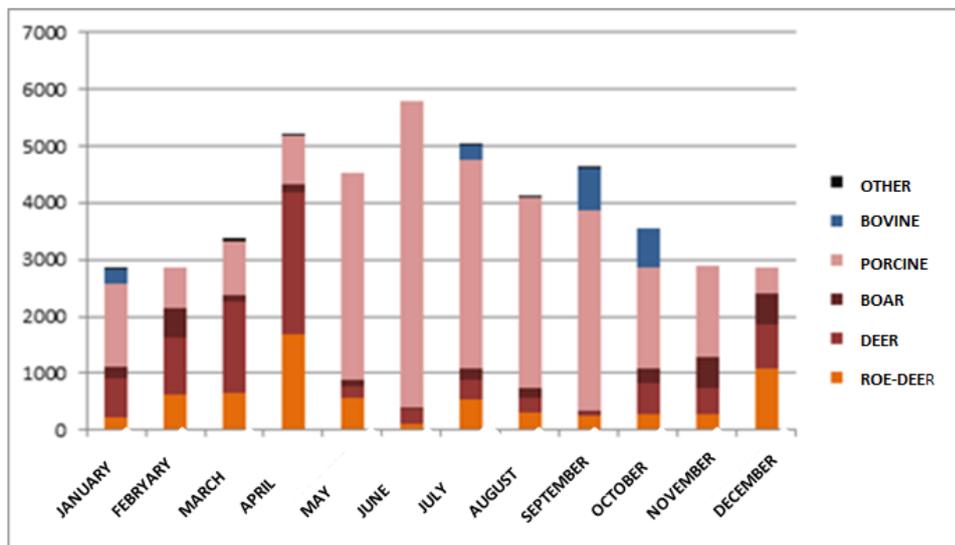


Figure 3. Animal species used on the feeding station in Cornino (Sušić 2014, unpublished data)

## **Spain**

Since 2011, the Royal Decree 1632/2011 is regulating the feeding of certain wildlife species with animal by-products not intended for human consumption. Feeding is regulated in fenced areas (called 'muladares') where carcasses are placed. Muladares are managed by governmental institutions, local communities, bird conservation organisations, or farmers. These areas are established for the feeding of birds of prey, and meat from different animal species is used, including cows and pigs. Feeding outside the dumps is permitted in extensive farming, as the Royal Decree 1632/2011 allows farmers to leave fallen stock in fields (not fenced) for protecting and promoting endangered or fragile populations of necrophagous bird species.

Spain has 235 registered feeding stations (Ministerio de Agricultura, public consultation).

## **France**

The traditional carcass dumps similar to the 'muladares' in Spain are not developed in France, where smaller individual feeding areas are preferred (around 200 in France). In such places, farmers are allowed to dispose of the dead animals from their own extensive farms, to lower the cost of disposal. Feeding stations are subject to authorisation. Only small ruminants (these are in practice essentially sheep) can be disposed of in such stations.

This system is efficient for providing complementary feeding to scavengers and it is also appreciated by the farmers in those areas where knackery premises are expensive.

There are also 8 feeding stations in France (associated with reintroduction programs) but they are of small sizes and are provided only with dead animals (small ruminants) collected from extensive farms.

## **Greece**

Greece has 3 active stations (Dadia forest, Meteora, Crete Island). Vultures are fed animal by-products from slaughter houses (mainly pig entrails). Fattening pigs are inspected by the authorities for drug residues. Unauthorised people are not allowed in the feeding stations.

### **2.3.3.2. Fallen stock**

Fallen stock refers to those animals that die in open pastures from natural causes and are left in fields to be disposed by vultures and other wild animals (Cuthbert *et al.* 2014). This route of exposure was the common one in the Indian subcontinent. Indeed, in India, the use of the product and numbers of dead animals fallen in open pastures differ from the numbers and treatment conditions in the European Union. Firstly, in India cattle can be used for milk production but the slaughter for human consumption is restricted or prohibited by religious practices in certain areas. In these areas there is a restriction on euthanasia on holy cows, hence it was common to use diclofenac as a palliative treatment to alleviate pain in old animals that may die shortly after. Dead cattle were disposed of by vultures and other wild animals as they were left in open spaces. However, it should be noted that the collapse of the vulture populations also occurred in other countries on the Indian subcontinent (i.e. Nepal, Bangladesh and Pakistan) where human consumption of cattle meat is not restricted by religious practices. On the other hand, in Europe, although the possibility of death of an animal kept in extensive pastures very soon after diclofenac treatment is low (given the number of consecutive treatments needed, the fact that animals are kept indoors or in a smaller plot during the treatment, and low probability of death for the targeted treatments), this possibility cannot be excluded. For example, diclofenac may be used in young animals for respiratory infections as a complementary treatment to an antibacterial. These animals may have a higher risk of mortality within 10 days after treatment and may be left in the field

or be taken to a carcass dump. Another difference that should be considered is also the difference in population sizes between the European Union and the Indian sub-continent. While in Asia vultures numbered millions, in Europe many of the necrophagous bird species are endangered and populations sometimes number only a few hundred birds. Therefore even though overall exposure can be low, the impact could be as significant.

In Europe, extensive farming is common in the Iberian, and Apennine Peninsula in the Alps, on the western Mediterranean and Adriatic islands, in the Dinaric Mountains, on the Balkan Peninsula and in the Aegean Sea. In these areas animals spend part or most of the year out in the field (common practice for rearing sheep, goats and donkeys). In some cases animals are not recovered, as they might get lost and die, be stolen, fall (from cliffs, etc.), be attacked by large carnivores or simply die on the field. In some uninhabited Adriatic Islands sheep are released to graze, but just lambs are gathered. If an animal dies, necrophagous birds are likely to find and eat its carcass in the field in a very short space of time.

Seasonal grazing in mountain pastures is common practice in the Alps, mostly for cattle. Cattle are taken to the high pastures at the beginning of the season, where they have extensive and unfenced areas for grazing. Usually the herd is led by the most experienced cow. In this situation, if an animal dies, it is left on the spot (the main cause of death is that they fall from cliffs). In grazing fields, animals do not belong to a single owner, but are collected from an entire village and taken together to a high pasture. Veterinary care can be provided during the winter season and before the herd leaves for seasonal grazing in mountain pastures.

In some countries, animals are reared in big enclosures. In Spain, for instance, half of the cattle is kept in open pastures. In this scenario, if an individual animal dies it can easily become a source of food for necrophagous birds, given that the large extension of land they inhabit would make it almost impossible for the animal to be found and disposed of in a short space of time.

A similar realistic scenario of exposure of necrophagous birds to carcasses could be considered for the free-range reared Iberian pig.

