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Review

Review of the factors affecting the decline of the European brown hare, *Lepus europaeus* (Pallas, 1778) and the use of wildlife incident data to evaluate the significance of paraquat

P.J. Edwards [a,*], M.R. Fletcher [b], P. Berny [c]

[a] *Zeneca Agrochemicals, Jealotts Hill Research Station, Bracknell, Berks RG42 6ET, UK*
[b] *Central Science Laboratory, Ministry of Agriculture Fisheries and Food, Sand Hutton, York YO41 1LZ, UK*
[c] *Laboratoire de Toxicologie, Ecole Nationale Veterinaire de Lyon, B.P. 83, 69 280, Marcy L'Etoile, France*

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Abstract

Hunting records indicate a decline in the populations of the European brown hare (*Lepus europaeus*) in the early 1960s. This paper summarises the likely reasons for the population decline. The emphasis on wildlife incident reports and paraquat is in response to the perception that it may present a risk to hares. The possibility for early incidents of European Brown Hare Syndrome (EBHS) being misdiagnosed as paraquat incidents are also considered.

The long-term decline in the hare population throughout Europe is widely believed to be due to changes in farmland management practices, resulting in the loss of crop/landscape diversity which affects nutrition. Predation and disease may lead to additional high mortality but probably do not influence the long-term population trend.

The decline in the hare population in England, as in Europe, started prior to the widespread introduction of paraquat. Wildlife incident schemes in the UK (WIIS) and France (SAGIR) confirm there have been very few hare deaths caused by paraquat. Research indicates that free living hares are likely to be deterred from foraging paraquat sprayed vegetation after an initial exposure.

Keywords: Hare; Wildlife; Population; Incidents; Disease; EBHS; Herbicides; Paraquat

1. Introduction

Wildlife incident data are a valuable source of post registration information to confirm the safety of pesticides in widespread commercial use. Hares are a medium sized high profile game species and as such are likely to feature strongly in incident schemes. Use of the herbicide paraquat started in Europe in 1964 and was followed by a number of unconfirmed reports of hare deaths linking the two. As a consequence Zeneca (formerly ICI), the manufacturer of paraquat, have monitored wildlife incident reports and carried out research in this area. As a precaution the label was changed to include a warning to minimise exposure to hares. A possible consequence of the new label and anecdotal reference to incidents in the agricultural

* Corresponding author. Tel.: +44-1344-414-158; fax: +44-1344-414-158.
E-mail address: peter.edwards@aguk.zeneca.com (P.J. Edwards)

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press was to increase awareness of paraquat and hare deaths. There are no published scientific data confirming hare incidents from paraquat use prior to incident reports published annually by the UK Ministry of Agriculture, Fisheries and Food, Wildlife Incident Scheme in 1976 and the French, Office Nationale de la Chasse Sanitary Surveillance Scheme for Wildlife in 1986. This paper is a review of these hare incident reports in the UK and France, research into the major causes of mortality and the significance of paraquat use for the European brown hare.

2. Population trends for the brown hare in Europe

The European brown hare originated on the open steppe grasslands of Eurasia and has adapted very successfully to mixed arable agriculture (Frylestam, 1980). It is an important small game species of mammal in Europe (Pielowski, 1976). As a result it has been possible to monitor long-term trends in its population through hunting records. Hunting records indicate a dramatic decline in the early 1960s in several European countries including Poland (Pielowski and Raczynski, 1976), Denmark (Strandgaard and Asferg, 1980) and the UK (Tapper and Parsons, 1984). The decline has been reviewed for Denmark, Germany, Austria, Switzerland and the UK by Mary and Trouvilliez (1995) (Fig. 1). These data indicate that up to 1989 the decline in the hare population may not have stabilised. Tapper and Parsons (1984) showed that hunting records were a reasonable means to provide temporal trends in the population. While there has been this decline in Europe the European hare has been successfully introduced into other countries such as New Zealand and Argentina (Flux, 1997; Bonino and Montenagro, 1997). Typically hunting records of brown hares shot can vary considerably from one year to the next depending on how good the breeding season has been, but there is no evidence of cyclical population fluctuations as has been found on some other lagomorph species (Tapper and Parsons, 1984). Hunting records in England, between 1938 and 1961, showed no consistent temporal trends, while after 1961, there has been a consistent decline (Tapper and Parsons, 1984). Marboutin and Peroux (1996) showed that hunting bags were synchronous between neighbouring districts in France indicating that trends are at least locally synchronous.

