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Appendix 1.
An evaluation of the risks to human health in the UK from lead derived from ammunition

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health”
by the Lead Ammunition Group, 2 June 2015.

Risk Assessment prepared by Professor Rhys Green and Dr Debbie Pain of the Primary Evidence and Risk Assessment Subgroup for consideration by the Lead Ammunition Group.

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Executive summary

We conclude that people can be exposed to and affected by lead from ammunition via four main exposure routes, which we refer to as linkage sets A – D. These are listed here.

A. The ingestion of whole lead shot or large fragments of lead shot or lead bullets in game meat, their subsequent retention in the appendix or bowel followed by absorption of lead into the bloodstream and/or disease caused by the presence of the foreign body.

B. The ingestion of lead derived from small fragments of lead shot or bullets in game meat from the ammunition used to kill the game animal and the absorption of lead through the intestine wall.

C. The ingestion and absorption of biologically incorporated ammunition-derived lead in game meat. This may occur when game animals have been exposed to lead from ammunition before being killed, and where this has become biologically incorporated within their tissues.

D. The inhalation or ingestion of lead aerosol/fume or dust from ammunition at firing ranges.

In this Risk Assessment we evaluate these four source-pathway links of exposure of humans to lead from ammunition. We then assess the potential for adverse effects on human health from exposure to dietary ammunition-derived lead, and estimate upper and lower bounds of the number of people that may potentially be affected. The evidence available on linkage sets B and C is reasonably comprehensive, but that for linkage sets A and D is relatively limited and our analysis of these two pathways is more cursory.

We conclude that:

For Linkage set A

1. Lead shot ingested with game meat can be retained in the appendix, resulting in elevated blood lead levels and associated negative health effects. While this is believed to be a rare occurrence in the general population of the UK, it may not be rare in subsistence hunting communities and in subsets of the UK population that frequently consume game. Very rarely, ingestion of shot may be associated with appendicitis. The potential risk due to appendicitis is generally believed to be small, but epidemiological assessment of the risk to high-level consumers of game meat in the UK compared with the general population has not been conducted and would be required to provide robust evidence. Retention of lead shot in the appendix may also result in increased absorption of lead. However, the measurements of lead concentrations in game meat referred to below for linkage set B include lead from this source. Hence, the potential level of exposure of human consumers to ammunition-derived lead described for linkage set B can be taken to include the lead from linkage set A.

For Linkage set B

2. Fragmentation of lead projectiles occurs frequently when they strike game animals. This occurs both when deer and other large game are shot with lead rifle bullets and when small game, such as birds are killed using shotgun cartridges containing lead shot.

   Some lead fragments will generally remain in meat from some parts of the carcass ingested by consumers from large game (e.g. deer or boar) shot with lead bullets after normal meat processing and culinary practice. Small lead fragments can be widely dispersed in the tissues of large game animals killed using lead bullets and these may occur 30 cm from the wound track. These could not all be removed unless a large proportion of edible tissues in body regions close to the wound site were to be discarded

during preparation. However, it is unlikely that animals shot in the head, neck or thorax would have significant amounts of ammunition-derived lead in the cuts of meat from the hind-quarters and most of the lumbar region, but meat from the neck and thorax is likely to be contaminated.

Some lead fragments will generally remain in meat from most parts of the carcass ingested by consumers from small game shot with lead gunshot after normal meat processing and culinary practice. Lead shotgun pellets often fragment and a small proportion of the shot volume will occur in the carcasses of shot animals as small particles of lead, many of which may be invisible to the human eye. Small radio-dense fragments have been found in the carcasses of gamebirds shot with lead gunshot, even in cases where no whole shot or large shot fragments are apparent. This is likely to have occurred when shot have passed through the animal. It is not feasible to remove many of these small fragments by practical food processing and culinary measures.

3. Mean lead concentrations in meat from both large and small game animals shot with lead ammunition are often elevated, and frequently considerably elevated, above the levels considered acceptable for meat derived from the muscle tissue of non-game animals under EU Regulation 1881/2006. Mean lead concentrations are likely to be generally higher in game meals made from small game shot with lead gunshot (e.g. gamebirds and waterfowl) than meals made from large game (e.g. deer) shot with lead bullets. However, meat and offal derived from tissues from the region of the body where a large game animal has been shot (such as the thoracic region) may have high lead concentrations.

4. The majority of the general population of the UK do not consume wild game frequently, but calculations based upon two independent surveys indicate that at least tens of thousands and possibly hundreds of thousands of people appear to be ‘high-level consumers’. The mean frequency of consumption of game meat by these high-level consumers may exceed one game meat meal per week, averaged over a whole year. Many more people will consume game less frequently.

5. Part of the ammunition-derived dietary lead from the tissues of game animals ingested by humans is absorbed in the alimentary tract and enters the bloodstream. However, regression analyses of data collected from humans and *in vitro* simulations of absorption both indicate that the absolute bioavailability of ammunition-derived lead is lower than that of lead in the general diet. The regression method suggests that bioavailability of ammunition-derived lead by adults is approximately 40% lower. However, absolute bioavailability of ammunition-derived lead remains substantial and capable of causing elevated blood lead concentrations.

For Linkage Set C

6. No detailed separate evaluation of linkage set C was considered to be required because comparatively little biologically incorporated lead is likely to be present in the muscles of game animals that have ingested lead from ammunition in the environment and, in any case, the measurements of lead concentrations in game meat referred to for linkage set B include lead from this source. Hence, the potential level of exposure of human consumers to ammunition-derived lead described for linkage set B can be taken to include any lead from linkage set C.

For Linkage Set D

7. It is concluded that there is a potential risk to people that use (primarily indoor) firing ranges from the inhalation of lead fume, aerosol and dust and ingestion of lead dust, especially to occupational workers, people that use firing ranges frequently, such as the military and the police, and hobby shooters. The level of risk is related to the frequency

of use and the adequacy of control measures. In the absence of readily available monitoring data for the UK we have not been able to evaluate this risk.

With respect to the potential for adverse effects on human health of exposure to dietary ammunition-derived lead, and numbers of people that might be affected, we conclude that:

8. There is no known requirement for lead by humans and therefore no known positive effect of exposure to low levels of lead. There is no evidence for a threshold of exposure such as dietary intake rate or of blood lead level below which lead-induced negative health effects, such as systolic blood pressure, chronic kidney disease and reduction in IQ score, can be considered to be completely absent.

9. A comprehensive recent study by the European Food Safety Authority (EFSA, 2010) found that the average adult in the EU appears to be at little risk of negative effects on the cardiovascular system or kidneys from current levels of dietary lead exposure. However, some frequent consumers of game (i.e. one x 200g meal per week) may potentially be at risk from these health effects as a result of exposure to dietary lead.

10. EFSA (2010) found that children are the group most vulnerable to health effects from lead exposure and that there is a potential risk that some children in the EU could incur a one point reduction in IQ as a result of feasible current levels of exposure to dietary lead. The increase in risk in those children that consume game frequently was not evaluated separately, but this additional exposure to dietary lead would increase the risk (see point 11).

11. The consumption of 200g of wild gamebird meat per week could increase the dietary exposure to Pb of adults in the UK by approximately 7-8 times over exposure from all other dietary components combined - i.e. 'background exposure' (FSA 2012, Green & Pain 2012). For toddlers (1.5-4.5 years old), the FSA (2012) estimate that consuming 60g of pheasant per week could result in 5 times the background dietary lead exposure and Green & Pain (2012) estimated that weekly consumption of 118g and 100g of gamebird meat could result in approximately 7.0 and 7.4 times the background dietary lead exposure in 6.9 year-old and 2.5 year old children respectively.

12. The UK Food Standards Agency (FSA) consider that the increased exposure resulting from high-level consumption of game, particularly gamebird meat, would be a concern in the case of toddlers, young children and pregnant women[88] in the UK, because of the neurotoxicity of lead to the developing brain. From estimates of dietary exposure to lead for adults, consumption of one or two 120 g venison meals per week is unlikely to be a health concern, whereas there is potential increased risk of kidney and cardiovascular effects with regular consumption of one or two 100g gamebird meals. Risks are lower for people who eat game meat occasionally.

Using scenarios of gamebird meat consumption which are hypothetical but cover the range established by surveys of high-level consumers of game in the UK, potential health risks associated with the consumption of gamebirds shot with lead were identified as listed below. The three Benchmark Responses (BMRs) included were selected by EFSA (2010) because such changes could have significant consequences for human health on a population basis.

- A potential neurodevelopmental risk (associated with a 1 point IQ reduction, the EFSA BMR) to children that consume 0.4 to 0.7 (40-70 g total weight) gamebird meals per week.

- A potential change in children's SATs writing tests scores equivalent to the EFSA BMR for IQ in children that consume 0.13 to 0.21 (12.7 to 20.4 g total weight) gamebird meals per week.

[88] Because of effects because of the foetus *in utero.*

- A potential risk of a 10% increased prevalence of chronic kidney disease (the EFSA BMR) in adults that consume 1.2 to 1.9 (240-380 g total weight) or 4.0 to 6.5 (800-1300 g total weight) gamebird meals per week (depending upon which of two alternative statistical models is used).

- A potential risk of a 1% increase in systolic blood pressure (the EFSA BMR) in adults that consume 3.2 to 5.2 (640-1040 g total weight) gamebird meals per week.

- A potential risk of 1% increase in the prevalence of spontaneous abortion in pregnant women that consume 2.8 to 4.6 (560-920 g total weight) gamebird meals per week.

In each of the bullet points above, the results for the higher assumed value of absolute bioavailability of ammunition-derived lead has been given first, followed by that for the lower value determined by a regression method. Both values are given because they probably bracket the true value. The higher value is that typically used for non-ammunition lead. It is likely to be too high because some of the metallic lead in ammunition fragments in game meat will not be absorbed. The regression-based estimate may be too low because of probable negative bias introduced by the statistical method.

Consumption of venison would probably lead to lower levels of ingestion of ammunition-derived lead than for gamebirds, but this is likely to be highly variable, according the cuts of meat consumed. It is also concluded that the risks are lower for people who eat game occasionally.

To summarise, we conclude that the consumption of meat from wild game animals killed using lead ammunition poses non-trivial risks to some high-level consumers of wild game in the UK, though risks are small for the general population who consume wild game infrequently. The number of high-level consumers subjected to elevated risk cannot be estimated precisely. However, approximate calculations indicate that he number is likely to be at least tens of thousands and is possibly hundreds of thousands. Potential adverse effects on health and function of high-level consumers include reduced intelligence and cognitive function of children, spontaneous abortion in pregnant women and cardiovascular effects and chronic kidney disease in adults. This assessment is based mainly on calculations using measured levels of contamination with ammunition-derived lead and absolute bioavailability estimates of lead from wild birds killed using lead shot. Consumption of venison from deer killed using lead bullets is likely to cause lower levels of ingestion of ammunition-derived lead than for gamebirds and be associated with a lower potential risk of adverse health effects, but this is likely to be highly variable, according to the cuts of meat consumed.

1 Background to the Risk Assessment and the procedure adopted

The Lead Ammunition Group (LAG) established under the auspices of the Department for the Environment, Food and Rural Affairs (DEFRA) and the food Standards Agency (FSA) appointed a sub-committee (the Primary Evidence and Risk Assessment Subgroup (PERASG) to prepare material for consideration by LAG. The terms of reference of PERASG, agreed in 2009, are as follows:

a. To gather and list sources of evidence for assessing the risks of lead in ammunition under the categories outlined below.

b. To advise on the quality, applicability and therefore inclusion of such evidence for risk assessment.

c. To propose a risk assessment method.

d. To use the proposed evidence sources to prepare an initial risk assessment under the categories outlined below:
   i. Risks to wildlife from ingested lead from ammunition. This will include welfare considerations, individual and population level risks.
   ii. Risks to human health from the ingestion of lead from ammunition. This will include both risks associated with the ingestion of lead gunshot/bullets or fragments thereof in game animals, and the ingestion of animals that have themselves ingested and assimilated lead from ammunition. (It may also include any other perceived risks arising from lead ammunition).
   iii. Risks to human health through livestock feeding in areas of lead shot deposition. This will include risks from lead deposited through inland shooting, including clay-pigeon and other target shooting.

This risk assessment (RA) seeks to identify and investigate the risks to human health from the ingestion of lead derived from ammunition. This will include both risks associated with the ingestion of lead gunshot/bullets or fragments thereof in game animals, and the ingestion of animals that have themselves ingested and assimilated lead from ammunition (assessment 1 in the list above). We have also included a brief evaluation of risks from inhalation/ingestion of lead at firing ranges although this has been covered in a more cursory fashion as it is not central to the assessment requested.

In preparing the RA use has been made of the publication “Green Leaves III Guidelines for Environmental Risk Assessment and Management” published by DEFRA and Cranfield University in November 2011 (DEFRA 2011). The general approach proposed in this document has guided the design of this RA. However, it should be noted that the terms of reference specified by the LAG for PERASG do not include the evaluation of options for reducing risk, which are the subject of chapters 4 - 6 of DEFRA (2011). Hence, this RA focuses only on the subject matter of chapters 2 and 3 of DEFRA (2011), which are formulating the problem and assessing the risk.

The approach to risk assessment adopted here requires the evaluation of scientific studies of the numerous steps in the Source-Pathway-Receptor (S-P-R) linkages relevant to the problem (see below). Hence, the holistic evaluation of previous studies which have conducted a complete risk assessment for effects on human health of ammunition derived lead only forms a small part of the assessment presented below. Instead, this assessment assembles and examines the evidence available for each of the many steps connecting the use of lead ammunition in game shooting to potential risks to human health. Only a small part of a particular study might be used in doing this, and several parts of a study might be used in evaluating disparate steps in a S-P-R linkage set. Where evidence is referred to in this assessment, it is considered sufficiently reliable for the use to which it is put. Significant limitations or caveats on the use of a particular piece of evidence for a

given purpose are mentioned as they occur. Use of the Klimisch approach (Klimisch *et al.* 1997) to the evaluation of the reliability of toxicological data was considered, but was judged to be inappropriate for general use in this RA. This approach involves scoring the reliability of whole studies on a four point scale, but it was not used here because the reliability of an entire study was so rarely relevant to this risk assessment. For this reason, Klimisch scores have not been assigned to any of the studies cited. However, the relevance and reliability of the elements of the studies cited are examined in the text. All the study elements used to draw conclusions here may be regarded as having Klimisch Reliability Score 1 (reliable without restriction) or Klimisch Reliability Score 2 (reliable well-documented study with acceptable restrictions).

2 Formulation of the problem

2.1 Framing the question

Based upon the categories set out in the terms of reference of the PERASG, the relevant question for this RA is “What are the risks to human health from the ingestion of lead from ammunition including risks associated with the ingestion of lead gunshot/bullets or fragments thereof in game animals, and the ingestion of lead-contaminated tissue from animals that have ingested and assimilated lead from ammunition?”. As requested in the terms of reference, the existence of any other perceived risks arising from lead ammunition was considered when drawing up the conceptual model of the problem (see below), but it was judged to be unlikely that other significant questions apply in this case. An exception is exposure to lead absorbed at indoor firing ranges, but little information is readily accessible for this in a UK context, so this pathway has been included but dealt with in a summary fashion.

2.2 Development of a conceptual model

Following DEFRA (2011), a conceptual model of the issues considered in the RA was developed. This includes the identification of S-P-R linkages which describe the route by which the potential hazard from ammunition-derived lead to human health might arise. The intention of this is to represent the scope of the problem, clarify assumptions about its underlying mechanisms, and set the boundaries of the risk assessment.

Based upon scientific literature, which is described and evaluated later in the RA, four plausible pathways by which ammunition-derived lead might affect human health were identified (linkage sets A, B, C and D). These are outlined in Table 1. Another candidate pathway was considered: the incorporation of lead derived from spent ammunition into the general environment and its uptake by plants, fungi and animals, leading to absorption by humans through water, dust and in the diet. This pathway was considered unlikely to be significant, except possibly for the parts of it routed through game animals and domesticated livestock and the consumption by humans of their meat and milk. The part of this routed through game animals is dealt with in the section on linkage set C and that through domesticated animals is dealt with comprehensively in another RA.

Table 1. Source-Pathway-Receptor linkages which might lead to lead derived from ammunition being taken up by humans and this leading to adverse effects on health and functioning.

S-P-R linkage label	Source	Pathway	Receptor
A	Whole projectiles or large fragments embedded in the tissues of game animals killed using lead ammunition.	Preparation of game meals which does not remove all large projectile remnants, followed by their ingestion by humans. Entry of whole gunshot or fragments of shot or bullets into the appendix.	Adverse effects on human health from sequestering (retention) of projectiles or fragments into the appendix or bowel, leading to disease.
B	Small fragments and particles of ammunition-derived lead from projectiles used to kill game animals and embedded in the animal's tissue. This may include fragments shot into the animal during previous episodes when it was shot but not killed.	Projectiles fragment upon impact with the tissues of the game animal and fragments and particles of metallic lead are embedded in the tissues. Some of the tissue thus contaminated is not removed during meat processing. Game animals are used for food by people who killed them, their associates and employees and the wider public, if game meat is sold. Lead compounds may be produced during contact with the game animal's tissues and this may be accelerated and enhanced by food processing techniques such as pickling, marinading and cooking. Meals produced from the game animals are ingested by humans. Ammunition-derived lead from the meal is absorbed in the alimentary tract and enters the bloodstream. Lead in the blood is distributed in various tissues and is redistributed among tissues subsequently.	Adverse effects on human health from effects of absorbed dietary lead on the functioning of various organ systems leading to reduced cognitive function and disease.

Table 1 continued.

S-P-R linkage label	Source	Pathway	Receptor
C	Lead in the tissues of game animals derived from spent ammunition that the animals ingested directly, in their food or from water, soil or sediment.	Projectiles from game shooting are deposited in soil, water and mud. They are ingested directly by game animals, sometimes after a considerable period during which lead compounds are formed on the surface of the projectile or nearby. Ammunition-derived lead compounds also pass into water and plant and animal foods of game animals and are ingested. Some of the ingested lead is absorbed in the alimentary tract of the game animal and distributed via the bloodstream into its tissues. The game animal is killed and eaten by people who killed them, their associates and employees and the wider public, if game meat is sold. Meals produced from the game animals are ingested by humans. Ammunition-derived lead from the meal is absorbed in the alimentary tract and enters the bloodstream. Lead in the blood is distributed in various tissues and is redistributed among tissues subsequently.	Adverse effects on human health from effects of absorbed dietary lead on the functioning of various organ systems leading to reduced cognitive function and disease.
D	Lead aerosol/fume or dust inhaled or ingested at firing ranges.	On firing a weapon, lead fume results from the hot gases produced by the propellant burning lead from the base of the bullet. As unjacketed bullets travel down the barrel lead particles are stripped from them and released into the air. Lead dust is produced when bullets impact bullet catchers at the end of the range. Lead ‘dust’ will be captured by filtration and extraction systems presenting a potential hazard to cleaning and maintenance staff. Lead fume and dust can be inhaled, enter the blood stream via the lungs; a proportion of inhaled lead can coat the mouth and throat and be swallowed entering the bloodstream via the alimentary canal. Lead dust can be transferred to the mouth via unwashed hands or the deposition on articles taken from the range to the home. Ingested ammunition-derived lead is absorbed via the alimentary tract and enters the bloodstream.	Adverse effects on human health from effects of absorbed inhaled or ingested lead from fume and dust on the functioning of various organ systems leading to reduced cognitive function and disease.