The IUCN Vulture Specialist Group provided information in the public consultation indicating that a vulture was found dead in Spain in 2012 with concentrations of a different NSAID in its tissues (flunixin), and also with severe visceral gout. This supports the hypothesis that the exposure routes described above are realistic. Moreover, in Spain diclofenac and flunixin are recommended for treatment of the same livestock species and for similar ailments (although VMPs containing flunixin are single dose treatments and might be favoured in extensive farming). Nevertheless, both products have similar withdrawal periods.

#### Description of extensive systems

The extensive systems are usually common in poor agricultural regions (Bernués *et al.* 2011), with big extensions of pasture land and minimum management of animals. The main features of these extensive systems are limited use of technology, low productivity per animal and per surface unit, feeding based on grazing and agricultural by-products, use of regional breeds and little use of chemicals (Boyazoglu 1998, Beaufoy *et al.* 1994). The extensive production should not be confused with the free-range breeding systems where animals are kept outdoors but in small plots, supplemented with feedstuff and in more controlled conditions.

In extensive systems (also known as low-input, agro-pastoral or grazing systems) the death of unattended animals that might become a food source for necrophagous birds is considered a realistic

exposure scenario. It is not possible to predict, at EU level, the amount of diclofenac treated stock that die before the diclofenac residues are below 3 µg/kg (estimated to 9–10 days after treatment for pigs and cattle, respectively), and that are consumed by necrophagous birds.

#### Expected use of VMPs containing diclofenac

The indications of the VMPs containing diclofenac are the following:

- Acute respiratory conditions.
- Acute metritis and mastitis.
- Lameness and other musculoskeletal disorders.

These conditions are more likely to appear in intensive production systems due to the existence of predisposing factors (e.g. overcrowding, high productivity index, presence of dust, ammonia or high moisture rate), which are absent in extensive production systems (e.g. animals have lower risk of mastitis). Furthermore, as the VMP has to be administered by injection daily for 3–5 days, it would be necessary to keep the animals enclosed in a pen during the treatment. There are other anti-inflammatory drugs for the same indications that can be administered in a single dose, which would be more suitable in extensive production systems.

Moreover, it should also be considered that the fallen stock scenario proposed in the report considers a risk if the animal dies before diclofenac is metabolized under a safe level. In principle, the conditions intended to be treated with this VMP have conditions that are unlikely to lead to death, but if diclofenac is used in young animals for respiratory infections as a complementary treatment to an antibacterial, a higher risk of mortality within 10 days after treatment and disposal to carcass dumps cannot be excluded.

The off-label use of products containing diclofenac in sheep and goats under the so-called cascade is expected to be very low at present. The economic value of these individual animals is low, hence individual treatments for these species are expected to be rare. The VMPs containing diclofenac are not suitable for herd treatment and, if treatment of larger number of animals would be necessary, single dose products would be preferred.

According to the marketing authorisation holders of the VMPs containing diclofenac (Fatro/Fatro Ibérica) across the EU/EEA most of the product is sold for treating cattle (up to 60%), and pigs (30%). The remaining 10% is sold for the treatment of horses. The majority of the product (90% for cattle and pigs) is reported to be used in intensive farming in EU/EEA countries (IFAH Europe; public consultation), whereas the remaining 10% would be used in grazing animals living freely. In the EU/EEA the use of the VMPs containing diclofenac has increased considerably and indeed, since the marketing authorisation of the two products in Spain in 2013, the sales volumes in the EU have tripled (Source: Fatro/Fatro Ibérica presentation to CVMP). Regardless, it cannot be foreseen what trends could be expected in sales volumes in the future and whether VMPs containing diclofenac would be authorised in other Member States and this assessment only evaluates the present situation.

The VMP is administered once a day during 3 days to cattle and pigs and during 3–5 days to horses. It should be dispensed under veterinary prescription. Administration should be conducted exclusively by the veterinarian (in the case of intravenous administration) or under the veterinarian's supervision. However, the use mainly in intensive farming is disputed by those stakeholders that state that in several countries, including Spain, up to 50% of cattle stay in large, open enclosures all-year-round. In the Iberian Peninsula, pigs are often kept in open pastures. Additionally, some stakeholders consider

that in Italy and in Spain the number of fallen stock is highly underestimated. According to the official statistics in Italy (Ministry of Health, animal register) and also the presentation from BirdLife International, during a period of 12 months a total of 29681 cattle have been deleted from the national database, yet based on the official database, 19230 of them have been lost in the field, and approximately 80% of those lost in the field are missing in areas of great biodiversity, also used for the establishment of feeding stations. No official statistics for deletions in national databases of pigs, sheep and goats are available.

As mentioned in section 2.2.2, only a very small proportion of diclofenac-treated carcasses has been estimated to trigger a decline of a metapopulation of vultures. Indeed, a simulation model from Indian vulture demography demonstrated that a very low number of contaminated carcasses in feeding stations (between 1:130 and 1:760) could lead to the extinction of an entire population (Green *et al.* 2004).

Moreover, the foraging behaviour of vultures is very effective and the average time for a vulture to find a fallen animal on a field/feeding station is an average of 31 minutes after the animal dies or is placed on the feeding station (Vulture Conservation Foundation - VCF; public consultation). Consequently, it is likely that necrophagous birds will feed on carcasses long before they are found by the farmers (European Association of Zoos and Aquaria; public consultation).

### **2.3.3.3. Conclusion on exposure**

In the European Union, there are two main scenarios in which necrophagous bird species can become exposed to diclofenac residues in feed: a) exposure at feeding stations and b) exposure through fallen stock. Although in Europe, compared to the Indian subcontinent, the likelihood of death of an animal kept in extensive pastures very soon after diclofenac treatment is very low (given the number of consecutive treatments needed, the fact that animals are kept indoors or in a smaller plot during the treatment, and low probability of death for the targeted treatments), this possibility cannot be excluded as discussed above.

In relation to the two main routes of exposure of necrophagous birds to diclofenac used in VMPs in the European Union the following was considered:

- **A. Exposure at feeding stations:**

Spain has 235 registered feeding stations (Ministerio de Agricultura, public consultation), which can be managed by governmental institutions, local communities, bird conservation organisations, or farmers. Seven feeding stations are registered in Italy (Federazione Nazionale Ordini Veterinari Italiani - FNOVI; public consultation), in nature reserve areas and at National Parks. Carcasses are mainly from domesticated animals and game (road-kills). In France, smaller individual feeding areas are preferred (around 200 exist) where farmers are allowed to dispose of the dead animals from their own extensive farms. There are also 8 carcass dumps in France (associated with reintroduction programmes) but they are of small size and are provided only with small ruminants collected from extensive farms. In Greece there are 3 feeding stations. These stations are stocked with animal by-products from slaughterhouses, mainly pig entrails.

