Bull. Environm. Contam. Toxicol. 27, 856–863 (1981)

Inhibition of Brain Acetylcholinesterase Activity in Songbirds Exposed to Fenitrothion During Aerial Spraying of Forests

G. A. Hamilton, K. Hunter, and A. D. Ruthven

Department of Agriculture and Fisheries for Scotland, Agricultural Scientific Services, East Craigs, Edinburgh, Scotland EH12 8NJ

In 1976, outbreaks of Pine Beauty moth (Panolis flammea) were recorded on plantations of Lodgepole pine (Pinus contorta) in northern Scotland and by 1977 attacks were so severe that chemical control was urgently required to avoid serious damage to large areas of forest. Aerial spraying of insecticide on the scale required had not been used previously in the United Kingdom and the preferred ULV technique had neither been tested nor approved for forestry use. In 1978 a programme of spraying with fenitrothion (O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate) was begun under limited clearance from the U.K. Pesticides Safety Precautions Scheme. A condition of this clearance was that any environmental impact resulting from the spraying programme should be assessed, including effects on forest songbirds. A limited study done in 1978 showed that residues of fenitrothion were present on birds of several species taken within a few days of the spraying (TILBROOK 1978). No major immediate effects on wildlife were observed but population studies by BROAD & DENNIS (1978) suggested some decrease in numbers in two of the seven most abundant songbird species in the period following spraying. However only a limited amount of residue analyses were done and no attempt was made to determine any sub-lethal effects of the spraying. In 1979 and 1980 further areas of forest were sprayed with fenitrothion using the ULV technique and on both occasions samples of various avian species were taken to determine any effect on brain acetylcholinesterase (AChE) activity and provide further information on the distribution of fenitrothion residues in the birds. The results of this work are reported here.

MATERIALS AND METHODS

In 1979 the study area was part of a forest in Grampian Region, Scotland. An area of 770 ha was sprayed on 10 June 1979 using a Pilatus Porter aircraft fitted with Micronair Spray Units. The formulation used was a 50% emulsifiable concentrate of fenitrothion diluted with butyl dioxitol to a concentration of 300 g/L active ingredient and applied at a rate of 1 L/ha. The two avian species, chaffinch (Fringilla coelebs) and coal tit (Parus ater) were chosen

0007-4861/81/0027-0856 $01.60

for this study because they were among the most abundant species present in this forest and had different feeding habits. Birds were collected by shooting on four occasions; several days before spraying and at intervals of 1, 4 and 11 days following spraying.

In 1980 the study area comprised parts of a number of adjacent plantations in Highland Region, Scotland. This area totalled 350 ha and was sprayed on 27 and 28 May 1980 using the same technique and formulation of fenitrothion as in 1979. The bird species sampled were chaffinch and willow warbler (Phylloscopus trochilus); collections were made on five occasions, pre-spray and 1, 2, 7 and 21 days after spraying. The birds were obtained by mist netting where possible or by shooting. Those captured in mist nets were killed by thoracic pressure.

All the samples were placed in separate clean glass jars, deep frozen within a few hours of collection and stored until analysis several weeks later. The head of each bird was separated from the body and the brain was removed for determination of AChE activity using the method of BUNYAN et al.(1968a). The activity was calculated as micromoles/min/g of brain tissue. The feet and tarsus were removed from each carcase and the bodies were skinned. The entire viscera was removed from the carcase and each of the three portions, skin plus plumage, viscera and carcase were analysed separately for residues of fenitrothion. Samples were chopped, dried by admixture with anhydrous sodium sulphate and extracted for 4 h with acetone using a Soxhlet apparatus. The volume of the extracts was adjusted as necessary and the fenitrothion content determined by gas chromatography (GC) with a phosphorus-specific flame photometric detector. A glass GC column (1.5 m x 2 mm i.d.) packed with 3% OV 225 on 100/120 mesh Chromosorb W-HP was used at a temperature of 220°C with oxygen-free nitrogen as the carrier gas at a flow of 40 mL/min. Recovery of fenitrothion was at least 75% and the limit of detection was 0.2 µg.

RESULTS

The whole body residues of fenitrothion and the brain AChE activities from all the samples are summarised in Table 1. The degree of AChE inhibition of individual birds at the various sampling times are shown in Figs. 1-4.