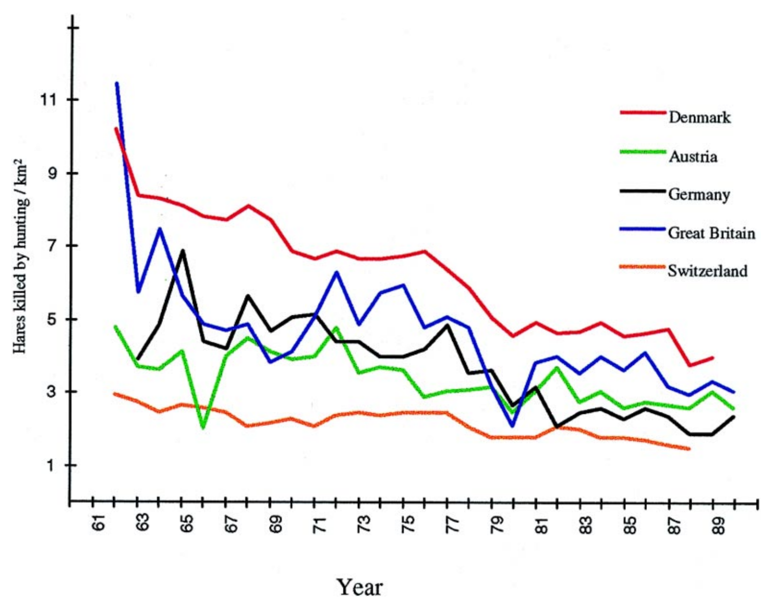

Fig. 1. Trends in the numbers of Hares shot/km^2 during the 1960s, 1970s and 1980s in five European countries (Mary and Trouvilliez, 1995).

3. Ecology of the European hare

As a consequence of the hare being a declining and important game species there has been considerable research done to understand the underlying causes to enable better management of the species. Hares are sedentary with home ranges typically ranging from 10 to 100 ha, (Broekuizen and Maaskamp, 1982; Tapper and Barnes, 1986; Kovacs and Buza, 1992; Reitz and Leonard, 1994). Within their home range, there needs to be available forage all year round so crop and landscape diversity are important (Tapper and Barnes, 1986). The most important forage for hares from October to May on arable farms in Ile-de-France are winter cereals (Chapuis, 1990) and this would be expected for most of Europe. Grass, particularly unstocked fields, is also an important forage for hares (Frylestam, 1976; Barnes et al., 1983) especially when winter cereals are not available during the summer and early autumn. Thus, on farmland with large fields and low crop diversity, the nutrition of the hare will be poor for part of the year limiting density. On lowland arable farms nutrition is poorest during the summer and autumn when the cereals are mature through harvest to emergence of the next winter wheat crop in October. Leverett production is dependent on good nutrition and weather and in a good breeding season can extend beyond the typical period of February to September (Tapper, 1987). During this time hares can produce as many as 3–4 litters of 2–3 leveretts (Hansen, 1992).

Hare populations in pastoral landscapes in the UK are very low compared to arable landscapes (McLaren et al., 1997) which is surprising considering that hares originated from open steppe grassland (Tapper, 1987). Reasons for this are unclear but may include grassland improvement, leading to higher stock density, leverett mortality from silage cutting and digestive problems from cultivated grasses (McLaren et al., 1997). This latter point is supported by Frylestam (1986) where data indicate that hares on pasture preferred wild grasses to perennial ryegrass, (*Lolium perenne* L.).

Red fox, *Vulpes vulpes*, is an important predator of leveretts and adult hares. The Game Conservancy in the UK estimate that a family group of foxes would consume 33 adult hares annually (in 'Hares and Arable Landscapes'). Predation may be more intense when small mammal populations are low, while the quality of cover (crops, hedgerows and woodland) is important to protect leveretts from predators (Pepin, 1989; Goszczynski and Wasilewski, 1992; Reynolds and Tapper, 1995).

Crop harvesting operations can be a further source of mortality where leveretts are using crops for cover (Milanov, 1996). Leverett losses were high in forage and grass fields with 44, 18 and 17% losses in lucerne (*Medicago sativa* L.), grass meadow and clover (*Trifolium* spp.) fields, respectively. Losses were much lower in arable crops, with 4 and 2% in spring barley (*Hordeum* spp.) and winter wheat (*Triticum* spp.), respectively (Kaluzinski and Pielowski, 1976). Harvesting operations do not appear to be a problem for adult hares (Marboutin and Aebischer, 1996).

