Secondary poisoning of non-target animals in an Ornithological Zoo in Galicia (NW Spain) with anticoagulant rodenticides: a case report

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ABSTRACT: The use of anticoagulants has increased in recent times as a method for controlling rodent populations. However, this increased use also provokes accidental and intentional ingestion for both animals and humans, triggering poisoning of non-target organisms. In the present report, a clinical case of secondary-poisoning of birds with anticoagulant rodenticides, which took place after a general rodenticide treatment in an Ornithological Zoological Park, is described. Three birds died as a result and samples were submitted to the Veterinary Hospital in Lugo (Galicia, NW Spain). After necropsy, samples of the birds, together with molluscs and faeces, were submitted to the Toxicology Unit of Caceres (Extremadura, W Spain) in order to detect possible chemicals. Results from HPLC analyses revealed the presence of the rodenticides difenacoum and brodifacoum. The present report shows that the risk of secondary exposure resulting from the scavenging of molluscs is likely to be significant. The potential routes of uptake by invertebrates include the consumption of rodent faeces, rodent carcases, the ingestion of soil-bound residues, and the direct consumption of poison baits.

Keywords: anticoagulants; birds; recovery centre; snails; poisoning

Rodent infestations continue to be an important cause of damage in both urban and rural farm lands. In this regard, the use of anticoagulants has increased in recent years as an effective method for controlling rodent populations and anticoagulant rodenticides are now probably the most commonly used anti-rodenticides worldwide. As their subchronic toxicity is much higher than their acute toxicity, death occurs only after repeated intake of small doses, which results in rodent extermination with greater efficiency and safety even in large areas (Matolcsy et al. 1988). It has been estimated that approximately 95% of all rodenticides used are anticoagulant baits (Murphy 2002). Commonly used rodenticides, such as brodifacoum, bromadiolone, and difethialone, are classified as second-generation anticoagulant rodenticides and are now more widely used than first-generation anticoagulant rodenticides such as warfarin, chlorophacinone, and diphacinone. Second-generation anticoagulants tend to be considerably more persistent in animal tissues (Erickson and Urban 2004) and have higher affinity for liver tissue (Albert et al. 2010). A caveat to the use of all these pesticides is associated with the fact that rodents develop bait shyness to pesticides which are generally of a disagreeable taste. Thus, death can occur rapidly upon acute poisoning, but if the dose is not lethal at the first ingestion, death does not occur (Matolcsy et al. 1988).

In general, anticoagulant rodenticides are classified into two principal groups, based on their chemical structure: derivatives of coumarin and indanediones. Coumarin preparations are more extensively used but both families have severe effects on vascular permeability, resulting in massive haemorrhages and the rapid death of rodents (Binev et al. 2005). In both cases, the increased

commercial availability of these compounds to control vertebrate populations has resulted in an increase in accidental and intentional ingestion for both animals and humans, and in the widespread accidental poisoning of non-target species. Such intoxications can appear as a result of primary contact (direct consumption) with poisoned baits, thus constituting one of the most important triggers of animal intoxications (Elmeros et al. 2011; Giorgi and Mengozzi, 2011). Moreover, secondary poisoning, i.e., cases in which a predator or scavenger consumes the tissues of poisoned target species, is also significant (Winters et al. 2010).

In this sense, the widespread use of bromadiolone, a second-generation anticoagulant, has been associated with substantial secondary poisoning of birds of prey in France. Moreover, secondary poisoning has also been observed in some water birds, such as the grey heron (*Ardea cinerea*) (Guitart et al. 2010) and mammals (i.e. red fox). In Great Britain, secondary rodenticide contamination is common in mammals, such as for example polecats (*Mustela putorius*) (Birks 1998), and raptors (Walker et al. 2008; Shore et al. 2011). Similar effects have been observed in different countries, for example, USA, Canada, Mongolia, Denmark and Spain (Winters et al. 2010; Elmeros et al. 2011; Thomas et al. 2011; Sanchez-Barbudo et al. 2012). Most studies investigating the indirect exposure of non-target species to anticoagulant rodenticides have focused on the consumption of poisoned rodents by predatory birds or mammals (Dowding et al. 2010). However, invertebrates can be a route of contamination for insectivorous and/or omnivorous vertebrates (Spurr and Drew 1999), and in this regard, the exposure of insectivorous birds has been scarcely reported (Borst and Counotte 2002; Dowding et al. 2006). The exposure and scale of contamination of these non-target species are likely to be more widespread that incident reports suggest, thus rendering clinical cases of such species of great importance.