In 1979 about 50% of all chaffinches taken up to 11 days after spraying showed marked depression of brain AChE and there was no evidence of recovery up to 11 days. The sample of coal tits obtained was very limited but birds taken 1 day after spraying all showed considerable depression of AChE and this was still evident in one of two birds obtained 11 days after spraying.

The chaffinches in 1980 had a high degree of AChE inhibition with more than 80% of the birds taken on three occasions up to 7 days after spraying showing severe depression. However at 21 days only 2 out of 12 birds showed some depression of AChE.

TABLE 1. Brain AChE activities (μ moles/min/g) and whole body residues (mg/kg) in songbirds as a result of aerial spraying of forests with fenitrothion.

Species	Sample Interval	No. of birds	Fenitrothion residue mean ± S.D.	Brain AChE mean ± S.D.	% Inhibition
Chaffinch	Pre-Spray	5		42.3 ± 3.3	
(1979)	1 day	7	0.80 ± 0.67	34.8 ± 9.5*	17.5
	4 days	4	0.33 ± 0.26	35.2 ± 4.2**	16.9
	11 days	10	0.11 ± 0.09	32.7 ± 8.0**	22.8
Coal Tit	Pre-Spray	6		41.8 ± 3.8	
(1979)	1 day	4	7.45 ± 3.09	22.2 ± 3.0**	47.1
	4 days	6	0.89 ± 0.88	28.3 ± 10.0*	32.2
	11 days	2	0.10 ± 0.05	36.8 ± 9.7**	12.1
Chaffinch	Pre-Spray	6		45.3 ± 3.2	
(1980)	1 day	13	2.40 ± 2.50	26.3 ± 10.7**	42.7
	2 days	10	0.62 ± 0.20	28.5 ± 6.0**	37.9
	7 days	11	0.25 ± 0.12	30.5 ± 3.8**	33.5
	21 days	12	0.04 ± 0.02	39.8 ± 3.3**	13.0
Willow Warbler	Pre-Spray	8		34.3 ± 2.3	
(1980)	1 day	12	1.90 ± 1.17	25.2 ± 5.3**	26.6
	2 days	12	1.01 ± 0.44	24.0 ± 5.8**	30.0
	7 days	12	0.14 ± 0.09	32.7 ± 1.7**	5.0
	21 days	13	0.25 ± 0.35	31.2 ± 4.3**	9.2

*Inhibition significant at $P < 0.02$
**Inhibition significant at $P < 0.001$

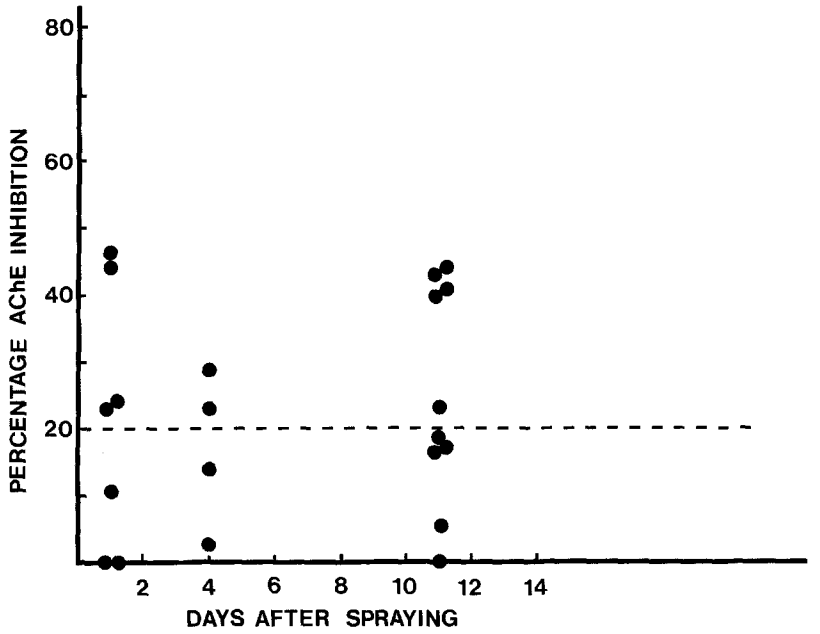

Figure 1. Inhibition of brain AChE activity in Chaffinches (1979)