3 Assessment of the risks

3.1 Approach to the assessment of the S-P-R linkages

Following DEFRA (2011), the probabilities of the hazard arising, the pathway steps which lead to exposure and the consequences for human health, conditional on exposure, were evaluated at least qualitatively and, wherever possible quantitatively. This was done separately for each of the linkage sets identified in Table 1. However for linkage sets B, C and D, and the part of linkage set A concerning absorption of ingested lead, the mechanisms by which health consequences arise in the receptor part of the linkage set can be assumed to be the same across all sets once absorption of ammunition-derived lead has occurred. All effects of dietary lead on health and functioning were assessed by the use of published relationships between health outcomes and measurements of blood lead concentration (B-Pb). In doing this, the approaches used in EFSA (2010) were followed in most cases. The assessment of human health consequences in the receptor phase of the linkage set will be dealt with together for linkage sets B and C in section 4 of this assessment.

3.2 Assessment of risks from linkage set A

Linkage set A represents the risks associated with whole gunshot and large shot fragments or bullet fragments entering and being retained in the appendix or bowel after ingestion by a human. A detailed assessment of the probability of whole gunshot and large fragments being present in game meat consumed by humans is presented below for S-P-R linkage set B and detailed consideration of this aspect is deferred until that section. Given that shot or bullet fragments are sometimes present in a meal made from game meat and may be swallowed, this section evaluates the potential risk of adverse effects on human health from shot entering the appendix or bowel. It appears to have long been thought that the majority of relatively intact lead shot and large fragments ingested while eating wild game pass through the alimentary canal and are voided in the faeces without any significant effects on health. For example, this is not mentioned as a route of exposure in the CODEX Alimentarius Code of Practice on reducing exposure to lead in food (Codex Alimentarius 2004) and Maximum Levels of lead in game have not been set either by Codex Alimentarius or within EU Regulation 1881/2006. However, shot may, on rare occasions, enter and be retained in the appendix or bowel. This can potentially affect health in two main ways. Firstly, it can result in prolonged absorption of lead and consequent elevated blood lead concentrations and related negative health consequences (for examples of shot retention and/or retention and lead absorption see Scott 1928; Carey 1977; Durlach *et al.*1986; Greensher *et al.* 1974, Reddy 1985; Madsen *et al*. 1988; Moore 1994; Gustavsson & Gerhardsson 2005; Hillman 1967, McKinney 2000; Treble & Thompson 2002; Sian & Lloyd 2003; Schep & Fountain 2006; Banner *et al*., 2012; Munipalle *et al*. 2013). Effects operating via this mechanism are included within the assessments of bioavailability of all ammunition-derived dietary lead examined below (S-P-R linkage set B) and in the assessment of the link between elevated blood lead concentration and health impacts. Secondly, entry of shot into the appendix may be associated with acute appendicitis (Larsen & Blanton 2000). Verbrugge *et al*. (2009) reviewed the literature on exposure of people to lead from ammunition in the circumpolar north and reported that in communities whose members frequently eat game shot with lead ammunition retention of lead pellets in the alimentary canal may be quite common. Tsuji and Nieboer (1997) found that 15% of 132 randomly selected radiographic charts from a hospital serving six native Cree communities in Northern Ontario (1990–1995) showed lead shot in the lumen of the intestine and/or appendix. Cox and Pesola (2005) stated that while most of the lead shot inadvertently ingested by Alaskan native people during the hunting season undoubtedly passes through the intestine over time, buckshot in the appendix is commonly seen. They published an extreme case of a radiograph from a native Alaskan elder with an appendix full of shot. Reddy (1985 - cited in Verbrugge *et al*. 2009) found that 62 patients in one Newfoundland hospital had from 1 – 200 lead shot in their appendices. The likelihood of retaining lead shot in the appendix, and suffering negative health effects as a consequence, will undoubtedly be related to the frequency with which wild game shot with lead ammunition is consumed and also on food preparation procedures. While lead shot retention in the

appendix has been reported incidentally in the UK (P. Green pers. comm.; Munipalle *et al.* 2013), the issue does not appear to have been investigated in communities in the UK or Western Europe in which individuals ingest wild-shot game meat frequently. Hence, although lead shot retention in the appendix or bowel may potentially influence human health in the UK, insufficient studies exist for this assessment to evaluate its effects quantitatively. The effects of lead retention in the appendix or bowel which arise from elevated blood lead concentrations are expected to be the same as those resulting from elevated blood lead concentrations from other lead ammunition routes examined below.

It is concluded that lead shot ingested with game meat may potentially be retained in the appendix or bowel, resulting in elevated blood lead levels and associated negative health effects. While this is believed to be a rare occurrence in the general population of the UK, it may not be rare in subsistence hunting communities and in subsets of the UK population that frequently consume game. Very rarely, ingestion of shot may be associated with appendicitis. It is not practical to conduct a quantitative separate assessment of the effects upon human health which may occur via linkage set A. However, the only consequence of this linkage set liable to be missed by this RA as a result is the increased incidence of appendicitis. The effects on blood lead concentration of absorption of ammunition-derived lead from shot within the appendix are included in the treatment of linkage sets B and C. The potential risk due to appendicitis is generally believed to be small, but epidemiological assessment of the risk of high-level consumers of game meat in the UK compared with the general population has not been conducted and would be required to provide robust evidence.

3.3 Assessment of steps in pathway of linkage set B

3.3.1 Fragmentation of projectiles shot into game animals

Previously it seems to have been supposed that exposure to elevated levels of dietary lead due to ingestion of meat from game shot with lead bullets and lead shot posed a minimal hazard to human health. For example, this is not mentioned as a route of exposure in the Codex Alimentarius Code of Practice on reducing exposure to lead in food (Codex Alimentarius 2004). This might be the case if nearly all of the mass of the projectiles striking the game animal remained in large pieces, which either passed through the carcass or were removed during food preparation or at the table. However, recent X-radiographic studies show that gamebirds and mammals shot either with lead gunshot or lead bullets often contained lead fragments which were small, numerous and widely dispersed in edible tissues away from the wound canals. Several examples of reliable evidence exist for substantial fragmentation of lead bullets within the carcasses of large game animals. Dobrowolska & Melosik (2008) measured lead concentrations in samples of muscle tissue from ten wild boar *Sus scrofa* and ten red deer *Cervus elaphus* obtained immediately after they had been shot. The samples were collected from around the entry and exit wounds, from around the bullet pathway at different sites along its length, from distances of about 5, 15, 25, and 30 cm from the bullet track and also from muscle as far away as possible from the bullet track. The results showed that lead concentration was consistently elevated in tissues close to the bullet track in all 20 animals. All individuals had higher mean concentrations of lead in tissue 5 cm and 15 cm from the bullet track than in muscle from the same animal distant from the bullet track and the mean lead concentration was also elevated at 25 and 30 cm from the bullet track. The degree of elevation of lead concentration near the bullet track was substantial, with geometric means of the ratio relative to the distant sample of 158 times the distant level at 5 cm from the bullet track, 28 times at 15 cm, 2.8 times at 25 cm and 1.8 times at 30 cm. The only plausible interpretation of these large and consistent increases in lead concentration in the vicinity of the bullet track is that fragments and particles of lead were embedded in the tissues at substantial distances from the bullet track.

Knott *et al.* (2010) performed X-radiography on carcasses of ten red deer and two roe deer shot in the thorax using copper-jacketed lead-cored bullets. The thoracic region of each of the eviscerated carcasses was X-rayed. An average of 356 radio-dense fragments per deer was visible on

radiographs of the thoracic region of the carcass. Tests using known particles of grit indicated that the presence of radio-dense particles on X-rays was not an artefact of the presence of grit on the pelage, so the fragments are likely to have been metallic and predominantly composed of lead. The weight of fragments was estimated by reference to an X-rayed scale of bullet fragments of known weight. The average total weight of radio-dense fragments, in the thoracic portion of the carcass, was estimated to be 1.2 g, which is 14% of the weight of the bullet.

Grund *et al.* (2010) performed X-radiography on carcasses of wild white-tailed deer *Odocoileus virginianus* killed using a rifle and on carcasses of domestic sheep *Ovis aries* which were euthanized and the carcass shot through the side of the thorax using a rifle to simulate shooting of deer. As the authors pointed out, the experiments on sheep were expected to give lower levels of fragmentation that the shooting of wild deer because the point of entry of the bullet was standardised and avoided the scapula, which was sometimes struck when wild deer were shot. Different types of lead-based bullets from centrefire rifles were compared in the experiment on the sheep carcasses. Centrefire bullets of two types designed to expand rapidly after impact both left large numbers of bullet fragments distributed widely in the carcasses (mean number of fragments detected per carcass, 60 in deer and 141 in sheep for one bullet type, 86 fragments per carcass, tested in sheep only, for the other bullet type). For two types of centrefire bullets designed to have more restricted expansion after impact, one type left a similar mean number of fragments in sheep to one of the rapid expansion types (mean 82 fragments), whilst the other type resulted in far fewer fragments (mean 9 fragments).

Hunt *et al.* (2009) X-radiographed the area of the wound channel in eviscerated carcasses of 30 white-tailed deer shot with conventional copper-jacketed lead hunting bullets and found metal fragments in all the deer. The median number of visible fragments was 136 per deer, with a maximum dispersion of 45 cm between fragments and a mean extreme distance between fragment clusters of 24 cm.

Substantial fragmentation of lead shot also occurs when gamebirds and waterfowl are killed using gunshot. A UK study (Pain *et al.* 2010) found small fragments on X-ray in 76% of 121 gamebirds and duck of six species examined. In this study wild-shot gamebirds obtained in the UK from selected supermarkets, game dealers or game shoots were X-rayed to determine the number of shot and shot fragments present. Most fragments were less than about a tenth of a shot in size. The small radio-dense particles sometimes appeared to follow the track taken by a shotgun pellet during passage through a bird, were sometimes clustered around bone, but sometimes appeared to be scattered throughout the bird. These authors calculated that approximately 0.3% of the mass of lead in the gunshot considered to have struck gamebirds in their study would need to have fragmented into small particles to account for the concentrations of lead subsequently found in the gamebird meat. This reflects the lead remaining *after* all of the larger fragments (visible to the naked eye) had been removed.

We were unable to find sets of studies in which the estimation of the proportion of the mass of the projectile striking the game animal which is turned into small fragments is directly comparable between studies of shot and bullets.

It is concluded that fragmentation of lead projectiles occurs frequently when they strike game animals. This occurs both when deer and other large game are shot with lead rifle bullets and when small game such as gamebirds and waterfowl are killed using shotgun cartridges containing lead shot.

3.3.2 Failure to remove all projectile fragments from meat during preparation

Hunt *et al*. (2009) studied prepared meat derived from 30 white-tailed deer shot in the USA with conventional copper-jacketed lead hunting bullets. Packages of minced meat (0.91 kg) and boneless steaks were prepared from the eviscerated carcasses by routine butchering, each carcass being processed by a different butcher. About one-third (32%) of the minced meat packs

(n=234) had one or more metal fragments detected in them by radiography. At least one metal fragment was detected in minced meat derived from 80% of the deer. Ninety-three percent of small samples of minced meat, selected from packages because a radio-dense metal fragment was visible in that part of the flattened package, contained lead at levels above background, indicating that the metal fragments detected by radiography were predominantly composed of lead. The two samples without lead contained copper, which was probably derived from the jacket of the bullet. Metal fragments were also found by radiography in loin steaks from 16 of the 30 deer selected because fragments had been seen by radiography of the carcass close to the spine. Of 49 packs of loin steaks, 8% had lead fragments in them, which had been derived from 25% of the carcasses in which fragments had been seen near the spine. This was 13% of all the deer, including those in which the steaks were not examined.

As described in the previous section, Dobrowolska & Melosik (2008) measured lead concentrations in samples of muscle tissue from ten wild boar and ten red deer obtained immediately after they had been shot. Arithmetic mean lead concentrations in muscle tissue were 31.0 mg/kg wet weight 5 cm from the bullet track, 6.3 mg/kg15 cm from the bullet track, 1.3 mg/kg 25 cm from the bullet track and 0.6 mg/kg 30 cm from the bullet track, compared with 0.2 mg/kg in muscle tissue as far as possible from the bullet track. This pattern of change in distance from the wound canal was similar in both species but the lead concentrations differed, being about double the concentration in deer than in boar at 5 and 15 cm from the wound canal, with these differences decreasing with distance from the wound canal. Hence, butchering and food preparation procedures on these boar and deer would require that a substantial proportion of muscle would have to be discarded if all tissue retained for human consumption was to have lead concentration within the limit set by the EU for non-game meat of 0.1 mg/kg for non-game meat (excluding offal).

Johansen *et al*. (2004) found that lead contamination of the meat of seabirds occurred even though shot was removed after cooking. Lead concentrations in shot eiders *Somateria mollissima* were about 44 times higher than in those that had drowned. This is most likely to arise because of small fragments of projectiles which were not detected and removed, though it may partly also be because formation of lead compounds during storage and cooking led to the redistribution of ammunition-derived lead into tissues away from locations of projectile fragments.

Many of the fragments of shotgun pellets detected in X-rays of carcasses of UK gamebirds by Pain *et al.* (2010) were less than about a tenth of a shot in size and likely to be too small and scattered to be detected and removed during gamebird preparation or at the table. The small radio-dense particles sometimes appeared to follow the track taken by a shotgun pellet during passage through a bird, were sometimes clustered around bone, but sometimes appeared to be scattered throughout the bird. In this study, to simulate likely exposure of humans, lead shot and large lead fragments were removed after cooking and before lead analysis, as large fragments and whole shot would usually be removed and not swallowed by the consumer. Statistical modelling of the concentration of lead in the resulting gamebird meals showed that both the number of shotgun pellets and large fragments, and the number of small fragments had significant separate effects on increases in the lead concentration in the cooked meal. High concentrations of lead occurred in some meals prepared from birds in which no whole pellets or large fragments were apparent on X-rays. The only plausible mechanism by which these relationships could occur is by lead and leadcompounds derived from large fragments and whole projectiles and small projectile fragments remaining in the meat after the removal of whole shot and large fragments, simulating normal culinary practice.

3.3.3 Lead concentrations in game meat

Evaluation of previous steps in the pathway in linkage set B indicates high probabilities that lead ammunition fragments upon striking a game animal killed by it and that fragments and particles of lead are embedded in the tissues. If these conclusions are correct, it would be expected that the edible tissues of game animals would contain elevated concentrations of ammunition-derived lead, compared with those present in meat from non-game animals. The previous section also indicated

that a proportion of the ammunition-derived lead shot into game animals would remain in the meat after butchery and food preparation. If this is the case, elevated concentrations of lead would be expected to be present throughout the edible tissues of all or the majority of small game[89] (e.g. gamebirds) and in meat from the region of the body in which large game were shot. This lead would be available for human consumption in meals prepared from game animals shot with lead ammunition. To test these conclusions, this section examines evidence concerning lead concentrations in game meat and meals prepared from it.

To give context to the evidence described below, it is notable that to protect human health, Commission Regulation 1881/2006 [EC 1881/2006] sets maximum levels (MLs) for certain contaminants, including lead, in food. Wild game is not currently included in the wide range of foodstuffs derived from domesticated and wild organisms listed within EC 1881/2006. MLs may be set at higher concentrations for food materials that are eaten less frequently as risk is associated with both concentrations in food and level of exposure of the group or population of concern. For indicative comparison, the current ML for lead permitted in bovine animals, sheep, pigs and poultry (excluding offal) is 100 ppb wet weight (0.1 mg/kg). These MLs are periodically reviewed by the Commission, following advice from the European Food Safety Authority (EFSA), in light of new evidence on the likely effects of lead at different levels of exposure.

Lindboe *et al*. (2012) collected 52 random samples from different batches of ground meat from moose killed in Norway with lead-based bullets. The lead content was measured by atomic absorption spectroscopy. Eighty-one percent of samples had lead concentration above the limit of quantification of 0.03 mg/kg, with a maximum of 110 mg/kg. The mean lead concentration was 5.6 mg/kg, which is 56 times the European Commission limit for lead in non-game meat (0.1 mg/kg).

As described above, Dobrowolska & Melosik (2008) measured lead concentrations in samples of muscle tissue from ten wild boar and ten red deer obtained immediately after they had been shot. Arithmetic mean lead concentrations in muscle tissue were 31.0 mg/kg wet weight 5 cm from the bullet track, 6.3 mg/kg 15 cm from the bullet track, 1.3 mg/kg 25 cm from the bullet track and 0.6 mg/kg 30 cm from the bullet track, compared with 0.2 mg/kg in muscle tissue as far as possible from the bullet track[90]. Hence, mean lead concentrations in a substantial fraction of the muscle tissue from deer and boar which could potentially enter the human food chain was above the European Commission ML for lead in non-game meat (0.1 mg/kg).

A survey was conducted in the UK by the Food Standards Agency (FSA) to gather information on the concentrations of metals and other elements in a variety of foods around the UK to allow more accurate and detailed intake estimates of metals and other elements to consumers and to provide current data for a future review of EC maximum levels (MLs) of metals in these foods. To protect human health Commission Regulation 1881/2006 [EC 1881/2006] sets MLs for certain contaminants, including lead, in food. The FSA (2007) study compared levels of lead and some other metals against relevant regulatory limits set within EC Regulation 1881/2006 and concluded that “Overall, estimated dietary exposure to metals and other elements measured in this survey does not pose a significant risk to consumer safety”. This referred to the average diet as a whole and not specifically to game meat or high-level consumers of game meat. The study analysed lead concentrations in 25 samples from game animals (these samples were from more than 25 individual animals as several were from a brace of pheasants, and several were of diced venison which could have come from several animals). Twelve samples analysed were from pheasant which were all presumably wild shot, and the remainder were venison samples, including both wild and farmed animals (i.e. not all will have been wild shot) introducing a negative bias to the data with respect to wild shot game. Shot were removed prior to analysis. The study itself concluded

[89] ‘Small game’ refers to game of the approximate size of gamebirds, rabbits and hares. ‘Large game’ referes to game of the size of wild boar and deer.

[90] This pattern of change in distance from the wound canal was similar in both species but the lead concentrations differed, being about double the concentration in deer than in boar at 5 and 15 cm from the wound canal, with these differences decreasing with distance from the wound canal. At the wound entrance mean concentrations were similar between the species, mainly because there was one boar sample with very elevated lead concentration.

that “Dietary exposure assessments for the venison and pheasant food group are not reliable as they are based on a limited number of consumers and these should be interpreted with caution.” However, the authors noted that “lead was present in some samples with a mean concentration of 0.23 mg/kg [230 ppb] and a maximum value of 1.63 mg/kg [1,630 ppb] in a sample of pheasant [which appears to have been from one animal rather than a brace). The arithmetic mean lead concentration in the 12 pheasant analysed, presumably all wild shot, was 0.414 mg/kg (414 ppb).] In both cases, these levels are higher than those previously seen in the composite meat food group of the TDS [Total Dietary Study]”” (FSA 2004) and concluded that “a possible source of lead is non-visible lead shot fragments”. While game meat is not included in Commission Regulation 1881/2006 which sets MLs of certain contaminants, mean concentrations in both pheasant samples and all game pooled considerably exceeded the EU ML for poultry (0.1 mg/kg or 100 ppb wet weight in tissue excluding offal). “The FSA wrote to the suppliers of these products encouraging the use [of] other types of shot available [so] as to lower the levels of lead present in the meat”. This study which included only a small proportion of samples from game animals does not provide sufficient information for a reliable assessment of the concentration of lead in game meat, though it indicates that some samples can have high levels.

