Ekológia (Bratislava) Vol. 30, No. 4, p. 405–413, 2011
doi:10.4149/ekol_2011_04_405

QUANTITATIVE DETERMINATION OF RODENTICIDE RESIDUES AND THE SUBSEQUENT EVALUATION OF POTENTIAL ECO-TOXICOLOGICAL RISK TO BIRDS OF PREY

RUDOLF HROMADA, SILVIA ONDRAŠOVIČOVÁ, DENISA KUDRÍKOVÁ, JURAJ HARKABUS, JAROSLAV LEGÁTH, JÁN KOŠČO, MILOSLAV ONDRAŠOVIČ

University of Veterinary Medicine and Pharmaci, Komenského 73, Košice, Slovak Republic;
e-mail: hromada@uvm.sk

Abstract

Hromada R., Ondrašovičová S., Kudríková D., Harkabus J., Legáth J., Koščo J., Ondrašovič M.: Quantitative determination of rodenticide residues and the subsequent evaluation of potential ecotoxicological risk to birds of prey. Ekológia (Bratislava), Vol. 30, No. 4, p. 405–413, 2011.

This study focuses on the observation of residues in mice after consumption of rodenticide BARAT G with the active ingredient of 0.005% brodifacoum and also KUMATOX with1% warfarin. These rodenticides were applied to cereal crops to control rodent infestation This is important in agricultural practice because the cereals are a food source for birds of prey. The feeding experiment was conducted on laboratory mice which were given the above-mentioned rodenticides and the respective residues found in the mice were used to calculate the ecotoxicological risk which was expressed as BAF and TER for the common kestrel (*Falco tinnunculus*) and common barn owl (*Tyto alba*).

Key words: residues, rodenticide, brodifacoum, warfarin, ecotoxicological risk

Introduction

The study of residues in the environment, including such an important group of substances as rodenticides,has attracted considerable attention from researchers (Atterby et al., 2005).

In field crops, and particularly cereal, there is frequently a problem with infestation of rodents which is important in agricultural practice. This most frequently involves the *Microtus arvalis* field mouse. In order to limit the damage caused by these rodents to field crops, control methods are applied using chemical rodenticides. Besides acute rodenticidal preparations, there are also rodenticides with slow onset of anticoagulant action. Following consumption of these rodenticides, rodents die after several days. Although specialized studies dealing with

mammal intoxication and treatment of affected non-target species are numerous, information concerning the ecological impact of currently used rodenticide residues is scarce. Because there is a lack of information on the potential occurrence of secondary rodenticide poisoning of birds of prey, this study focuses on the determination of residual levels of these substances in dead rodents and the subsequent assessment of eco-toxicological risk to these birds. BARAT G and KUMATOX were selected from the large range of rodenticidal preparations and these were fed to laboratory mice to determine the risk of development of secondary poisoning in the common kestrel (*Falco tinnunculus*) and the common barn owl (*Tyto alba*).

Material and methods

Material

KUMATOX S – a powder containing 1% warfarin,- INEKON SLOVAKIA, s.r.o. Trenčín
BARAT G – a 0.005% brodifacoum rodenticide bait
JJJ – DDD, Slovakia
LABORATORY MICE /ICR/ 30 animals, central breeding at LF UPJŠ, Košice /SK CH 55004 /open system/ individually non-interchangeable.

Methods

Feeding test

Three groups of 10 mice were collected under laboratory conditions and marked K1, K2 and K3.

The mice from group K1 were given rodenticide bait consisting of 5g Kumatox and 500 g barley meal. The concentration of warfarin in this mixture was 0.05%. Group K2 were fed rodenticide bait Barat G containing 0.005% brodifacoum and group K3 was used as the control.

Laboratory mice were kept in individual cages and K1 and K2 were daily supplied with 10 g of the respective rodenticide bait and also separately 10g of Top Dovo feed which is a mixed feed for mice and rats. The mice in group K3 received only 20 g of feed daily and water was supplied to all groups ad libitum.

