5.3: Wildlife population ecotoxicology

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5.3.1. Forensic investigation into crash of Asian vulture populations

Author: Nico van den Brink

Reviewers: Ansje Löhr, John Elliott

Learning objectives:

You should be able to

- describe how forensic approaches are used in ecotoxicology
- critically reflect on the uncertainty of prospective risk assessment of new chemicals

Keywords: Pharmaceuticals, uncertainty, population decline, retrospective monitoring

Introduction

Historically, vulture populations in India, Pakistan and Nepal were too numerous to be effectively counted. In the mid-1990s numbers in northern India started to decline catastrophically, which was evidenced in the Keoladeo National Park (figure 1, Prakash 1999). Further monitoring of population numbers indicated unprecedented declines of over 90-99% from the mid-1990s to the early 2000s for Oriental White-backed vultures (*Gyps bengalensis*), Long-billed vultures (*Gyps indicus*) and also Slender-billed vultures (*Gyps tenuirostris*) (Prakash 1999).

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Figure 1. Populations of White-backed vultures in Keoladeo National park in different years. Redrawn from Prakash (1999) by Wilma IJzerman.

In the following years, similar declines were observed in Pakistan and Nepal, indicating that the causative factor was not restricted to a specific country or area. Total losses of vultures were estimated to be in the order of tens of millions. The first ideas about potential causes of those declines focussed on known infectious diseases or the possibility of new diseases to which the vulture population had not been previously exposed. However, no diseases were identified that had shown similar rates of mortalities in other bird species. Vultures are also considered to have a highly developed immune response given their diet of scavenging dead and often decaying animals. To obtain insights, initial interdisciplinary ecological studies were performed to provide a basic understanding of background mortality in the species affected. These studies started in large colonies in Pakistan, but were literally races against time, as some populations had already decreased by 50%, while others were already extirpated, (Gilbert et al., 2006). Despite those difficulties it was determined that mortalities were occurring principally in adult birds and not at the nestling phase. More in depth studies were performed to discriminate between natural mortality of for instance juvenile fledglings, which may be high in summer, just after fledging. After scrutinising the data no seasonality was observed in the abnormal, high mortality, indicating that this was not related to breeding activities. The investigations also revealed another important factor that these vultures were predominantly feeding on domestic livestock, while telemetric observations, using transmitters to assess flight and activity patterns f the birds, showed that the individual birds could range over very long distances to reach carcasses of livestock (up to over 100 km).

Since no apparent causes for mortality were obtained in the ecological studies, more diagnostic investigations were started, focussing on infectious diseases and carried out in Pakistan (Oaks, 2011). However, that was easier said than done. Since large numbers of birds died, it was deemed essential to establish the logistics necessary to perform the diagnostics, including post-mortems, on all birds found dead. Although high numbers of birds died, hardly any fresh carcasses were available, due to remoteness of some areas, the presence of other scavengers and often hot conditions which fostered rapid decay of carcasses. Post-mortems on a selection off birds revealed that birds suspect of abnormal mortality all suffered from visceral gout, which is a white pasty smear covering tissues in the body including liver and heart. In birds, this is indicative for kidney failure. Birds metabolise nitrogen into uric acid (mammals into urea) which is normally excreted with the faeces. However, in case of kidney failure the uric acid is not excreted but deposited in the body. Further inspections of more birds confirmed this, and the working hypothesis became that the increased mortality was caused by a factor inducing kidney failure in the birds.

Based on the establishment of kidney failure as the causative factor, histological and pathological studies were performed on several birds found dead which revealed that in birds with visceral gout, kidney lesions were severe with acute renal tubular necrosis (Oaks et al., 2004), confirming the kidney failure hypothesis. However, no indications of inflammatory cell infiltrations were apparent, ruling out the possibilities of infectious diseases. Those observation shifted the focus to potential toxic effects, although no previous case was known with a chemical causing such severe and extremely acute effects. First the usual suspects for kidney failure were addressed, like trace metals (cadmium, lead) but also other acute toxic chemicals like organophosphorus and carbamate pesticides and organochlorine chemicals None of those chemicals occurred at levels of concern and were ruled out. That left the researchers without leads to any clear causative factor, even after years of study!

