Griffon Vultures at a feeding station in Lleida, Spain.

SCIENCE AND REGULATION

One Health approach to use of veterinary pharmaceuticals

Weak environmental assessments undermine regulations

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POLICY

An estimated 6051 tons of active substances went into the production of veterinary pharmaceuticals (VPs) for the treatment of food animals in the European Union (EU) in 2004, including 5393 tons of antibiotics and 194 tons of antiparasitics (*1*). With global meat production projected to increase (*2*) and the growing market for companion animal pharmaceuticals (*3*), the use of VPs will continue to increase. Although VPs may benefit the health and welfare of domestic animals and the efficiency of food animal production, they can contaminate the environment through manufacturing, treatment of animals, and disposal of carcasses, offal, urine, feces, and unused products (*4*) (see the chart). This contamination is a threat to nontarget species, including humans. With Spain having recently authorized marketing of a VP that was banned in South Asia in the past decade in light of environmental impacts, we recommend strengthening of current procedures and addition of a more proactive, holistic, One Health approach applicable to all VPs.

VULNERABLE VULTURES. In the 1980s, the three *Gyps* vulture species endemic to South Asia were the most abundant large raptors in the world, but their populations were reduced to near extinction in the 1990s (*5*). The nonsteroidal anti-inflammatory drug (NSAID) diclofenac was identified (in 2004) as the primary cause of rapid declines in Pakistan, India, and Nepal. Low-cost diclofenac-based products were being widely administered to livestock. Sufficient residues remained in carcasses of treated animals to cause acute renal failure and death of vultures feeding on them. Lethal contamination of just 0.3 to 0.7% of ungulate carcasses could account for observed decline of one vulture species at 50% per year (*6*).

The government of India enacted a ban on production, importation, and sale of veterinary diclofenac products in 2006. Similar measures were taken in Pakistan, Nepal, and Bangladesh. This was facilitated by the identification of meloxicam as a suitable alternative drug that was safe for *Gyps* vultures. Over the past 8 years, vulture population declines in South Asia have slowed, and may have reversed in some areas (supplementary materials) (*7*, *8*).

Despite this history, the government of Spain authorized marketing of diclofenac as a VP for use in cattle, pigs, and horses in 2013. This authorization was in compliance with current EU guidelines. Spain is important for global conservation of avian scavengers, as it holds >95% of the European population of vultures, the entire population of the globally threatened Spanish Imperial Eagle (*Aquila adalberti*), and important numbers of Red Kites (*Milvus milvus*) (table S1 and fig. S1).

Spanish law allows carcasses of farm animals to be left in the field in some protected areas or to be taken to "muladares" (vulture feeding stations) to provide food for wildlife. By law, diclofenac should only be administered under veterinary supervision and should not be given to animals that are likely to enter the natural food chain. But Spain's livestock industry has around 25 million pigs and 5.7 million cattle, and diclofenac is licensed for use against many clinical conditions that occur in these animals. There has been a dramatic increase in veterinary use of NSAIDs in recent decades (*9*). Vulture populations are very sensitive to even very low levels of contamination. Thus, despite existing regulations, given the scale of use and the reality of imperfect compliance with regulations, it seems reasonable that diclofenac could still find its way into the vulture food chain, with potentially harmful outcomes.

Vultures have traditionally provided important ecosystem services, helping control disease and pests, recycling nutrients, and providing cultural inspiration and recreational value. It has been estimated that Spanish vultures remove >8000 tons of livestock carcasses per year alone, preventing release of greenhouse gases and providing economic savings estimated at €1.5 million ($1.86 million) (*10*).

European countries have important populations of other endangered avian scavengers, and these depend heavily on livestock carcasses in some areas (*11*). Consequences of use of NSAIDs are likely to occur beyond the borders of individual countries, as many species show pronounced seasonal and erratic movements (*12*) (fig. S2). The toxicity of diclofenac to most accipitriforms is largely unknown, but an eagle species, *Aquila nipalensis*, may be susceptible (*13*). The risk to avian scavengers has not been evaluated adequately, thus diclofenac should be suspended for veterinary use in the EU.