After each 24 h period, the offered feed and bait was re-weighed, the health status of mice was evaluated and the food and respective bait was replenished up to a dose of 10g. After 10 days, the K3 mice were euthanised with ether, while the experimental K1 and K2 mice died gradually.

According to the requirements for determination of rodenticide residues by the HPLC method, the weight of any dead mice was determined and it was stored in a freezer until all animals from the respective group died.

The obtained composite material was used to carry out two parallel examinations for each experimental group. Theses consisted of samples 1 and 2 for group K1 and samples 3 and 4 for group K2 while sample 5 served as the control group.

Laboratory mice used in this experiment were supplied by a certified experimental breeding centre SK PCH 54004 and the experiment was approved by Decision Ro 755/07-221/3 of the State Veterinary and Food Administration of SR, in accordance with current regulations for the protection of laboratory animals.

Determination of residues of brodifacoum and warfarin and the ananalytic procedure by a liquid chromatograph Wares, equipped with DAD detector

The sample of frozen mice was thawed and homogenized. From each group, 10g of homogenized sample was weighed into a 250 ml Erlenmayer flask and extracted twice with a 1:1 mixture of chloroform and acetone and these extracts were filtered through filter paper.

The pooled extracts were evaporated in a rotating vacuum evaporator at 40°C and the residuum was dissolved in l ml methanol, and l ml of the mobile phase was added to remove co-extracted proteins.

This obtained solution was transferred to a centrifugation tube containing n-hexane and centrifuged for 10 minute at 2000 r.p.m. The clear bottom layer was filtered and used for HPLC analysis.

Chromatographic analysis was carried out on a column LiChrospher ® 100 RP-18 (5µm) with mobile phase acetonitril (A) and acetate buffer, pH 4.6, (B) (50+50) for warfarin and (60+40) for brodifacoum, at a flow rate of l ml.min^{-1}, injecting a 10µl aliquot. UV detection was performed at 265 nm for brodifacoum and 310 nm for warfarin, and the determined mean values of residues of individual active ingredients were used to assess potential risks.

Results

Residue results of warfarin and brodifacoum in the laboratory mice are presented in Table 1. The following residues of warfarin were found: sample 1 – 0.29 mg.kg^{-1}, sample 2 – 0.22 mg.kg^{-1} with a mean of 0.25 mg.kg^{-1}. Brodifacoum was found at lower levels in sample 3 – 0.036 mg.kg^{-1}and sample 4 – 0.014 mg.kg^{-1} with a mean of 0.025 mg.kg^{-1}. The control sample.5 was included to compare respective findings. The eco-toxicological risk was as described in the September 25th, 2002 final Manual SANCO/4145/2000,using the bioaccumulation factor (BAF) to calculate this risk. This factor represents the ratio of the quantities of the respective substance retained in an organism and that supplied in the feed. The BAF level has direct correlation with residue accumulation.

BAF = C organism/ C food

C organism = level of the substance bound in an organism (mg.kg^{-1})

C food = substance in food (mg.kg^{-1})

Calculations showed that warfarin BAF was 0.04 and brodifacoum was 0.05, indicating low substance accumulation potentials.

According to the above manual, an additional parameter for risk assessment is TER which is calculated from the ratio of toxicity and exposure. Toxicity (LD_{50}), necessary for TER calculation of warfarin and brodifacoum levels, has only been reported in avail-

T a b l e 1. Residues of warfarin and brodifacoum in laboratory mice.

Sample No.	Applied preparation (conc. of active ingredient)	Mean quantity of accepted bait (g)	Mean time of mice death(day)	Mean detected residuum of anticoagulant in laboratory mice(mg.kg $^{-1}$)
Ø No. 1 and 2	KUMATOX, active ingredient warfarin (0.05%)	24.5	7	warfarin = 0.25
Ø No. 3 and 4	BARAT G, active ingredient brodifacoum (0.005%)	4.45	7.5	brodifacoum = 0.025
5	control: mixed feed TOP DOVO	0.0	10	0.0

able sources for individual classes of animals such as. birds (www.the-piedpiper.co.uk, U.S.EPA, 1998).