In the European Union, official veterinarians are assigned to some feeding stations, and in order to identify those animals or by-products unsuitable for feeding vultures they have to rely on medicines records. Not all feeding stations have official veterinarians.

#### *A.1 Exposure from animal by-products from slaughterhouses:*

Animals that are intended for human consumption are sent to slaughterhouses. Animal by-products from slaughterhouses can be then used in feeding stations. The withdrawal periods for these species are 15 days for cattle and 12 days for pigs. Therefore, based on the data provided animals taken to slaughterhouses (after the withdrawal period is completed) should have residue concentrations below 3 µg/kg in all animal by-products, and therefore be safe to enter the food chain of necrophagous birds.

#### *A.2 Exposure from animals that have died from natural causes/illnesses and are not sent to slaughterhouses:*

In intensive production and on pastures animals can die from natural causes. As mentioned, in principle the conditions intended to be treated with a diclofenac-containing VMP are conditions that are unlikely to lead to death. However, diclofenac may be used in young animals for respiratory infections as a complementary treatment to an antibacterial and the risk of mortality within 10 days after treatment of these animals might be higher. These carcasses may be left in the field or be taken to a carcass dump by the farmer to avoid higher disposal costs. It is also important to note that horses not intended for human consumption are generally categorised as farm animals and their carcasses must be promptly disposed of at an approved site, in the same way as any other farm stock. Diclofenac might be used in this species for palliative purposes and the risk of mortality for treated animals might be higher within days after being treated.

- **B. Exposure from fallen stock:**

Based on the information reported by stakeholders, different views exist on whether exposure of necrophagous birds to diclofenac through fallen stock is a likely route of exposure. There are discrepancies on whether animals can be treated with diclofenac before they are sent for extensive pasture or while they are on an extensive pasture. Common practice seems to indicate that when animals are sick, they are predominantly contained in pens rather than being treated and being left on pastures, as the treatment and the follow up of the animal's condition would not be feasible to monitor in large open spaces (IFAH-Europe; public consultation). Extensive farming is common in the Mediterranean region and in the Alps. In these areas, animals spend part or most of the year out in the field. In the Alps, animals are let on summer pastures. In other countries, animals are kept in open enclosures all the year around. In Spain alone, about the half of the 6 million beef cattle stock is bred in open enclosures. On the Iberian Peninsula, sometimes pigs are reared in open enclosures. A potential exposure route for necrophagous birds could be considered from fallen free range pigs from extensive pastures (e.g. Iberian pig), fallen cattle, horses living in large enclosures or semi-wild bred, and from donkeys, sheep and goats which die on the pasture.

The above exposure scenarios are considered realistic. In particular, and as mentioned above, the exposure of vultures or other necrophagous birds to carcasses from fallen stock after their treatment with diclofenac is possible, given that it has been reported that in Spain a vulture was found dead with concentrations of a different NSAID in the tissues (flunixin), and also with severe visceral gout (Zorrilla *et al.* 2014). How this wild bird was exposed to flunixin is unknown. In Spain, diclofenac and flunixin are recommended for treatment of the same livestock species and for similar ailments.

## 2.3.4. Risk assessment

### 2.3.4.1. Risk characterisation

As described under section 2.3.1.4, with a meat consumption of 1.023 kg per meal and an average weight of 4.75 kg for Oriental white-rumped vulture (Green *et al.* 2007), the diclofenac residue concentration in food at the LD<sub>50</sub>, i.e. the LC<sub>50</sub> (diclofenac concentration in food at which 50% of the exposed animals would die) is 1.044 mg/kg diclofenac residues in feed. The calculated LC<sub>10</sub> is 0.343 mg/kg, and the LC<sub>1</sub> is 0.138 mg/kg.

### 2.3.4.2. Assessment of the actual risk

The actual risk for the Oriental white-rumped vulture can be determined by comparing the LC<sub>1</sub> (138 µg/kg food) with the exposure levels from the residue depletion studies. As the residue concentrations in cattle present the worst case, the risk assessment is focussed on cattle. The concentrations of diclofenac in cattle (injection site muscle in particular) are above the LC<sub>1</sub> in the Oriental white-rumped vulture, until 10 days after treatment.

At 8 days after treatment, the amount of diclofenac at injection sites is still higher than the LC<sub>10</sub>, which is the concentration established to cause the death of 10% of Oriental white-rumped vultures if they were to eat 1.023 kg of injection site muscle. No data are available for the period between administration and 8 days after treatment.

Taking the estimated amount at the injection site into account (Table 6), it can be assumed that up until 7 days after treatment, the diclofenac concentration at the injection site is higher than the LD<sub>50</sub>, which is the dose established to cause the death of 50% of the birds.

From 10 days after injection onwards, the concentrations in all tissues are well below the LC<sub>1</sub>. It should be noted that the LC<sub>1</sub> should *not* be regarded as a safe level, it is an estimate of 1% mortality, which can have further consequences on (meta)population level. It can be concluded that the Oriental white-rumped vulture is at risk, in particular when cattle have been treated less than 10 days before being consumed by these birds.

The actual risk for other necrophagous birds cannot be derived directly from the data. However, assuming that the sensitivity of other necrophagous birds is similar (other vulture species in particular, see also table 3), it can be estimated that the risk for mortality will mainly exist within the first 10 days after treatment of cattle. Therefore, it is concluded that other scavenging birds, especially vulture species, are also at risk, in particular when cattle have been treated less than 10 days before being consumed by these birds.

It should be noted that the residue data were generated with 200 kg cattle. Treatment of heavier cattle will result in higher and perhaps more persisting injection site residue concentrations. This means that in practice the period of risk can be longer.

### 2.3.4.3. Assessment of risk for other necrophagous bird species and all age groups

The assessment of the actual risk for the Oriental white-rumped vultures presented above does contain some uncertainty, because there is no information on the differences in sensitivity between bird species and on chronic toxicity. In order to take these uncertainties into account, the assessment of the potential risk makes use of assessment factors. The outcome of the assessment of the potential

risk is a concentration of diclofenac in the tissues of carcasses that can be considered as safe for necrophagous birds.

As explained in section 2.3.1.4 above, an assessment factor of 100 should be applied to the LC<sub>10</sub> to safeguard all potentially affected necrophagous birds, resulting in a concentration of 3 µg/kg feed that can be assumed to be safe to these birds.

These values can be compared to the residue values (see section 2.3.2), in brief:

For residues in cattle, data show that at the injection site and in liver, levels are higher than the safe value of 3 µg/kg food until 10 days after the last administration. (The animals received three doses, however the injection site data are from the third injection only and would therefore also cover a single administration). For the other tissues, residues after 8 days are below 3 µg/kg.