With these considerations, the aim of the present study was to describe a clinical case of secondary-poisoning of birds with anticoagulant rodenticides, which took place after a general rodenticide treatment in an Ornithological Zoological Park which did not affect raptor but rather omnivorous species. Analytical results are associated with the clinical findings, in order to confirm the toxicological process, and in the hope that similar scenarios may be prevented in the future.

Case description

Clinical case. "Avifauna Zoo" (Centro de Interpretación) is an Ornithological Zoological Park located in Galicia (NW Spain), in operation since 1983. Its facilities house over 200 different species of birds, from five continents, spread over an area of almost 30 000 m². This Ornithological Zoological Park (LU-2010/01-PZ) was designed to offer guided visits through the botanical garden where guests can see a variety of birds from the whole world.

In summer 2012, the Clinical Hospital of the Veterinary Faculty of Lugo (University of Santiago de Compostela, NW Spain) received three birds from "Avifauna", according to a cooperative agreement between the two institutions: a Grey-necked wood-rail (*Aramides cajanea*), which was already dead when received, and two Black grouses (*Tetrao tetrix*). The Black grouse male was previously observed to be ill in the Zoo, while the female of the same species was suddenly found dead in an adjacent cage. Both grouse specimens had excreted very characteristic stools in blue and green colour. The live animal had no externally visible haemorrhage or bruising but showed a slight depression and pale mucous membranes. At the Veterinary Hospital, the male was treated using vitamin K, fluid support and was handfed with worms. Nevertheless, it died the next day.

Together with bird samples, commercial baits used against rodents at "Avifauna" were supplied. Those baits, consisting of different formulations of anticoagulant rodenticides, were used by workers some days before the animals fell ill and/or died. It should be noted that the baits were not in direct contact with birds, in order to avoid unnecessary risks. In order to investigate the suspicion of secondary-poisoning associated with anticoagulant rodenticides, samples of dead snails, as well as their faeces (typically coloured), which were observed in the different bird cages, were also supplied (Figure 1). Several small dead mice were observed close to where these samples were taken from indicating the effectiveness of the pesticide treatment. However, these were not analysed.

Necropsy observations. Postmortem examinations were performed within 24 h of death. When considering the Black grouse, the most interesting findings were the following (Figure 2A). Plumage from the pericloacal area showed traces of blue-green faeces. Subcutaneous and abdominal fat deposits were clearly observed. There was no food in the crop, ventriculus, and proventriculus, the

Figure 1. Baits used as rodenticides, and dead slug found close to those baits (**A**); sample of faeces from snails, submitted for chemical analysis (**B**); different molluscs internally coloured with the pigments of rodenticides (**C**); dead mouse located in the cages, with coloured faeces on it (**D**)

last showing intense yellow bile pigments. The ventricular mucosa was brightly green, with small traces of grit and plant remains. Intestinal handles showed moderate bloating. Mucous enteritis was also observed, with bloody fluid content, abundant mucus and some undigested food. Intestinal mucosa was haemorrhagic and dotted. The male lung had a macroscopically normal appearance, but bled profusely when cut. The kidney was congestive, very dark and grainy, and also bled when cut. Liver lesions (plaques) manifested as concentric and lighter in colour ("cooked") and as necrosis (not nodules). The small intestine of the male presented with moderate bloating, bloody fluid content and some undigested food.

With respect to the Grey-necked wood-rail, a large haematoma in the lateral and ventral area of the body wall, infiltrating the connective tissue and the surface of the pectoral muscles, was observed. Bruising on the lateral and ventral neck, in the vicinity of the jugular veins, was also identified (Figure 2B). Other internal organs were completely normal in appearance; there was no content in the intestines.

Chemical analysis. After bird necropsy at the Hospital, selected samples were sent to the Toxicology Unit of the Veterinary School of Caceres (University of Extremadura, W Spain) for chemical analysis. Analyses were performed upon arrival to the laboratory because the analytical results were of critical importance to the owners and to the normal function of the Zoo.