A study by Pain *et al*. (2010) deals specifically with gamebirds in the UK and that of EFSA (2010) with game from EU countries. In Pain *et al*. (2010) wild-shot gamebirds (six species) obtained in the UK from selected supermarkets, game dealers or game shoots and were X-rayed to determine the number of shot and shot fragments present, and cooked using typical methods. Shot were then removed to simulate realistic practice before consumption, and lead concentrations determined for the resulting gamebird meals. This study found that a high proportion of meals prepared from gamebirds (29-67% according to species) in the UK exceeded the EU ML for regulated meats of 0.1 mg/kg (100 ppb), in some cases by more than two orders of magnitude. Gamebirds containing five or more shot had high tissue lead concentrations, but some with fewer or no shot also had high lead concentrations, supporting X-ray results indicating that small lead fragments remain in the flesh of birds even when the shot exits the body. The mean concentration of lead in 121 gamebirds was 1.181 mg/kg with no significant variation among the six species. This was approximately one third of that found in 2,521 samples of game from EU countries presented in EFSA (2010 – Table 14; 3.1 mg/kg). This difference may have been due to differences in the game animal species included and the methods used to prepare the game meat before sampling and analysis. The preparation methods of game analysed in the EFSA report are not specified, and it is not clear whether visible shot fragments were removed prior to analysis. Data from the Veterinary Medicines Directorate (VMD) Statutory Surveillance Programme documenting lead levels in raw tissues of wild gamebirds and deer, without shot being removed, are also presented in the Pain *et al*. (2010) study but the extent to which these levels resemble those to which consumers of game meat are typically exposed is uncertain because shot were not removed and tissues were not cooked. Concentrations reported from game in other parts of the world are broadly comparable with these levels, although some exceed them. For example, Johansen *et al*. (2004) reported arithmetic mean lead concentrations of 6.1 mg/kg wet weight (6,100 ppb) in cooked whole breast tissue of common eiders and 0.71 mg/kg (710 ppb) in thick-billed murres *Uria lomvia* killed with lead shot in Greenland. Visible lead pellets were identified by X-ray and removed by dissection prior to analysis in this study, thus simulating human exposure.

*It is concluded that mean lead concentrations in meat from game shot with lead ammunition are often elevated, and frequently considerably elevated, above the levels considered acceptable for meat derived from the muscle tissue of non-game animals under EU Regulation 1881/2006. Mean lead concentrations are likely to be generally higher in game meals made from small game (*e.g. *gamebirds and waterfowl) shot with lead gunshot than meals made from large game (*e.g. *deer) shot with lead bullets. However, meat and offal derived from tissues from the region of the body where a large game animal has been shot (such as the thoracic region) may have high lead concentrations.*

3.3.4 Extent to which meat from game animals killed using lead ammunition is eaten by humans in the UK

3.3.4.1 Background

Precise and reliable estimates are not available for the total amount of wild-shot game consumed per year in the UK and hence the mean per capita consumption rate. However, it is apparent that average per capita consumption must be low. The National Diet and Nutrition Survey (NDNS 2002) indicates a mean daily per capita consumption of all meat from species shot as game animals in the UK of 0.7 g per day (approximately 250 g/year), but this includes meat from farmed animals as well as wild-shot game.

This consumption level appears to be far lower than suggested by a survey of a sample of UK adult consumers claimed to be representative of the population by Mintel (2007) which found that 5% of adults claim to eat game fairly regularly when in season. However, there was no definition of "fairly regularly" and the proportion of wild-shot and farmed game is uncertain.

From this limited evidence, it seems likely that the average per capita consumption of wild-shot game in the UK is low and probably less than 1% of average per capita consumption of all meats. However, people involved in game shooting and their family members and associates are likely to eat substantially more game meat than this low average per capita level. The following section attempts to assess the levels of consumption of wild-shot game meat by these high-level game consumers and to make order of magnitude estimates of how many of them there might be.

A more reliable appraisal can be made of the proportion of available wild-shot game that is killed using lead ammunition rather than other types. Most game animals in the UK are killed using lead ammunition. Sales of other types of ammunition are low. Use of lead ammunition is permitted throughout the UK away from wetlands and for species not associated with wetlands[91]. However, even though the use of lead ammunition to shoot ducks has not been lawful in England since 2009, a recent study found that 70% of wild duck carcasses bought from game dealers in England had been shot using lead ammunition (Cromie *et al.* 2010).

3.3.4.2 Consumption of game meat by high-level consumers

The purpose of this section of the RA is to establish, as far as possible, the extent to which wild-shot game animals are used for food by the people who killed them, their associates and employees and the wider public, as proposed in the Source-Pathway-Receptor linkages B and C in Table 1. Information is available on the approximate total amount of game meat consumed per capita annually in the UK, averaged over the whole population. Several lines of evidence indicate that this is small. However, it may be that significant numbers of people consume much more game meat than this overall average. Hence, evidence concerning how many of these high-level consumers there are and how much game they typically eat is of key importance to this risk assessment. There have been no scientifically rigorous fully quantitative attempts to establish the numbers of high-level consumers of game in the UK and their consumption patterns, but several studies provide evidence which provides constraints to the upper and lower limits of these quantities. This evidence is assessed in the following subsections.

[91] In England the use of lead shot for any shooting over the foreshore, over specified wetland SSSIs and for the shooting of all ducks and geese (and coot and moorhen) anywhere has been banned since September 1999. Similar regulations were introduced in Wales in September 2002. Slightly different regulations, banning the use of lead shot for all shooting activities over any wetlands were implemented in Scotland in 2005 and Northern Ireland in 2009.

3.3.4.2.1 British Association for Shooting and Conservation (BASC) Questionnaire Survey

BASC (2010) conducted a survey of the consumption of game meat by its members. Questionnaires were sent to 2,217 BASC members living in north-west England and north-east Wales in 2008. Of these 1,141 returned questionnaires, of which 1,138 were completed in full and considered valid. The responding households were identified as containing 2,721 people of which 1,114 were BASC members and 1,607 were other members of the household. Hence, there was an average of 2.38 individuals per responding household. Of the 2,263 persons in responding households whose age was reported, 15% were children (ages undefined).

In response to the question “In a typical year approximately how often do you/other members of your household eat game meat?", just under a quarter (23%) of households reported that game meat other than venison and wild boar was eaten at least once per week. Consumption rates for venison and wild boar were also reported, but were considerably lower (5% of respondents reported eating meat from deer and boar one or more times per week) and this might well include meat from farmed, rather than wild-shot, deer and boar. Hence, we do not include these estimates in our later calculations. This makes our estimate of the frequency of eating wild-shot game conservative, because some of the deer and boar would have been wild-shot. By contrast, we assume that virtually all game meat, other than venison and boar meat, consumed by BASC members was wild-shot. It seems unlikely that much meat from farmed galliform birds, such as quail, would be included by BASC members when answering this question.

In response to the question “Is your and/or your household's consumption of game meat typically a) during the shooting/deer seasons or b) all year round?”, 57% of respondents reported that they ate game meat all the year round. Although this answer provides no evidence about the frequency of consumption, it provides corroboration for the results from the question about the year-round average frequency of consumption of game meat, discussed above. Had less than 23% of respondents said that they ate game meat throughout the year, then considerable doubt would have been cast on the accuracy of the response to the question about year-round frequency of consumption at levels at or above once per week. However, since the answers to the two questions are consistent with one another and we accept the estimate of 23% of respondents eating game meat more than once per week averaged across the whole year as likely to be accurate. Given the large sample size in this survey, the precision of the estimates is high. The exact binomial 95% confidence interval for the estimate of 23% of respondents eating game meat more than once per week is 20 – 26% and that for the estimate of 57% who said that they ate game meat throughout the year is 54% – 60%.

If these findings are assumed to be representative of households of BASC members throughout the UK, it would imply that about 30,000 households which include BASC members eat game meat at least once per week and that about 74,000 households which include BASC members eat some game meat throughout the year. This is based upon a UK-wide BASC membership of 129,000. Assuming 2.38 persons per household, all consuming game at the frequency given for the household as a whole, the estimated number of persons associated with households of BASC members eating game meat at least once per week may be up to about 71,000 and the number of persons eating some game meat throughout the year may be up to about 175,000. Assuming that the reported proportion of children is accurate and representative of households of all BASC members in the UK, the estimated number of children eating game meat at least once per week is about 11,000 and the number of children belonging to households of BASC members who eat some game meat throughout the year is about 26,000. It seems reasonable to assume that some high-level consumers of game meat are not members of BASC, so these estimates are conservative in that respect.

3.3.4.2.2 Food Safety Agency in Scotland (FSAS) Survey of Habits and Behaviours of High-level Consumers of lead-shot wild-game meat in Scotland

FSAS (2012) reported a survey of game consumption rates derived from quantitative questionnaires administered to respondents during semi-structured interviews conducted in Scotland in 2011. People involved in the management and use of wild game were contacted and asked to participate in the study. These contacts included butchers, game dealers, members of shooting clubs, farmers, gamekeepers, beaters and gun shop proprietors. These people also identified others known to them who eat wild game frequently and who were then also asked to participate. These people were not necessarily working in the same types of enterprises as the initial contacts. In total, 311 subjects were asked about their level of consumption of wild game and the interviews showed that 200 of these reported consuming wild game at least once per week during the shooting season. This level of consumption was taken by FSAS (2012) to represent the definition of a high-level consumer of wild game and the following analysis applies only to them. Our further calculations are only performed on the results from the 200 high-level consumers defined in this way.

Of the high-level consumers of wild game, 79% reported eating wild game once or twice per week during the shooting season and 21% ate wild game more frequently (three or more times per week) during the shooting season. All but two of the 200 high-level consumers also reported on their consumption of wild game outside the shooting season. Thirty-two percent of these high-level consumers reported eating wild game once or twice per week outside the shooting season and 9% ate wild game more frequently (three or more times per week) outside the shooting season. Raw data from the survey kindly provided to the PERASG by FSAS, show that 41% of high-level consumers reported eating wild game at least once per week throughout the year (both within and outside the shooting season) and 9% ate wild game at least three times per week throughout the year.

The raw data from the FSAS (2012) survey can be used to make an estimate of the mean number of wild game meals consumed per week throughout the year by high-level consumers. To do this it is first necessary to estimate the proportion of high-level consumers eating wild game during the shooting season on average 1.0 – 2.0 times per week, 2.0 – 3.0 times per week, and so on up to 6.0 – 7.0 times per week. It is assumed that wild game was not eaten on more than seven occasions per week. Since the proportion of high-level consumers eating wild game on 1.0 – 3.0 occasions per week is much higher (79%) than the proportion eating game on 3.0 – 7.0 occasions per week (21%, see above), it seems plausible that the proportion of consumers eating game at each progressively higher number of occasions per week diminishes exponentially (i.e. by the same proportion) for each stepwise increase in consumption rate of one game meal per week. If this is the case, the proportions of high-level consumers eating wild game during the shooting season 1.0 – 2.0 times per week, 2.0 – 3.0 times per week, and so on up to 6.0 – 7.0 times would be 54%, 25%, 12%, 5%, 3% and 1% respectively. These proportions were obtained by calculating numerically the rate of exponential decline per occasion in the proportion of consumers in each one occasion per day category which would result in 79% being in the 1.0 – 3.0 occasions per week category and 21% being in the 3.0 – 7.0 occasions per week category. Outside the shooting season, the proportions of high-level consumers reporting wild game consumption in the categories never, less often than once a month, at least once a month, at least once a fortnight, at least once per week and 3 or 4 times per week or more are 20%, 6%, 26%, 16%, 31% and 1% respectively for consumers who ate wild game once or twice per week during the shooting season. The equivalent proportions of out-of-season consumption for consumers who ate wild game three or more times per week during the shooting season are 7%, 0%, 5%, 10%, 37% and 41% respectively. These results for consumption within and outside the shooting season were combined by converting them to mean daily consumption rates (game meals per day) for the two periods and multiplying by the number of days in the shooting season and outside it. For this purpose, the duration of the shooting season was taken to be 124 days, which is the season for pheasant shooting. Had the shooting seasons for all game animals been merged, their combined duration would have been larger than this. However, because pheasants comprise the majority of wild-shot birds eaten by people in the UK (PACEC 2006), adopting their season alone seems reasonable.

However, it should be noted that this is a conservative assumption. Because the frequency of consumption of game is higher during the shooting season than outside it, the total annual game meal consumption rate would have been higher if we had adopted the combined duration of the seasons for more than one game species. Based upon these assumptions, the estimate of the mean consumption rate of wild game averaged over the whole year for the FSAS sample of high-level consumers was 1.64 game meals per week. Confidence limits for this estimate were obtained by bootstrap resampling from the raw data provided by FSAS. We drew 10,000 bootstrap samples of 200 at random from the 200 real data and performed the same set of calculations upon each of the bootstrap sets as described above. We then took the values bounding the central 9,500 of these bootstrap estimates as the 95% confidence interval. The bootstrap 95% confidence interval for the estimate of 1.64 game meals eaten per week, year-round, is 1.49 – 1.84 meals per week. Hence, subject to the assumptions made about the duration of the shooting season and other issues, this survey provides reasonably precise estimates of the rate of consumption of game meals by this sample of high-level consumers in Scotland.

A limitation of the FSAS (2012) survey is that the size of the cohort of people, beyond the sample interviewed, to which the game consumption rate described above applies, cannot be ascertained. The method used to select people likely to eat large amounts of game meat did not identify a subset of the whole population expected from demographic or occupational data to have high levels of game consumption and then draw a random sample to be contacted from that defined subset. Had it done so, the mean rate calculated above could have been applied to the whole of the population from which the sample was drawn. Thus the survey provides no information about the number of people in Scotland to which its results apply, except that they apply to at least the 200 people identified from the survey responses as high-level consumers. If we make the extreme and unrealistic assumption that there were no high-level consumers in Scotland other than those identified by the FSAS survey and also assume that the patterns of game consumption are similar in other parts of the UK to those in Scotland, then our best minimum estimate of the number of high-level consumers of game meat in the UK is 2387. This figure was obtained by dividing 200 by 8.4%, which was the proportion of the UK population located in Scotland at the time of the 2011 national census (ONS 2012). Whilst this estimate is clearly unrealistic, it is also an obvious underestimate and therefore provides a constraint on the minimum number of high-level consumers of game in the UK. Hence, based upon this evidence, this group is numbered at least in thousands.

3.3.4.2.3 Public and Corporate Economic Consultants Study of the Economic and Environmental Impact of Sporting Shooting in the UK

An approximate range for the rate of consumption of meat derived from wild-shot gamebirds, both for the whole population and by people involved in game management, can be derived from data on game and pest shooting and numbers of people involved in sport shooting in PACEC (2006). This report gives estimates of the number of wild gamebirds and waterfowl shot in the UK in 2004 as just under 19 million, of which about 79% were pheasants. This total excludes woodpigeons, which PACEC (2006) treats as pests, rather than game. Results from game bag records collected by the Game and Wildlife Conservation Trust and presented by Aebischer (2013), show that numbers of pheasant, red-legged partridge, grey partridge and mallard shot in 2011 were 12 – 23% higher than they were in 2004, with the scale of increase varying among the four species. Because of the preponderance of pheasants in the national bag of gamebirds and waterfowl, we took the value for the increase in bag of this species (12%) to represent the recent increase in bag for all gamebirds and waterfowl combined. Multiplying by the 2004 total of 19 million gives an estimated UK total for 2011 of 21.3 million gamebirds and waterfowl shot, excluding woodpigeons. PACEC (2006) reports that 3.6 million pigeons were shot, not as part of a job, in 2004 and that 53% of the total number of pigeons shot were killed not as part of a job. Hence, the estimated total number of pigeons shot is 6.8 million. Adding these to the total of other birds shot and assuming that the 2004 pigeon total also applies to 2011, gives a total of 28.1 million birds shot in 2011. This is a conservative estimate because we used the lowest of the four species estimates of the 2004 – 2011 increase in bag. Multiplying the species totals by mean body weights from Snow and Perrins

(1998) gives a total of 25.4 million kilograms (25,400 tonnes) for the total annual weight of the bag of these quarry bird species. PACEC (2006) reported that 99% of the gamebirds and waterfowl and 90% of the pigeons were intended to be used for food by humans. Using these proportions, and assuming that the birds were actually used as food, we calculated the total annual unprocessed intact weight of gamebirds, waterfowl and pigeons from which food for human consumption was obtained at 24.7 million kg (24,700 tonnes), derived from 27.3 million individual wild-shot birds. It seems probable that some of these birds were not used as food in the UK because their carcasses were rejected or because they were exported. The proportions of birds rejected and exported are unknown, as is the extent to which exports were compensated for by imports.

We estimated the mean weight of unprocessed gamebird carcasses required for a serving of a main course game meal using recipes published on the internet by the British Broadcasting Corporation (http://www.bbc.co.uk/food/recipes/). We used the number of birds required by the recipe and converted this into the weight of unprocessed bird carcasses required using body weights from Snow and Perrins (1998). In doing this, we took into account whether male or female birds were specified. We divided the total unprocessed weight of game required by the recipe by the number of portions this was said to provide. We avoided recipes which did not use the whole bird. We found ten eligible recipes for galliform gamebird meals (four pheasant, three partridge, three grouse). The mean weight of unprocessed carcass per served portion was 0.50 kg (1 SE = 0.06 kg). Assuming that this conversion rate is typical for gamebirds, we calculate that the 27.3 million wild-shot birds intended for human food per year from PACEC (2006) result in 54.6 million portions of game meat derived from gamebirds.

The calculations described above make it clear that the mean number of gamebird-derived meals consumed per year by the average person in the UK is small. Given that the population of the UK in 2011 was 63.2 million people (ONS 2012), the estimated per capita number of gamebird-derived meals was 0.86 meals per person per year (0.017 meals per week). However, there is evidence, described in the sections above, that people involved in sport shooting of game, their friends and their dependents may consume a substantial proportion of the total number of gamebird-derived meals eaten. High-level consumers of game meat are likely to be a subset of this sector of the population. Mintel (2007) estimated that 14% of game shot in Britain is given away to guns and people involved in shoot management, whereas PACEC (2006) reported that 44% of wild-shot gamebirds and wildfowl were sold to dealers and indicated that the remaining 56% were taken by shoot participants, employees and associates. Similarly, PACEC (2006) found that 57% of wild-shot pigeons used as food were not sold to dealers. The Mintel and PACEC estimates of this proportion are quite different (i.e. 14% and 56%) and it is uncertain which is nearer to the truth. The empirical basis for the Mintel (2007) estimate is less clear than that of PACEC (2006), but nonetheless we use both estimates to calculate a plausible range for the number of informally-sourced wild-shot gamebird-derived meals consumed by people associated with sport shooting. These estimates are 7.6 million (Mintel) and 30.6 million (PACEC) gamebird-derived meals per year.

The numbers of people consuming any of the informally-sourced gamebird-derived meals considered above cannot be estimated precisely, but it can be assigned plausible low and high bounds. PACEC (2006) gives the number of full-time equivalent jobs of shoot managers, gamekeepers, beaters and loaders in the UK as 31,000 and the number of people receiving payment for such jobs as 390,000. When unpaid work is also included, PACEC (2006) estimated that 600,000 persons were involved in work required to provide shooting opportunities. Neither of these figures includes guns who do not work on the shoots, but are also recipients of informally-sourced gamebirds. However, as PACEC (2006) points out, the larger figure (600,000) also included family members and associates of paid workers and it is unlikely that whole of the 600,000 eat large amounts of game. For this reason, 600,000 seems a reasonable upper bound to the number of people consuming informally-sourced wild-shot birds. Taking the lower number of informally-sourced gamebird-derived meals in the previous paragraph (7.6 million per year) as being consumed by 390,000 people gives a weekly consumption rate, averaged over the whole year, of 0.4 game meals per person per week, whilst the estimate is 0.2 meals per person per week if the number of consumers of informally-sourced gamebirds is 600,000. Taking the higher

number of informally-sourced gamebird-derived meals in the previous paragraph (30.6 million per year) as being consumed by 390,000 people gives a weekly consumption rate of 1.5 game meals per person per week, whilst the estimate is 1.0 meals per person per week if the number of consumers of informally-sourced gamebirds is 600,000. Hence, these calculations indicate that the number of people potentially eating some informally-sourced wild-shot birds frequently is at least hundreds of thousands and that the year-round mean frequency of consumption for these high-level consumers may lie between 0.2 and 1.5 game meals per person per week. The number of the people who are high-level consumers of informally-sourced wild-shot birds, defined by FSAS (2012) as consuming an average of more than one game meal per week, is a subset of these hundreds of thousands. Although this number cannot be estimated with precision, there are probably at least tens of thousands of high-level consumers of informally-sourced wild-shot birds in the UK, given the range of mean consumption rates for all consumers of informally-sourced wild-shot birds presented above.