For pigs, except for the muscle tissue, the safe concentration of 3 µg/kg is exceeded 7 days after treatment. From 9 days after the final injection onwards, the residue concentrations of diclofenac were below 3 µg/kg in all tissues.

Because the dose is expressed as mg/kg bw heavier animals of all species will receive greater amounts of diclofenac in the injection site than the ones used for the studies. Moreover, the target animals are treated on three consecutive days, therefore three injection sites per carcass may be available for consumption. In addition, injection sites may overlap, which could result in higher local residue concentrations.

#### **2.3.4.4. Discussion on risk**

Based on the calculations above, in the worst case diclofenac residues in animal carcasses or animal by-products would be at levels which are toxic for necrophagous birds for at least 10 days following treatment of the animal.

The VMPs containing diclofenac authorised in the EU are indicated for cattle, pigs and for horses not intended for human consumption.

Considering the above, the following two main scenarios are established for the risk assessment:

- **A. Exposure at feeding stations:**

*A.1. Use of slaughterhouse material in feeding stations:*

If there is compliance with the withdrawal periods for cattle and pigs of 15 and 12 days, respectively, there is no risk for vultures feeding on slaughterhouses' animal by-products from these two species in feeding stations, considering that by-products will mainly consist of organs and not of injection sites. With regard to use of the VMPs in species for which they were not authorised ('cascade' use), the standard withdrawal period to be applied is at least 28 days. Despite the lack of data in other species, it could be assumed that this withdrawal period would also be safe for necrophagous birds. Additionally, sheep and goats are usually farmed extensively and the considerations on this practice described below would equally apply to those species.

*A.2. Exposure through dead treated animals in intensive production transported to feeding stations:*

The risk of exposure of necrophagous birds to diclofenac is considered high in situations where animals have been treated with diclofenac and die in intensive farming practices. For example, diclofenac can be used in young animals for respiratory infections, as a complementary treatment to an antibacterial. In this situation, an animal can die in farms shortly after treatment, before the residue levels of

diclofenac have depleted below 3 µg/kg (the level which is considered safe for necrophagous birds). Also, non-food producing horses are generally categorised as farm animals and diclofenac might be used in this species for palliative purposes, with a higher risk of mortality. Therefore animal carcasses or by-products with toxic levels of diclofenac might be disposed of by farmers in carcass dumps.

- **B. Exposure through fallen stock in extensive production:**

Some extensive producing farms can be authorised to leave dead animals in the field for the consumption of carrion birds. These carcasses are not controlled. The Federation of Veterinarians in Europe also confirmed that the monitoring of fallen stock in remote areas is very difficult. Animals that are kept in pastures are considered less likely to be treated with diclofenac given that the conditions diclofenac is prescribed for are not common for animals under this farming practice. Moreover, if animals happened to show the conditions intended to be treated with diclofenac-containing VMPs, these have conditions that are unlikely to lead to death. In addition, the dosing regimen of the products, which requires injecting the animal on three consecutive days, might not be practical in free grazing animals, except when the farms have adjacent small plots where they keep and treat diseased animals. Overall, this would contribute to the infrequent use of VMPs containing diclofenac in extensive farming conditions and thus reduce the risk from exposure of necrophagous birds to toxic levels of diclofenac residues in fallen stock in the field. A risk would exist in situations where extensively reared animals are treated with VMPs containing diclofenac and die in the field or get lost and subsequently die. A vulture might spot the carcass within 30 minutes, whereas it might take the farmer a day or two to discover it.

#### **2.3.4.5. Conclusion on risk**

The only available LD<sub>50</sub> for necrophagous birds (derived for the oriental white-rumped vulture) is 0.225 mg/kg bodyweight, for a single exposure. From the dose response data an LC<sub>1</sub> of 138 µg/kg food is derived, which can be used to determine the actual risk for the Oriental white-rumped vulture when compared with the exposure levels from the residue depletion studies. In summary, from 10 days after injection onwards, the concentrations of diclofenac in all cattle tissues, which is considered the worst-case scenario, are well below the LC<sub>1</sub> for cattle weighing below 200 kg of which the injection sites do not overlap. For heavier animals and for animals to which injections are given in overlapping injection sites, this period will be longer. It should be noted that the LC<sub>1</sub> should *not* be regarded as a safe level, it is an estimate of 1% mortality, which can have further consequences on a metapopulation level. It can be concluded that the Oriental white-rumped vulture is at risk, in particular when cattle have been treated less than 10 days before being consumed by these birds.

The actual risk for other birds cannot be derived directly from the available data. However, assuming that the sensitivity of other birds is similar (the other vulture species in particular, see also table 3), it can be estimated that the risk for mortality will mainly exist within the first 10 days after treatment of cattle weighing less than 200 kg and not having overlapping injection sites. Therefore, it is concluded that other scavenging birds, especially vulture species, are also at risk, in particular when cattle have been treated less than 10 days before being consumed by these birds. The actual risk assessment presented above does contain some uncertainty, because there is no information on the differences in sensitivity between bird species and on chronic toxicity. In order to take these uncertainties into account, the potential risk assessment makes use of assessment factors. The outcome of the potential risk assessment is a concentration of diclofenac in the tissues of carcasses that can be considered as safe for necrophagous birds. When an assessment factor of 100 is applied to the LC<sub>10</sub> to safeguard all potentially affected necrophagous birds, a concentration of 3 µg/kg food results, which can be assumed

to be safe to these necrophagous birds species, also a level of residue achieved after 10 and 9 days for cattle and swine, weighing less than 200 and 60 kg, respectively, and of which the injection sites do not overlap. For heavier animals and/or animals with overlapping injection sites, these periods will be longer.

Considering the above, the following scenarios are established for the risk assessment:

- **A.1. Use of slaughterhouse material in feeding stations:** If there is compliance with the withdrawal periods for cattle and pigs of 15 and 12 days, there is no risk for vultures feeding on animal by-products of cattle and pigs from slaughterhouses used in feeding stations, considering that the animal by-product consists mainly of organs and not of injection sites.
- **A.2. Exposure through dead treated animals in intensive production transported to feeding stations:** The risk of exposure of necrophagous birds to diclofenac is considered high in situations where animals have been treated with diclofenac and die in intensive farming practices shortly after treatment and before the levels of diclofenac have reached the safe concentration of 3 µg/kg.
- **B. Exposure through fallen stock in extensive production:** Some extensive producing farms can be authorised to leave dead animals in the field for the consumption of the carrion birds. A risk would exist in situations where extensively reared animals are treated with VMPs containing diclofenac and die in the field or get lost and subsequently die, in which case a vulture might spot the carcass within 30 minutes.