As indicated, and according to the general findings, anticoagulant analyses were performed in different samples by the evidence of time-space associations between anticoagulant treatments, bird incidents and observation of dead snails in and close to the cages. Therefore, different aliquots were taken in order to fully investigate the suspicion of poisoning with anticoagulant rodenticides. Samples received at the Toxicology Unit consisted of:

– baits (blocks of several colours, paper with a blue past and a syringe),
– molluscs (snail and slugs) and their faeces,
– Black grouse: male and female (faeces, liver and stomach content),
– Grey-necked wood-rail (faeces and stomach content).

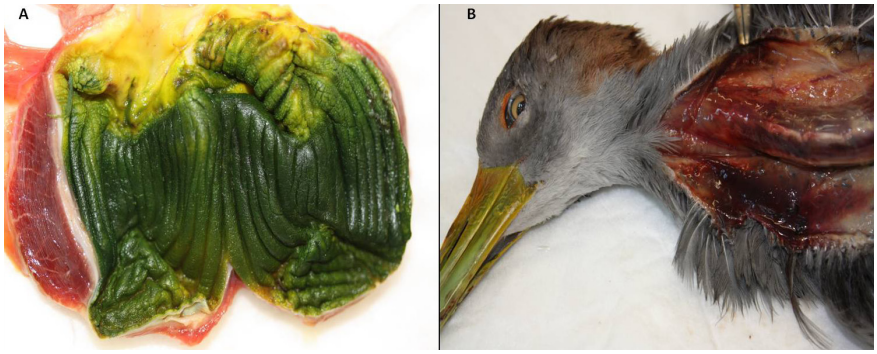

Figure 2. Ventricular mucosa of Black grouse (**A**); Grey-necked wood-rail with haematoma (**B**)

The extraction procedure was modified from Shore et al. (2003). Samples were ground in a mortar with anhydrous sodium sulphate (1 : 10). The homogenate was transferred to a Teflon-capped-glass tube with 15 ml of a mixture of dichloromethane : acetone (70 : 30), horizontally shaken for 10 min and sonicated for 5 min. The sample was filtered and liquid recovered in another tube. The extraction step was repeated with a further 15 ml of the solvent mixture and the filtered sample was pooled with the previous one. Subsequently, this extract was cleaned-up in a neutral alumina column (ALN 500 mg/3 ml, Upti-clean Interchrom, Montlucon, France). The solid phase extraction (SPE) column was conditioned with 5 ml of dichloromethane and 10 ml of dichloromethane : acetone (70 : 30), following the protocol described by Sanchez-Barbudo et al. (2012). After eluting the anticoagulant rodenticides with 3 ml of methanol : acetic acid (95 : 5), the solvent was evaporated under N_2 flow and the extract reconstituted in 0.5 ml of methanol and filtered through a 0.2 µm nylon membrane.

Analyses for coumarines using HPLC-FLD were performed according to Fauconnet et al. (1997). The injection volume was 30 µl. The chromatographic conditions of analysis consisted in a gradient elution of two solvents (A: methanol; B: buffer-ammonium acetate 40mM, acetic acid 0.2% and triethylamine 0.2%, pH: 5.2) with a flow rate of 0.8 ml/min. The initial conditions were 62% A and 38% B, reaching 82% A and 18% B at min 4. This was maintained until min 12, returning to the initial conditions by min 17. Then, the column was stabilised until min 25 before the next sample injection. Anticoagulants were detected using a KSOD2-12QK column (CART QK Lichrospher SOD2 125 × 4.6 mm).

Standard stock solutions were purchased in methanol at a concentration of 10 µg/ml from Dr. Ehrenstorfer (Augsburg, Germany). Standards were injected separately as well as in a mixture of all of them. The recovery of the analytical procedure was calculated with samples spiked with a mixture of standards. Those spiked samples were processed as normal samples. The recovery rate with the extraction method and analyses described here was >70%.

