Accidental poisoning of wild geese in Perthshire, November 1971

S. BAILEY, P. J. BUNYAN, G. A. HAMILTON, D. M. JENNINGS and P. I. STANLEY

Introduction

During the latter half of the 1950's the use of persistent chlorinated hydrocarbon insecticides for cereal seed dressing led to the deaths of increasing numbers of seed-eating birds, particularly following the use of dressed seed for spring sowing. Investigations (Turtle *et al.* 1963) by the Pest Infestation Control Laboratory (P.I.C.L.) of the Ministry of Agriculture, Fisheries and Food (M.A.F.F.) showed that aldrin, dieldrin and heptachlor used to dress cereals sown in spring were almost certainly responsible for the incidents. As a result of these investigations and the concern of the general public and various ornithological and naturalist organisations, it was agreed in 1965 that these dressings should not be used on spring-sown cereals, and that in autumn they should be restricted to those areas where there was a real danger of attack by wheat bulb fly *Leptohylemyia coarctata.* Although the agreement reached with manufacturers and distributors of pesticides under the "Pesticides Safety Precautions Scheme" is voluntary it appears to work well in practice, and reports of mass bird deaths of the type previously seen are now relatively rare. However, partly because of continuing minor incidents, successive official reports (for example Cook 1964) have urged the development of less persistent seed dressings and a few alternatives are now available. Two cereal dressings based on the more acutely poisonous, but less persistent organophosphorus pesticides, chlorfenvinphos (2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate) and carbophenothion (S-(4-chlorophenylthiomethyl) diethyl phosphorothiolothionate) have recently come into use. We wish to report the poisoning of some hundreds of Greylag Geese *Anser anser* as a result of the use of one of these pesticides and thereby draw attention to hazards which might arise following the use of such seed dressings in certain special circumstances.

Field details and background to the incident

Boyd and Ogilvie (1972) report that the large majority of the Greylag Geese wintering in Britain come from the population breeding in Iceland and that the Greylags enter Britain during October. In the autumn, up to two-thirds of the wintering stock is concentrated in east central Scotland particularly the counties of Angus, Perth and Fife. Counts in November each year reveal that the present wintering population amounts to approximately 60,000 to 65,000 birds. In east central Scotland, the Greylags typically roost inland on small areas of water and feed on agricultural land within a few miles of the roost. Strathmore, an area of low-lying agricultural land well provided with standing water in the form of numerous small lochs and rivers, holds a large wintering Greylag population including at least 3,000 birds on the River Isla near its confluence with the Tay.

The incident took place on arable land bordering the lower part of the River Isla. It has been normal practice for these fields to be sown at the end of October with winter wheat and barley and for a seed-dressing to be used to protect the grain against wheat bulb fly and fungal attack. On about 40 hectares of winter wheat and barley sown in this area between 25th and 27th October 1971 at least 200 geese had died by 1st November. Birds still alive were observed to have foam emerging from the beak and to have blood in their droppings. The presence of grain on the surface of these fields was also reported. Six geese were sent at this time to the Veterinary Investigating Officer (V.I.O.), East of Scotland College of Agriculture, Perthshire, for post-mortem examination. A further six corpses were obtained on 8th November by the East Craigs Laboratory of the Department of Agriculture for Scotland (D.A.F.S.).

Geese continued to die for at least a week, 500 being a reasonable estimate of the total number of deaths. Most of the geese were buried by the farmers on whose land they died so that the exact numbers and species are not known. However, samples sent for examination were Greylag Geese with the exception of one Pink-footed Goose *Anser brachyrhynchus* and it seems likely that the incident involved predominantly Greylags.

A selection of the tissues, including the crop contents, brain and liver from six birds was received by P.I.C.L. from the V.I.O. (1-6) and one whole goose (7) was obtained from D.A.F.S. who also sent two specimens to the Veterinary Laboratory, M.A.F.F., Lasswade, for post-mortem examination. The warden of the Loch Leven National Nature Reserve kindly supplied two shot Greylag Geese for comparative purposes (8 and 9).