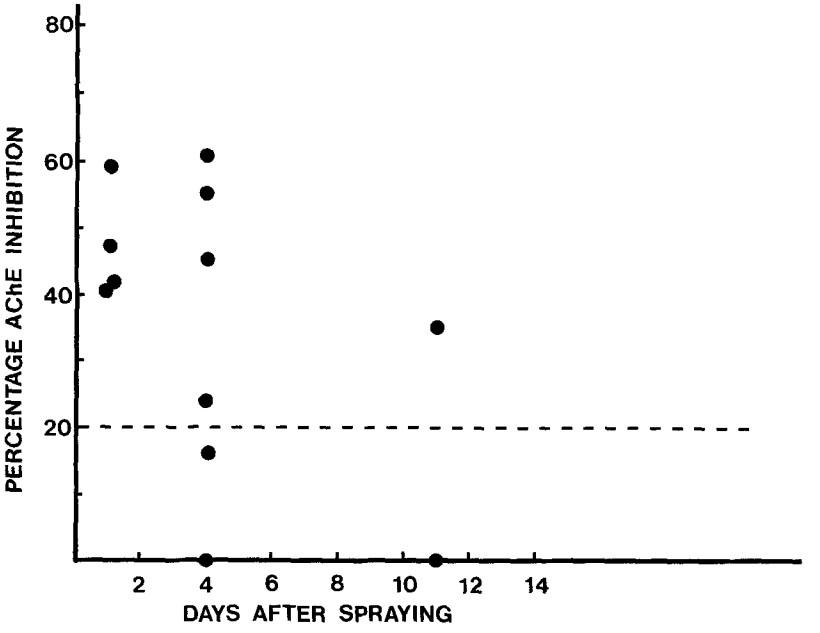

Figure 2. Inhibition of brain AChE activity in Coal Tits (1979)

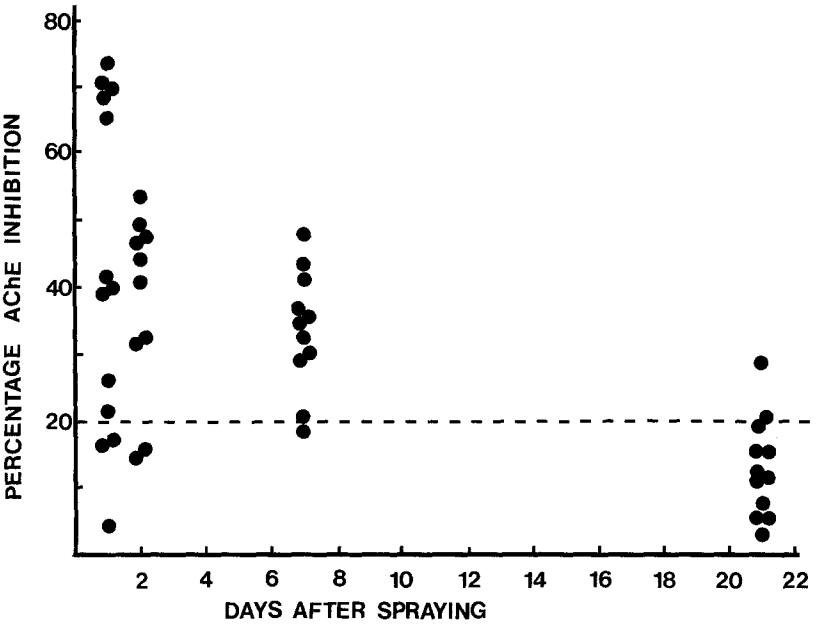

Figure 3. Inhibition of brain AChE activity in Chaffinches (1980)