Figure 3A shows the chromatogram of a mixture of the four anticoagulant rodenticides that are more

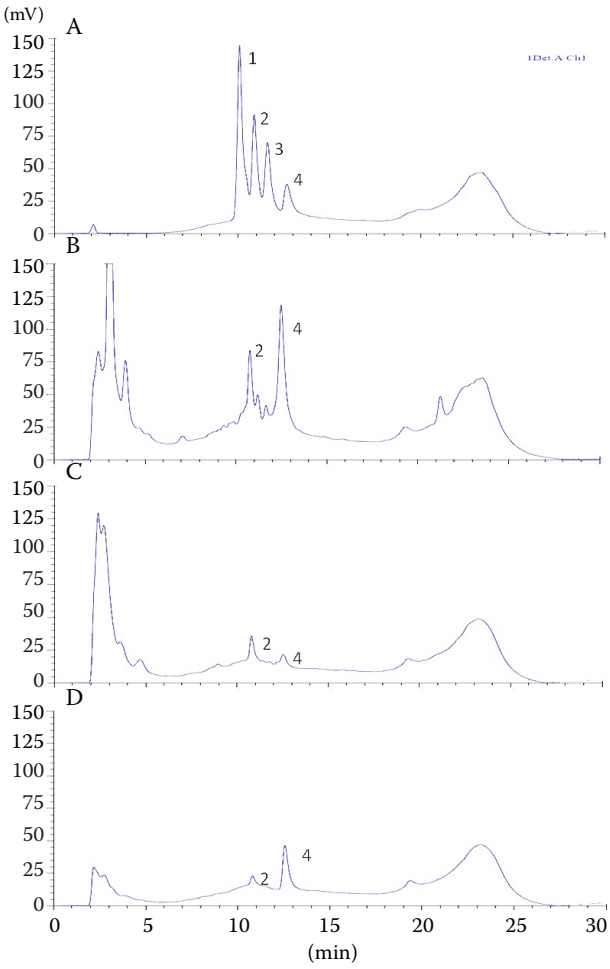

Figure 3. HPLC profile corresponding to the different samples: (**A**) rodenticide standards: 1 = bromadiolone, 2 = difenacoum, 3 = flocoumafen, 4 = brodifacoum; mollusc faeces (**B**); stomach content of Grey-necked wood-rail (*Aramides cajanea*) (**C**); liver of Black grouse (*Tetrao tetrix*) (**D**)

widely used in commercial products on the Spanish market: bromadiolone, difenacoum, flocoumafen and brodifacoum. The HPLC profile clearly demonstrates the perfect separation of the four tested compounds.

Anticoagulant rodenticides were detected in several samples. In the commercial baits, both difenacoum and brodifacoum were clearly identified (HPLC results not shown). Mollusc faeces showed two clear peaks corresponding to both identified rodenticides (Figure 3B). Stomach contents from the Grey-necked wood-rail and the liver sample from the Black grouse (Figure 3C and D, respectively) were also positive, thus confirming the exposure to anticoagulant rodenticides. No quantification was considered, associated with the fact that there is a lack of clear association between anticoagulant levels in inner organs and signs of toxicosis in birds, and it is possible that individual variation play a role in susceptibility to toxicosis (Murray 2011). Moreover, given the fact that the birds had died, and that there was no requirement for therapeutic measures, this quantification was considered unnecessary.

DISCUSSION AND CONCLUSIONS

The use of chemicals in pest control is common in zoological parks worldwide. Nevertheless, the use of such biocides can trigger the opposite result, affecting non-target animals. This effect can appear as primary intoxication (through direct contact with chemicals), but also by ingestion of intoxicated animals, leading to secondary poisoning.

Since 1998 some researchers have identified the possibility of secondary poisoning of non-target animals (Birks 1998), and have therefore recommended the extension of monitoring schemes against those rodenticides in national programs. Indeed, international authorities have stressed the need for environmental risk assessment regarding the use of these pesticides in the field, especially with regard to their transfer in food chains. Moreover, anticoagulant residues in wildlife have been shown to be increased by survey programs over the last years, leading to greater concerns of non-target effects (Sage et al. 2008).