3.3.4.2.4 A study by Haldimann et al. on game consumption in Switzerland

Information on game meat consumption by high-level consumers in Switzerland is available from a study by Haldimann *et al.* (2001). Questionnaire surveys revealed that during the hunting season, hunters consumed on average 2.2 (0.3 – 6.0) wild game meals per week amounting to an average daily intake of about 50g of wild game. Consumption of game outside the hunting season was not established. Although game consumption patterns are not expected to be the same in Switzerland and the UK, it is nonetheless striking that comparison of this result with the UK findings presented above indicates that the number of occasions per week on which game was eaten by high-level consumers was similar in these two European countries.

It is concluded that the majority of the general population of the UK do not frequently consume wild game, but calculations based upon two independent surveys indicate that at least tens of thousands and possibly hundreds of thousands of people appear to be 'high-level consumers', The mean frequency of consumption of game meat by these high-level consumers may exceed one game meat meal per week, averaged over a whole year. Many more people will consume game less frequently.

3.3.5 Bioavailability of ammunition-derived lead present in game meat and the effect of its ingestion on blood lead concentration

In this section evidence for the bioavailability to humans of dietary lead derived from ammunition and the extent to which consuming game meat containing the remains of spent lead ammunition causes an increase on the concentration of lead in the blood (B-Pb) are considered. It is necessary to do this to establish the probability associated with the next link in the pathway of linkage set B. The link between the amount of ammunition-derived lead ingested and elevation of B-Pb is necessary because assessments of the effects of lead on human health generally rely upon associations between B-Pb and health outcomes. The previous sections established that lead derived from lead ammunition used to kill a game animal is likely to result in elevated concentrations of lead being present in game meat derived from such an animal (see section 3.3.3). However, some of this lead might not be absorbed by consumers of game meat if it is in a form which is not readily taken up in the human alimentary tract.

Dietary lead ingested by people who eat the meat of birds and mammals shot with lead ammunition includes fragments of the metal from ammunition used to kill the animal. As described in previous sections, both lead shot and lead bullets fragment when fired into quarry animals and produce pieces of lead of a wide range of sizes which are embedded in the tissues. Some of these are at a considerable distance from the wound and remain after butchery and food preparation. Gamebird meat may also contain a small amount of ammunition-derived lead not shot-in from ammunition (from pathway C in Table 1) and lead derived from sources other than ammunition, but

no available studies examine the absorption by humans of this fraction separately to that of shot-in lead. Consumers also ingest lead from the general diet.

Several studies indicate elevation in the concentration of B-Pb of people who eat game animals killed using lead ammunition. A recent survey of 736 individuals with different levels of game consumption in North Dakota found that, after adjusting for potential confounders, people who consumed wild game had geometric mean B-Pb levels higher by 0.3 µg/dL (36%) in people that consumed wild game than in those that did not (geometric mean B-Pb of 0.84 µg/dL for non-consumers of game), and by 0.5 µg/dL (60%) in people that had consumed three types of game (Iqbal 2009). However, it should be noted that all groups in the North Dakota study had B-Pb lower than the US average at the time (CDC 2005). Adult Inuit people in arctic Canada showed elevation of blood lead levels, the degree of which was positively correlated with the quantity of hunted waterfowl in the diet (Dewailly *et al*. 2001). Analysis of stable isotope ratios of lead in blood samples indicates that exposure to ammunition is the main cause of elevated B-Pb in indigenous people in Canada (Tsuji *et al*. 2008). Other studies carried out in Greenland also show an association between consumption of wild-shot game and elevated B-Pb, but these more quantitative studies are examined in detail below.

A study by Haldimann *et al*. (2001) in Switzerland did not find elevation of B-Pb associated with intake of lead from game meat. The authors took blood samples from 31 hunters and compared these with samples from 42 control blood donors. Hunters completed questionnaire surveys on game consumption and other lifestyle and socioeconomic characteristics that could influence exposure to lead. The authors reported that mean lead concentrations did not differ between the two groups and blood lead concentrations did not correlate with mean number of weekly game meals reported. Hence, they concluded that game consumption had no significant effect on blood lead levels. However, this conclusion is likely to be unreliable because the control group was not a true control as the individuals in it did not complete questionnaire surveys. Consequently, controls may or may not have been hunters, consumers of game, and occupationally or otherwise exposed to lead. In addition, statistical analyses did not control for potential sources of non-game lead exposure (drinking, smoking etc.), although some individuals considered that they may have been occupationally exposed to lead, were heavy drinkers or smokers. Given the number of confounding variables, the sample size was probably too small to draw firm conclusions about differences in blood lead levels in relation to exposure. Finally, no data were collected on the timing of reported game consumption compared with the timing of blood sampling; blood samples were taken in early February while the peak hunting season was reported to be September-November. Hence, lead ingested in game meals during the hunting season may have been largely in tissues (bone liver and kidneys) other than blood at the time of sampling. The half life of lead in blood is around 30 days (as cited in U.S. ATSDR 2007).

Two recent European studies have found elevated blood lead concentrations in people that regularly consume meals of the meat of large game animals shot with lead ammunition. These were published after the HHRA was presented to the LAG and are outlined in Section 7.2.

Hunt *et al*. (2009) performed an experiment on pigs to measure the degree to which lead derived from bullets shot into wild deer was absorbed when they were fed on the deer meat. Each of 30 carcasses of deer shot by hunters with lead bullets was processed by a different butcher and fluoroscopy revealed that 32% of resulting packages of minced meat contained at least one fragment of metallic lead. Meat from packages known to contain fragments was then fed to pigs (considered a good model for gastrointestinal absorption by humans), and statistically significant increases in their B-Pb were observed compared with controls fed on meat that contained no fragments. Mean blood lead concentrations in pigs peaked at 2.29 µg/dL two days following first ingestion of fragment-containing venison, which was 3.6 times higher than that of controls (0.63 µg/dL). The concentration of lead in the blood of the pigs fed on fragment-containing venison returned to within 10% of the value for controls nine days after first ingestion. This does not indicate elimination from the pigs' bodies. Depuration (progressive reduction over time) of lead from the blood of mammals largely reflects its incorporation into other tissues, such as bone. Isotope ratios of lead in the meat matched those of the lead in the bullets used to shoot the deer,

supporting the contention that the absorption by the pigs was of dietary lead derived from the ammunition. The quantity of lead in the meat fed to the pigs was not measured, so this experiment could not be used to measure the absolute bioavailability of bullet-derived lead, which is the proportion of the ingested bullet-derived lead which was absorbed.

The consensus finding from these studies is that B-Pb tends to increase in association with consumption of game meat containing ammunition-derived lead due to absorption of ammunition-derived lead from the alimentary canal. However, without further analysis, these studies do not indicate what proportion of the ammunition-derived lead ingested is absorbed or how much B-Pb is increased per unit of dietary lead ingested. Such estimates require either *in vitro* gastrointestinal simulation experiments which attempt to simulate conditions in the human alimentary canal or empirical studies in which both the intake of lead and the elevation of B-Pb are measured. Studies of this type are described below.

The absolute bioavailability of dietary lead derived from ammunition (the proportion of the ingested amount which is absorbed and enters the blood) might be expected to be lower than that of lead in the general diet because some of the ingested ammunition lead may remain as metallic fragments after cooking and processing in the alimentary canal. Metallic lead, especially that remaining in large fragments, will not be as soluble nor be absorbed in the intestine as readily as more soluble lead salts and complexes (Barltrop & Meek, 1975; Oomen *et al*., 2003). The absolute bioavailability in humans of dietary lead from meals of wild game has been estimated from the results of *in vitro* gastrointestinal simulation experiments by Mateo *et al*. (2011). These authors used cooked meat from partridges *Alectoris rufa* killed with lead shot and showed that the proportion of lead in the cooked gamebird meat that was bioaccessible (soluble and available for absorption) in simulated gastrointestinal conditions was considerably greater when a recipe containing vinegar was used than when wine was used, and that much less of the lead in uncooked partridge meat was bioaccessible. These *in vitro* gastrointestinal simulation experiments with partridge meat provided estimates of absolute bioavailability of 0.157 and 0.236 for the non-acidic and acidic recipes respectively (Mateo *et al*. 2011).

However, the reliability of estimates from *in vitro* gastrointestinal simulation experiments depends on the uncertain degree to which the experiment mimics human digestion and absorption (Zia *et al*. 2011). In addition, in the case of the study by Mateo *et al*. (2011), the cooking methods used recipes with considerable quantities of vinegar and wine, which are prevalent in Spain but somewhat different from those most frequently used in the UK. Green & Pain (2012) therefore used observations from two studies of Greenland adults (Bjerregaard *et al*. 2004; Johansen *et al*. 2006) to derive an empirical relationship between the mean daily intake of dietary lead from the meat of birds killed using lead shot and the mean concentration of B-Pb. Bjerregaard *et al*. (2004) measured the B-Pb of adults in Greenland and also administered a dietary questionnaire which asked each subject to report their consumption of various foods, including meat from shot wild seabirds (mostly thick-billed murre) and ducks (mostly common eider) killed using guns and mostly with lead gunshot. They found that B-Pb was positively related to the frequency of consumption of wild bird meals, but multiple regression analysis did not find any significant effects on B-Pb of the frequency of consumption of other foods (fish, meat from whales and seals, and various imported food items).

Bjerregaard *et al*. (2004) provided mean B-Pbs, adjusted for the effects of age and sex using a regression model, for each of six categories of subjects defined according to their reported intake of wild bird meals. Green & Pain (2012) calculated a mean daily consumption rate of wild bird meals for each category and multiplied by the mean quantity of lead in a meal of cooked wild bird meat to obtain the mean daily quantity of dietary lead per person from wild bird meat. Green & Pain (2012) then performed a weighted ordinary least squares regression of the adjusted mean B-Pb for the six categories of wild bird meal consumption in µg/dL on the mean daily intake rate of lead from this source, with the number of subjects being used as weights. They also performed a similar set of calculations on data from a similar study by Johansen *et al*. (2006) who measured the B-Pb of adult men in Greenland and also asked each subject to report his consumption of various foods. They found that B-Pb was positively related to the frequency of consumption of wild bird meals

comprising the same species as in the other study. They converted the frequency of consumption data so that lead intake per meal would be that expected if meals were made only from auks (mostly thick-billed murre). This conversion takes account of the higher concentration of lead in meals made from common eider. In this study no adjustment of B-Pb for age was made and no multiple regression analysis of effects of consumption of other foods was reported. Johansen *et al*. (2006) provided mean B-Pbs, for each of five categories of subjects defined according to their reported monthly intake of guillemot-equivalent meals. For each category Green & Pain (2012) calculated a mean daily consumption rate of guillemot equivalent meals, using the midpoints of frequencies for those categories defined by a range, and then multiplied the mean number of meals consumed per day by the amount of lead in a guillemot meal to obtain the mean daily quantity of dietary lead per person from wild bird meat. They then performed a weighted ordinary least squares regression of the adjusted mean B-Pb for the five categories of wild bird meal consumption in µg/dL on the mean daily intake rate of lead from this source, with the number of subjects being used as weights. The regression coefficients from the two studies were similar. There was a strong relationship in the data from both Greenland studies between mean B-Pb and the estimated mean rate of intake of dietary lead from meals of cooked wild bird meat across the categories of adults defined according to their wild bird meat intake. The weighted mean of the estimates of the regression coefficients was taken to represent the mean increase in B-Pb for each additional µg/d of dietary lead derived from wild bird meat. The two Greenland investigations appear to be the only other published studies in which dietary lead intake rates from humans eating wild birds shot with lead ammunition and B-Pb had both been measured.

The regression coefficient *b* from the analysis by Green & Pain (2012) of the data from Greenland adults is directly comparable to the coefficient used in the method of Carlisle & Wade (1992) to calculate the effect of the mean daily quantity of dietary lead in the ordinary diet on the B-Pb of adults. When intake rate is in µg/d and B-Pb is in µg/dL the value of the coefficient recommended by Carlisle and Wade for lead in the ordinary diet is 0.04. The value of *b* obtained by Green & Pain (2012) was 0.02448, indicating that lead from spent gun shot is absorbed by humans, but that its effect on B-Pb is 39% lower than that expected for lead not derived from ammunition. It should be noted that this regression method is subject to a known bias. Least squares regression assumes that the independent variable (in this case the dietary lead intake rate) is known without error. This is not the case because the intake rate means used were determined from sample estimates with attached errors which cannot be fully quantified and adjusted for. The direction of this bias on the slope of the fitted regression is negative, meaning that the true absolute bioavailability of lead may be larger than that estimated by this method.

There appear to be no published studies in which B-Pb was related to ingestion rates of ammunition-derived lead in children. The bioavailability of lead in the ordinary diet is considerably higher in children than in adults (Mushak 1998; IEUBK Win). Green & Pain (2012) used the relative absorption rate from the study of Greenland adults to estimate the absolute bioavailability of ammunition-derived dietary lead in children by assuming that the ratio of their estimate of *b* from the regression study for Greenland adults to the value 0.04 for Carlisle and Wade's bioavailability coefficient can be taken to be the same as the ratio of the absolute bioavailability of dietary lead from cooked wild bird meat to that for lead from the ordinary diet in both adults and children. By this method they estimated the absolute bioavailability of dietary lead from cooked wild bird meat for children by multiplying a widely-used value for the absolute bioavailability to children of dietary lead from the ordinary diet (0.5, from Mushak 1998; IEUBK Win) by the quantity *b*/0.04. The absolute bioavailability in children of dietary lead derived from the cooked meat of wild birds was estimated by this method as 0.3060. The same caveat about probable negative bias in this estimate applies as that described above for adults.

The conclusion from the evidence considered here is that part of the ammunition-derived dietary lead from the tissues of game animals ingested by humans is absorbed in the alimentary tract and enters the bloodstream. However, regression analyses of data collected from humans and in vitro simulations of absorption both indicate that the absolute bioavailability of ammunition-derived lead is lower than that of lead in the general diet. However, absolute bioavailability of ammunition-derived lead remains substantial and capable of causing elevated blood lead concentrations.

3.4 Assessment of risks from linkage set C

By describing linkage set C in Table 1, this RA recognises that part of the ammunition-derived lead which is present in the tissues of small game animals occurs because of ingestion by the small game animal of shot from spent ammunition present in the environment. This is in addition to the lead from the projectile(s) shot into the game animal when it killed (linkage set B). In addition, the carcasses of small game animals consumed by humans regularly contain lead ammunition which was in the alimentary tract at the time of death (see Wildlife Risk Assessment). However, it seems improbable that this would be ingested by humans given that these parts of the animal are rarely eaten in the UK, except in the case of woodcock *Scolopax rusticola* and snipe *Gallinago gallinago*. This assessment therefore only considers the lead ingested by game animals that is absorbed by them. The extent to which shot is deposited in the environment and ingested by wild animals, including game, is established in the Wildlife Risk Assessment. That evidence is not repeated here. No evidence was found which allows the fractions of the lead concentrations found in human foodstuffs derived from the tissues of game animals to be quantified separately for the lead routed through pathways B and C. The evidence about lead concentrations in tissues of game animals described above in section 3.3.3 refers to combined total of lead routed through both pathways. Concentrations of lead in edible tissues, such as muscle, liver and kidney, of game species tend to be low, most often <0.1 mg/kg w.w., though considerably higher levels are often found, primarily in the liver and kidneys, in animals which have recently ingested shot (Eisler 1988). Even in animals that have become poisoned through lead shot ingestion muscle levels are likely to be far lower than those in liver and kidney so most of the lead measured in those tissues of game animals frequently eaten by humans is likely to be from the ammunition which killed the animal. This contention is supported by the positive relationship found between the concentration of lead in meals prepared from wild-shot gamebirds and the quantities of small and large particles of radio-dense material in the carcass from which the meal was prepared, as observed by X-radiography (Pain *et al.* 2010). In any case, accurate discrimination between the lead in game animals derived from pathways B and C is unnecessary for the purposes of this assessment. The potential level of exposure of human consumers to ammunition-derived lead (section 3.3.4) can be taken to refer to the lead from both pathways B and C combined. Similarly, because the estimates of absolute bioavailability of ammunition-derived lead used in this RA were obtained from empirical regressions of B-Pb on the concentration of all lead from both pathways in game meals, the evidence on bioavailability can also be taken to apply to lead derived from both pathways. For these reasons, no detailed separate evaluation of linkage set C is considered to be required.

3.5 Assessment of risks from linkage set D

Considerable information exists on exposure of humans to lead from rifle and pistol ammunition at firing ranges. Individuals potentially at risk to elevated lead exposure from this source include occupational workers (i.e. staff that run, clean and train others at firing ranges), people that use firing ranges frequently, such as the military and the police, and hobby shooters. The risks arising from the inhalation (and ingestion) of lead dust and aerosol at indoor (and covered outdoor) firing ranges have long been recognised. Numerous studies have investigated exposure to lead at firing ranges in the USA and in several other countries (e.g. Ross *et al*. 1960; Landrigan *et al*. 1975; Anderson *et al*.1977; Fischbein *et al*. 1979; Seitzinger & Wittman 1983; Jackson 1987; Novotny *et al*. 1987; Brewer 1989; Chau *et al*. 1995; Fischbein *et al*. 1995; Stromness *et al*. 2000; Bonanno *et al*. 2002) and elevated blood lead levels are typically reported in people exposed to elevated airborne lead at firing ranges (e.g. Tripathi *et al*. 1991; Fischbein, A. 1992; Svensson *et al*., 1992; Abudhaise, *et al*. 1996; Mancuso *et al* 2008; Vivante *et al*. 2008).

On firing a weapon, lead fume results from the hot gases produced by the propellant burning lead from the base of the bullet. As unjacketed bullets travel down the barrel lead particles are stripped from them and released into the air. Lead dust is produced when bullets impact bullet catchers at the end of the range. Lead dust will be captured by filtration and extraction systems presenting a potential hazard to cleaning and maintenance staff. While all people working in or using indoor ranges are exposed to the lead hazard produced by firing, exposure can be significantly reduced

by effective ventilation systems and following strict health and safety protocols. However, while the health risks, especially associated with indoor shooting ranges, have been widely recognised for more than 50 years, in many places risks persist. Several recent reports provide evidence of this in the USA (Anon 2013a,b,c), and, even where control measures are considered and applied, these are not always adequate to protect human health. A recent report (Anon 2013b) by the Centres for Disease Control and Prevention (CDC) in the USA highlights the insufficiencies still found in many indoor ranges from a health standpoint. In recognition of the potential health risks associated with indoor shooting ranges in the USA, at the request of the Department of Defence, the National Research Council recently evaluated potential health risks from recurrent lead exposure of firing-range personnel, including cumulative exposure to lead. The outputs of this study (Anon 2013a) will help to inform decisions about setting new air exposure limits for lead on firing ranges in the USA and on the design lead-surveillance programmes for range personnel. Similarly, The National Institute for Occupational Safety and Health (NIOSH), a US federal agency that is part of the CDC, produced a document (Anon 2013c) highlighting the need for employers and firing range operators to take steps to protect their workers and shooters from exposure to hazardous lead levels at indoor firing ranges. Workers potentially exposed to hazardous amounts of lead at firing ranges in the USA include thousands of employees at the firing ranges as well as more than a million Federal, State, and local law officers who train regularly at these facilities. In addition to workers, 20 million active target shooters in the USA are potentially exposed to lead hazards at indoor firing ranges. Occupational workers are likely to be exposed to the highest risks, and this includes not only trainers and shoot-workers but also professions that frequently fire weapons. For example, Vivante *et al*. (2008) found that mean B-Pb concentration increased significantly in 30 soldiers in the Israel Defence Forces after a 6 week period of exposure to lead dust through intensive target practice in indoor firing ranges. Lofstedt *et al*. (1999) found B-Pb concentrations in Swedish police officers to be positively correlated with the number of bullets fired annually both on and off duty.