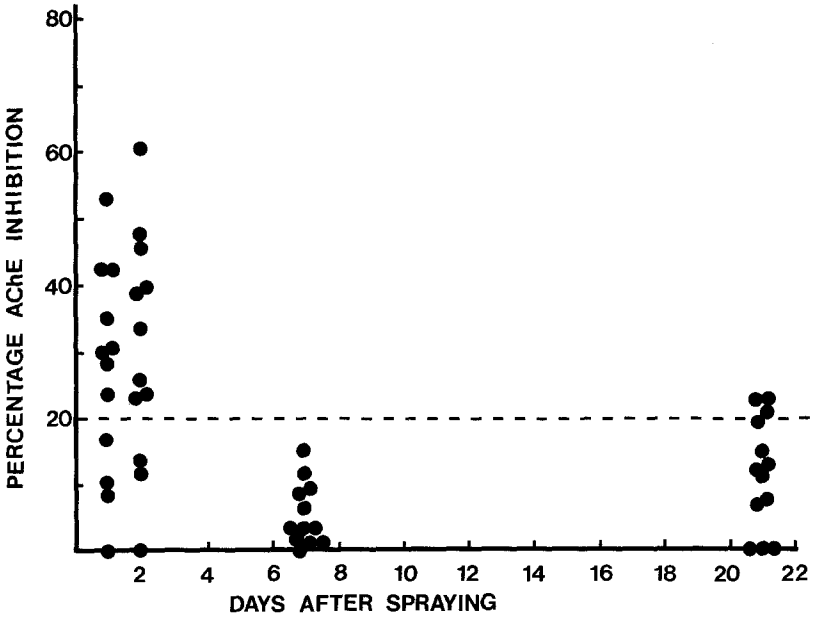

Figure 4. Inhibition of brain AChE activity in Willow Warblers (1980)

The willow warblers taken in 1980 showed that there was marked inhibition in 70% of the birds taken up to 2 days after spraying but subsequent samples indicated recovery since only 12% of these birds showed some degree of AChE inhibition.

Fenitrothion residue analysis of the 3 portions of the birds showed that large amounts were concentrated on the skin and plumage of many birds and that this residue formed at least 95% of the total body burden in each group of samples. In all the groups the maximum residue was found in birds taken 1 day after spraying and this declined rapidly with the later samples showing only low residues.

DISCUSSION

Measurement of inhibition of brain AChE in avian species has been widely reported as an indicator of exposure to organophosphorus pesticides and as a means of assessing the effect of such exposure (BUNYAN et al. 1968b, LUDKE et al. 1975, WHITE et al. 1979, BUSBY et al. 1981). Based on some of these results and their own observations, ZINKL et al. (1979) suggest that brain AChE inhibition of at least 80% is necessary for birds to die from a single oral dose of an organophosphorus pesticide, whereas 50% inhibition resulting from continuous exposure may cause death.

LUDKE et al. (1975) considered that inhibition of 20% was indicative of exposure to organophosphorus pesticides. A modification of this concept by ZINKL et al. (1979 and 1980) used the lower of two values, either the mean activity of the control birds minus 2 standard deviations or the mean activity of the control birds minus 20% of the mean. Birds with AChE activities below this level were judged to be significantly inhibited. In all four of the pre-spray sample groups reported here the mean activity minus 20% was the lower value and has been used as the indication of exposure.

The numbers of chaffinches and coal tits obtained in 1979 were limited, partly because of the relatively small populations present in the area and also because many birds were severely damaged by shooting and it was not possible to recover suitable tissue samples from them. Nevertheless it was evident that brain AChE activity was significantly inhibited in many birds and the maximum depression may not have occurred until several days after spraying. The samples taken 11 days after spraying showed that activity was still significantly depressed in 50% of the birds with several showing inhibition greater than 40%. There was no apparent difference observed in the results from the two species sampled.

The sampling done in 1980 was more extensive and the use of mist nets or shooting with fine grade shot made it possible to recover tissues from every bird. The sampling times were extended to 21 days after spraying. The results from these samples show a similar but more marked effect on brain AChE activity. Over 70% of the birds taken during the first 2 days after spraying had significant

inhibition of brain AChE with many exceeding 40% inhibition. At 7 days after spraying there was no evidence of recovery in the sample of chaffinches but there was a substantial change of activity in willow warblers with no birds showing significant inhibition in this sample. The final samples of both species, taken 21 days after spraying showed that recovery had taken place in chaffinches also, but some inhibition was still evident in both species.

BUSBY et al. (1981) reported a lower proportion of songbirds showing significant but smaller reductions in brain AChE following forest spraying with fenitrothion at a similar rate of 280 g/ha. However, sampling in this study was limited to a period of 48 h after spraying. ZINKL et al. (1979) showed that some insecticides may cause maximum AChE inhibition several days after exposure and that the effect can still be present for periods up to 33 days. Our results suggest that severe inhibition occurs in the first 2 days after initial exposure and that this may persist for several days. Moreover significant reductions in AChE activity may be present in certain species for periods of several weeks.