Disease is a major source of hare deaths. Hare populations are at their highest in the autumn, comprising a high proportion of juveniles. At this time of year disease can lead to heavy mortality (Lamarque et al., 1996). Concentration of hares, especially juveniles, onto the remaining foraging areas in the autumn may promote disease transmission. Lamarque et al. (1996) showed that >50% of hares found dead in France between 1986 and 1994 died from disease, principally European Brown Hare Syndrome (EBHS), Yersiniosis (Pseudo-tuberculosis), Pasteurellosis and Coccidiosis. Coccidiosis is enhanced in wet weather whereas EBHS tends to have epidemic years following high leverett production. Juveniles, after loosing their protective maternally derived antibody, probably become susceptible to EBHS (Duff et al., 1997) with disease outbreaks killing most hares in September through December.

Hare populations have been shown to be resilient to heavy hunting pressure of between 40–50% of the population during the autumn when juvenile:adult ratios prior to hunting were between 1.2–2.5 (Pepin, 1989). This resilience must depend largely on high juvenile productivity. On good shooting estates autumn quotas are set to ensure shooting does not over exploit the population. However, if this is not practised, overshooting may be an additional factor.

In conclusion, evidence suggest that the loss of crop and landscape diversity is primarily responsible for the long-term decline in hare populations in Europe (Pielowski and Raczynski, 1976; Tapper and Barnes, 1986).

4. Paraquat use

Of all farmland wildlife, the hare must be one of the most exposed species to pesticides because they forage almost exclusively in fields. The introduction of paraquat as a contact herbicide used for total vegetation control in 1964 revolutionised farming in the 1960s. The rapid non-systemic foliar activity made it the ideal 'chemical plough' for soils susceptible to erosion through cultivation in a practice called 'direct drilling' (Hood, 1965). This practice not only made arable farming on fragile soils sustainable but also improved water quality by reducing soil run-off (Boon, 1973). Thus, paraquat is a significant Integrated Crop Management tool. Its non-systemic activity makes it ideal for use in row crops 'a chemical hoe' and for weed control in dormant (perennial) crops (Calderbank, 1968). Its strong adsorption to soil prevents run-off and leaching and furthermore makes it unavailable to soil organisms (Riley and Wilkinson, 1976). The principal uses and timing of paraquat in Europe are listed in Table 1.

Since 1964 the use of paraquat has steadily increased world-wide, as the benefits of this herbicide have been recognised by farmers. The major areas of expansion are in row crops and plantations. Over this period paraquat use for weed control in stubbles (prior to sowing spring crops) has declined with the increase in winter cropping in Western Europe and increased competition from non-selective systemic herbicides. This trend for paraquat usage in the UK can be seen in Fig. 2. This decline has not occurred for weed control in lucerne in France during the winter when it is dormant.

Indices in Fig. 2 show that the hare decline was most pronounced before paraquat was first sold in 1964 and that there was no major change in the population in the early 1970s when paraquat usage increased dramatically. The hare population in the UK appears to have stabilised at the lower level established in the 1980s. The trend in the decline of hare population in Denmark between 1961 and 1988 is similar to that in the UK (Mary and Trouvilliez, 1995). Unlike the UK paraquat used in arable crops has been negligible throughout this entire period in Denmark amounting to less than 0.1 times that used in the UK.

Table 1
Principle uses of paraquat and timing in European agriculture

Use	Season
Prior to drilling winter crops	August–October
Prior to sowing spring crops	January–April
Dormant lucerne	November–January
Row crops and plantations	Spring–Summer
Grass	June–September

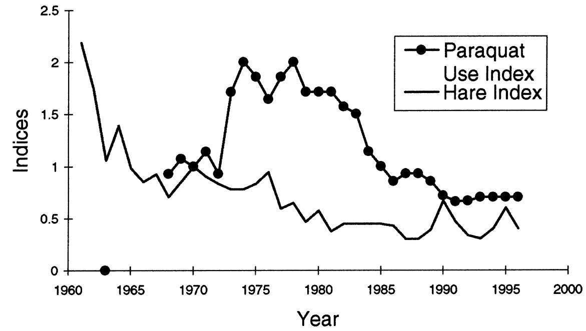

Fig. 2. UK indices for the hare from 1961 to 1996 (National Game Bag Census) and for paraquat sales volume from 1968 to 1996 (Indices relative to 1 in 1970). The year 1970 was chosen as a reference in Fig. 2 because it was close to the mid point for numbers of hares in the National Game Bag Census. National Game Bag Census data are courtesy of U.K Game Conservancy.