However, elevated B-Pb concentrations are also found in non-occupational (hobby) shooters (e.g. Tumpowsky *et al*. 2000). Gelberg & DePersis (2009) reviewed the New York State Heavy Metals Registry for information on people with lead exposure from target shooting. Overall, 598 individuals were reported with exposures from target shooting of which more than a half were non-occupational. People with non-occupational exposures were reported more frequently with elevated blood lead levels (over 40 µg/dL) than those with occupational exposures. These authors concluded that hobby target shooters were at significant risk of having elevated blood lead levels.

In Sweden, while blood lead concentrations in Swedish police officers were found to be positively correlated with the number of bullets fired annually (Lofstedt *et al*. 1999), B-Pb concentrations were relatively low (5 and 3.7 µg/dL in male and female officers). These authors considered that while occupational and recreational lead exposure from firing ranges remained a source of lead exposure in Swedish police officers, it did not appear to be a significant health risk, and that lead-free ammunition and well-ventilated indoor firing ranges may have been responsible for the relatively low blood lead levels.

The US government NIOSH includes ‘work on firing ranges as an occupation where workers may be exposed to lead, and the recent reports described above (Anon 2013 a, b, c) describe the problem and measures that can be taken to reduce this. Working with lead ammunition is not included as an example of a main occupation with a lead risk, or an example of an activity that places individuals at particular risk by the UK Health and Safety Executive (Hazards, 2009; HSE INDG305(rev2)).

We have been unable to find readily available information on B-Pb concentrations in those working on or frequently using firing ranges in the UK (although these may exist). We have also been unable to find publications dealing with lead exposure at indoor (or outdoor) firing ranges in the UK. However, as an indication of the number of individuals that might be involved, in England & Wales

141,820 firearm[92] certificates were on issue on 31 March 2012 covering 477,888 firearms - the highest number since these figures were first collected in 1995 (HOSB 2012). In addition, 562,696 shotgun certificates were on issue on 31 March 2012 (HOSB 2012). In the absence of readily accessible monitoring data we have been unable to evaluate the level of risk that these activities present.

It is concluded that there is a potential risk to people that use (primarily indoor) firing ranges from the inhalation of lead fume, aerosol and dust and ingestion of lead dust, especially to occupational workers, people that use firing ranges frequently, such as the military and the police, and hobby shooters. The level of risk is related to the frequency of use and adequacy of control measures and in the absence of readily available monitoring data for the UK we have not been able to evaluate the UK level of risk.

[92] A **firearm** means a lethal barrelled weapon of any description from which any shot, bullet or other missile can be discharged. A shotgun is defined as a smooth-bore gun (not being an air gun) which: (i) has a barrel not less than 24 inches in length and does not have any barrel with a bore exceeding two inches in diameter; (ii) either has no magazine or has a non-detachable magazine incapable of holding more than two cartridges; and (iii) is not a revolver gun (HOSB 2012).

4 Assessment of adverse effects on human health of exposure to ammunition-derived lead.

This section addresses the evidence for effects on human health of ammunition-derived lead routed through the linkages identified in Table 1. In doing this, the RA makes use of the substantial body of scientific research on the association between human B-Pb concentration and health outcomes. It therefore assumes that a given elevation of B-Pb caused by exposure to ammunition-derived lead would produce the same effects on health as the same elevation of B-Pb caused by exposure to lead from other sources. This is considered appropriate because of the widespread use in medical science of the same principle when the effects on health of other routes of exposure to lead are evaluated.

While we assume that a given blood lead concentration has similar effects irrespective of the source of that lead, we do not assume that bioavailability of lead (the proportion of the ingested amount which is absorbed and enters the blood) from ammunition sources is similar to bioavailability of lead from other dietary sources. This topic was dealt with in section 3.3.5.

4.1 Background

Lead has many industrial and domestic uses. It has long been mined and smelted and consequently has become widespread in the environment. Lead is a toxic metal that has a wide range of health effects on humans. While some metals are essential for biological functioning at low concentrations, but may become toxic to body systems at high concentrations, there is no known requirement for lead (i.e. it is considered as non-essential). No minimum B-Pb threshold has been defined for the toxic effects of lead, i.e. there is no known blood lead concentration below which it is considered that no negative physiological effects of lead occur (EFSA 2010; ACCLPP 2012). In recent decades, information on the human health effects of lead has increased steadily. Much research has been based upon studies of humans with different occupational and environmental exposure levels, rather than upon theoretical calculations or extrapolations from laboratory studies, hence risks are well documented and established. Experimental studies with non-human animals generally support the effects observed in humans. Lead affects reproduction, the immune system and a range of organs, although effects on some systems are currently considered only to be apparent at higher lead exposures (U.S. ATSDR 2007). Experiments show that high doses of lead can induce tumours in rodents, and possibly humans, and the International Agency for Research on Cancer classified inorganic lead as *probably carcinogenic to humans* (Group 2A)[93] in 2006 (IARC 2006).

Some body systems are particularly sensitive to low levels of exposure to lead. Those most sensitive to chronic effects that occur at relatively lower exposures are the hematopoietic, nervous, cardiovascular and renal systems (EFSA 2010).

Inorganic lead can be absorbed following inhalation or ingestion, and, far less efficiently, through the skin. The amount and rate of absorption of ingested lead through the gastrointestinal tract depends upon the individual (age, nutritional status etc.) and the physical and chemical characteristics of the material ingested. Children absorb proportionately more ingested lead than adults. Once absorbed, lead is transported around the body in the bloodstream, primarily in red blood cells, from which it can be excreted or transferred to soft tissues such as the liver and kidneys. Lead is also transferred to bone tissue where it accumulates with age. Half lives of lead in body tissues vary from approximately 30 days in blood to several decades (approximately 27 years in bone). Approximately 94% of the total lead body burden in adults is in the bone, compared with

[93] Group 2A — The agent (mixture) is probably carcinogenic to humans.
The exposure circumstance entails exposures that are probably carcinogenic to *humans.* This category is used when there is *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in experimental animals.

about 73% in bone in children. In adults, the majority of lead absorbed that is not stored in bones will be eliminated within a few weeks – although less is eliminated in children. Accumulation of lead in body tissues, especially bone, occurs with continuous or episodic exposure.

Lead is accumulated in the body over the lifetime of an individual and it is generally released only slowly. In times of physiological stress (such as pregnancy or poor nutrition) lead can be mobilised from bone resulting in elevated blood lead concentrations. Lead is excreted primarily via faeces and urine. A comprehensive recent account of the toxicology of lead is provided by the U.S. Agency for Toxic Substances and Disease Registry, which is fully referenced and from which much of the preceding paragraph has been summarised (ATSDR 2007).

Lead in blood is the most widely used measure of, mainly recent, exposure and most of the information on human exposure to and the health effects of lead is based on B-Pb data. As the knowledge base on the health effects of lead and the sensitivity of analyses have increased, B-Pb concentrations shown to be associated with human health effects have correspondingly decreased. Also, as human health concerns have resulted in the removal of Pb from many significant sources of human exposure, and background B-Pb concentrations have correspondingly decreased, it has become possible to detect significant associations between individual sources of lead and their effects. This has also facilitated the detection of the effects of lead at lower concentrations that would previously have been possible. This has resulted in a progressive decrease in the B-Pb concentrations proposed as thresholds for action and these are now one sixth or less of those considered as protective of human health in the 1960s.

Most lead absorption is through the lungs or alimentary canal. However, the removal of lead from vehicle fuel across Europe has resulted in a substantial decrease in lead absorbed through the lungs from the atmosphere. Today, the majority of lead exposure in the general population across the EU is from the diet (EFSA 2010).

The consequences for human health of exposure to dietary lead have been considered in great detail by the appropriate authorities of several individual countries and by EFSA, which is the competent authority in the EU. Given the areas of specialism and expertise of these agencies, it is considered to be both unnecessary and inappropriate to conduct a separate evaluation of the evidence for the receptor links in the right-hand column of the S-P-R linkage sets in Table 1 which link elevated blood lead concentration to health outcomes. The next section of this RA therefore summarises the results of the evaluation recently undertaken by the CONTAM Panel of EFSA (2010).

4.2 A risk assessment for lead in food conducted in the European Union by the European Food Safety Authority (2010)

4.2.1 Background

The recent EFSA Panel on Contaminants in the Food Chain (CONTAM) Scientific Opinion on Lead in Food (EFSA 2010) is considered to be the most authoritative recent evaluation of risks to human health from dietary lead, and is of fundamental importance for the current RA. A summary and some comments on this report are presented below.

EFSA were requested by the European Commission (in accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002) to produce a scientific opinion on the risks to human health related to the presence of lead in foodstuffs. In particular, EFSA was asked to consider new developments regarding the toxicity of lead since the European Commission's Scientific Committee for Food (SCF) opinion of 1992 in order to assess whether the 1986 Joint FAO/WHO Expert Committee on Food Additives (JECFA) provisional tolerable weekly intake (PTWI) of 25 µg Pb per kilogram body weight (µg/kg b.w.) was still appropriate. EFSA was asked to include an updated exposure assessment for dietary and non-dietary lead and to consider the exposure situation for specific groups of the population (including infants and children and people following specific diets) and an

indication of the age group in which children would be most susceptible to the toxic effects of lead. Updated scientific evaluation is of great importance to provide a basis for review of MLs for lead in foodstuffs set within Regulation (EC) No 1881/2006 of 19 December 2006. EFSA's scientific opinion was published in 2010 (EFSA 2010).

The scientific opinion included an assessment of exposure to lead of different groups based on data on the occurrence of lead in food and of food consumption levels across the EU, an analysis of the literature on the human health effects of lead and the establishment of health based guidance for exposure to dietary lead and an assessment of the health risks to different groups from dietary exposure to lead.

Existing data from peer-reviewed literature and from various EU databases as outlined below were evaluated and used in the scientific opinion. In addition the CONTAM Panel used new data from a call for data on lead in food from 2003-2008 (DATEX-2008-0002) that EFSA issued in April 2008. The CONTAM Panel also used data from a refined analysis of a complete dataset from seven studies on the neurodevelopmental effects of lead on children that it requested the University of Copenhagen to conduct.

We consider this review to be largely comprehensive, authoritative and of high reliability. Relatively minor issues of interpretation, of relevance to exposure to lead from ammunition, are described in the summary below. The summary outlines the main methods and conclusions of the report, specifically as these relate to the assessment of risk from lead from ammunition. The EFSA opinion is well-referenced and consequently we only cite original references of major importance in the summary below. The summary below has been taken from the EFSA report without checking of the primary literature to which it refers, as this has already been evaluated by acknowledged experts in the field on the EFSA CONTAM Panel.

4.2.2 Human exposure assessment

The CONTAM Panel undertook a detailed analysis of human exposure to lead. The majority of lead exposure in the general population across the EU is from the diet, although soil and house dust can be an important additional source of exposure in children, as can environmental tobacco smoke (see EFSA 2010, Table 29, page 61). Assessment of human exposure to dietary lead requires detailed information on lead concentrations in foodstuffs and on the amounts of different foodstuffs consumed.

4.2.2.1 Lead concentrations in foodstuffs

Lead is a contaminant in food, regulated in the EU. Food lead concentrations have decreased significantly in recent decades, primarily due to the phase-out of leaded petrol, and the resulting decrease in lead air pollution.

Decreases in food lead concentrations over time have been noted in the UK (Food Standards Agency, 2009). In 2006, 310 food samples were analysed for lead in a study commissioned by the Food Standards Agency (Food Standards Agency, 2007). The highest level recorded was in game meat (a pheasant) at 1.63 mg/kg.

Data on food lead concentrations are available from an EU-wide study undertaken in 2002 (SCOOP, 2004). EFSA collected new data covering the years 2003 to 2008 (the DATEX-2008-0002 call), receiving 94,126 results from food testing that were suitable for inclusion in the calculation of lead concentrations in food. Data were received from 14 Member States, Norway and three commercial operators. Germany contributed almost half of the data, and Great Britain a very small proportion (ca. 2%).

Tables summarising lead concentrations in different food groups are presented in the EFSA Report (Tables 5-19). As several food subcategories were aggregated to give lead concentrations for food categories, sampling adjustment factors (SAF) were used, largely to take account of the estimated dietary proportions of each sub-category. A low arbitrary SAF was assigned to some rarely consumed food sub-categories. For the food subcategory of "Meat, meat products and offal" an SAF of 0.2% (0.002) was used for game, compared with, e.g. 41.9% (0.419) for pig meat.

Five samples that had lead concentrations at the very high end of the range, more than ten times higher than the next highest result in the subcategory, were considered as outliers and removed from the calculations. Mean and adjusted mean lead concentrations in food categories are presented in Table 20.

The CONTAM Panel conducted a separate specific analysis of the distribution of samples with a lead content of more than 1 mg/kg. In this analysis 771 samples (0.8%) had a lead concentration > 1 mg/kg. Of these, 17 samples with very high lead concentrations, characterised as outliers, were excluded from further analyses. The justification given by the CONTAM Panel for excluding these samples was their very high values. With exposure from lead gunshot or bullets, some of the lead from ammunition is likely to have been small metal fragments/particles, and it is possible that these outliers may have reflected true lead concentrations. If these results were a true reflection of lead concentrations, their retention would have resulted in an almost doubling of the estimated arithmetic mean concentration. Of the remaining 754 samples, 14.1 % exceeded 10 mg/kg with a maximum of 867 mg/kg in a wild pig muscle sample. Game meat had both the highest proportion of elevated (> 1mg/kg) lead concentrations, and the highest overall lead concentrations, followed by non-algae based food supplements and fungi, and algae based food supplements. The CONTAM Panel considered that of the top ten groups, most are rarely consumed or consumed in small amounts, although no data were provided to substantiate this or suggest to which of these groups this statement refers. Although not highlighted by the CONTAM Panel, it should be noted that game meat, the category with the highest proportion of elevated concentrations, may be consumed relatively frequently by specific user groups across the EU (see section 4.3.3 for information on game consumption levels in the UK).

4.2.2.2 Food consumption levels and calculation of dietary exposure to lead

Food consumption levels were based upon the EFSA Concise European Food Consumption Database (a summary can be found at CEFCD - http://www.efsa.europa.eu/en/datexfooddb/docs/fooddbuk.pdf - individual data are not available), with national data provided by 19 countries. Data were aggregated into 15 broad food groups and certain subgroups, giving a total of 28 separate subgroups. The database is intended as a screening tool for exposure assessment and is subject to limitations as the food categories defined are broad and different countries employ different data collection methods.

The CONTAM Panel used data at an individual level from the Concise European Food Consumption Database (CEFCD) to calculate lead exposure. This included information on sex, age and body weight. For each individual recorded in the database, the average daily consumption of each food group was linked with the corresponding average lead concentration (Table 20 of EFSA 2010 - adjusted mean columns) and exposure in µg/kg b.w. was calculated by summing that from all different food groups divided by the body weight of the individual. Mean ('average base diet') and 95th percentile ('high base diet') lead dietary exposures were calculated separately for each country (Table 22 of EFSA 2010). Due to differences in methods, it is not appropriate to derive a mean of European exposure from these data.

UK data in the EFSA CEFCD are based on the National Diet and Nutrition Survey 2000-2001 from 1,724 individuals aged 19-64 over a seven day period. This illustrates the broad-brush nature of data used from the CEFCD e.g. the contributions of different foodstuffs to an individual's diets will be related, among other factors, to the time of year and duration of survey relative to the seasonality of product availability.

4.2.2.3 Specific diets: consumers of one meal of game per week

The CONTAM Panel specifically tested the influence of a diet containing a long-term average exposure approximating to one weekly meal of 200g of game meat. This was assumed to represent 'high' game consumption, although the choice of one meal per week was arbitrary in the absence of data on game consumption of population subgroups. No change was assumed in median exposure via the base diet, of 0.36 and 1.24 µg/kg b.w. per day, respectively, for lower and upper bounds for average base diet consumers (from Table 22 of EFSA 2010). To ensure that this calculation retained a total constant weekly meat consumption level and to give an estimate of lead exposure to reflect this, the CONTAM Panel could have subtracted the concentration of lead in a 200g portion of other meat. However, the amount of lead contained in 200g of other meats is very small (ca. 0.006 mg from adjusted means for 'all meat and meat products and offal' in Table 20) so this would have a negligible effect on the CONTAM Panel's lead exposure estimates for game consumers. The method used by the CONTAM Panel is therefore appropriate in this respect.

The impact of game consumption on lead exposure of high base diet consumers was not calculated. The CONTAM Panel stated that "It was not considered likely that a high consumer of the base diet would also regularly consume the specific products". However, no data were presented to substantiate this, and we can see no reason to assume that consumers of a high base diet would be more or less likely to be game consumers than those with an average base diet. Consequently, while lead exposure levels in high game consumers can be compared with those of consumers of an average base diet, it may be misleading to compare them with those with a high base diet. Table 38 and the summary of the EFSA (2010) report should therefore be interpreted in this context.

EFSA (2010) investigated the impact of a range of specific diets, not only game, on dietary exposure to lead and stated that "The impact of the specific diets on dietary exposure was modest in most cases with a 2.5 fold increase of exposure only for the game meat diet." Table 27 of EFSA (2010) suggests that this may sometimes be higher than a 2.5 fold increase; consumers of a game diet have lead exposure from the total diet between 5.5 times that for the base diet (lower bound base diet) and 2.0 times that for the base diet (upper bound base diet).

4.2.3 Hazard identification and characterisation

Hazard identification determines the toxic effects of lead and hazard characterisation quantifies the amount of lead required to cause a stated toxic effect. The CONTAM Panel conducted a detailed assessment of both (section 8, pages 61-100 of EFSA 2010).

Lead has a wide range of health effects on humans. These have been observed in a range of studies across cross-sections of the population, and over time. Experimental studies with non-human animals generally support the effects observed in humans. Lead affects reproduction, the immune system and a range of organs, although effects on some systems are currently considered only to be apparent at higher lead exposures (U.S. ATSDR, 2007). Experiments show that high doses of lead can induce tumours in rodents, and possibly humans, and the International Agency for Research on Cancer classified inorganic lead as *probably carcinogenic to humans* (Group 2A) in 2006 (IARC 2006).

Some body systems are sensitive to low levels of exposure to lead. Those most sensitive to chronic effects that occur at relatively lower exposures are the nervous, hematopoietic, cardiovascular and renal systems. The CONTAM Panel thus based their dose-response modelling on chronic effects in humans with detailed dose-response assessments focussed on neurotoxicity, cardiovascular effects and renal toxicity. Effects of lead on these systems are briefly outlined below. While the EFSA report describes mechanisms of action, where known, these are not described in our report.