A threshold value of TER > 10 indicates an acceptable risk, and where the TER <10 the risk is unacceptable. To evaluate the risk of rodenticide residues, the male barn owl and the common kestrel were selected ,and also the respective female during her egg laying period. It was assumed that in free nature these preferentially feed on rodents. In order to calculate the TER, it is first necessary to determine the relative intake of feed in grams per day in relation to the body weight of the predator in grams (FIR).

TER = toxicity/ exposure

toxicity = The LD_{50} for the respective class of animal in $mg.kg^{-1}$

exposure = residues in the body of the dead laboratory mice x FIR

The warfarin TER for the Barn Owl was 618.56 and for the common kestrel it was 571.43 for the male and 4285.7 for an egg-laying female. The brodifacoum TER determined was lower with 26.6 in the owl, 24.76 in the male falcon and 45.61 in the female falcon (Table 2). Data in Masman et al. (1986) concerning the relative intake of feed and body weight of the male and female common kestrel was used herein to calculate the eco-toxicological risk. Obtained results indicate that brodifacoum toxicit is higher than that of warfarin for selected non-target species, such as. birds of prey. However, since all values were over 10, the risk of poisoning for these birds resulting from the consumption of rodents with the respective levels of rodenticide residues in their bodies could be considered ecologically acceptable. Table 3 shows the changes in feed intake and eco-toxicological risk to the barn owl with increasing ambient temperature (Hamilton, 1985). Although feed intake was lower at increased temperatures, the eco-toxicological risk decreased with accompanying higher TER levels.

T a b l e 2. Comparison of TER for warfarin and brodifacoum in selected predators.

Tested species	**Type of feed**	**Body weight of the species (g)**	**Intake of feed (g/day)**	*FIR*	**Toxicity LD_{50}(mg.kg $^{-1}$)**	***TER*
Common kestrel male (*Falco tinnunculus*)	small mammals	**188**	**79**	0.42	warfarin = 60 brodifacoum = 0.26	**571.3 24.76**
Common kestrel female in egg laying period (*Falco tinnunculus*)	small mammals	**305**	**70**	0.229	warfarin = 60 brodifacoum = 0.26	**4285.7 45.61**
Common barn owl (*Tyto alba)*	small mammals	**294**	**112**	0,38	warfarin = 60 brodifacoum = 0.26	**618.56 26.6**

Note: **TER* = the value <10 presents non-acceptable risk (risk of poisoning is minimal) >10 acceptable risk.

T a b l e 3. Comparison of the influence of ambient temperature on feed intake and TER in the common barn owl (Hamilton, 1985).

Tested species	Type of feed	Body weight of the species (g)	Intake of feed (g/day)	Ambient temperature	*FIR*	Toxicity LD_{50} (mg.kg $^{-1}$)	*TER*
Common barn owl (*Tyto alba*)	small mammals	294	**116.9**	**5**	0.39	warfarin = 60 brodifacoum = 0.26	**615.38 26.6**
Common barn owl (*Tyto alba*)	small mammals	294	**96.8**	**15**	0.329	warfarin = 60 brodifacoum = 0.26	**729.48 31.7**
Common barn owl (*Tyto alba*)	small mammals	294	**76.9**	**25**	0.261	warfarin = 60 brodifacoum = 0.26	**923.07 40**

Discussion

Economic losses to field crops caused by rodents can be estimated in the millions. For example, Stenseth et al. (2003) reported that feed consumed by rodents each year in China would suffice to feed 200 million people, and in Indonesia, 15% of rice production losses are attributed to rodents. According to Margaletić et al. (2007), the stability of forest ecosystems have changed as a result of crop pests, climate change and hydro-amelioration activities.