Some essential pieces of information were available, however:

1) acute renal failure seemed associated with the mortality,

2) no infectious agent was likely to be causative pointing to chemical toxicity,

3) since exposure was likely to be via the diet the chemical exposure needed to be related to livestock (the predominant diet for the vultures), pointing to compounds present in livestock such as veterinarian products,

4) widespread use of veterinarian chemicals had started relatively recently.

After a survey of veterinarians in the affected areas of Pakistan, a single veterinarian pharmaceutical matched the criteria, diclofenac. This is a non-steroid anti-inflammatory drug (NSAID) since long used in human medicine but only introduced since the 1990s as a veterinarian pharmaceutical in India, Pakistan and surrounding countries. NSAIDs are known nephrotoxic compounds, although no cases were known with such acute and sever impacts. Chemical analyses of kidneys of vultures confirmed that kidneys of birds with visceral gout contained diclofenac, birds without signs of visceral gout did not. Also kidneys from birds that showed visceral gout and that died in captivity while being studied, were positive for diclofenac, as was the meat they were fed with. This all indicated diclofenac toxicity as the cause of the mortality, which was validated in exposure studies, dosing captive vultures with diclofenac. The species of *Gyps* vultures appeared extremely sensitive to diclofenac, showing toxic effects at 1% of the therapeutic dose for livestock mammalian species.

This underlying mechanism for that sensitivity has yet to be explained, but initially it was also unclear why the populations were impacted to such severe extent. That was found to be related to the feeding ecology of the vultures. They were shown to fly long ranges to search for carcasses, and as a result of that they show very aggregated feeding, i.e. a lot of birds on a single carcass (Green et al., 2004). Hence, a single contaminated carcass may expose an unexpected large part of the population to diclofenac. Hence, a combination of extreme sensitivity, foraging ecology and human chemical use caused the onset of extreme population declines of some Asian vulture species of the Gyps genus, or so called "Old World vultures".

This case demonstrated the challenges involved in attempting to disentangle the stressors causing very apparent population effects even on imperative species like vultures. It took several years of different groups of excellent researcher to perform the necessary research and forensic studies (under sometimes difficult conditions). Lessons learned are that even for compounds that have been used for a long time and thought to be well understood, unexpected effects may become evident. There is consensus that such effects may not be covered in current risk assessments of chemicals prior to their use and application, but this also draws attention to the need for continued post-market monitoring of organisms for potential exposure and effects. It should be noted that even nowadays, although the use of diclofenac is prohibited in larger parts of Asia, continued use still occurs due to its effectiveness in treating livestock and its low costs making it available to the farmers. Nevertheless, populations of Gyps vultures have shown to recover slowly.

References

Green, R.E., Newton, I.A., Shultz, S., Cunningham, A.A., Gilbert, M., Pain, D.J., Prakash, V. (2004). Diclofenac poisoning as a cause of vulture population declines across the Indian subcontinent. Journal of Applied Ecology 41, 793-800.

Gilbert, M., Watson, R.T., Virani, M.Z., Oaks, J.L., Ahmed, S., Chaudhry, M.J.I., Arshad, M., Mahmood, S., Ali, A., Khan, A.A. (2006). Rapid population declines and mortality clusters in three Oriental whitebacked vulture *Gyps bengalensis* colonies in Pakistan due to diclofenac poisoning. Oryx 40, 388-399.

Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B.A., Shivaprasad, H.L., Ahmed, S., Chaudhry, M.J.I., Arshad, M., Mahmood, S., Ali, A., Khan, A.A. (2004). Diclofenac residues as the cause of vulture population decline in Pakistan. Nature 427, 630-633.