In response to concerns raised by mem-

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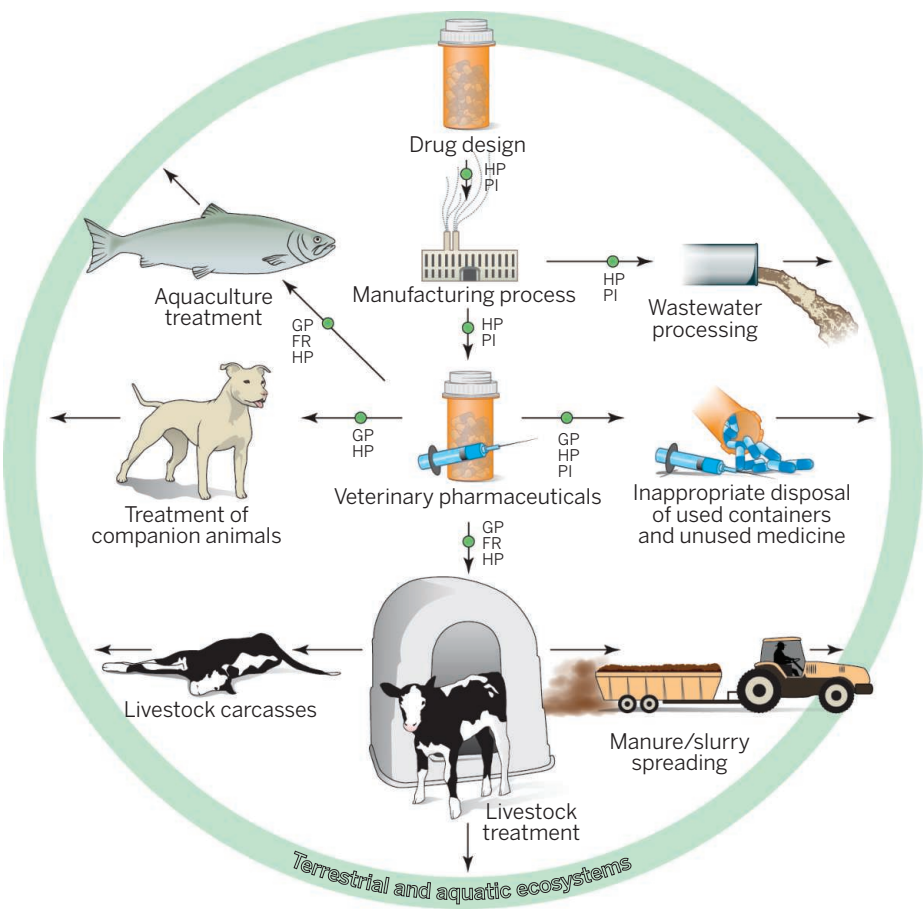
PHOTO: JORDI BAS

bers of the public, politicians, and conservation organizations, the European Commission will consider scientific advice on possible effects of veterinary medicines containing diclofenac on avian scavengers. This may lead to withdrawal of diclofenac for veterinary use in the EU and, it is hoped, convince other countries to follow.

A FLAWED APPROACH. Environmental risk assessment for new VPs is necessary for national licensing in EU countries. But there are approximately 2000 VPs in use in the EU, most of which have never been fully tested (*14*). VPs are exempt from assessment if they are used in a nonfood species, in a minor food species (i.e., all species except cattle, pigs, chickens, sheep for meat, and Atlantic salmon) if reared the same way as a major species for which an assessment already exists, or belong to certain product types (considered to be used for "a small number of animals in a herd or flock"): anesthetics; sedatives; injectable antibiotics (except those used for pigs, respiratory disease in cattle, or foot rot in sheep); injectable corticosteroids; hormones (except those that have a zootechnical use); and injectable NSAIDs (*15*). Assessment does not account for several key issues, many of which we remain largely ignorant of, including effects on species other than the few tested, low-dose effects, chronic effects, interactive effects after exposure to multiple pharmaceuticals, exposure during vulnerable stages (such as gestation and development), rate of degradation of pharmaceuticals, and toxicity of metabolites (*4*).