Under our conditions, higher levels of field-mouse infestation are observed periodically every 3–5 years. This periodicity is particularly related to temperature and snow cover during winter months. One of the reasons may be the failure to observe agro-technical techniques, such as delayed cutting after the seeds of principal grasses start to fall off as shown in Fig. 1. These over-ripe grasses and their seeds serve as both a food source and a hiding place for rodents.

Henderson et al. (2000) investigated the distribution of the common kestrel, the common barn owl and other predators, and they reported an approximate density of 0.01 bird per hectare. The common barn owl hunts mostly in meadows while the principal hunting areas of the common kestrel are pastures and fields. Approximately 3,000 field-mice per hectare is considered a high level of infestation.

Previously, warfarin preparations represented the first generation of anticoagulant rodenticides, and their use in controlling rodents was agriculturally important.

According to the List of registered preparations for the protection of plants and other preparations noted in the Bulletin of Ministry of Agriculture SR, 2006, the following rodenticides can be used: COMMANDO, RODENTIC BLESK and STUTOX which is based on zinc phosphide (OHS Database, 1994).

Of the acute rodenticides, zinc phosphide based baits such as STUTOX have been preferred to control field-mice (Ondrašovič et al., 2000). It's commercial preparation is a yellow-green coloured bait containing 4% zinc, and this specific colouring should make

Fig. 1. Location with neglected grass cover.

it unattractive to birds. According to Staples et al. (2003), the acceptance of zinc phosphide based baits by rodents is generally high while they are relatively safe regarding development of secondary poisoning in natural predators and they do not leave significant residues in cereals, soil, water and the atmosphere.

Anticoagulant rodenticides are also used to control field-mice. The currently used anticoagulants are divided into 3 generations according to the concentration of active ingredient in the respective bait and also the amount of bait needed to achieve a lethal effect (William, Douglas, 2002, 2004). The open field application of the 2nd generation anticoagulant rodenticide BROMADIC H which is based on brodifacoum is also possible.

Although anticoagulant preparations are generally considered effective (Saxna et al., 1992), according to current knowledge their residues persist in the environment. This issue has been discussed by a number of authors including Smith and Cox (1990) and Eason and Spurr (1995). Residues of pesticides and heavy metals entering the ecosystem through soil, atmosphere, water, plants and animals result in undesirable changes in the environment (Debski et al., 2007; de Vries et al., 2005).

This study has focused on the determination of residues of brodifacoum and warfarin. Brodifacoum is a bromylate derivative of hydroxycoumarin which was used for the first time in 1975 to control rodent resistance to coumarin.

Determinations in laboratory mice indicated that this rodenticide leaves residues in their bodies correlated to the applied dose of BARAT G and KUMATOX. Table 1 and the related HPLC measurements showed that the concentration of brodifacoum was only 0.005% com-

pared to warfarin concentration which reached 0.05%, and therefore brodifacoum residues were lower. This was also confirmed by calculation of BAF and TER which were used to assess the risk of secondary poisoning in non-target species, in our case in the common barn owl and common kestrel. Because the threshold level for TER is >10 the respective value in both predators was below the limit set by ANNEX VI of the Council Directive 91/414/EEC. The respective value for the falcon is considerably higher which indicates a lower risk of poisoning. The presence of brodifacoum and warfarin residues in the tissues of a wide range of predators can be anticipated, but their levels are not always necessarily toxic. According to Berny et al. (1997) this is also supported by results from the United Kingdom which showed the presence of trace amounts of these anticoagulants in the liver of fifty analyzed ferrets (*Mustela putorius*) but these died for different reasons. Moreover, the level of brodifacoum changed relative to the season together with the availability of feed, so the ferrets collected in the first half of the investigated year exhibited higher rodenticide level in their liver than those which died in the second half of the same year.