5. Diagnosis of paraquat incidents

To be confident in the diagnosis of paraquat as the cause of death it is important to look for evidence of paraquat toxicity and exposure by residue analysis. Paraquat toxicity can be separated into local and systemic effects. Local effects are characterised by ulceration of the mucous membranes (nose, lips, tongue and stomach) and complete recovery is normal. Systemic effects are characterised by lung and kidney damage. In the lung, congestion, haemorrhage, oedema and fibrosis (thickening of the alveoli walls with connective tissue) which can lead to death, are typical (Clark et al., 1966). However, following acute exposure, death may occur before fibrosis has developed, which may explain why Lavaur et al. (1973) and Rosmini et al. (1980) did not observe fibrosis in lung tissue of experimentally poisoned hares. Typical of kidney damage in the rabbit is proximal tubular necrosis (unpublished data). Paraquat is a polar compound and readily excreted in the urine by animals, thus the kidney is the most appropriate organ for residue analysis in poisoning incidences as any paraquat will be concentrated there. The typical limit of detection for paraquat in tissue is 0.1 mg/kg and this should be sufficient to detect residues in the kidneys 5 days after a hare has received a lethal dose assuming the excretion kinetics are similar to the rat (Murray and Gibson, 1974). Further unpublished data for the rabbit support this conclusion. Paraquat residues in the kidney of rabbits 3 days after receiving a median lethal acute oral dose were 2.7 mg/kg (wet weight). Residues in the kidneys of rabbits 7 days after receiving a much lower non-lethal dose were 0.02 mg/kg (wet weight). It is difficult to be certain that paraquat is or is not the cause of death from residue analysis alone. Animals which receive a median lethal dose of paraquat may take 3–5 days to die, thus a positive residue in the kidney could indicate either recent low non-lethal exposure or be the result of earlier high lethal exposure. In isolation, the typical paraquat lung effects (congestion, oedema and haemorrhage) could be confused with post-mortem change and EBHS pathology, therefore confirmation of the presence of paraquat is required if the death is to be attributed to the compound. The detection of residues of paraquat in the kidney, ulceration of the mucous membranes (nose, lips and tongue) and the absence of liver damage should be sufficient to separate paraquat from EBHS. The presence or absence of antibodies is not diagnostic of the cause of death from EBHS because seropositive hares may have survived the disease. Clinical signs of paraquat toxicity in laboratory rats include subdued behaviour and difficulty in breathing where damage led to respiratory failure (Clark et al., 1966).

6. Incidents and research

6.1. Historical incidents

Between 1964 and 1971 there were 19 unpublished reported hare incidents from France and the UK associated with the use of paraquat. Typically incidents involved about 20 hare deaths (range 5–120). Clinical observations are mostly not available. In one incident the hare was described as behaving in an unusual way prior to death and was well nourished. In another incident many hares were found dead in village gardens where they are not usually seen. In the UK a total of nine incidents involved spraying stubble or grass while in France 10 incidents involved spraying dormant lucerne. Paraquat residues were detected in hares from nine of these 19 incidents, stomach contents (five), urine (two), liver (one) and kidney (one). These analyses confirm exposure but not necessarily the cause of death. Herbicides, particularly paraquat, through association of their timing of use and the high frequency of hare deaths in the autumn (principally from disease) has led to the belief that herbicides are responsible.

6.2. Research

In response to these incidents, field observations were made in November 1965; paddock enclosure studies conducted with INRA, France in 1972 and 1980 and label recommendations made to reduce exposure in 1981. Field observations were made on five farms in Berkshire, UK in November 1965. In total approximately 32 hares were counted in close proximity to 110 ha of stubble, sprayed with paraquat to control weeds. Only live hares were observed and no dead hares were found (unpublished data). In 1973 a no choice paddock enclosure study was conducted in co-operation with INRA. Eight hares were exposed for 2 days in enclosures to lucerne and grass which had been freshly sprayed with paraquat. Five hares died and the three survivors showed post-mortem signs of local and systemic paraquat toxicity. One of the hares died from disease (Lavaur, et al., 1973). In a second paddock enclosure study, two hares were each exposed to the following four treatments: repellent only (ammonium sulphate), paraquat only, paraquat+repellent and an untreated control (Grolleau, 1981). In these paddocks hares had a choice of treated and untreated areas of lucerne. The study was conducted under wet conditions then repeated under dry conditions. No hares died in any treatment group under either weather conditions. It was noteworthy that even paraquat alone reduced feeding on tillers. This effect might be expected if the mucous membranes of the mouth were irritated by paraquat (local toxicity) and hares have free access to untreated as well as treated areas.

