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Use of Two Halogenated Biphenyls as Indicators of Non-Target Exposure during Rodenticide Treatments

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The introduction and use of anticoagulant rodenticides added a new perspective to rodent control (Hadler and Buckle 1992). Both acute and multi-dose studies (Crabtree and Robison 1952; Papworth 1958) showed warfarin to be less toxic to birds than rodents but there were indications that the potential for secondary poisoning existed (Crabtree and Robison 1952; Prier and Derse 1962). The post-Approval monitoring undertaken in the UK between 1964 and 1983 did not identify any secondary poisoning problems associated with the use of anticoagulant rodenticides (Hardy et al 1986), though it is possible that cases of poisoning went undetected due to the low sensitivity of the methods then available for determining anticoagulant rodenticides. The second-generation compounds are in general relatively more toxic to birds than warfarin (Anon 1981; Lund 1981) and the presence of residues in wildlife is the cause of some concern (Greig-Smith et al 1990; Newton et al 1990). Residues in owls and birds of prey are most likely to have resulted from secondary exposure, although this is less certain in the case of cats, dogs and the red fox.

In order to undertake risk assessments for secondary poisoning from rodenticides information is required as to their fate in the environment during poison treatments (Cox and Smith 1990). Some studies of the food intake of predators and scavengers have been made by 'marking' their potential prey, and a range of agents have been used (Savarie et al 1992). The use of non-toxic markers allows studies to be conducted independent of any poison and in circumstances where the use of pesticides is proscribed, for example in studies of the risk to rare or endangered species. The use of visual markers reduces the need for sophisticated analytical equipment but is less satisfactory than the use of chemicals which may prove to be a better model for the toxicant. We have investigated the use of decachloro-biphenyl (DCBP) and 2,4,5,2',4',5'-

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hexachlorobiphenyl (HCBP), which although persistent are among the least toxic of the PCB congeners (Mizutani et al (1980), to monitor non-target exposure alongside a project monitoring feeding behavior of rats during anticoagulant treatments on farms (Quy et al 1992).

MATERIALS AND METHODS

A pair of farms to be treated for up to 7 weeks with a pin-head oatmeal bait containing 50 ppm difenacoum and either 100 ppm DCBP (weeks 1-3) or 100 ppm HCBP (weeks 4-7) was selected from those used by Quy et al (1992) as part of an extensive study of rat feeding behavior. Our study was started on 28 October 1991. Farm 1 in Sussex was about 4 ha and consisted of a few buildings and a meadow for rearing young stock. During the treatment, 7.1 kg of bait was used at 20 points laid around the outside of the buildings. Farm 2 was in Hampshire and extended to over 100 ha, forming part of an estate used for game rearing. Rodent control was undertaken within the farm buildings and around the farm yard using a total of 6.1 kg of bait at 59 bait points. At weekly intervals during the rodent control programme, small mammals were trapped from both within and outside the areas containing the bait boxes. Traps were laid singly about 5 m apart in and around the farm buildings and along hedgerows up to about 100 m from the nearest bait point. Longworth traps were set outdoors at both farm 1 (50) and farm 2 (103) and in addition 23 'Trip-traps' were set inside 2 buildings at farm 2 where there were signs of house mouse (*Mus domesticus)* activity. All traps were examined within 24 h and the species and sex of the animals caught were recorded. As it was of interest to retain small mammals on the farms throughout the trials approximately two-thirds of the animals caught were released close to the point of capture, and the remainder killed and returned to the laboratory. In the laboratory, the bodies were given a visual post-mortem examination and the liver removed for a separate study. The remainder of the carcase was stored at -18°C until analysed. While still frozen, the tail was removed and the body was sliced and finely minced with anhydrous sodium sulphate. The presence of DCBP and HCBP were determined essentially as per Buckle et al (1987) using capillary gas chromatography and electron-capture detection.

RESULTS AND DISCUSSION

The total numbers of animals caught in relation to the trapping efforts are shown in Table 1. As might be expected, the wood mouse (*Apodemus sylvaticus*) was the most abundant species and accounted for 64% of animals caught. Within each site there was variation in the distribution of

Table 1 Data for total number of animals trapped, including number sampled or found dead and in brackets the number containing residues of at least one marker. A total of 59 traps (3.7%) were found closed but contained no animal.