Although field evidence of secondary poisoning with anticoagulant compounds is quite limited, nocturnal raptors and carnivorous mammals were reported as groups with higher prevalence of secondary exposure, especially to second generation anticoagulant rodenticides. Brodifacoum and difenacoum were detected in very low concentrations (< 0.5 mg/kg) in 10% of all barn owls found dead in a survey period of five years in a British field study (Newton et al. 1990). In that study, only one animal presented haemorrhages and higher liver concentrations of brodifacoum, an anticoagulant compound known to persist for up to several months in mammalian liver and, thus, highly likely to induce secondary poisoning (Berny et al. 1997). Similarly, Merson et al. (1984) measured brodifacoum in pellets rejected by Screech owls (*Otus asio*) and concluded that raptors were exposed to brodifacoum, via contaminated preys. None of the animals developed signs of anticoagulant poisoning. On the other hand, when considering anticoagulant rodenticides belonging to the first generation, granivorous birds showed the highest relation with this group in a study carried out in Spain (Sanchez-Barbudo et al. 2012). The same study, carried out with a large number of species, showed liver to be the tissue with the highest residues of anticoagulants. Moreover, and according to the obtained results, for a broad spectrum of species the exposure to rodenticides was considered as the main reason of death. Similarly, an American study determined that 86% of birds of prey admitted to a wildlife clinic that died or were humanely euthanised due to their presenting injuries had anticoagulant rodenticide residues in liver tissue (Murray 2011).

With respect to the risk of use of such rodenticides, it is important to note that pest managers tend to argue that the risk of secondary poisoning of non-target species during control operations is negligible. This fact can be accepted when rodent species tend to die below this ground, but on many occasions this is not the reality. Moreover, this argument does not consider the attractiveness of rodenticide baits for molluscs, as presented in this paper. Invertebrates have different blood-clotting mechanisms to vertebrates and so are less susceptible to anticoagulant rodenticides than birds and mammals (Shirer 1992; Johnston et al. 2005; Dowding et al. 2010). Nevertheless, these animals can easily access and feed on rodenticides, including baits, and retain ingested compounds in their bodies for four weeks or longer (Booth et al. 2001; Craddock 2002). Thus, the predation of such contaminated invertebrates is likely to be a major pathway by which insectivorous animals are exposed to anticoagulant rodenticides (Dowding et al. 2010).

In conclusion, if anticoagulant baits be not removed, the risk of primary non-target poisoning can potentially be reduced by protecting baits, and placing them in places only accessible to the target rodent (i.e., burrows). However, when considering insectivorous birds, the risk of secondary exposure resulting from the scavenging of molluscs is likely to be significant, as showed in the present study. The potential routes of uptake by invertebrates include the consumption of rodent faeces, the consumption of rodent carcases, the ingestion of soil-bound residues, and the direct consumption of poison baits. In all cases, the exposure and/or contamination of invertebrates can constitute a serious risk for species that feed on them.

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REFERENCES

Albert CA, Wilson LK, Mineau P, Trudeau S, Elliott JE (2010): Anticoagulant rodenticides in three owl species from Western Canada, 1988–2003. Archives of Environmental Contamination and Toxicology 58, 451–459.

Berny PJ, Buronfosse T, Buronfosse F, Lamarque F, Lorgue G (1997): Field evidence of secondary poisoning of foxes (Vulpes vulpes) and buzzards (Buteo buteo) by bromadiolone, a 4-year survey. Chemosphere 35, 1817–1829.

Binev R, Petkov P, Rusenov A (2005): Intoxication with anticoagulant rodenticide bromadiolone in a dog – a case report. Veterinarski Arhiv 75, 273–282.

Birks JDS (1998): Secondary rodenticide poisoning risk arising from winter farmyard use by the European polecat Mustela putorius. Biology Conservation 85, 233–240.

Booth LH, Eason CT, Spurr EB (2001): Literature review of the acute toxicity and persistence of brodifacoum to invertebrates. Science for the Conservation 177, 1–9.

Borst GHA, Counotte GHM (2002): Shortfalls using second-generation anticoagulant rodenticides. Journal of Zoo and Wildlife Medicine 33, 85.

Craddock P (2002): Aspects of the ecology of forest invertebrates and the use of brodifacoum. [PhD Thesis.] University of Auckland, New Zealand.

Dowding JE, Lovegrove TG, Ritchie J, Kast SN, Puckett M (2006): Mortality of northern New Zealand dotterels (Charadrius obscurus aquilonius) following an aerial poisoning operation. Notornis 53, 235–239.

Dowding CV, Shore RF, Worgan A, Baker PJ, Harris S (2010): Accumulation of anticoagulant rodenticides in a non-target insectivore, the European hedgehog (Erinaceus europaeus). Environmental Pollution158, 161–166.