Oaks, J.L., Watson, R.T. (2011). South Asian vultures in crisis: Environmental contamination with a pharmaceutical. In: Elliott, J.E., Bishop, C.A., Morrissey, C.A. (Eds.) Wildlife Ecotoxicology. Springer, New York, NY. pp. 413-441.

Prakash, V. (1999). Status of vultures in Keoladeo National Park, Bharatpur, Rajasthan, with special reference to population crash in *Gyps* species. Journal of the Bombay Natural History Society 96, 365-378.

5.3.1. Question 1

Which ecological traits make vulture populations extremely vulnerable to exposure to chemicals like diclofenac?

5.3.1. Question 2

What indirect effects do you expect from the chemical induced crashes of the Asian vultures?

5.3.2. Otters, to PCB or not to PCB?

Author: Nico van den Brink

Reviewers: Ansje Löhr, Michiel Kraak, Pim Leonards, John Elliott

Learning objectives

You should be able to:

- explain the derivation of toxic threshold levels by extrapolating between species
- critically analyse implications of risk assessment for the conservation of species

Keywords: Threshold levels, read across, species specific sensitivity

The European otter (*Lutra lutra*) is a lively species which historically ranges all over Europe. In the second half of last century populations declined in North-West Europe, and at the end of the 1980s the species was declared extinct in the Netherlands. Several factors contributed to these declines, exposure to polychlorinated biphenyls (PCBs) and other contaminants was considered a prominent cause. PCBs can have different effects on organisms, primarily Ah-receptor mediated (see section on Receptor interactions). In order to assess the actual contribution of chemical exposure to the extinction of the otters, and the potential for population recovery it is essential to gain insight in the ratios between exposure levels and risk thresholds. However, since otters are rare and endangered, limited toxicological data is available on such thresholds. Most toxicological data is therefore inferred from research on another mustelids species the mink (*Mustela vison*) (Basu et al., 2007) a high trophic level, piscivorous species often used in toxicological studies. Several studies show that mink is quite sensitive to PCBs, showing e.g. effects on the length of the baculum of juveniles (Harding et al., 1999) and induction of hepatic enzyme systems and jaw lesions (Folland et al., 2016). Based on such studies, several threshold levels for otters were derived, depending on the toxic endpoints addressed. Based on number of offspring size and kit survival, EC_{50} were derived of approximately 1.2 to 2.4 mg/kg *wet* weight (Leonards et al., 1995), while for decreases in vitamin A levels due to PCB exposure, a safety threshold of 4 mg/kg in blood was assessed (Murk et al., 1998).

To re-establish a viable population of otters in the Netherlands, a program was established in the mid-1990s to re-introduce otters in the Netherlands, including monitoring of PCBs and other organic contaminants in the otters. Otters were captured in e.g. Belarus, Sweden and Czech Republic. Initial results showed that these otters already contained < 1 mg/kg PCBs based on wet weight (van den Brink & Jansman, 2006), which was considered to be below the threshold limits mentioned before. Individual otters were radio-tagged, and most were recovered later as victims of car incidences. Over time, PCB concentrations had changed, although not in the same direction for all specimen. Females with high initial concentrations showed declining concentrations, due to lactation, while in male specimens most concentrations increased over time, as you would expect. Nevertheless, concentrations were in the range of the threshold levels, hence risks on effects could not be excluded. Since the re-introduction program was established in a relatively low contaminated area in the Netherlands, questions were raised for re-introduction plans in more contaminated areas, like the Biesbosch where contaminants may still affect otters .