6.3. Recent incidents (after 1974)

Wildlife and game incident schemes in the UK and France, respectively have been used to interrogate recent trends.

6.3.1. United Kingdom

In the UK the Ministry of Agriculture, Fisheries and Food (MAFF) and other government agricultural departments administer a Wildlife Incident Investigation Scheme (WIIS). One of the objectives of WIIS is to monitor incidents, should they occur, following recommended (labelled) use once products are registered and used commercially (Fletcher and Grave, 1992). WIIS data are available for every year from 1974 to 1997 in annual reports (e.g., Fletcher et al., 1997). After an incident is reported to the Scheme, a field biologist makes a field enquiry and a post-mortem is carried out. An incident may involve several animals. If there is no obvious cause of death i.e., trauma or disease, and the enquiry indicates pesticide use, residue analysis is attempted to confirm exposure or likely cause of death. In the case of paraquat, residue analysis was conducted if there were post-mortem signs of paraquat toxicity (mouth and lungs) and/or the field enquiry indicates paraquat was used in the vicinity of the incident. Over a 23 year period 8887 incidents (wildlife and companion animals) have been reported to WIIS. During this period there have been 104 hare incidents of which two were confirmed paraquat incidents (2%) (Table 2). In both paraquat incidents several hares were found dead on or very close to the sprayed fields of grass and potato in January and August, respectively.

The frequency of autumn hare incidents reported in 1984 and 1989 was much higher than for other years, following good breeding seasons. The 1984 incidents were widely distributed throughout southern, central and eastern England. Incidents typically involved up to 20 individuals with some reports of numerous and heavy losses. In 80% of incidents hares were in apparent good physical condition at the time of death. With one exception (shot hare) kidneys from all hares were analysed for paraquat. No paraquat was detected in any of the kidneys. Paraquat use was reported in association with only two of these incidents in 1984 and was not diagnosed as the cause of death. Field enquires in 1984 revealed that farmers associated deaths with pesticides used, especially herbicides. The readiness to blame pesticides is not new. The disappearance of free living hares in Croatia was wrongly associated with the use of fungicidal wheat seed treatments (Sostaric et al., 1991). Following the recognition and diagnosis of EBHS in England in 1989 and retrospective analysis of the clinical and post-mortem histories, we suspect the majority of these incidents in 1984 and 1989 were due to EBHS.

Table 2
Wildlife Incident Investigation Scheme (WIIS) incidents involving hares and paraquat reported from 1974 to 1997 in the UK

Year	Hare incidents	Hare incidents involving paraquat
1974	0	0
1975	2	0
1976	4	1
1977	2	0
1978	3	0
1979	2	0
1980	4	0
1981	8	0
1982	4	0
1983	3	0
1984	18	0
1985	4	0
1986	4	0
1987	2	0
1988	6	0
1989	18	0
1990	5	1
1991	3	0
1992	3	0
1993	2	0
1994	2	0
1995	2	0
1996	2	0
1997	1	0
Total	104	2

6.3.2. France

In France the National Hunt Office administers a Sanitary Surveillance Scheme for Wildlife (SAGIR). While SAGIR has not been running so long as WIIS in the UK it is particularly valuable because of the large number of game species submitted. In SAGIR an incident involves a single animal. Hares feature in this monitoring scheme because they are an important game species in France. However, a disproportionate number of hares come from a small number of regions (Departments). The cause of hare deaths has been well documented since 1986 in SAGIR annual reports, e.g., Barrat et al. (1994) and principal diagnoses have been reviewed by Lamarque et al. (1996). In respect to diagnosis of paraquat, the process of diagnosis is similar to that of WIIS. If paraquat use is reported or there are post-mortem signs i.e., lung oedema, then paraquat residue analysis is conducted on the gut contents.