Our department has already been previously involved in investigations of secondary poisoning. The study was conducted on Beagle laboratory dogs and this showed disturbances in blood coagulation after the animals consumed meat from rats poisoned with the rodenticide TALON, whose active ingredient was 0.05% brodifacoum (Ondrašovič et al., 1987).

Riedstra et al. (1998) investigated the daily intake of feed and body weight of the common kestrel males, and females in their egg laying period. This predator feeds mostly on rodents, particularly on field-mice. The authors observed a considerable difference in body weight between males and females, particularly during the egg laying period when the mean weight of females reached 305 g while the males weighed only 188 g. When the ecotoxicological risk was calculated, in relationship to the above mentioned facts, the risk of female poisoning from either warfarin or brodifacoum was found to decrease as the respective value of TER increaseed (Table 2).

The chemical characteristics of brodifacoum, including its insolubility in water, its 228–230 °C melting point and solubility in acetone and chloroform, support the deduction that its residues can persist for a long time in rodent bodies. When the soil becomes contaminated with brodifacoum its decontamination is difficult (Legáth et al., 1997).

According to current legislative provisions, although rodenticide concentrates are included among extra-hazardous poisons, this does not apply to prepared rodenticide baits. Because of their high toxicity for animals, their influence on non-target animal species has become the subject of the following investigations; (1) Booth et al. (2001) described the acute toxicity of brodifacoum in invertebrates including snails and terrestrial species of crabs, (2) Eason et al. (2001) investigated poisoned feral pigs and goats for residues of brodifacoum, and (3) In 2003, Booth et al. observed a toxic action of brodifacoum on earthworms. These results, together with previous findings, highlight the necessity not only for the investigation and assessment of the effect of anticoagulants on invertebrates but also for the effects of residue development in plants when anticoagulant rodenticides are applied in free nature.

Translated by the authors
English corrected by R. Marshall

Acknowledgements

The study was supported financially by the project VEGA No.1/3485/06 and the National Reference Laboratory for Pesticides at UVM in Košice.

References

ANNEX VI Nariadenia 91/414/EEC.

Atterby, H., Kerins, G.M., MacNiccoll, A.D., 2005: Whole-carcass residues of the rodenticide difenacoum in anti-coagulant-resistant and – susceptible rat strains (*Rattus norvegicus*). Environ. Toxicol. Chem., 24: 318–323.

Berny, P.J., 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 : 1871–1829.

Booth, L.H., Eason C.T., Spurr, E.B., 2001: Literature review of the acute toxicity and persistence of brodifacoum to invertebrates. Science for Conservation, 177A: 1–9.

Booth, L.H., Fisher P., Heppelthwaite, V., Eason, C.T., 2003: Toxicity and residues of brodifacoum in snails and earthworms. DOC Science Internal Series, 143: 5–14.

Bulletin of the Ministry of Agriculture of the Slovak Republic of March 20th, 2006, – section 6. 25. List of registered preparations for plant protection and of other preparations (in Slovak).

Debski, B., Feloks Kania, B., Kuryl , T., 2007: Transformations of diazinon, an organophosphate compound in the environment and poisoning by this coumpound. Ekológia (Bratislava), 26, 1: 68–82.

De Vries, W., Čurlík, J., Groenenberg, B.J., Reinds, G.J., Šefčík, P., Murányi, A., Kádár, I., 2005: Long-term impacts of different fertiliser scenarios on metal leaching and metal uptake in Slovakian and Hungarian agricultural soils. Ekológia (Bratislava), 24, 3: 314–335.

Eason, C.T., Spurr, E.B., 1995: Review of the toxicity and impacts of brodifacoum on nontarget wildlife in New Zealand. N. Z. J. Zool., 22: 371–379.