With the existing information it is not possible to determine if, to date, in the European Union any vultures have been intoxicated from eating carcasses from food-producing species that have been treated with VMPs containing diclofenac. Pharmacovigilance reports of diclofenac-related deaths of necrophagous birds are not easily obtained if such event would occur, given that if a necrophagous bird is found dead, the diagnosis of the cause of death is complicated by the fact that birds intoxicated with diclofenac will not die close to the contaminated carrion, as would be the case for acute poisoning. Indeed, the intoxicated animal might die a few days after exposure and a considerable distance away from the contaminated carcass. Moreover, *post mortem* examinations determining tissue residue levels of chemicals and toxins are usually not conducted on wildlife. This is a major data gap for the Committee's conclusion.

However, although pharmacovigilance data on diclofenac are not available for events in any of the above scenarios, other available data indicates that the suggested scenarios are realistic, as a report available from a vulture intoxicated with tissue residues of an NSAID and found dead in the south of Spain indicates that exposure from contaminated carcasses to necrophagous birds can happen (reported by IUCN Vulture Specialist Group).

It should be borne in mind that a very small proportion of diclofenac-treated carcasses is needed to trigger a decline of a metapopulation of vultures. The study on the modelled impact of intoxication revealed that a very low number of contaminated carcasses in feeding stations (between 1:130 and 1:760) can have serious effect on the metapopulation of the birds.

## 2.3.5. Risk management and risk mitigation measures

### 2.3.5.1. Risk management measures in place in EU Member States

The risk management measures that are in place in the EU and also those under discussion are presented below. The information has been obtained from:

- The responses given by those EU countries for which the use of Category 1 material has been granted for the feeding of vultures and that replied to the Commission request to provide information (by 1 August 2014), on potential derogations according to Article 18 of Regulation (EC) No 1069/2009, associated implementing measures and measures in place to avoid the exposure of vultures to diclofenac.
- The product information on authorised VMPs containing diclofenac that the Member States provided to the Agency.
- The assessment report carried out by the Spanish Ministry of Agriculture, Food and Environment and the Spanish Medicines Agency: "Analysis of the risk of using veterinary medicines with diclofenac in relation to the populations of vultures in Spain".
- Information from the United Kingdom's Veterinary Medicines Directorate.

Based on the above, the risk management measures listed in table 8 are already in place or under discussion in the certain Member States.

**Table 8:** Overview of risk management measures in Member States

Member State	In place/under discussion	Risk management measure
Bulgaria	Under discussion	Avoid the authorisation of any VMP containing diclofenac.
Cyprus	In place	Animal by-products of animals treated with VMPs are not permitted for use in feeding stations.
France and Italy	Under discussion	Create a list of substances (including diclofenac) banned for use in animals which will be disposed of in feeding stations. <i>Italy only.</i> Carrying out of official checks on the place of origin of the by-products used in feeding stations to ensure that no by-products containing drug residues are used (diclofenac, aceclofenac, flunixin and enrofloxacin) to avoid exposure.
Italy	In place	Include risk mitigation measures in the product literature of authorised products: <ul style="list-style-type: none"> <li>• providing advice regarding the use in pasture animals limiting the graze of treated animals</li> <li>• forbidding the use in non-authorised species</li> </ul>
Italy and Spain	In place	Include risk mitigation measures in the product literature of authorised products in order to prevent the use of bodies or parts of bodies containing residues of diclofenac for the feeding of wild animals.
Portugal	In place	Restrict the use of animal by-products and derived products coming from intensive farming in feeding stations.

Member State	In place/under discussion	Risk management measure
Spain	Under discussion	Establishing a monitoring programme in feeding stations to assess the presence of diclofenac in by-products. Additionally, the distribution of an explanatory note to the prescribers (the VMP is a prescription-only medicine) informing of the risks to vultures and the conditions of use.
United Kingdom	In place	Import/export restrictions - not approve any requests from veterinarians to import products containing diclofenac or issue any export certificates which name diclofenac-containing products in the list of products to be exported.

### 2.3.5.2. Other considerations on risk management measures

#### The toxicity potential of NSAIDs other than diclofenac

A review of a total of 34 veterinarians and institutions responses providing information on over 870 cases of NSAID treatment for 79 species of birds including necrophagous birds as well as owls, storks and carinas reveals that meloxicam did not show toxic effect to treated birds. There was uncertainty regarding the safety of ketoprofen and dexamethasone and more information would be required. High toxicity was observed in treatment with diclofenac, carprofen and flunixin (Cuthbert *et al.* 2007).

The CVMP also noted the following Opinions from the Council of the European Union (2007) and the United Nations Environmental Programme (2014):

#### Problem of necrophagous birds in Spain because of shortage of food: a serious threat to Biodiversity (Council of the European Union, 2007)

“Spain has the largest populations of necrophagous birds of prey in Europe and, in the case of certain globally endangered species, the largest in the world. ... All these species are substantially dependent on carrion from livestock, be it from animals that die in the fields, with a territorial distribution similar to that of wild ungulates, or from waste dumps and feeders where carcasses of farm animals were thrown out. This source is important for young and unskilled animals and for feeding chicks in the nest. The effect that Regulation (EC) No 1774/2002 laying down health rules concerning animal by-products not intended for human consumption had on the number of available carcasses in fields has been very significant, in that it has deprived them of one of their main food sources. ... The options offered by the derogations from Regulation 1774/2002 are necessary but not sufficient to ensure the survival in favourable conditions of necrophagous species in Spain, some of which are endangered worldwide. ... A network of artificial feeders, however dense it might become, could never replace the supply of carcasses of extensively farmed livestock. ... In short, solutions must be sought such that human health protection measures do not bring in their wake avoidable negative effects on the populations of species which are protected by national and Community legislation and for the protection of which significant resources are being used. ... Add to this the problem of the artificial nature of the feeders and their effect on the behaviour of the birds; birds of prey have very large minimum habitat areas, which frequently involve movements of 100 km or more in a straight line. In these circumstances, it is not desirable for these birds, some of which are endangered, to be concentrated in large numbers in very particular areas, where they are undoubtedly more vulnerable to uncontrolled hunting by unscrupulous hunters or, worse still, users of poisoned baits. It is preferable to retain an uneven and unpredictable spread of carrion, so that necrophagous birds have to keep up their food search and

detection skills. Networks of feeders designed for highly endangered species like the bearded vulture are another matter; they need these additional food provisions (mainly bones) to maintain viable population levels.”