Diclofenac would be exempt from environmental impact assessment because it is an injectable NSAID, despite its known toxicity in nontarget species such as vultures. Other NSAIDs used in the EU may also pose a risk to avian scavengers. Ketoprofen is nephrotoxic in African *Gyps* vultures at doses likely to be encountered when feeding on carcasses of ungulates given a standard veterinary dose (*16*), and carprofen and flunixin may also be nephrotoxic to *Gyps* vultures (*5*, *17*). NSAIDs are one of several categories of VPs that may pose a risk to nontarget species through environmental contamination. Others include parasiticides and their nontarget impact on vertebrates and invertebrates in dung, soil, and watercourses (*18*); and more broadly, the human health implications of antimicrobial resistance in environmental bacteria associated with large-scale antibiotic use in food animal production (*19*).



Major pathways of release of veterinary pharmaceuticals into the environment. Green dots represent control points where environmental contamination can be prevented or minimized. GP, general public; FR, food retailers; HP, health professionals and scientists, including veterinarians, pharmacologists, farmers, animal scientists, ecologists, and environmental scientists; PI, pharmaceutical industry. Based in part on (*4*).

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A ONE HEALTH MINDSET. We may never have the knowledge required to adequately assess environmental risk of VPs. Whereas we need to strengthen current systems of environmental impact assessment where possible, we also need to foster the precautionary principle and aim to prevent environmental contamination with VPs in the first place. We advocate "cradle-to-cradle" stewardship that promotes environmental responsibility, involves all sectors of society and considers environmental effects during production, use, and disposal (see the chart).

General public. Increase public education regarding environmental effects of pharmaceuticals and personal care products [e.g., a brochure (www.vulpro.com) for South African farmers and land owners, explaining risks of VP-contaminated carcasses]; promote take-back programs at pharmacies and veterinary clinics to facilitate appropriate disposal of unused medication [patients informed of environmental consequences of pharmaceuticals were more likely to return unused medicines for proper disposal (*20*)]; use consumer purchasing power to encourage environmentally sustainable food animal production (e.g., eating-better.org).

Food retailers and restaurants. Source and promote food products of animal origin that are generated under environmentally sustainable conditions. Organizations like sustainweb.org give practical guidelines for restaurants and caterers to adopt a sustainable approach to food.

Professionals and scientists. Stimulate collaboration among veterinarians, pharmacologists, farmers, animal scientists, ecologists, and environmental scientists, who are often unaware of each other's work. These disciplines are integral for design, dispensing, and application of VPs and for creating animal husbandry systems that promote biosecurity and principles of hygiene and that contribute to the health of food animals and their consumers, while maintaining the integrity of the environment and safety for nontarget species. The Swedish Environmental Classification and Information System for Pharmaceuticals brings together the pharmaceutical industry, Swedish Medical Products Agency, regional authorities, and physicians to provide tools for prescribing drugs in an environmentally conscious way (*21*).

Pharmaceutical industry. Practice "green pharmacy" (*22*) by considering the environment at all stages of the pipeline: drug de-

sign, delivery, packaging, advertising, and marketing. The pharmaceutical industry already has moved toward products and processes that are more environmentally sustainable, e.g., by use of enzymes for some transformation reactions and use of continuous processes for primary and secondary pharmaceutical production (*23*).

Such stewardship for VPs would mirror similar programs proposed for human pharmaceuticals and personal care products (*22*, *24*) and would help restrict effects of pharmaceuticals to where they belong: in the target species. This integrated effort to link the health of people, animals, and the environment is a good example of the One Health approach, an important step toward a more sustainable society. ■