4.2.3.1 Neurotoxicity

A large number of studies spanning many decades have examined the relationship between the concentration of lead in whole blood (B-Pb) and measures of nervous system function in children and adults. Earlier studies concerned occupational groups of lead-exposed workers where serious manifestations of neurological effects were regularly reported before lead regulations became tightly enforced. In more recent decades non-clinical neurological effects have been studied in adults and children. A variety of methods and study designs have been used. These have included examining the effects of Peak B-Pb, Lifetime mean B-Pb, Early childhood B-Pb and concurrent B-Pb (i.e. where blood lead measurements were taken at the same time as clinical tests such as intelligence quotient (IQ) were performed). Studies are generally correlative, with the relationship between B-Pb and clinical outcome examined after correction for other variables that may also affect the clinical outcome. A number studies have involved the same group of people examined at several points in time, often over long periods (longitudinal studies), allowing for assessments of both the magnitude of effects and their expression over time. These longitudinal studies tend to be more informative than cross-sectional studies.

Toxic effects of lead upon the nervous system in adults include impairment of central information processing, especially for visuospatial organisation and short-term verbal memory, psychiatric symptoms and impaired manual dexterity. While there is evidence that both the developed and the developing brain are susceptible to the effects of lead exposure, the developing brain appears to be more vulnerable. Evidence of lead affecting the developing nervous system (i.e. causing developmental neurotoxicity) is clear, with effects reported at exposures that correspond to B-Pb concentrations as low as 20 µg/L [2 µg/dL]. In addition, the effects of lead do not appear to be linearly related to exposure level (dose). Proportionately greater impacts are seen per unit exposure to lead at very low lead exposures than at higher lead exposures. This is illustrated by a meta-analysis (Lanphear *et al.* 2005), where the results of seven studies addressing the impacts of lead were combined to give a more powerful indication of the effect size. This pooled study estimated decreases in IQ points as B-Pb increased from 24 to 100 µg/L [2.4-10 µg/dL] , 100 to 200 µg/L [10-20 µg/dL] and 200 to 300 µg/L [20-30 µg/dL] of 3.9 (95 % CI, 2.4 to 5.3), 1.9 (95 % CI, 1.2 to 2.6) and 1.1 (95 % CI, 0.7 to 1.5), respectively. Note that, since the standard deviation of variation in IQ among individuals is 15 points, these are not negligible effects. The effects of lead on the developing nervous system appear to persist, at least until late teenage years.

Outcomes associated with the effects of persistent low level lead exposure on the developing nervous system include: lower perceptual scores, reduced reasoning ability, reduced short-term memory and attention, increased likelihood of attention deficit hyperactivity disorder (ADHD), decreased cognitive function, reduced IQ and lower scores on tests of academic performance and related skills.

4.2.3.2 Cardiovascular effects

Long term low-level exposure to lead is associated with increased blood pressure in humans and experimental studies conducted on other animal species support the plausibility of this relationship.

In humans, this association has been illustrated in ‘cross-sectional’ studies, where the association between B-Pb and blood pressure is assessed at one point in time across a representative subsection of the population, and in prospective studies, where study individuals are first selected and then followed over time (i.e. longitudinally) to examine the relationship between B-Pb and the subsequent occurrence or absence of cardiovascular effects. Particularly notable findings include those of the large cross-sectional U.S. National Health and Nutrition Examination Surveys (NHANES)[94]. Mortality follow-up analyses of the first and second NHANES showed statistically

[94] NHANES is a programme of studies designed to assess the health and nutritional status of adults and children in the United States http://www.cdc.gov/nchs/nhanes.htm. The study was widely cited in the EFSA (2010) report but no specific published reference was provided.

significant effects of high B-Pb on adult cardiovascular-related mortality, although there was a marked decline over time in B-Pb levels in the general population. The validity of some of the studies conducted is limited by methodological issues and uncontrolled confounding variables and measurement error. However, meta-analyses of the epidemiological findings support a relatively weak, but significant, association between B-Pb levels and systolic blood pressure, amounting to an increase in systolic blood pressure of approximately 1 mmHg with each doubling of B-Pb, without any clearly identifiable threshold.[95]

4.2.3.3 Nephrotoxicity

Glomerular filtration rate (GFR), the flow rate of filtered fluid from the blood through the kidney, is a good indicator of kidney function. Serum creatinine is a by-product of muscle metabolism that is excreted unchanged mainly by the kidneys and primarily via glomerular filtration. When kidney filtration is deficient, serum creatinine levels rise, and can be used to estimate GFR. Although serum creatinine concentration is not very sensitive in detecting lead-induced renal changes (EFSA 2010), EFSA chose to use it as a range of cross-sectional and prospective longitudinal studies have been conducted to examine the relationship between serum creatinine and B-Pb. These include, for example, analyses of cross-sectional data collected on B-Pb and serum creatinine concentrations from >15,000 US residents (of 20 years of age or older) for the third NHANES. Results suggested an increased likelihood of elevated serum creatinine concentrations and chronic kidney disease as B-Pb increased above that of the lowest quartile (7 to 24 µg/L [0.7-2.4 µg/dL]). A stronger effect was found in people with hypertension.

Prospective longitudinal studies have included analyses of data collected as part of the Normative Aging Study in the United States. The studies presented similarly found associations between B-Pb and serum creatinine concentrations, again with possible interactions between B-Pb, kidney function and hypertension.

In general, the literature suggests that lead contributes to kidney disease, even at low B-Pb levels (below 50 µg/L [5 µg/dL]). EFSA (2010) reviewed several cross-sectional and prospective studies that established strong links between B-Pb levels in adults and kidney function. The CONTAM Panel concluded that nephrotoxic effects are real, that they may be greater in men than women and that they are exacerbated by concurrent diabetes or hypertension.

4.2.4 Establishing a health-based guidance model

4.2.4.1 Establishing BMDL values

Following a detailed analysis of the toxicological information, the CONTAM Panel based their dose-response modelling on chronic effects in humans, with detailed dose-response assessment focussed on neurotoxicity, cardiovascular effects and renal toxicity.

Four measures of dose were available from published studies: B-Pb (µg/L); concurrent B-Pb with health measurements; early childhood B-Pb; average or peak B-Pb over the study period.

Responses were defined as the most suitable 'critical endpoints' (sensitive, relevant and measurable responses to lead exposure). These were: full scale IQ points for neurodevelopmental effects; systolic blood pressure for cardiovascular effects; the presence or absence of chronic kidney disease for renal effects.

The Benchmark Dose (BMD) approach was used to evaluate risk. The BMD is the B-Pb concentration associated with a pre-specified change in response (i.e. a specified loss of IQ, increase in systolic blood pressure, increased incidence of chronic kidney disease), the Benchmark

[95] See EFSA (2010) page 86 for references to the primary literature.

Response (BMR). The lower one-sided 95 % confidence bound of the BMD, denoted BMDL, is then taken as the reference point. [In general, the use of the BMDL as a reference point is a conservative approach that takes account of uncertainty due to sparse data or variation among studies and is thus considered to be health protective.] The CONTAM Panel pre-specified a 1% change in outcome as BMRs for IQ (a 1% change in full scale IQ score which equates to a one point change in IQ) and systolic blood pressure (1% is equivalent to a 1.2 mmHg change in systolic blood pressure), and a 10% change in incidence of chronic kidney disease. A 1% change is described as a BMD_{01} and $BMDL_{01}$ (i.e. for IQ and systolic blood pressure) and a 10% change a BMD_{10} and $BMDL_{10}$ (i.e. for chronic kidney disease).

The three BMRs were selected because such changes were within the range of observable values and could have significant consequences for human health on a population basis. For example, the BMR for neurological effects would impact the socioeconomic status of a population and its productivity. Studies in the USA have related a 1 point reduction in IQ to a 4.5% increased risk of failure to graduate from high school and a 2% decrease in productivity in later life (Schwartz 1994; Grosse *et al.*, 2002). Studies have related the BMR for cardiovascular effects (an increase of systolic blood pressure of 1.2 mmHg) with an increase in the percentage of the population treated for hypertension by 3.1%, and a 2.6% or 2.4% increase in expected annual mortality from cerebral stroke or myocardial infarction respectively (Selmer *et al.*, 2000).

The CONTAM Panel identified a range of studies for the quantification of dose-response relationships. These are described in some detail in the report with details of statistical calculations in the Appendices. For changes in IQ, the CONTAM Panel used the analysis of Lanphear *et al.* (2005) who determined the association between IQ scores and B-Pb in 1,333 children taken from seven studies published between 1989 and 2003. The CONTAM Panel also requested a refinement/reanalysis of the same individual data by the University of Copenhagen (Budtz-Jørgensen, 2010). Four studies involving 7,640 individuals were identified for the quantification of a dose-response relationship between systolic blood pressure and B-Pb (Glenn *et al.* 2003; Vupputuri *et al.* 2003; Nash *et al.* 2003; Glenn *at al.* 2006). The CONTAM Panel defined renal toxicity of lead by using the chronic kidney disease and B-Pb data of the most recent cross-sectional study (NHANES 1999-2006) and fitting the dose-response models recommended by EFSA.

The following BMDLs were defined:

1. The BMD_{01} for developmental neurotoxicity (IQ) = 18 µg/L [1.8 µg/dL] (B-Pb) and the $BMDL_{01}$ = 12 µg/L [1.2 µg/dL] (B-Pb)

2. The BMD_{01} for systolic blood pressure in adults = 61 µg/L [6.1 µg/dL] (B-Pb) and the $BMDL_{01}$ = 36 µg/L [3.6 µg/dL] (B-Pb)

3. The BMD_{10} for chronic kidney disease in adults = 15.9 µg/L [1.59 µg/dL] (B-Pb) and the $BMDL_{10}$ = 15 µg/L [1.5 µg/dL] (B-Pb)

4.2.4.2 Estimating the relationship between B-Pb and dietary lead intake

For a given BMDL B-Pb concentration it is possible to calculate a corresponding dietary intake (the BMDL intake value). The CONTAM Panel used the Integrated Exposure Uptake Biokinetic (IEUBK) Model for lead in children (IEUBKwin version 1.1) and an equation from Carlisle and Wade (1992) [[food exposure (µg/kg b.w. per day)*b.w. *0.4]+[soil and dust lead level (mg/kg)*0.025*0.18]+[air lead level (µg/m^3)*16.4] = B-Pb (µg/L)] for lead in adults. The IEUKB is a widely validated exposure assessment model applicable to children of up to seven years old that mathematically describes movement of absorbed lead through the body. In contrast, the Carlisle and Wade model estimates B-Pb using exposure from ingested lead and an empirically determined ratio of B-Pb level and lead intake. This has been successfully applied to adults but is less suitable for children. It should be noted here that the CONTAM Panel assumed that the bioavailability of dietary lead directly derived

from ammunition was the same as for other sources of dietary lead. A later analysis by Green & Pain (2012) suggests that the biovailability of dietary lead derived from ammunition shot into birds may be 39% lower than that assumed in the CONTAM Panel calculations. If this lower bioavailability estimate is correct, the elevation of B-Pb and associated potential health risks are expected to be less than the CONTAM Panel's estimates. However, further independent empirical estimates of the bioavailability of ammunition-derived lead would be desirable before drawing firm conclusions about this.

Both models estimate exposure from the concentration of lead in the environmental media to which an individual is exposed, multiplied by the amount of contact an individual has with that medium (e.g. concentration of lead in food multiplied by daily food consumption).

These calculations give the dietary lead exposure levels that would be associated with BMDLs (Table 2).

Table 2. Dietary lead exposure levels that would be associated with BMDLs. This is Table 37 from EFSA (2010).

Endpoint	Population	BMDL B-Pb µg/L	Corresponding dietary Pb exposure (BMDL intake value for effects)	
			µg/kg b.w. per day	µg/person per day
Developmental neurotoxicity	Children	12 [1.2 µg/dL]	0.50	10[a]
Nephrotroxicity	Adults	15 [1.5 µg/dL]	0.63	37.5[b]
Cardiovascular effects	Adults	36 [3.6 µg/dL]	1.50	90[b]

(a) Based on a 20 kg child; (b) Based on a 60 kg adult; B-Pb: blood lead; BMDL: benchmark dose lower confidence limit; b.w.: body weight.

4.2.5 Risk characterisation

The CONTAM Panel's analysis of the data is in agreement with a large number of recent studies in concluding that there is no evidence for a minimum B-Pb threshold below which effects on IQ, systolic blood pressure and chronic kidney disease do not occur. Consequently it is not possible to define a safe exposure level to lead and the CONTAM Panel used a Margin of Exposure (MOE) approach. This identifies relative levels of risk. The MOE is calculated by dividing the BMDL intake value for effects (µg/kg b.w./day) by the dietary exposures estimated for adults, children and those with specific diets (such as game meat consumption - Table 29 of the EFSA report). When the MOE is greater than one, there is a relatively lower risk that estimated population intakes of lead would result in some people incurring the defined effects (BMR changes in IQ, systolic blood pressure and chronic kidney disease). When the MOE is less than one, it is relatively more likely that some individuals within the study population would incur the BMR effects. The lower the MOE, the higher the risk of a proportion of the exposed population incurring pre-specified health effects (Table 3). The risk to children of frequently consuming game, or of the high base diet group frequently consuming game was not evaluated by the CONTAM Panel.

For those that do not frequently consume game, from these MOEs, the CONTAM Panel concluded that if the exposure to lead is closer to the lower end of the range of estimates, the potential risk of cardiovascular effects as a result of exposure to lead is very low to negligible. However, if exposure is closer to the upper end of the range, it is possible that some consumers of the high base diet could incur increased systolic blood pressure as a result of exposure to lead. For

nephrotoxicity, the MOEs were small, and the CONTAM Panel concluded that it is possible some people with both high and low base diets (i.e. at the high and low end of the exposure ranges) could potentially incur chronic kidney disease as a result of exposure to dietary lead.

Table 3. Estimated Margins of Exposure (MOE) for different endpoints by type of population (data selected from Table 38 of EFSA 2010).

Population/ Diet	Endpoint	Average base-diet consumer	Average base-diet + one game meal (200g) per week	High base-diet consumer	High base-diet + one game meal per week
Adult	Cardiovascular effects[a]	1.2-4.2	0.76-0.61	0.62-2.1	Not calculated
	Nephrotoxicity[b]	0.5-1.8	0.32-0.26	0.26-0.86	Not calculated
Children:					
1-3 years	Developmental neurotoxicity[c]	0.16-0.45	Not calculated	0.09-0.29	Not calculated
4-7 years		0.19-0.63	Not calculated	0.10-0.38	Not calculated

(a) For cardiovascular effects the MOE was calculated by dividing the BMDL01 intake value of 1.50 µg/kg b.w. per day by the respective dietary exposure estimates taken from Table 29; (b) For nephrotoxicity the MOE was calculated by dividing the BMDL10 intake value of 0.63 µg/kg b.w. per day by the respective dietary exposure estimates taken from Table 29; (c) For neurodevelopmental toxicity, the MOE was calculated by dividing the BMDL01 intake value of 0.50 µg/kg b.w. per day by the respective dietary exposure estimates taken from Table 29.

For consumers of an average base-diet but frequent consumers of game, the CONTAM Panel concluded that there is a potential risk that some people could incur cardiovascular and nephrotoxic effects as a result of exposure to lead. The changes in MOE of people consuming game over those with an average diet illustrate that the potential risk of some people incurring effects on systolic blood pressure and chronic kidney disease is substantially increased over people not frequently consuming game. While the effect of game consumption on those consumers with a high base diet was not estimated, some of these people may potentially already be at risk of cardiovascular and nephrotoxic effects from lead exposure, and exposure to lead from game would obviously further exacerbate these risks.

The CONTAM Panel estimated MOEs for developmental neurotoxicity in infants and children and these were very low. They therefore concluded that there is a potential risk that some children in both average and high consumer groups could incur reductions of a full IQ point as a result of exposure to dietary lead. It was not possible to provide estimates of the potential numbers of children who might be affected - even in average consumers the MOE was <1. The potential impact of children being exposed to additional lead through frequent consumption of game was not evaluated, but this would further exacerbate the risks in a group in which some children may already incur reduced IQ as a result of exposure to lead.

To give a comparative UK perspective it is appropriate to cite the results of the FSA (2012) assessment. They estimated that consuming one 200g portion of pheasant meat per week could increase the dietary exposure to Pb of adults by 8 times over exposure from all other dietary components combined (average exposure of 0.1 ug/kg bw in the diet plus 0.704 in a 200g portion of pheasant). For toddlers (1.5-4.5 years old), dietary exposure of consuming 60g of pheasant per week could be 5 times the background exposure (average exposure of 0.25 ug/kg bw in the diet and 1.106 in 60g of pheasant). These estimates assume that visible shot is not removed.

In their overall study conclusion, the CONTAM Panel considered both limitations of epidemiological data and health significance of observed changes associated with blood lead levels, and

concluded that the potential risk of clinically important effects on either the cardiovascular system or kidneys of adult consumers, at current levels of lead exposure is low to negligible. However, the CONTAM panel did not draw a specific conclusion relating to the level of risk to consumers of game, or evaluate this risk in those on a high base diet or in children.

In infants, children and pregnant women, the CONTAM Panel concluded that there is potential concern at current levels of exposure to lead for effects on neurodevelopment. They concluded that protection of children and women of child-bearing age against the potential risk of neurodevelopmental effects would be protective for all other adverse effects of lead, in all populations.

4.2.6 Conclusions with respect to the JECFA PTWI

Following detailed evaluation of the literature, the CONTAM Panel concluded that the previously-endorsed JECFA PTWI of 25 µg/kg b.w. is no longer appropriate as there was no evidence for a threshold for a number of critical endpoints, including developmental neurotoxicity and nephrotoxicity in adults. The CONTAM Panel considered it appropriate to calculate MOE, using a dose-response relationship as described above, to support the risk characterisation.

Subsequently, based on dose-response analyses, JECFA estimated that the PTWI of 25 µg/kg body weight is associated with a decrease of at least 3 IQ points in children and an increase of approximately 3 mmHg in systolic blood pressure in adults, and concluded that the PTWI could no longer be considered health protective and withdrew it (JECFA 73/sc, 2010). These recent analyses follow the trends observed over the last 60 years of lowering lead exposure levels of concern and B-Pb thresholds for action as further evidence of the chronic effects of low level exposure to lead becomes accepted by the scientific and regulatory communities.

4.2.7 Overall conclusions of relevance from EFSA 2010

There is no evidence for a minimum threshold (of exposure such as weekly intake or of blood lead level) below which negative health effects of lead on IQ score, systolic blood pressure and chronic kidney disease cannot be considered to occur.

The potential risk of clinically important effects on either the cardiovascular system or kidneys of adult consumers, at current average levels of lead exposure, was considered to be low to negligible. However, there was considered to be a potential risk that some frequent consumers of game (one x 200g meal per week) could incur cardiovascular and nephrotoxic effects as a result of exposure to lead.

There is a potential risk that some children could incur reductions of a full IQ point as a result of current levels of exposure to dietary lead in the EU. The potential impact of children being exposed to additional lead through frequent consumption of game was not evaluated separately, but this additional exposure to dietary lead would increase the risk.

4.3 Assessment of the potential consequences for human health for high-level consumers of wild-shot game meat in the UK

Two quantitative assessments are available of potential health risks for high-level consumers of game meat in the UK from consumption of game shot with lead ammunition. They are described and assessed in the following sections.