United Nations Environmental Programme/Conservation of Migratory Species of Wild Animals (2014)

The United Nations Environmental Programme (UNEP) published in November 2014 a review and guidelines to prevent the risk of poisoning of migratory birds. In this guideline a number of legislative and non-legislative recommendations are proposed to address the risk of veterinary pharmaceuticals, particularly NSAIDs (with special mention to diclofenac), to migratory birds. The report highlights the impact diclofenac could have by seriously jeopardizing the last remaining large populations of vultures in the EU (95% of the total numbers of vultures in Europe being in Spain) given that in this country, new regulations (Royal Decree 1632/2011) allow livestock carcasses to be consumed by wild scavengers in the field or at supplementary feeding stations, thus being directly consumed by vultures from dead cattle. The same strategy of allowing livestock carcasses being offered to consumption by wild vultures would be of key importance also for the remaining and critically endangered Italian populations of European griffon, Egyptian and bearded vultures.

*Non-legislative recommendations include:*

1. Enhance surveillance of ungulate carcasses in high risk areas for diclofenac: Vulture Safe Zones should be introduced (a focus on breeding sites), with the aim is to secure a 100 km diameter diclofenac-free (and other harmful NSAIDs) area. Presently, there are seven provisional safe zones across Nepal, India and Pakistan and none in the high-risk area of Bangladesh.
2. Raise stakeholder awareness on alternatives to diclofenac: promote product stewardship and voluntary withdrawal of NSAIDs toxic to scavenging birds

*Legislative recommendations include:*

1. Prohibit the use of veterinary diclofenac for the treatment of livestock and substitute with readily available safe alternatives, such as meloxicam.
2. Introduce mandatory safety-testing of NSAIDs that pose a risk to scavenging birds, including multi-species testing using in-vitro and read-across methods, with burden of proof on applicant; VICH/OECD to evaluate and provide guidance on wider risks of veterinary pharmaceuticals to scavenging birds.

## **2.4. Considerations of the CVMP**

The CVMP is of the opinion that based on field and laboratory data available from the Oriental white-rumped vulture, and residue data available from the marketing authorisation holder for cattle and pigs, a risk is identified for vultures feeding on carcasses of food-producing animals that have been treated with diclofenac within 10 days (or longer) before their death (section 2.3.4), given that from 0-10 days the concentration of residues in diclofenac treated food-producing animals are expected to be above 3 µg/kg. Tissue residues of diclofenac below 3 µg/kg in food-producing species have been assessed to pose no risk to necrophagous birds. However, this conclusion is based on data for cattle below 200 kg and pigs below 60 kg and with no overlapping injection sites. Heavier animals and/or animals with overlapping injection sites will need a longer time period for residue depletion to below 3 µg/kg, but it is unknown how much longer.

The crucial point in the assessment is whether it is likely that necrophagous birds in the EU will be exposed to animal carcasses within 10 days (or longer) after treatment with diclofenac. Evidence of

exposure in the EU is lacking at the moment, given that no pharmacovigilance reports or any other type of communication is available that would indicate that a vulture in the European Union has died as a result of feeding on carcasses from animals treated with diclofenac.

Nevertheless, the Committee is of the opinion that to ensure the safety of necrophagous bird populations, additional risk management measures are needed to prevent that a food-producing animal treated with diclofenac, within 10 days (or longer) after treatment will enter the food chain of necrophagous birds in the European Union.

The Committee has identified two main exposure scenarios for necrophagous bird species exposure to diclofenac treated animals for the European Union:

#### **A. Exposure at feeding stations**

- A.1. Ingestion of animal by-products from slaughterhouses
- A.2 Ingestion of animals that have died from natural causes and are taken directly to feeding stations

#### **B. Exposure through fallen stock.**

A.1. Exposure of necrophagous birds to animal by-products in feeding stations is considered to pose no risk, given that only those animals intended for human consumption are taken to the slaughterhouses. Only animals that comply with the withdrawal periods for the treatments received are taken to the slaughterhouses. Thus, cattle and pigs will only be sent to slaughter 15 and 12 days, respectively, after diclofenac treatment. As at these times residues concentrations in the organs will be below 3 µg/kg, it is considered that necrophagous birds feeding on these by-products will have no risk of mortality. Consequently, no additional risk management measures are necessary to be implemented regarding the safety of necrophagous bird populations from their feeding in this scenario, as no risk is identified from necrophagous birds ingesting animal by-products in feeding stations coming from slaughterhouses.

A.2. The CVMP acknowledges the risk that can arise from animals that have died from natural causes or illnesses on farms. It is considered that the conditions intended to be treated with diclofenac are unlikely leading to death. However, diclofenac may be used in young animals for respiratory infections as a complementary treatment to an antibacterial. These animals may have a higher risk of mortality after treatment and may be left in the field or be taken to a carcass dump. In several Member States, farmers are able to dispose of fallen stock by leaving the carcass on the field or taking it to a feeding station. As a result the CVMP considers that in this scenario risk management measures are needed to ensure that necrophagous bird populations are not at risk if feeding from animals in feeding stations that are not coming from slaughterhouses.

B. The third exposure scenario is exposure through fallen stock. This situation would be relevant for animals in extensive pastures only, and it is important to take into account that according to approximately 10% of diclofenac is used for such animals, during treatment they will be kept indoors or in a pen given that at least 3 consecutive daily injections are needed and the condition of the animal is likely to have to be supervised. Although, the risk through fallen stock is considered limited, the Committee is of the opinion that additional risk management measures are needed for this scenario.

Based on available peer reviewed data, the CVMP acknowledges that modelling studies have indicated that less than 1% of contaminated carcasses were needed in India to trigger the collapse of the vulture populations assuming that birds would feed only on livestock carcasses. Whether this value would be

the same for the European populations is unknown, but this estimate is the result from considering a group feeding behaviour typical for vulture species. This feeding behaviour is typical for European as well as for other populations from the Indian subcontinent.

The CVMP considers that the risk management measures that have to be implemented for the two exposure scenarios where a risk is identified (feeding stations from non-slaughterhouse material (A.2) and fallen stock (B)) have to ensure that no contaminated carcasses will enter the food chain.

The Committee considered a wide range of risk management measures (see table 9) and discussed their practicalities and impact. However, the effectiveness of some of the measures could not be evaluated, as data to quantify the effectiveness is lacking. Besides, several of them do not fall within the remit of the CVMP. Consequently, it was not possible to make a recommendation at this stage on which of them would be most appropriate. Such a recommendation could only be made once the feasibility and effectiveness of all of the possible measures proposed has been assessed by those responsible for veterinary controls before and after death of animals. The risk management measure that would be most effective to ensure safety from all exposure scenarios is the discontinuation of the use of diclofenac in veterinary medicine. Nevertheless, off-label use of human medicinal diclofenac might still be available under the cascade for veterinary use. It is important to keep in mind that no field data on intoxication of European necrophagous birds by diclofenac has been identified. The Committee acknowledges that the fact that a vulture was found dead in the south of Spain with residues from a different NSAID in its tissues, is an indication that the exposure scenarios and risk levels identified are possible. The CVMP is of the opinion that additional risk management measures are needed and efforts should focus on determining suitable and effective risk management measures, particularly applicable for those scenarios for which a risk has been identified.