Over an 11 year period from 1986 to 1996 a total of 13 588 hares incidents have been investigated to SAGIR. In 7437 incidents disease was diagnosed as the cause of death. Lamarque et al. (1996) reviewed incidents from 1986 to 1994 and described the major causes of death, EBHS (19%), yersinosis (pseudo-tuberculosis) (15%), trauma (14%), pasteurellosis (8%), coccidiosis (7%) and tularaemia (3%). Out of 13 588 hares submitted eight were confirmed paraquat incidents (0.06%) (Table 3).

The proportion of incidents remaining undiagnosed were largely due to poor condition of the animal at post-mortem. The combination of information on paraquat use, post-mortem findings and residue analysis provides confidence in diagnoses where animals are in good condition.

Table 3
Sanitary Surveillance Scheme for Wildlife (SAGIR) incidents involving hares and paraquat reported from 1986 to 1996 in France

Year	Hare incidents	Disease	EBHS[a]	Poisoning	Paraquat
1986	500	182	36	NR[b]	NR
1987	1399	828	393	12	3
1988	1525	843	163	40	2
1989	1672	814	250	18	0
1990	2289	1366	731	72	1
1991	684	481	172	6	0
1992	1100	702	312	7	2
1993	1129	618	160	9	0
1994	1018	554	117	11	0
1995	900	558	99	1	0
1996	1372	491	188	36	0
Total	13588	7437	2621	212	8

[a] EBHS: European Brown Hare Syndrome.
[b] NR: Not reported by SAGIR.

7. European Brown Hare Syndrome

Prior to the discovery of EBHS, deaths due to EBHS may have been misdiagnosed as paraquat incidents. Death from EBHS results from liver damage (Marcato et al., 1991). Typical lesions consist of necrotic hepatitis and congestion, haemorrhage and oedema in lungs and trachea. The presence of EBHS seropositive antibodies indicates that hares have been exposed to the disease or something very similar, but is not diagnostic of the cause of death because hares may survive the disease. EBHS was not diagnosed until 1989 (Gavier-Widen and Morner, 1989) following retrospective analysis back to 1980. Paraquat incidents are unlikely to be confused with EBHS since it was described in 1989, however before 1989 it is possible that EBHS was mistaken for paraquat poisoning if hepatitis was not observed.

The emergence of EBHS in the UK has been investigated (Chassey and Duff, 1990; Duff et al., 1994, 1997). The first clinical cases of EBHS in the UK were diagnosed retrospectively in animals which died from the disease in 1982, suspected clinical cases were identified in 1976 and antibodies of EBHS detected from 1962–1971. Detection of seropositive antibodies in 1962–1971 does not necessarily imply the disease was expressed clinically (Duff et al., 1997). However, it is interesting that the major decline in hares during the early 1960s coincides with the first detection of seropositive hares in England. Hares may have been seropositive to EBHS prior to 1960. The general view is that EBHS is a significant disease, particularly where it occurs as local epidemics, but is probably not the cause of a major reduction in the hare populations (Gavier-Widen and Morner, 1993; Frolich et al., 1996).

Clinical investigations indicate that death from EBHS is frequently rapid with affected hares usually in good physical condition. Affected hares have been observed uncoordinated and attempting to run away but dying after paralysis within a few minutes. A reported feature was ‘lack of fear’ (Duff et al., 1994) and many carcasses were collected in atypical places for hares, such as close to houses and roads (Sostaric et al., 1991). These clinical observations were not dissimilar from two unconfirmed paraquat incidents in 1967 and 1969. Unfortunately we can only speculate on when the earliest clinical case of

EBHS occurred and the possibility of confusion with paraquat.

8. Conclusions

European hare hunting bags have declined throughout Europe during the 1960s, 1970s and 1980s and probably reflect the population trend. Changes in agricultural management, primarily through loss of crop/landscape diversity effecting nutrition, are the most likely factors responsible for the long-term decline. The apparent stabilisation in the hare population at a lower level compared to the pre-1960s in the UK may have resulted from stabilisation in crop and landscape diversity. However, data are currently not available to test this hypothesis.

Significant hare mortalities from EBHS probably first appeared in the 1970s although earlier deaths cannot be ruled out. Deaths initially suspected as being caused by the ingestion of paraquat could have been EBHS cases. Analysis of recent incidents reported to SAGIR (France) and WIIS (UK) show that paraquat is not a significant cause of hare deaths or decline in hare populations.

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