Species	Farm 1			Farm 2		
	Total	Sampled	Dead	Total	Sampled	Dead
A. sylvaticus	72	28(6)	2(0)	98	49(16)	1(0)
A. flavicollis	11	2(0)	0	11	2(0)	2(0)
C. glareolus	4	2(1)	0	27	4(0)	7(0)
M. agrestis	3	0	0	2	0	0
M. domesticus	1	1(1)	0	7	6(3)	1(1)
M. minutus	1	0	0	2	0	0
Sorex sp.	3	0	3(2)	8	0	7(1)
Unknown	6	0	0	10	0	0
Total	101	33	5	165	61	18
Total trap nights	500			1091		

small mammals, with traps in the hedgerows generally producing the larger number of animals. It was found that despite putting fly pupae in the traps to prevent starvation, the majority of shrews were found dead.

Overall, at both farms 26% of the samples examined contained at least one bait marker. The proportions of samples with marker collected from inside the areas containing bait points were 83% and 67% for farms 1 and 2 respectively and were higher than those (16% and 17%) from outside the rodenticide treatment areas. The results showed that animals trapped in the hedgerows, including some woodmice trapped up to 80 m from the treatment area on farm 2, had fed at the bait points. Animals containing marker were trapped throughout the study period and although variations in the proportions containing markers occurred there were no obvious trends that could be related to changes in the rat populations. The changeover in marker occurred after 3 weeks and was based on the normal treatment time to achieve rodent control using anticoagulant rodenticides against susceptible populations. The use of the two markers made it possible to identify animals which only started feeding after 3 weeks (2/16) as well as those feeding during both phases (6/16). The design of the experiment did not allow estimates of the total small mammal populations to be made.

Using the analytical method described, the limit of detection for either marker was 0.05 mg/kg, and the residue levels found ranged from 0.05-241 mg/kg body weight with the median 3.0 mg/kg. These values were used to estimate bait intake on the assumption that the storage ratio (body residue/daily oral intake) was 0.5 for both markers. This is based on values of approximately 0.51 for DCBP and 0.61 for HCBP in studies with the rat (Quy et al 1992). Slightly lower values were reported by Mizutani et al (1980) for laboratory mice, but this may be due to differences in bait formulation. In the present study livers were excluded from the body analyses, although unlike Quy et al (1992) the gastro-intestinal tract was included.

For the wood mouse (*A. sylvaticus*) the estimated bait consumption ranged from 0.01-35 g corresponding to an estimated difenacoum intake 0.05-135 mg/kg and a median of 2.9 mg/kg. House mice (*M. domesticus*) showed similar variation with 3 animals estimated to have consumed difenacoum equivalent to 60-240 mg/kg and 2, 0.1-1.0 mg/kg. It appeared that difenacoum bait had been consumed by a bank vole (*Clethrionomys glareolus)* resulting in an estimated difenacoum intake of 0.06 mg/kg but it is not possible to determine whether the intake by 3 shrews (*Sorex spp.*) 0.05, 8.7 and 18.1 mg/kg was through primary or secondary exposure.

The single dose, oral toxicity (LD_{50}) of difenacoum for laboratory mice was reported as 0.8 mg/kg and the 5-day multi-dose as 0.07 mg/kg/day equivalent to a total difenacoum intake of 0.35 mg/kg body weight (Bull 1976). If this value is representative of the toxicity to wild small mammals, then animals containing >0.35 mg/kg bait marker (74% of all positive samples in our study) may have received an LD_{50} dose of difenacoum. However, it is likely that significant variation on toxicity exists as Rowe et al (1981) reported wild-trapped house mice able to survive accumulative dietary intakes of 144 mg/kg.