To assess potential risks of PCB contamination in e.g. the Biesbosch for otter populations a modelling study was performed in which concentrations in fish from the Biesbosch were modelled into concentrations in otters. Concentrations of PCBs in the fish differed between species (lipid rich fish such as eel greater concentrations than lean white fish), size of the fish (larger fish greater concentrations than smaller fish) and between locations within the Biesbosch. Using Biomagnification Factors (BMFs) specific for each PCB-congener (see section on Complex mixtures), total PCB concentrations in lipids of otters were calculated based on fish concentrations and different compositions of fish diets of the otters (e.g. white fish versus eel, larger fish versus smaller fish, different locations). Different diets resulted in different modelled PCB concentrations in the otters, however all modelled concentrations were above the earlier mentioned threshold levels (van den Brink and Sluiter, 2015). This would indicate that risks of effects for otters could not be ruled out, and led to the notion that release of otters in the Biesbosch would not be the best option.

However, a major issue related to such risk assessment is whether the threshold levels derived from mink are applicable to otter. The resulting threshold levels for otter are rather low and exceedance of these concentrations has been noticed in several studies. For instance, in well-thriving Scottish otter populations PCBs levels have been recorded greater than 50 mg/kg lipid weight in livers (Kruuk & Conroy, 1996). This is an order of magnitude higher than the threshold levels, which would indicate that even at higher concentrations, at which effects are to be expected based on mink studies, populations of free ranging otters do not seem to be affected adversely. Based on this, the applicability of mink-derived threshold levels for otters may be open to discussion.

The case on otters showed that the derivation of ecological relevant toxicological threshold levels may be difficult due to the fact that otters are not regularly used in toxicity tests. Application of data from a related species, in this case the American mink, however, may be limited due to differences in sensitivity. In this case, this could result in too conservative assessments of the risks, although it should be noted that this may be different in other combinations of species. Therefore, the read across of information of closely related species should therefore be performed with great care.

References

Basu, N., Scheuhammer, A.M., Bursian, S.J., Elliott, J., Rouvinen-Watt, K., Chan, H.M. (2007). Mink as a sentinel species in environmental health. Environmental Research 103, 130-144.

Harding, L.E., Harris, M.L., Stephen, C.R., Elliott, J.E. (1999). Reproductive and morphological condition of wild mink (*Mustela vison*) and river otters (*Lutra canadensis*) in relation to chlorinated hydrocarbon contamination. Environmental Health Perspectives 107, 141-147.

Folland, W.R., Newsted, J.L., Fitzgerald, S.D., Fuchsman, P.C., Bradley, P.W., Kern, J., Kannan, K., Zwiernik, M.J. (2016). Enzyme induction and histopathology elucidate aryl hydrocarbon receptor-mediated versus non-aryl receptor-mediated effects of Aroclor 1268 in American Mink (*Neovison vison*). Environmental Toxicology and Chemistry 35, 619-634.

Kruuk, H., Conroy, J.W.H. (1996). Concentrations of some organochlorines in otters (*Lutra lutra* L) in Scotland: Implications for populations. Environmental Pollution 92, 165-171.

Leonards, P.E.G., De Vries, T.H., Minnaard, W., Stuijfzand, S., Voogt, P.D., Cofino, W.P., Van Straalen, N.M., Van Hattum, B. (1995). Assessment of experimental data on PCB-induced reproduction inhibition in mink, based on an isomer- and congener-specific approach using 2,3,7,8-tetrachlorodibenzo-p-dioxin toxic equivalency. Environmental Toxicology and Chemistry 14, 639-652.

Murk, A.J., Leonards, P.E.G., Van Hattum, B., Luit, R., Van der Weiden, M.E.J., Smit, M. (1998). Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). Environmental Toxicology and Pharmacology 6, 91-102.

Van den Brink, N.W., Jansman, H.A.H. (2006). Applicability of spraints for monitoring organic contaminants in free-ranging otters (*Lutra lutra*). Environmental Toxicology & Chemistry 25, 2821-2826.

5.3.2. Question 1

Name three reasons why assessment of risk of PCBs to otters is relatively complicated

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5.3.2. Question 2

How is it possible that although toxicity threshold levels are exceeded in some otter populations, for example in Scotland, the population seem to thrive really well?