4.3.1 Potential health risks to adults and children in the UK from exposure to dietary lead in gamebirds shot with lead ammunition

Green & Pain (2012) used data on lead concentrations in UK gamebirds, from which gunshot had been removed following cooking to simulate human exposure to lead (Pain *et al.* 2010). They combined this with UK food-consumption and lead concentration data to evaluate the number of gamebird meals (of 200 g for adults; 118g for a 6.9 yr old and 100g for a 2.5 year old child) consumed weekly that would be expected, based upon published studies, to result in changes, over and above those resulting from exposure to lead in the base diet, in intelligence quotient, systolic blood pressure and chronic kidney disease. These health effects were considered in the recent opinion of the EFSA (EFSA 2010) to be significant at a population level. Green & Pain (2012) also used the same approach to evaluate potential effects of consumption of gamebird meat on Standard Assessment Test (SAT) scores and in rates of spontaneous abortion, which were not evaluated by EFSA. The SAT score study was not published in time to be evaluated by EFSA, whilst the spontaneous abortion study by Borja-Aburto *et al.* (1999) was available to EFSA but not mentioned. Although it has recently been suggested by ACOG (2012) that the results of the Borja-Aburto study were inconsistent with those of another study by Vigeh *et al.* (2010) of the relationship between B-Pb concentration and the rate of spontaneous abortion, this is actually not the case. The Vigeh study included many fewer cases than the Borja-Aburto study (15 cf. 35 cases respectively) and had a more limited distribution of B-Pb. For these reasons, the Vigeh study had a much lower statistical power to detect effects of blood lead. Hence, the decision by Green & Pain (2012) to use the results of the study by Borja-Aburto *et al.* (1999) as the best available statistical model of the relationship of the rate of spontaneous abortion to blood lead concentration seems justified. To estimate the effect on blood lead concentration of ingestion of a given quantity of ammunition-derived lead, Green & Pain (2012) used both the commonly used standard estimate of the bioavailability of dietary lead and new estimates they obtained as part of the study of the bioavailability of ammunition-derived lead.

The results indicated that, in the UK context, the consumption of 0.4 to 0.7 gamebird meals (40-70 g total weight) per week may be associated with a 1 point decrease in the IQ of children, the BMR identified by EFSA (2010). Chandramouli *et al.* (2009) reported a negative association of academic test results of UK schoolchildren at four different Key Stage 1 (KS1) Standard Assessment Tests (SATs) taken at 7-8 years of age with B-Pb measured at 30 months of age. The study of Chandramouli *et al.* (2009) had not been published when the EFSA opinion was written and therefore EFSA (2010) did not evaluate the study or calculate a Benchmark Response (BMR) for SATs scores. However, EFSA (2010) defined the BMR for IQ as 1 IQ point, which is one-fifteenth of the population standard deviation for IQ. To calculate an equivalent change in SATs KS1 writing score to that identified as the BMR for IQ, we obtained the maximum-likelihood mean and standard deviation of SATs scores for children in England in 2010 as given in Table 3b, downloaded from the website https://www.gov.uk/government/publications/national-curriculum-assessments-at-key-stage-1-in-england-academic-year-2009-to-2010-provisional. The calculated values were 1.90 SATs grade points for the mean and 0.60 SATs grade points for the standard deviation, where the SATs grades run from 0 (working towards Level 1) to 4 (Level 4). Hence, we take the equivalent BMR for the SATs KS1 writing grade score to that used by EFSA (2010) for IQ to be 0.60/15 = 0.04 SATs grade points. Green & Pain (2012) used the relationship between the mean outcome of the SATs writing test and blood lead shown in Figure 3 and Table 2 of Chandramouli *et al.* (2009). Because the relationship of SATs grade to blood lead concentration may be non-linear, Green & Pain (2012) took the mean SATs grade values calculated by Chandramouli *et al.* (2009) for the bins of blood lead 5 – 10 µg/dL and > 10 µg/dL, with B-Pb assumed to be at the mean value for the children studied in each of these bins. They then used linear interpolation between these values to estimate SATs grade scores for the expected B-Pb values obtained from various gamebird meat consumption scenarios. Using this relationship, we calculated the expected SATs KS1 grade score for a child with a blood lead concentration equal to the mean observed for all children studied by Chandramouli *et al.* (2009), which was 3.92 µg/dL, and then calculated the blood lead concentration required to give a SATs test result one BMR lower than that score. This blood lead concentration was 4.25 µg/dL. We then used the same two absolute bioavailability values as were used by Green & Pain (2012) to calculate the number of gamebird meals and their weight required

to be eaten per week to give this change in blood lead concentration. These calculations indicate that consumption of 0.13 gamebird meals per week (12.7 g of gamebird meals per week) would be associated with the BMR for the SATs KS1 writing test if bioavailability was assumed to be the same for all dietary lead, and the equivalent was 0.21 gamebird meals per week (20.4 g of gamebird meals per week) if a lower value for bioavailability derived by Green & Pain (2012) based upon consumption of ammunition-derived lead was used. Higher levels of game consumption may be associated with commensurately greater impacts on SATs scores, as illustrated in Green & Pain (2012).

The consumption of 2.8 to 4.6 (560-920 g total weight) gamebird meals per week may be associated with a 1% increase in the prevalence of spontaneous abortion in pregnant women. The consumption of 1.2 to 1.9 (240-380 g total weight) or 4.0 to 6.5 (800-1300 g total weight) gamebird meals per week (depending upon the statistical model used) may be associated with a 10% increased prevalence of chronic kidney disease in adults, the BMR identified by EFSA (2010). The consumption of 3.2 to 5.2 (640-1040 g total weight) gamebird meals per week may be associated with a 1% increase in systolic blood pressure in adults, the BMR identified by EFSA (2010). In each case, the results for the higher assumed value of bioavailability have been given first first, followed by that for the lower value.

The BMR for IQ, systolic blood pressure and chronic kidney disease were selected by EFSA because such changes could have significant consequences for human health on a population basis. Studies in the US have related a 1 point reduction in IQ to a 4.5% increased risk of failure to graduate from high school and a 2% decrease in productivity in later life (Schwartz, 1994; Grosse *et al.* 2002). Studies have associated the BMR for cardiovascular effects (an increase of systolic blood pressure of 1.2 mmHg) with an increase in the percentage of the population treated for hypertension by 3.1 %, and a 2.6 % or 2.4 % increase in expected annual mortality from cerebral stroke or myocardial infarction respectively (Selmer *et al.*, 2000).

Many of the assumptions and approximations involved in the calculations affect the level of dietary exposure to lead from components of the diet other than gamebird meals. These uncertainties include the true lead concentrations in foods for which the reported concentration was below the limit of quantification (LOQ), the degree to which the diet composition of individuals and age, sex and social groups differ from that of the average diet upon which our calculations are based and the applicability to the situation in the UK of the ratio of the lead intake for children and adults in Germany. Green & Pain (2012) performed a sensitivity analysis of the effects of halving or doubling the calculated level of this background dietary exposure and showed that it had minor effects for most health effects, with their direction and size depending upon the form of the model that related the health effect to B-Pb. The doubling and halving of background dietary exposure in the sensitivity test is probably greater or of a similar order to the uncertainties in the calculations. For example, dietary exposure from non-meat diet components would have been 1.6 times the value used if concentrations for all foods with concentrations less than the LOQ had been set at the LOQ and 0.4 times the value used if concentrations for all of these foods had been set at zero. It therefore appears that the results of this study are generally robust against failures in its assumptions and approximations in calculating dietary lead exposure from foods other than gamebird meat.

The main limitations of this study are that it is confined to gamebirds and it evaluates potential health effects for a set of hypothetical scenarios of game consumption rather than estimating health effects for observed levels of game meat consumption by high-level consumers of game. Green & Pain (2012) were unable to estimate the proportion of people in the UK exposed to sufficient dietary lead from game meat consumption to cause responses exceeding the BMR because they had little information on game consumption levels in the UK. The levels of game consumption in some of the hypothetical scenarios are certainly not typical for the population at large. However, the range of game meal scenarios they used are shown by data which have become available subsequently to overlap those of substantial numbers of high-levels game meat consumers. This information on consumption is combined with their findings in section 4.3.3.

A further limitation is that Green & Pain (2012) estimated BMDs but did not estimate BMDLs, as was done by EFSA (2010). This is because of the difficulties associated with including uncertainties in the additional elements used in their calculations, such as bioavailability. Had BMDLs been calculated they would have indicated that consumption of considerably smaller quantities of gamebird-derived meals would result in BMDL doses than those resulting the the BMD doses.

It is concluded that, in a UK context, there are potential health risks associated with the regular consumption of gamebirds shot with lead as follows:

- *A potential neurodevelopmental risk (of the EFSA (2010) BMR- a 1 point IQ reduction) to children that consume 0.4 to 0.7 (40-70 g total weight) gamebird meals per week and potential reductions in children's SATs writing tests scores equivalent to the EFSA BMR for IQ from the consumption of 0.13 to 0.21 (12.7 to 20.4 g total weight) gamebird meals per week.*

- *A potential risk of a 10% increased prevalence of chronic kidney disease in adults that consume 1.2 to 1.9 (240-380 g total weight) or 4.0 to 6.5 (800-1300 g total weight) gamebird meals per week (depending upon the statistical model used).*

- *A potential risk of a 1% increase in systolic blood pressure in adults that consume 3.2 to 5.2 (640-1040 g total weight) gamebird meals per week.*

- *A potential risk of 1% increase in the prevalence of spontaneous abortion in pregnant women that consume 2.8 to 4.6 (560-920 g total weight) gamebird meals per week.*

In each case, the results for the higher assumed value of bioavailability have been given first, followed by that for the lower value.

4.3.2 Risk to human health from exposure to lead from lead bullets and shot used to shoot wild game animals

A quantitative risk assessment was conducted by the Food Standards Agency (2012). This risk assessment combined information on levels of consumption of game meat by high-level consumers from data collected by FSAS (2012) with data on lead levels in game from FSA (2007), the National Surveillance Scheme (NSS), EFSA (2010), and Pain *et al.* (2010) and other published sources and game meat portion sizes from FSA (2002) to assess the same potential health effects as those considered by EFSA (2010), that is, neurodevelopment of children and cardiovascular effects and chronic kidney disease in adults.

The assessment concluded that lead concentrations were generally higher in gamebird meat than in venison and that the majority of consumers use acidic media in cooking, which they suggested would make the lead more bioavailable. The assessment used NSS data on the concentrations of lead in deer meat but excluded three high measurements from their calculations on the grounds that these were from close to the wound channel. This led to an estimated mean concentration of lead in deer meat of 195 µg/kg (0.195 mg/kg w.w.). The assessment also used NSS data on the concentrations of lead in pheasant meat. This led to an estimated mean concentration of lead in pheasant meat of 1870 µg/kg (1.87 mg/kg w.w.). The assessment does not explain why this value was selected, in which lead pellets were not removed from the meat before taking samples for analysis, in preference to those of Pain *et al.* (2010) in which gamebirds were cooked and then pellets and large fragments removed, in line with normal culinary practice.

Portion sizes of game consumed per meal were taken from FSA (2002) as 120 g for venison and 100 g for gamebird meat, assuming the portion size to be the same as for chicken. Children 1.5 – 4.5 years old were assumed to eat portions of 40 g for venison and 30 g for gamebird meat.

Exposure to dietary lead was then calculated for consumption rates of one or two game meals per week.

The average exposure of children 1.5 – 4.5 years old from one venison meal (40 g) per week was 0.077 µg/kg bw which is less than dietary lead levels associated with a 1 point decrease in IQ as calculated by EFSA (0.5 µg/kg bw/day). It would require seven venison meals (280g) per week to attain that level. However, the average exposure of children 1.5 – 4.5 years old from one gamebird meal (30 g) per per week was 0.553 µg/kg bw which slightly exceeds the dietary lead levels associated with a 1 point decrease in IQ as calculated by EFSA (0.5 µg/kg bw/day). Consumption of two gamebird meals (60 g) per week would result in exposure of 1.105 µg/kg bw, which is more than twice dietary lead levels associated with a 1 point decrease in IQ. The assessment took these results to indicate that the effect of lead on the neurodevelopment of toddlers regularly consuming gamebirds would not be negligible even if there were no other sources of exposure to lead.

In the case of adults, the estimated increased exposure to lead from one venison meal (120 g) per week was 0.044 µg/kg bw, but EFSA estimated that dietary lead intake values of 1.50 µg/kg bw/day could result in a 1% increase in systolic blood pressure and 0.63 µg/kg bw/day could result in a 10% increase in the prevalence of chronic kidney disease. Hence, consumption of one or two venison meals (120-240 g) per week would result in exposure that is considerably lower than the risk levels identified by EFSA and is unlikely to increase the risk of cardiovascular and kidney effects. The estimated increased exposure to lead from one gamebird meal (100 g) per week (0.352 µg/kg bw) or two gamebird meals (200 g) per week (0.704 µg/kg bw) would also be less than the EFSA BMDL for cardiovascular effects, but consuming two gamebird meals (200 g) per week would lead to the BMDL for chronic kidney disease being exceeded.

The assessment concluded that the increased exposure resulting from high-level consumption of game, particularly gamebird meat, would be a concern in the case of toddlers, young children and pregnant women[96]*, because of the neurotoxicity of lead to the developing brain. From estimates of dietary exposure to lead for adults, consumption of one or two 120 g venison meals per week is unlikely to be a health concern, whereas there is potential increased risk of kidney and cardiovascular effects*[97] *with regular consumption of one or two 100g gamebird meals. It also concluded that the risks are lower for people who eat game occasionally.*

4.3.3 Synthesis of conclusions from the quantitative risk assessments and available information on game meat consumption by high-level consumers

Since the study of Green & Pain (2012) was conducted, further information has been released on levels of game meat consumption of high-level consumers from the studies of BASC (2010) and FSAS (2012). Hence, the hypothetical game consumption scenarios of Green & Pain (2012) can now be combined with estimates of game consumption by high-level consumers in the UK to give an improved risk assessment for these consumers.

As described in section 3.3.4, the surveys by BASC (2010) and FSAS (2012), indicate that the mean rate of consumption of meat (of unknown weight but probably in the range of 100-200g per meal) from game in high-level consumers, averaged over the whole year, is in the range one to two game meals per week. In the case of the FSAS study this refers to wild game, nearly all of which can be assumed to have been killed using lead ammunition. In the case of the BASC survey, the data may include some farmed game, but most of this is probably removed from the reported consumption rates by the exclusion of game other than venison and wild boar. According to the results of the survey conducted by FSAS (2012), 41% of high-level consumers ate wild game at

[96] Because of effects because of the foetus *in utero.*

[97] FSA concluded that there is a potential increased risk of cardiovascular effects with regular consumption of one or two x 100g gamebirds meals. We note that for cardiovascular effects exposure at this consumption level would remain below, but in the order of, that considered by EFSA to be associated with a 1% increase in systolic blood pressure.

least once per week throughout the year and 9% ate wild game at least three times per week throughout the year.

It is uncertain how large the UK cohort of high-level consumers of game meat is because no sufficiently powerful survey has been conducted covering the whole of the UK. However, two separate analyses of data from BASC (2010) on the one hand and PACEC (2006) and Mintel (2007) on the other indicate that high-level consumers of wild game in the UK may comprise at least tens of thousands of people. From the data collected by BASC (2010), this includes at least thousands of children. Many more people than this of all ages consume wild game less frequently and game consumption in recent years has shown a rapidly increasing trend (Mintel 2007; Shooting Gazette, 2012).

Rates of game meat consumption may be somewhat lower for young children than adults (FSAS 2012). However, the potential risk of damage to neurological and cognitive development, as measured by the risk of reduction in the IQ of young children, of high-level consumers of wild game appears substantial. The evaluation by Green & Pain (2012) of the potential effects of consumption of gamebird meals by young children on subsequent IQ indicates a risk of a loss of one IQ point at levels of consumption of 0.4-0.7 meals per week (40-70 g total weight). The evaluation in this risk assessment, based upon Chandramouli *et al*. (2009) and Green & Pain (2012), of the potential effects of consumption of gamebird meals by young children on subsequent performance in writing tests also indicates a potential change in children's SATs writing tests scores equivalent to the EFSA BMR for IQ from the consumption of 0.13 to 0.21 (12.7 to 20.4 g total weight) gamebird meals per week.

Green & Pain (2012) concluded that consumption of 4.6 gamebird meals (920 g total weight) per week would be required to increase the potential risk of prevalence of abortion to 1% above that if no game meat was consumed. Data collected by FSAS (2012) indicate that 9% of high-level consumers of game in Scotland report eating three or more than game meals per week throughout the year. Hence, a non-trivial proportion of high-level consumers may be exposed to this risk, which was not evaluated by FSA (2012) though it did conclude that increased exposure from two or more x 100g gamebird meals per week would be a concern in the case of pregnant women for a different reason: the neurotoxicity of lead to the developing brain.

Both Green & Pain (2012) and FSAS (2012) assessed potential effects of ammunition-derived dietary lead exposure on cardiovascular effects and chronic kidney disease in adults. In both assessments the risk of effects evaluated as significant by EFSA (2010) was lower than for the risks to neurodevelopment in children described above. However, for gamebird meals, significant potential risks were identified within the range of consumption reported by high-level consumers of game in Scotland according to data collected by FSAS (2012).

It is concluded that consumption of meat from wild game animals killed using lead ammunition poses non-trivial risks to some high-level consumers of wild game in the UK, though risks are small for the general population who consume wild game infrequently. The number of high-level consumers subjected to elevated risk cannot be estimated precisely. However, approximate calculations indicate that the number is likely to be at least tens of thousands. Potential adverse effects on health and function of high-level consumers include reduced intelligence and cognitive function of children, spontaneous abortion in pregnant women and cardiovascular effects and chronic kidney disease in adults. This assessment is based mainly on calculations using measured levels of contamination with ammunition-derived lead and absolute bioavailability estimates of lead from wild birds killed using lead shot. Consumption of venison from deer killed using lead bullets is likely to cause lower levels of ingestion of ammunition-derived lead than for gamebirds and be associated with a lower potential risk of adverse health effects, but this is likely to be highly variable, according to the cuts of meat consumed.

5 Information gaps

The following information gaps were identified during the preparation of this RA.

1. Data on consumption levels of wild shot game in the UK. Accurate data on the size, type and frequency of wild game meals consumed in England and the other UK countries would improve the accuracy and precision of the estimate that at least tens of thousands of people are high-level consumers of game meat. However, we consider that this estimate is likely to be of the correct order of magnitude.

2. While few records exist of appendicitis associated with lead retention in the appendix, an epidemiological assessment comparing high-level consumers of wild game meat in the UK with the general population would be required to accurately evaluate the level of potential risk.

3. The extent to which lead is retained in the appendix by high-level game consumers, with subsequent health consequences, has not been evaluated by studies conducted in the UK. The level of risk from this source could not therefore be accurately evaluated.

4. We could not readily find published monitoring data on levels of exposure to and absorption of lead aerosol and dust from firing ranges in the UK and could not evaluate the level of risk from this source.

6 References

All the references below, cited in this RA, are considered as being of appropriate quality for the purpose stated in the main text. Shortcomings are, where necessary, described in the text. The findings used are regarded as being sufficiently well-documented with acceptable restrictions on use. It is considered to be unnecessary and inappropriate to give any reliability score to entire studies for reasons given in section 1. A classification will be added later of the predominant geographical location(s) from which the material from each study which is used in the Risk Assessment originated.

For the majority of, primarily, case studies cited in linkage sets A (3.2 - lead in the appendix) and D (3.5 - lead exposure at firing ranges) we have accessed and cited abstracts rather than primary literature. We do not consider this to present a problem as few data exist for these topics in the UK thus no conclusions have been drawn from the evidence available.

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7 Supporting information

7.1 Abbreviations, definitions and units used

7.1.1 Units of concentration

We have generally cited the units used by the authors of publications, and where we consider that it will help with data interpretation, we have also cited alternative units of concentration in brackets. To simplify comparison where this has not been done, please note that:

1 mg/kg = 1 ppm = 1000 ppb

7.1.2 Definitions

This risk assessment relates primarily to chronic disease (lead-poisoning) associated with long-term low-level exposure to lead. 'Chronic' disease is that which is persistent or long-lasting in its effects (i.e. generally lasting for three months to life). This is different from 'Acute' disease which has rapid onset and can be more severe in the short-term. 'Low-level' exposure refers to exposure to levels of lead that are unlikely to cause 'Acute' lead poisoning. 'Long-term', 'regular' or 'persistent' exposure in the context of this risk assessment refers to an ongoing exposure throughout the year. This would not necessarily have to be on a weekly basis, or last for the whole year, but it implies more than just occasional exposure. Some effects of low-level exposure to lead could potentially be realised following several months of exposure (e.g. in pregnant women), and some may require longer periods of exposure. We interpret long-term as suggesting that exposure should persist for at least months and often longer.