**Table 9.** Summary of risk management measures to be considered

Proposed risk management measure	CVMP considerations
Information to veterinarians	<ul style="list-style-type: none"> <li>• The product might only be supplied with a veterinarian prescription.</li> <li>• Veterinarians should be knowledgeable about the risks of the product to necrophagous birds to ensure no exposure of vultures to carcasses from treated animals containing diclofenac residues.</li> <li>• Information should also be transferred to the animal owners, as once the animal is medicated the fate of the carcass will not be under the control of the veterinarian.</li> </ul>
Warnings in the product literature	<ul style="list-style-type: none"> <li>• The SPC and package leaflet, which are addressed to the veterinarian and animal holder (farmer), respectively, should contain highlighted warnings indicating the desired protection, and restriction of use. Contraindications for extensive pasture animals could be considered. Additionally, horses not intended for human consumption are likely to end up in a feeding station, and this should be reflected.</li> <li>• Given that all current authorisations are by the national procedure, the national authorities would be responsible to implement this measure.</li> </ul>
Change in the administration pattern of the VMP	<ul style="list-style-type: none"> <li>• Exclusive administration of the product by the veterinarian (not only under his/her supervision) to prevent that animals at risk of being consumed by vultures receive treatment.</li> <li>• The veterinarian would have to visit the animal daily for 3–5 days.</li> <li>• Alternatively, only the exact dosage for the treatment could be kept in the farm for its administration by the farmer under veterinary supervision.</li> <li>• It is recognised that once the animal is medicated the fate of the carcass will not be under the control of the veterinarian.</li> </ul>
Changes in the food chain information	<ul style="list-style-type: none"> <li>• FCI<sup>3</sup> should contain additional information specifying if the animal has been treated with diclofenac during its life.</li> <li>• The content of the FCI, detailed in EU legislation, should be amended.</li> <li>• The farm veterinarian could be involved by making it compulsory for him to issue the FCI instead of the farmer.</li> <li>• Slaughterhouse operators should be informed that if the withdrawal period of the animal has not been respected, the animal cannot be taken to a feeding station.</li> </ul>

<sup>3</sup> FCI: Food chain information, as described in Regulation 853/2004 is a document that the farm operator has to fill in and sign in order to guarantee an adequate public health protection.

Proposed risk management measure	CVMP considerations
Increase controls in farms intentionally leaving dead animals in the field	<ul style="list-style-type: none"> <li>• The Regulations 1069/2009 and 142/2011 allow farmers to leave fallen stock on fields. The farms that can do this have to meet certain requirements and have to be authorised to do so by the competent authorities.</li> <li>• As these farms are known and controlled, it would be a good measure to increase the frequency of controls in these farms and the checks of the farm book looking for the use of diclofenac.</li> <li>• Contraindications for extensive pasture animals could be considered. Additionally, horses not intended for human consumption are likely to end up in a feeding station, and this should be reflected as well.</li> </ul>
Accompanying document for by-products	<ul style="list-style-type: none"> <li>• A new document can be developed to be prepared by the farmer (when supply was direct from the farm) or slaughterhouse operator (when supply was from the slaughterhouse) and to make it available to the responsible person/body in charge of the feeding station, which states that the by-products dispatched come from animals not treated with diclofenac.</li> <li>• This information could also be used to trace back the origin of the by-products in order to apply corrective actions if necessary.</li> </ul>
Sampling scheme	<ul style="list-style-type: none"> <li>• It would not be feasible to sample all the material used to feed necrophagous birds, but developing a sampling scheme with statistical purposes would be useful for detecting an eventual breach of the protocols and for applying the necessary corrective actions.</li> <li>• The information provided by this scheme would provide a firm indication on whether exposure in the EU is likely and to predict its extent.</li> <li>• The sampling scheme could be included as a condition on the marketing authorisation of VMPs containing diclofenac, leaving the marketing authorisation holder to bear the costs, at least partly.</li> </ul>
Withdraw diclofenac products from the EU market	<ul style="list-style-type: none"> <li>• This measure would ensure a negligible risk. However, exposure under the 'cascade' by parenteral human medicinal products containing diclofenac might still be possible.</li> <li>• This measure would negatively affect the availability of medicines, however animal welfare will not be affected as alternatives are available.</li> <li>• The toxicity of most other NSAIDs to necrophagous birds is unknown and the risk of their use cannot be estimated.</li> <li>• It has been clearly proven that diclofenac represents a potential threat to necrophagous birds, however no cases of diclofenac-related deaths of necrophagous birds have been reported so far in the European Union.</li> <li>• A withdrawal of the marketing authorisation should be based on a benefit-risk analysis. It should be considered that there is a limited availability of active substances for the relief of inflammation and pain.</li> </ul>

### 3. Overall summary of the scientific evaluation

The Committee, having considered the matter, reviewed data from published literature, answers provided by stakeholders during the public consultation including data received from the marketing authorisation holders, information from the presentations that the Committee received from Fatro S.p.A, Fatro Ibérica and BirdLife International, and personal communications, came to the following conclusions:

The Committee confirmed that vultures and other necrophagous birds in the European Union may be at risk due to residues of diclofenac if they feed on carcasses of animals that have been treated with this medicine. In the European Union, there are two main scenarios in which necrophagous bird species can become exposed to diclofenac residues:

**A. Exposure at feeding stations** (place where animal by-products and/or carcasses of domestic livestock or wild mammals are put out for vultures and other scavengers)

- A.1. Ingestion of animal by-products from slaughterhouses
- A.2 Ingestion of animals that have died from natural causes and are taken directly to feeding stations

**B. Exposure through fallen stock** (animals that die in open pastures from natural causes and are left in fields to be disposed by vultures and other necrophagous birds and wild mammals).