Although haemorrhaging is generally accepted as indicative of exposure to anticoagulant rodenticides it is not infallible. In our study, animals for laboratory examination were killed in the field. In week 1 this was by cervical dislocation following diethyl ether anaesthesia, causing some internal bleeding. To prevent mis-diagnosis, exposure to diethyl ether vapour alone was used to kill the 78 animals sampled during weeks 2-7. Of the 26 animals containing marker trapped in weeks 2-7, only 58% showed haemorrhages. There was no significant correlation between the presence of haemorrhage and the level of marker residue ($P>0.05$) and

as over 40% of marked animals did not show haemorrhaging these markers appear to be more sensitive indicators of exposure. In addition, the study suggested that haemorrhaging is not a reliable indicator for exposure to anticoagulant rodenticidesis as haemorrhages were found in 46% of the animals sampled whereas only 33% contained bait markers. Further evidence to support this cnclusion comes from an analysis of data for incidents reported as possibly involving rodenticides during operation of the Wildlife Incident Investigation Scheme (Hardy et al 1986) . In the period 1981-1990 confirmation of anticoagulant residues in tissue samples was obtained in only 24% of mammalian and 18% of avian incidents in which post-mortem veterinary investigation reported haemorrhages.

During weeks 2-6, 7 voles (*C. glareolus*) were found dead in the traps and although 2 showed signs of haemorrhaging neither contained marker and it was considered likely that all had succumbed to hypothermia due to the lack of suitable food. Similarly, although 5 of the 6 mice found dead in the traps had haemorrhages, only a house mouse (*M. domesticus*) also contained high levels of bait marker. Shrews often succumb in Longworth traps, but they are also reported to be extremely sensitive to warfarin poisoning, following either primary or secondary exposure (Harradine, 1976). From the sample of 9 dissected, 3 were found to contain bait marker, one of which also had haemorrhages and can be considered to have died as a result of the anticoagulant rodenticide treatment.The halogenated biphenyls used as bait markers belong to a class of compounds known to induce liver microsomal enzyme systems, although the response shows a wide variation among the congeners. In this study the number of animals restricts any comparative investigation of relative liver weight (liver weight per 100 g body weight, RLW) to *A. sylvaticus*. The mean RLW ($\pm$SE) of animals of this species containing bait marker was 6.57 ± 0.21 ($n=22$) and this is significantly higher ($t=5.20$, $p<0.001$) than the mean value (5.12 ± 0.19, $n=58$) found for animals in which no marker was detected. As there was no difference in the body weight of both sets of animals it was concluded that some induction had occurred. The potential influence of this on pesticide toxicity should be considered when interpreting the results of studies using chemical bait markers.

The disadvantage of physical markers is that they only allow animals that have eaten bait in the previous 24 h to be identified. They have been used to show that non-target species have had access to bait (Fellows et al 1988) and are most appropriate to studies of acute poisons. The use of pollen grains by Gemmeke (1990) in conjunction with pellet collection showed that two tawny owls resident on a farm during a bromadiolone treatment had ingested prey exposed to the rodenticide.

Hegdal and Blaskiewicz (1984) used a chemical marker (DMTC) incorporated into brodifacoum bait used for control of commensal rodents. Pellets from barn owls in the treatment areas did not contain marker, but here the target species constituted only a small proportion (6%) of the diet of the owls. In another study with barn owls (Harrison et al 1988) pellets were collected from an area in Eire where rodent control was being undertaken by farmers. The pellets were found to contain rodent remains indicating that rats and house mice constituted 45% of the food of barn owls in this area, but no rodenticide residues were detected despite the reported rodenticide usage in their study area. This study was directed mainly at obtaining information regarding flocoumafen but studies by Gray et al (1992) with barn owls and difenacoum and brodifacoum, and by Townsend et al (1981) with tawny owls and warfarin indicated that residues of these compounds should have been detected if present.

Our study has shown that halogenated biphenyls can be used to study feeding behavior of non-target primary species and could be expected to be present in owl pellets following ingestion of contaminated prey. These compounds extend the available range of techniques for studying potential exposure independent of a specific pesticide and use of poison. When only a single marker is required then given that the storage ratios of DCBP and HCBP are similar, the use of DCBP would be preferable as the retention time on analysis is longer and subject to less interference from co-extracted material. In addition DCBP produces less changes in liver microsomal enzymes. This study also shows that non-target small mammals are significantly exposed during outdoor use of difenacoum. Furthermore, Cox and Smith (1990) showed an effect of outdoor bromadiolone use on woodmouse survival. Given that such species represent a higher proportion of the diet of predators such as owls than target rodents, then this route of exposure needs to be examined in more detail if risk management methods are to be identified.

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