Methods and results

Post-mortem examination

The post-mortem examinations carried out by the East of Scotland Agricultural College, Lasswade, and the P.I.C.L. revealed that birds picked up during the early part of the incident had died fairly quickly with little deterioration in general condition. The crop and gizzard contents included considerable quantities of soil and wheat, some of which showed evidence of a red dye. Birds dying later in the incident showed loss of condition and had almost empty crops and gizzards. At least two of the specimens had haemorrhages into the large intestine. Bacteriological examination and examination for parasites revealed nothing of pathological significance.

Organochlorine pesticide residues

Portions of the livers from three geese were extracted by the method of Taylor *et al.* (1964) and analysed on a Pye 104 gas-liquid chromatograph (g.l.c.) equipped with a ^{63}Ni electron-capture detector. The analysis was carried out on a 3 ft. × 4 mm. internal diameter (i.d.) glass column packed with 2.5% silicone oil and 0.25% epikote on 100/120 mesh celite and maintained at 160°. Two birds had no detectable residues whilst the third contained 0.1 parts per million (ppm) p,p'DDT, 0.1 ppm p,p'DDE and 0.04 ppm p'p'DDD.

Total mercury residues

Combined organic and inorganic mercury was measured in portions of the livers from three geese and in portions of the combined crop and gizzard contents from six geese. The samples were digested with nitric acid and hydrogen peroxide (Monk 1961) and the inorganic mercury thereby formed, estimated by a flameless atomic absorption method (Hatch and Ott 1968) with the final measurement on an EEL 240 atomic absorption spectrophotometer. Results are given in Table I, in milligrammes (mg.) and parts per million (ppm).

Organophosphorous pesticide residues

Portions of three livers and six crop and gizzard contents were admixed with anhydrous sodium sulphate to give a friable powder and continuously extracted with hexane. Analysis of the crop extracts, on a Pye 114 g.l.c. equipped with a phosphorous-specific thermionic detector using a 5 ft. × 4 mm. i.d. glass column packed with 5% diethylene glycol succinate on 100/120 mesh chromosorb W maintained at 230°, showed one major peak identified as carbophenothion. The identity was confirmed on two other columns by comparison of the retention times with an authentic sample. The liver extracts showed no peaks on the thermionic detector. By addition of an internal standard it was demonstrated that carbophenothion was unstable in the hexane extracts and therefore possibly in the crop contents. Thus results shown in Table I are almost certainly an underestimate of the quantities of carbophenothion remaining in the crop and gizzard at death.

Thin layer chromatography (t.l.c.) was also carried out on the hexane extracts of the crop contents. Silicagel G (500 micron) coated glass plates were run to a length of 150 mm. with 5% acetone in toluene and developed by the method of Mendoza *et al.* (1968) which is specific for esterase inhibitors such as the organophosphorus pesticides. A major and a minor cholinesterase inhibiting spot (Rf 0.74 and 0.22) was found in all the extracts and in the standards, and an additional spot (Rf 0.14) was found in the crop contents. The latter was probably

Table I. Total carbophenothion and mercury content of dead Greylag Geese.

Goose	*Carbophenothion Total crop and gizzard content (mg.)*	*Mercury Liver content (ppm)*	*Mercury Crop and gizzard content (ppm)*
1	17		3.0
2	1.2	0.8	1.1
3	0.7	0.6	
4	1.2		1.1
5	1.7		0.7
6	0.6		0.1
7		0.3	1.1

an oxidative metabolite of the pesticide. The liver extracts showed none of the above spots but did contain another cholinesterase inhibiting compound (Rf 0.02) which also appeared to be an oxidative metabolite. These results were confirmed on a second t.l.c. system.

Mass spectral confirmation

The 70 eV mass spectrum of the major component of the hexane extract of the crop and gizzard contents of goose 1 was obtained using a Pye 104 g.l.c. coupled to an AEI MS 30 mass spectrometer. It was found to be identical with the mass spectrum of carbophenothion.

Esterase determinations

Cholinesterase measurements were carried out on spun extracts of brain from all the geese by the standard method in use in the laboratory (Bunyan *et al.* 1968a) using a Radiometer automatic titration apparatus. Because measurements had not previously been made on tissue from Greylag Geese, two control birds (8 and 9) were also examined. All measurements were checked for linearity with both time and concentration. Results are shown in Table II. Brain protein levels were measured by the method of Lowry *et al.* (1951).