Elmeros M, Christensen TK, Lassen P (2011): Concentrations of anticoagulant rodenticides in stoats Mutela erminea and weasels Mustela nivalis from Denmark. Science of the Total Environment 409, 2373–2378.

Erickson W, Urban D (2004): Potential risks of nine rodenticides to birds and non-target mammals: a comparative approach. US Environmental Protection Agency. Washington DC. 225 pp. http://www.fluoridealert.org/wp-content/pesticides/EPA-HQ-OPP-2006-0955-0005.pdf

Fauconnet V, Pouliquen H, Pinault L (1997): Reversed-phase HPLC determination of eight anticoagulant rodenticides in animal liver. Journal of Analytical Toxicology21, 548–553.

Giorgi M, Mengozzi G (2011): Malicious animal intoxications: poisoned baits. Veterinarni Medicina 56, 173–179.

Guitart R, Sachana M, Caloni F, Croubels S, Vandenbroucke V, Berny P (2010): Animal poisoning in Europe. Part 3: Wildlife. Veterinary Journal 183, 260–265.

Johnston JJ, Pitt WC, Sugihara RT, Eisemann JD, Primus TM, Holmes MJ, Crocker J, Hart A (2005): Probabilistic risk assessment for snails, slugs, ad endangered honeycreepers in diphacinone rodenticide baited areas on Hawaii, USA. Environmental Toxicology and Chemistry24, 1557–1567.

Matolcsy G, Nadasy M, Andriska V (eds.) (1988) Pesticide Chemistry. Elsevier, New York.

Merson MH, Byers RE, Kaukeinen DE (1984): Residues of the rodenticide brodifacoum in voles and raptors after orchard treatment. Journal of Wildlife Manage 48, 212–216.

Murphy MJ (2002): Rodenticides. Veterinary Clinics of North America: Small Animal Practice 32, 469–484.

Murray M (2011): Anticoagulant rodenticide exposure and toxicosis in four species of birds of prey presented to a Wildlife clinic in Massachusetts, 2006–2010. Journal of Zoo and Wildlife Medicine 42, 88–97.

Newton I, Wyllie I, Freestone P (1990): Rodenticides in British barn owls. Environmental Pollution 68, 101–117.

Sage M, Coeurdassier M, Defaut R, Gimbert F, Berny P, Giraudoux P (2008): Kinetics of bromadiolone in rodent populations and implications for predators after field control of the water vole, Arvicola terrestris. Science of the Total Environment 407, 211–222.

Sanchez-Barbudo IS, Camarero PR, Mateo R (2012): Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. Science of the Total Environment 420, 280–288.

Shirer M (1992): In poison's defence. Terra Nova 17, 3.

Shore RF, Birks JD, Afsar A, Wienburg CL, Kitchener AC (2003): Spatial and temporal analysis of second-generation anticoagulant rodenticide residues in polecats (Mustela putorius) from throughout their range in Britain, 1992–1999. Environmental Pollution 122, 183–193.

Shore RF, Malcolm HM, Wienburg CL, Turk A, Horne JA, Dale L, Wyllie I, Newton I (2011): Wildlife and pollution: 1999–2000 Annual report, No. 321. JNCC Report.

Spurr EB, Drew KW (1999): Invertebrates feeding on baits used for vertebrate pest control in New Zealand. New Zealand Journal of Ecology 23, 167–173.

Thomas PJ, Mineau P, Shore RF, Champoux L, Martin PA, Wilson LK, Fitzgerald G, Elliott JE (2011): Second generation anticoagulant rodenticides in predatory birds: probabilistic characterization of toxic liver concentrations and implications for predatory bird populations in Canada. Environment International 37, 914–920.

Walker LA, Turk A, Long SM, Wienburg CL, Best J, Shore RF (2008): Second generation anticoagulant rodenticides in tawny owls (Strix aluco) from Great Britain. Science of the Total Environment 392, 93–98.

Winters AM, Rumbeiha WK, Winterstein SR, Fine AE, Munkhtsog B, Hickling GJ (2010): Residues in Brandt's voles (Microtus brandti) exposed to bromadiolone-impregnated baits in Mongolia. Ecotoxicology and Environmental Safety 73, 1071–1077.

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