Eason, C.T., Wright, G.R.G., Milne, L.M., Morriss, G.A., 2001: Laboratory and field studies of brodifacoum residues in relation to risk of exposure to wildlife and people. Science for Conservation, 177: 11–23.

Hamilton, K.L., 1985: Food and energy requirements of captive barn owls *Tyto alba*. Comp. Biochem. Physiol. A., 80, 3: 355–358.

Henderson, I.G., Cooper, J., Fuller, R.J., Vickery, J., 2000 : The relative abundance of birds on set – aside and neighbouring field in summer. J. Appl. Ecol., 37, 2: 335–347.

Legáth, J., Bláha, K., Čermáková, T., Dolinay, Š., Hufnagelová, B., Kočišová, A., Košuth, P., Kotleba, J., Legáth, Ľ., Majláthová, Ľ., Markovič, J., Mlynarčíková, H., Murín, M., Ondrašovič, M., Prosbová, M., Škulínková, K., Sokol, J., Toporčák, J., 1997: Assessment of risk to domestic, farm and free-living animals, bees and water fauna resulting from chemical substances (in Slovak). DataHelp, Košice, 102 pp.

Margaletić, J., Božić, M., Jazbec, A., Čavlović, J., Lukić, N., 2007: Small rodents as the cause of decrease in young narrow-leaved ash tree growth (*Fraxinus angustifolia* V a h l). Ekológia (Bratislava), 26, 4: 371–380.

Riedstra, B., Dijkstra, C., Daan, S., 1998: Daily energy expenditure of male and female marsh nestlings. Auk, 115, 3: 635–641.

OHS Database, 1994: Occupational Health Services, Inc. 1994 (June) MSDS for Warfarin. OHS Inc., Secaucus, NJ.

Ondrašovič, M., Rosocha , J., Vargová, M., Ondrašovičová, O., Miško, J., 1987: Secondary poisoning related to the use of rodenticide Talon. Proc. Scientific Congress on Animal Hygiene. High Tatras, p. 107–108.

Ondrašovič, M., Para, Ľ., Vargová, M., Bis-Wencel, H., Novák, P., Ntimpirangeza, M., 2000: The use of rodenticides for the control of harmful rodents (in Slovak). Bioklimatológia a životné prostredie. XIII. Bioklimatologická konferencia SBkS a ČBkS, Košice.

SANCO/4145/2000 – final, 25. September 2002, Europea Commission, Health & consumer protection directorate - general, working document – guidance document on risk assessment for birds and mammals under Council Directive 91/414/EEC.

Saxna, Y., Kumar, D., Blandari, T., Bhasin, H., 1992: Laboratory and field evaluation of difethialone, a new antikoagulant rodenticide. Pest. Conf. Proc. Vertebr. Pest. Conf., Lincoln, p. 175–177.
Smith, R.H., Cox, P.R., 1990: Rodenticide ecotoxicology: systems analysis and simulation. Pest. Conf. Proc. Vertebr. Pest. Conf., Lincoln, p. 47–54.
Staples, L., Smith, M., Pontin, K., 2003 : Use of zinc phosphide to overcome rodent infestations. Proc. Austral. Postharvest Techn. Confer. Canberra, p. 110–115.
Stenseth, C.N., Leirs, H., Skonhoft, A., Davis, S.A., Pech, R.P., Andreassen, H.P. et al., 2003: Mice and rats: the dynamics and bioeconomics of agricultural rodents pests. Frontiersen Ecology and the Environment, 7: 367–375.
William, E., Douglas, U., 2002: Potential risks of nine rodenticides to birds and nontarget mammals: a comparative approach. U.S. Environmental Protection Agency, Washington, 202 pp.
William, E., Douglas, U., 2004: Potential risks of nine rodenticides to birds and nontarget mamals: a comparative approach. U.S. Environmental Protection Agency, Washington, 230 pp. www.the-piedpiper.co.uk/th15(k).htm
U.S.EPA, 1998: Registration Eligibility Document for Rodenticides.