Although the control sample (8 and 9) was small, brain cholinesterase is generally a reliable parameter with a small deviation and we feel justified in concluding that all the geese from the incident exhibited depressed brain cholinesterase levels. However, whilst most of the values approached 90% inhibition which is our normal criterion of death due to organophosphorus poisoning (Bunyan *et al.* 1968b), only one (5) fully met the criterion. It may be that spontaneous reactivation occurs following the inhibition of cholinesterase by carbophenothion and further investigation is required to resolve this matter. However, starch gel electrophoresis of brain extracts coupled with histochemical staining for cholinesterase and α-naphthyl acetate esterase activity using methods described elsewhere (Bunyan and Taylor 1966) gave isoesterase patterns consistent with inhibition by comparison with patterns from the two controls. Unfortunately only in one case (7) was a wider range of tissues available to compare with the controls. However, measurements of triacetin esterase, α-naphthyl acetate esterase and phenyl benzoate esterase in brain, liver and kidney as well as starch gel electrophoresis of liver extracts from this bird all indicated a reduction of esterase activity consistent with organophosphorus pesticide poisoning.

Table II. Brain cholinesterase levels in Greylag Geese.

Goose	*Brain cholinesterase**
1	0.82
2	1.03
3	1.60
4	0.70
5	0.32
6	1.99
7	0.66
8	4.85
9	3.65

* μmol acetylcholine hydrolysed/h/mg. protein

Gas-liquid chromatographic analysis using electron capture and phosphorous specific thermionic detectors at the Agricultural Scientific Services Laboratory, D.A.F.S., confirmed the presence of carbophenothion in liver, breast muscle, crop and gizzard contents from other Greylag Geese involved.

Conclusion

It would appear that the dead geese had consumed considerable quantities of grain dressed with carbophenothion, a mercurial fungicide and a red dye. The agreement between the quantities of mercury and carbophenothion found in the crop contents is consistent with this conclusion and a commercial dressing having these three constituents is now available. The levels of mercury found in the liver are unlikely to have been lethal. There are no published data on the toxicity of carbophenothion to birds but the esterase measurements made on these geese support the conclusion that death was due to organophosphorus poisoning.

Significantly grain was still visible on the surface of the newly sown fields after the incident and it is also notable that a second smaller wave of deaths occurred after a nearby area was sown. Almost all of the grain eaten by Greylags is picked up from the surface and there is no evidence that they dig for properly sown grain (Kear 1963). Sowing under the most favourable conditions can leave some grain on the surface so that the presence of large numbers of geese in this particular part of the country can give rise to special problems. Greylags frequent the area in very large flocks at the time of autumn sowing and continue to be present in smaller numbers until April, so that it would be difficult to sow when geese are absent. Carbophenothion, although it may be generally preferable to a chronic and persistent poison like dieldrin, is an acute poison and might present a greater hazard to geese under these conditions. In areas regularly visited by geese such factors as the type and degree of insecticidal treatment required, and the sowing practices may repay attention, while scarers properly used (D.A.F.S. 1971) could prevent birds from feeding on newly sown fields. It is also known (Hutson and Hathway 1967; Bunyan *et al.* 1971) that certain organophosphorous compounds whilst generally of low toxicity can be highly toxic to a few species and it may be an unfortunate coincidence that carbophenothion is unduly toxic to Greylags. The apparent absence of casualties among other seed-eating species known to frequent the area lends support to this suggestion. More work will be required to clarify this point and to test how far species variation in toxicity extends among the organophosphorus pesticides currently in use. It thus becomes important to ensure by methods such as those described that, with the introduction of less persistent seed dressings, isolated incidents of the type described are avoided.

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Summary

Following the autumn sowing of wheat dressed with carbophenothion and a mercurial fungicide in an area of Perthshire visited by Greylag Geese *Anser anser* some hundreds of the birds were found dead. Analytical and biochemical investigations gave results which were consistent with poisoning by carbophenothion. Conclusions are drawn about this incident and and some suggestions made to reduce risks in future.

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S. Bailey, P. J. Bunyan, D. M. Jennings and P. I. Stanley, Pest Infestation Control Laboratory, Ministry of Agriculture, Fisheries and Food, Hook Rise South, Tolworth, Surrey.

G. A. Hamilton, Agricultural Scientific Service, Department of Agriculture for Scotland, East Craigs, Edinburgh 12.