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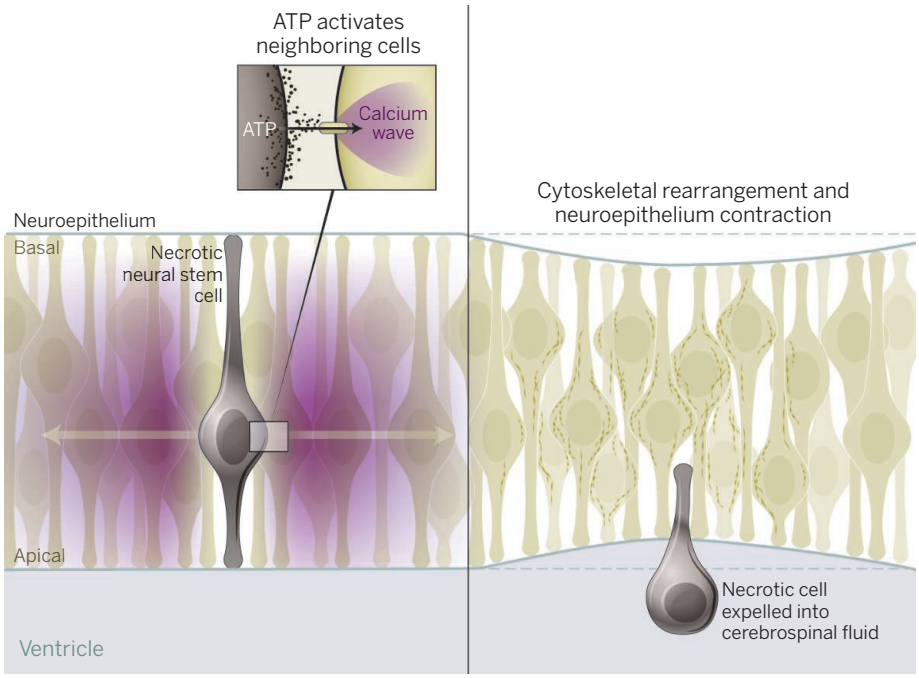
ACKNOWLEDGMENTS

We thank C. Meteyer and M. Lindsjö for manuscript assistance. The views expressed in this article are those of the authors and do not necessarily represent those of their institutions. A.M. was supported by a Ramón y Cajal research contract from the Ministerio de Economía y Competitividad (RYC-2012-11867). J.A.D. and J.A.S.-Z. were supported by projects CGL2012-40013-C02-01-02 and RNM1925.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/346/6215/1296/suppl/DC1

Published online 4 December 2014
10.1126/science.1260260



Ejected. A necrotic neural stem cell in the neuroepithelium of a developing vertebrate brain releases ATP. This activates purinergic receptors on neighboring stem cells, which triggers a calcium wave. Subsequent cytoskeletal rearrangement causes the neuroepithelium to contract, ejecting the necrotic cell.

NEURODEVELOPMENT

Tossed out to save the masses

Dead stem cells are expelled from the vertebrate brain

By **Federico Calegari**

During the 6th century, the Syriac historian John of Ephesus described how the citizens of Constantinople disposed of the corpses of the deceased inhabitants after the outbreak of a pandemic spreading through the Byzantine and Sassanid empires. Unable to cope with the numbers, all mass graves overflowing, the emperor Justinian ordered the dead to be thrown into the waters of the northern shore of the Golden Horn (*1*). Fifteen centuries later, Herrgen *et al.* (*2*) describe a similar behavior by neural stem cells of the developing vertebrate brain: burying their dead at sea by throwing them into the ventricular cerebrospinal fluid.

During development of the central nervous system in all vertebrates, neural stem cells form a densely packed and highly elongated sheet of tissue called the neuroepithelium (*3*). Within this tissue, the nuclei of neural stem cells continuously move from the boundary with the ventricle (apical) to the pial surface (basal) and then back again, completing a round of migration at every cell division cycle (*4*, *5*). This dynamic arrangement of cells (cell motion and shape) has long been proposed to represent a key step toward increasing brain size during evolution. It optimizes the number of neural stem cells that can fit within an apical unit area of tissue (*6*, *7*).

In such crowded whereabouts, within the midbrain of a developing tadpole (*Xenopus laevis*), Herrgen *et al.* killed single neural stem cells. This was accomplished by inserting a glass micropipette through the apical, ventricular-facing side of the neuroepithelium. This cell damage coincided with the onset of a calcium wave—an increase in intracellular calcium that propagated in a wave-like fashion across many cells. The wave was of reproducible origin, speed, and duration and was specifically caused by the death of the pierced cell; when a target cell was not killed and the micropipette merely contacted the cell, no calcium wave was generated. Moreover, the correlation between cell death and calcium waves was faithfully reproduced by more sophisticated means of killing that did not require physical contact, such as electric shocks or laser irradiation.

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