In relation to the two main routes of exposure of necrophagous birds to diclofenac used in VMPs in the European Union the following was considered in respect to the risk to vultures and other necrophagous bird populations in connection with the use of veterinary medicinal products containing the substance diclofenac:

- All populations of birds that display a necrophagous feeding behaviour in the European Union have been considered species of concern and fall within the scope of the assessment. The Oriental white-rumped vulture (*Gyps bengalensis*) has been chosen as the model organism for the risk assessment given that laboratory and field toxicity data are available for this species.
- As many of the species considered at risk from veterinary medicines containing diclofenac are included in the International Union for the Conservation of Nature Red List of Threatened Species, and also considering their reproductive strategy, the level of protection for the risk assessment has been established at the individual level (i.e. probability of death of a single individual). This is the common methodology adopted internationally for assessing environmental risk to endangered species.
- The assessment of the risks to vultures and other necrophagous birds from the use of veterinary medicinal products containing diclofenac, as a result of ingesting carcasses containing diclofenac residues in feeding stations, or through fallen stock in pastures, is not a standard environmental risk assessment. Given that the CVMP guidelines for the assessment of environmental risks cannot be used for this particular environmental risk assessment, and in the absence of suitable default parameters, the Committee decided to apply an *ad-hoc* approach by identifying the most suitable species to use as a model organism for the assessment, as well as determining the most adequate inter- and intraspecies extrapolation factors based on expert judgement.

- Based on laboratory and field toxicity data from the Oriental white-rumped vulture and considering its feeding patterns, the maximum concentration of residues of diclofenac in tissue to ensure the safety of vultures and other necrophagous birds has been assessed to be 3 µg/kg.
- After the recommended three day treatment of pigs and cattle with VMPs containing diclofenac, the highest residue concentrations are found in injection sites. Residue concentrations in tissues and in the injection site decline to below 3 µg/kg after 9 and 10 days for pigs and cattle, respectively. These conclusions are based on data from cattle weighing below 200 kg and pigs below 60 kg, and having no overlapping injection sites.
- The withdrawal periods for diclofenac products are 12 days for pigs and 15 days for cattle.

Based on the above the risk to vultures and other necrophagous birds from the use of veterinary medicinal products containing the substance diclofenac has been assessed as follows:

#### **A. Exposure at feeding stations:**

##### *A.1. Ingestion of animal by-products from slaughterhouses.*

No risk to vultures and other necrophagous birds is identified from their feeding in stations supplied by animal by-products from slaughterhouses. Animals sent to slaughterhouses are intended for human consumption and therefore their withdrawal periods after being treated have to comply with those indicated in the SPC. For pigs and cattle the withdrawal periods are 12 and 15 days, respectively. Taking into account residue data, after 9 days for pigs and 10 days for cattle, diclofenac residue levels in animal by-products are below 3 µg/kg, the concentration established as safe and therefore considered not to pose a risk to vultures and other necrophagous birds if they are consumed.

##### *A.2. Ingestion of animals that have died from natural causes and are taken directly to feeding stations.*

A risk to vultures and other necrophagous birds is identified if feeding in stations supplied by animals that have died from natural causes and are taken directly (by farmers) to feeding stations. In principle, the conditions that are treated with diclofenac are unlikely to lead to death. However, diclofenac can also be used in young animals for respiratory infections as a complimentary treatment to an antibacterial, and consequently the risk of mortality after treatment of these animals might be higher. These carcasses may be left in the field or be taken to a carcass dump by the farmer to avoid higher disposal costs. As a result the CVMP considers that in this scenario further regulatory action (e.g. additional risk management measures) are needed to ensure that necrophagous bird populations are not at risk if feeding from animals in feeding stations that are not coming from slaughterhouses. Also, non-food producing horses are generally categorised as farm animals and their carcasses might be taken to feeding stations. For older horses diclofenac might be used for palliative purposes, and thus with a higher risk of mortality.

- **B. Exposure through fallen stock:**

A risk to vultures and other necrophagous birds is identified from their feeding on fallen stock in open pastures. A risk would exist in situations where extensively reared animals are treated with VMPs containing diclofenac and die in the field, or get lost and subsequently die shortly after treatment in which case a vulture might spot the carcass before it is discovered by the owner. Some extensive producing farms can be authorised to leave dead animals in the field for the consumption of carrion birds. Animals that are kept in pastures are considered less likely to be treated with diclofenac given that the conditions diclofenac is prescribed for are not common for animals under this farming practice. Moreover, conditions intended to be treated with diclofenac are unlikely to lead to death. In addition,

the dosing regimen of the products, which requires injecting the animal on three consecutive days, is not very practical in free grazing animals. This would contribute to the infrequent use of VMPPs containing diclofenac in extensive farming conditions and thus reduce the risk from exposure of necrophagous birds to toxic levels of diclofenac residues in fallen stock in the field. It is worth noting that common practice seems to indicate that when animals are sick, they are predominantly contained in pens rather than being treated and being left on pastures, as the treatment and the follow up of the condition of the animals would not be feasible to monitor in large open spaces. Based on the information provided by the marketing authorisation holders, approximately 10% of diclofenac is used for animals in extensive pastures. Although the risk through fallen stock is considered low, the Committee considers that additional regulatory action is needed (e.g. risk management measures) for this scenario.

Consequently, The CVMP is of the opinion that based on field and laboratory data available from the Oriental white-rumped vulture, and residue data available from the marketing authorisation holders for cattle and pigs, a risk is identified for vultures feeding on carcasses of livestock that have been treated with diclofenac.

The crucial point in the assessment is whether it is likely that necrophagous birds in the EU will be exposed to food-producing animal carcasses within 10 days (or longer) after treatment with diclofenac. Evidence of exposure in the EU is lacking at the moment, given that no pharmacovigilance reports or any other type of communication is available that would indicate that a vulture in the European Union has been exposed or died as a result of feeding on carcasses from food-producing animals treated with diclofenac. This is a major data gap for the Committee's conclusions.

There are two exposure scenarios from the three identified above where a risk has been identified for vultures and other necrophagous birds (feeding stations from non-slaughterhouse material (A.2) and fallen stock (B)). Hence, the CVMP considers that for these two scenarios further regulatory action is needed (e.g., implementation of additional risk management measures).

Based on available peer reviewed data, the CVMP acknowledges that modelling studies have indicated that less than 1% of contaminated carcasses were needed in India to trigger the collapse of the vulture populations. Whether this value would be the same for the European populations is unknown, but this estimate is the result from considering a group feeding behaviour typical for vulture species and from vultures feeding on diclofenac-contaminated carcasses only. This feeding behaviour is typical for European and Indian populations of vulture species.

The Committee considered a wide range of risk management measures and discussed their practicalities and impact. The Committee was not in a position to evaluate the effectiveness of all of the proposed measures as several of them cannot be quantified at present or do not fall within the remit of the CVMP. Therefore, it was not possible to make a recommendation at this stage on which of them would be most appropriate.

In conclusion, the CVMP is of the opinion that additional risk management measures are needed and efforts should focus on determining the most suitable and effective ones to ensure that contaminated carcasses do not end up in the food chain of vultures and other necrophagous birds.

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