In both the 1979 and 1980 studies many birds had a reduction in AChE activity of 50% or more, and even 7 and 11 days after spraying several chaffinches showed inhibition near this level. It is possible that some birds with this degree of AChE inhibition may have died (ZINKL et al. 1979), but searches of the sprayed area for casualties were unlikely to be successful because of the density of the tree planting and ground vegetation. In 1980 one chaffinch was found on a forest roadway about 24 h after spraying. It showed unco-ordinated movements and was unable to fly and post-mortem examination revealed no obvious evidence of disease or injury. This bird had a reduction in brain AChE activity of 50% and a whole body residue of 1.38 mg/kg of fenitrothion.

Detectable residues of fenitrothion were found on every bird taken after the spraying in both years. The residues found on skin and plumage were very high from birds taken during the first few days after spraying but these declined fairly rapidly to a low level in later samples. Fenitrothion residues in viscera samples were initially much lower and not detectable after a few days from spraying. Very low residues were found in the remainder of the carcase of a few birds but these residues could have resulted from contamination from plumage. The residue results indicate that contamination of the plumage is an important feature in accumulation of fenitrothion from forest spraying. This may be followed by ingestion during preening or direct absorption through the skin. Insectivorous species will also take in fenitrothion during feeding because many of the invertebrate populations in sprayed forests will be contaminated by the spray. Analyses of P. flammea larvae on two occasions soon after spraying showed mean residues of 2.7 and 1.3 mg/kg. Analyses of some other invertebrates showed a range of residues of fenitrothion from 0.5 to 25.0 mg/kg.

No attempt was made to assess either direct mortality or behavioural effects on the bird populations following the forest spraying in 1979 and 1980. On an earlier occasion BROAD & DENNIS (1978) reported a reduction in populations of robins (Erithacus rubecula) and willow warblers following a similar spraying programme. No significant changes were noted in populations of several other species but no measurements of brain AChE activities were made on that occasion. The marked inhibition of AChE activity reported here suggests that some mortality may have occurred in certain species as a result of the fenitrothion spraying in 1979 and 1980. Reduced singing activity, nest desertion, increased nest predation, adult and nestling mortality and decreased nestling growth were associated with 40-50% AChE inhibition in white-throated sparrows, (Zonotrichia albicollis) following a similar spraying programme (BUSBY et al. 1981). More than 25% of the birds examined after the 1979 and 1980 spraying showed AChE inhibition above 40% and this suggests that similar sub-lethal effects may have occurred. It is likely that the sampling methods would have selected birds which were less affected by the spray because sick birds would be less active and not easily seen or caught. The present study shows that aerial spraying of forests with fenitrothion may present a considerable hazard to some songbird species, but a longer-term study of populations and behaviour following spraying would be necessary to define the nature and extend of this effect.

ACKNOWLEDGEMENTS

We thank staff of the Forestry Commission, Nature Conservancy Council and Department of Agriculture and Fisheries for Scotland for their assistance in this work and in particular, J.H. Cuthbert, S.R. Baillie and D.S. Whitaker for much of the sampling.

REFERENCES

BROAD, R. A. AND R. H. DENNIS: In: Control of Pine Beauty moth by Fenitrothion in Scotland 1978. U. K. Forestry Commission, Edinburgh. pp. 143-175 (1978).

BUNYAN, P. J., D. M. JENNINGS AND A. TAYLOR: J. Agric. Food Chem. 16, 326 (1968a).

BUNYAN, P. J., D. M. JENNINGS AND A. TAYLOR: J. Agric. Food Chem. 16, 332 (1968b).

BUSBY, D. G., P. A. PEARCE AND N. R. GARRITY: Bull. Environm. Contam. Toxicol. 26, 401 (1981).

LUDKE, J. L., E. F. HILL AND M. P. DIETER: Arch. Environ. Contam. Toxicol. 3, 1 (1975).

TILBROOK, P. J.: In: Control of Pine Beauty Moth by Fenitrothion in Scotland 1978. U. K. Forestry Commission, Edinburgh. pp. 133-141 (1978).

WHITE, D. H., K. A. KING, C. A. MITCHELL, E. F. HILL AND T. G. LAMONT: Bull. Environm. Contam. Toxicol. 23, 281 (1979).

ZINKL, J. G., C. J. HENNY AND P. J. SHEA: In: Animals as Monitors of Environmental Pollutants. National Academy of Sciences, Washington, D. C. pp. 356-365 (1979).

ZINKL, J. G., R. B. ROBERTS, C. J. HENNY AND D. J. LENHART: Bull. Environm. Contam. Toxicol. 24, 676 (1980).