7.1.3 Abbreviations

ATSDR (U.S.)	Agency for Toxic Substances and Disease Registry
BASC	British Association for Shooting and Conservation
B-Pb	Blood lead
CODEX Alimentarius	The Codex Alimentarius Commission, established by FAO and WHO in 1963 develops harmonised international food standards, guidelines and codes of practice to protect the health of the consumers and ensure fair practices in the food trade.
BMD	Benchmark Dose
BMDL	Benchmark Dose Limit
BMR	Benchmark Response
CDC	Centres for Disease Control and Prevention
CEFCD	Concise European Food Consumption Database
DEFRA	Department for the Environment, Food and Rural Affairs
EFSA	European Food Safety Authority
EU ML	European Union Maximum Level (of a contaminant in food)
FAO	Food and Agriculture Organisation
FSA	Food Standards Agency
FSAS	Food Standards Agency Scotland
IQ	Intelligent Quotient
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LAG	Lead Ammunition Group
LOD	Limit of Detection
LOQ	Limit of Quantification
NHANES	U.S. National Health and Nutrition Examination Surveys
PACEC	Public & Corporate Economic Consultants
PERASG	Primary Evidence and Risk Assessment Subgroup (of the Lead Ammunition Group)
PTWI	Provisional Tolerable Weekly Intake
RA	Risk Assessment
SATs	Standard Assessment Tests
VMD	Veterinary Medicines Directorate
WHO	World Health Organisation

7.2 Relevant studies of blood lead in Europeans in relation to levels of consumption of large game animals published after presentation of the HHRA to the LAG but prior to an assessment of risk reduction measures

Hunting is an important recreational activity in Norway undertaken by about 3% of the population during the hunting season 2011/2012 (Statistics Norway, 2012) and game meat is a major food resource for families involved in hunting. Lead based bullet ammunition is permitted and is the ammunition of choice in cervid hunting in Norway. Recently, high concentrations of lead in samples of minced moose meat were reported in a Norwegian study (Lindboe *et al.,* 2012; see section 3.3.3).

Meltzer *et al.* (2013) investigated blood lead level among Norwegians with varying frequencies of cervid game consumption. Other factors that may potentially affect blood lead concentrations were also investigated. Those who reported eating cervid meat regularly (monthly) or often (weekly) had a mean blood lead concentration of 21.7 µg /L (n=104) compared with 14 µg /L (n=43) in those that reported never or rarely eating cervid meat. Blood lead concentrations varied with sex and age, but when these were adjusted for, blood lead concentrations remained significantly higher in participants that reported eating cervid meat monthly or weekly than those that reported seldom or never eating cervid meat. Consumption of cervid game meat once a month or more was associated with an approximately 31% increase in blood lead concentrations, and this seemed to be particularly associated with the consumption of purchased minced cervid meat. Of the higher consumption group, 68% had blood lead concentrations greater than the EFSA $BMDL_{01}$ of 15 µg/L for increased blood pressure and 11% had blood lead concentrations greater than the EFSA $BMDL_{10}$ of 36 µg/L for chronic kidney disease. These figures fell to 35% and 0% respectively in people reporting seldom or never eating cervid meat. The models also suggested that there may be a significant effect of wine intake on blood lead, possibly relating to increased lead absorption, as increased blood lead levels in those eating minced game and drinking wine could not be explained by the wine lead content alone. Other factors that helped explain the variance in blood lead included the number of bullet shots per year, years with game consumption, self-assembly of bullets and smoking.

Bjermo *et al.* (2013) reported on blood lead levels among Swedish adults, and investigated the association between these and diet and other lifestyle factors. The study was based on a subgroup (n = 273) of the national survey Riksmaten 2010–2011 (4-day food records and questionnaire). The authors reported a median value (5–95th percentiles) of blood lead of 13.4 (5.8–28.6) µg/L. Blood lead levels were positively associated with intakes of game and alcohol.

Appendix 2.
The risks to human health from livestock feeding in areas of lead gunshot deposition

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health” by the Lead Ammunition Group, 2 June 2015.

Risk assessment prepared by Peter Green[1] on behalf of the Primary Evidence and Risk Assessment Subgroup of the Lead Ammunition Group.

March 2013

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1 Abstract

The Primary Evidence and Risk Assessment Sub-Group of the Lead Ammunition Group (LAG) was asked to prepare three risk assessments, of which this is one. It seeks to assess the risk to human health through livestock feeding or foraging in areas of lead shot deposition in England.

The risk assessment has been prepared in line with the recommendations of the “Green Leaves III Guidelines for Environmental Risk Assessment and Management” published by DEFRA and Cranfield University in November 2011. Primary evidence from the previously agreed Primary Evidence List, together with other primary, secondary and tertiary sources has been used to determine whether a proposed Source-Pathway-Receptor model of lead from shooting to humans via animal-derived food is credible and whether it can be established from the reliable, relevant and adequate scientific information.

Primary evidence is reviewed.

The risk assessment concludes that the risk to human health through lead shot deposition on livestock feeding areas in the general pastoral environment where there is no or only limited shooting activity in the UK is negligible.

There is a risk to the health of livestock and poultry feeding or foraging upon heavily lead-contaminated shooting areas such as clay pigeon shooting sites or feeding upon silage and haylage harvested from such sites and therefore a low but not negligible risk of onward transfer of lead to humans consuming products from these animals.

2 Introduction

2.1 Background

The Lead Ammunition Group (LAG) established under the auspices of DEFRA and FSA appointed a sub committee (the Primary Evidence and Risk Assessment Subgroup (PERAG)) to prepare material for consideration by LAG. The terms of reference of PERAG, agreed in 2009, are as follows:

a. To gather and list sources of evidence for assessing the risks of lead in ammunition under the categories outlined below.

b. To advise on the quality, applicability and therefore inclusion of such evidence for risk assessment.

c. To propose a risk assessment method.

d. To use the proposed evidence sources to prepare an initial risk assessment under the categories outlined below:

    1. Risks to wildlife from ingested lead from ammunition. This will include welfare considerations, individual and population level risks.

    2. Risks to human health from the ingestion of lead from ammunition. This will include both risks associated with the ingestion of lead gunshot/bullets or fragments thereof in game animals, and the ingestion of animals that have themselves ingested and assimilated lead from ammunition. (It may also include any other perceived risks arising from lead ammunition).

    3. Risks to human health through livestock feeding in areas of lead shot deposition. This will include risks from lead deposited through inland shooting, including clay-pigeon and other target shooting.

This draft risk assessment (dRA) seeks to identify and investigate the risks to human health from the deposition of lead shot on livestock feeding areas ((d) (3) above) and will be referred to as dRA3 in the text of this document.

A Primary Evidence List (PEL) was prepared by PERAG and agreed by LAG and is to be used as the basis for the Risk Assessments. It has been agreed that this initial list would form the platform or starting point for the information necessary to produce the dRAs, but that the list was not intended to be closed or definitive. All relevant published material with sufficient weight and credibility may be included in the source material and added to the PEL.

2.2 Materials and methods

There are many approaches to the preparation of risk assessments and several schemes or models are in use in the environmental, veterinary and public health sectors. This dRA3 has been prepared by reference to the publication “Green Leaves III Guidelines for Environmental Risk Assessment and Management “published by DEFRA and Cranfield University in November 2011 (DEFRA Green Leaves III (2011)). This peer-reviewed guidance is a revision and update of the [former] Department of Environment Transport and the Regions (DETR) Guideline for Environmental Risk Assessment and Management published in 2000.

DEFRA Greenleaves III (2011) provides a structured framework for preparing a risk assessment and for the management options that flow from it. This framework consists of four proposed

components within a cyclical model: Formulate the problem, Assess the risk, Identify and appraise the management options and Address the risk with the selected management option. The scope of this dRA will include the first two components of this process only – Formulate the problem and Assess the risk, since any consideration of management options is not within the remit of PERAG, furthermore the guidance makes it clear that if the assessment of the risk gives rise to a conclusion that the risk is absent or negligible, the process need not proceed.

DEFRA Greenleaves III (2011) suggests that in the process of formulating the problem or potential problem the conceptual model of Source-Pathway-Receptor (S-P-R) is considered, whereby the actual or potential hazard [the source] is linked in theory to the actual or potential organism, population or system at risk [the receptor] by a credible pathway. This may be achieved by the construction of a conceptual flow-diagram. The parameters of this dRA3 are relatively tight and the conceptual models that suggest a hazard to human health by the deposition of lead shot on livestock feeding grounds are therefore simple:

Hazard	**Source**	**Pathway(s) ⇒**		**Receptor**
Lead	Spent ammunition on feeding areas	Livestock consuming contaminated feed	Humans consuming contaminated animal derived food products	Human consumers
		Livestock consuming contaminated soil		
		Livestock drinking contaminated water		
		Livestock consuming lead pellets or shot on the ground		

The identification of the hazard must be supported by evidence that exposure to the hazard will cause harm to the receptor. In the case of this dRA3, there is no doubt that metallic lead and lead compounds can be toxic to humans (the potential receptors). This dRA3 does not seek to establish this as a fact or to review the considerable body of evidence that shows the toxicity of lead to humans. There is, however, no evidence for a threshold for a number of critical endpoints of human health (i.e. no current evidence of a minimum level of blood lead below which negative health effects cannot be detected - EFSA 2010). Medical opinion has altered in the past fifty years and the thresholds of acceptability, in terms of both exposure and human circulating blood levels, have been successively revised downwards. This trend results from both increased sensitivity in analytical techniques, and evidence of the negative health effects of lead at the lower concentrations that can now be measured. The trend seems likely to continue. This is well documented in EFSA (2004), SCOOP (2004) and EFSA (2010) and will not be reviewed again here.

In attempting to formulate the actual or potential problem of lead deposition on food-animal feeding areas both the temporal and spatial components of exposure must be considered. In all mammals lead can be both an acute and also a cumulative toxin (Lonergan & Gould 2002) and the use of lead in ammunition has been widely practiced in Europe for centuries (Bana 2004, Sneddon et al 2009). Levels of lead may therefore accumulate over time both on the feeding areas where shooting occurs and in the herbivorous animals themselves. The spatial distribution of lead on the feeding areas where shooting occurs will affect the exposure and intake of the toxin by the herbivores.

In the initial stages of constructing the Risk Assessment, DEFRA Greenleaves III (2011) makes it clear that risk screening should be employed, whereby factors such as the plausibility of linkages between the source of a hazard and the receptor should be evaluated and the relative potency of the hazard and vulnerability of the receptor should be assessed. These screening assessments

are at best semi-quantitative and may be wholly qualitative; as LAG has tasked PERAG with preparing risk assessments, no such screening on these bases has been performed in this dRA3.

Having identified the hazard (lead), assessed the potential consequences (harm to human health by consumption of food products derived from animals exposed to lead shot deposition) and identified possible pathways (food-producing animals consuming contaminated forage or water), the Risk Assessment should consider the probability or likelihood that the adverse impacts will occur. This part of the assessment can be divided into three stages: the probability of the initiating event occurring, the probability that the receptor(s) will be exposed to the hazard via the identified pathways and the probability of the receptors being adversely affected by the hazard. Examination of the evidence from the Primary Evidence List (PEL see 2.3 below] and other sources will be necessary to attempt to establish these probabilities. In order to compute or calculate probabilities in a semi-quantitative way solid, reliable and agreed data of exposure levels and thresholds of toxicity are required such as levels at which no detectable harm occurs (the No Observable Adverse Effect Level (NOAEL) or the Predicted No-Effect Concentrations (PNEC)). It is currently considered that (unlike many other contaminants) there are no identified NOAEL or PNEC levels for lead. EFSA (2010) states that for developmental neurotoxicity in children and nephrotoxicity in adults there is no evidence for any threshold below which there is no concern and gives extensive details of human health risks associated with exposure to lead at low levels.

A Risk Assessment based upon absolutely nil exposure of humans to lead is impossible to prepare given the ubiquitous presence of elemental lead and lead salts in the crust of the earth and in the natural environment unaffected by anthropogenic influences. EFSA (2010) indicates that remote, non-industrialised soils contain 10-30 mg/kg lead from the natural environment. For these reasons this dRA3 will have no alternative but to assess probabilities qualitatively by informed consideration of the hard data provided by the PEL and other sources.

For the purposes of this dRA3 “livestock” is taken to mean:

- Dairy cattle and goats producing milk.
- Cross-bred dairy calves reared for beef and dairy cull-cows consigned to the human food chain.
- Beef cattle bred and fattened for beef.
- Sheep bred and fattened for lamb and mutton.
- Free range pigs bred and fattened for pork.
- Free range poultry producing eggs and/or chicken carcasses for human consumption.
- Other free range avian species (ducks, geese, quail etc.) from which human food products are derived.

2.3 The evidence used in preparation of this dRA

The evidence on the original PEL provided a useful starting point for the risk assessment, and has been supplemented by other similarly appropriate sources of data and peer-reviewed information as agreed by the LAG.

The quality of data used for risk assessment is extremely important. Evaluation of the quality and reliability of available information or data can be approached in several ways. DEFRA Greenleaves III (2011) tabulates two possible approaches, one that ranks six indicators of evidence reliability and another that scores data in the light of epistemic uncertainty. Within the peer-reviewed scientific press such indices as the Thomson Reuters Impact Factor, the University of Washington

Eigenfactor or other journal ranking schemes attempt to map and rank scientific papers with varying degrees of success; in experimental science factors such as randomisation and controls are critical in determining quality and value of any published data (Pandit &Yentis 2005).
Within the Life Sciences information is categorised as:

Primary This is published information obtained by experiment, investigation or observation. Raw data is provided with full description of how it was obtained together with details of statistical and analytical interpretations. The reader has access to all the information necessary to weigh the conclusions reached by the authors of the report.

Secondary This is information in reviews, reports, literature surveys and studies based upon a number of primary data sources.

Tertiary This is information in text books and in-depth educational articles.

The Chairman of PERAG suggested that the data in the various sources on the PEL might be evaluated by means of the Klimisch method (Klimisch et al 1995), which is applicable to the assessment of data for purposes of hazard and risk assessment. The option of using this approach was agreed by all members of PERAG. Klimisch proposed that data is ranked for reliability and assessed for relevance and adequacy. The Klimisch system is specifically applicable to the evaluation of “substances” in respect of possible hazardous or toxic effects. In applying a ranking or scoring for reliability, such objective assessments as compliance with the principles of Good Laboratory Practice [GLP] or with internationally accepted test guidelines of the EU, FDA or OECD are used, so that the data on a given substance is ranked as Category 1 (Reliable without restriction) when all such standards are met. There are three categories of reliability, in decreasing rank of reliability and a fourth category of “Not assignable”. Secondary data and literature is ranked as Category 4 (Not assignable), since it relies upon primary data as its source.

The Klimisch approach has some merit for the purposes of this dRA3, and all primary data sources used have been ranked for reliability in the reference section of the paper, but as the nature of the data is so variable and the methods by which the data has been obtained are so diverse, it is inevitable that scoring the data is based upon subjective criteria, not upon objective standards. The relevance and adequacy can also only be assessed subjectively. This will provide scope for discussion or disagreement, but the authors have sought to be as independent as possible in assigning value to the primary data upon which this dRA3 is based. What cannot be disputed is that secondary and tertiary data (reviews, text books, abstracts, literature surveys and summaries) are usually to be classed as Category 4 (Not assignable) since they provide insufficient detail of the precise methods by which data was obtained and rely upon the published work of others. However, this does not imply that they are not as relevant as data scored as Category 1, or that they are unreliable. In medical science (and other scientific fields), the evaluation of likely risks and disease impacts, and therefore disease management and policy decisions, are based on reviews and meta-analyses of all of the literature available in the round. Indeed, the risk assessments that we are conducting here would all fall into Category 4 as we evaluate and rely upon the published and where relevant unpublished work of others rather that raw data. Exceptions to this rule are standard reference data for well-studied substances that provide values for physico-chemical parameters published in internationally accepted reference manuals such as the Merck Index. These can be assigned Category 2 of reliability (Reliable with restrictions).

Category 2- 4 material may therefore have significant “Relevance” or “Adequacy” under the Klimisch scoring system and thus usefully inform the judgments made in the dRA.

The PERA Group is therefore aware of possible confusion between the “Primary” Evidence List (the PEL) and what, in Life Science terms is called “Primary” Information. Clearly the Group will rely heavily upon such publications as EFSA (2010), which reviews in depth the risks to human consumers of lead in food, and is therefore an important element of the PEL, but because it is in effect a review and metastudy, can only be classed as Secondary in terms of peer reviewed

information. EFSA (2010) may be classified as Category 4 under the Klimisch system, but it represents an analysis conducted by a group of the most experienced scientists in their field in Europe, thus the Group will accept its general conclusions and recommendations, and view it as authoritative and reliable since it is based upon resources, expertise and knowledge beyond the scope or expertise of this group to dispute.

When assigning KL scores in the reference lists we have also used an additional symbol - NPR for literature not in a peer-reviewed journal or medium. Conference proceedings and other documents have been listed as NPR because their standard of review is variable.

There is considerable variation in the published literature between the units chosen to express levels of lead in soil, plant tissue, blood and animal tissue. Lead levels are expressed in μg/g (Clements 1997, Stansley & Roscoe), in mg/kg (Mellor & McCartney 1994, Sneddon et al 2009], in ppm [Mellor & McCartney 1994), in "percentage mean soluble lead" (Rice et al 1987) in μmol/L (Rice at al 1987) in μg/L (EFSA 2010, Braun et al 1997) and in μg/dL (Quy 2010). Others report lead levels mg/L (Galey et al 1990) in μg/kg (Philips et al 2003) and in μmol/kg (Sharpe & Livesey 2006). Certain of the units of concentration are directly equivalent(eg μg/g = mg/kg = ppm) and others can be accurately converted to equivalence or near equivalence to provide conformity. However, some levels are reported on a dry matter (DM) and some a wet matter (WM) basis. Here conversions between the two can only be made approximately based upon average wet to dry weights for the materials in question, but occasionally authors do not specify which has been used.

In the UK the laboratories of the Animal Health Veterinary Laboratories Agency (AHVLA) accept that for soft tissues (meat and offal) the conversion factor for wet weight to dry weight is a factor of 3.1 and that for bone a factor of between 3.9 and 4.5 is appropriate, depending on the proportions of dense cortex and soft medulla present in the sample (AHVLA Langford pers. com.). The lead content of dry matter will therefore be 3.1 times higher than wet matter in the case of meat and offal and approximately 4 times higher in the case of bone because the removal of water will concentrate the sample.

Units expressed in micro-mols (μmol) can be converted to parts per million by reference to the atomic weight of lead (207.2). EFSA (2010) recommends that for foodstuffs one litre is equivalent to one kilogram. The following table will assist in comparison of results expressed in different units.

Unit	Symbol	is equivalent to	Conversion factor
parts per million	ppm	mg/kg & μg/g	1
milligrams per kilogram	mg/kg	μg/g & ppm	1
micro grams per gram	μg/g	mg/kg & ppm	1
micromols per kilogram	μmol/kg	ppm 207.2 X 1000	4.83
micromols per kilogram	μmol/kg	μmol/L	1
micromols per litre	μmol/L	μmol/dL X 10	10

Exact conversions

- One deciliter = 100mls
- To convert mols to grams multiply by 207.2 (i.e. 1mol = 207.2g)
- To convert grams to mols divide by 207.2 (i.e. 1g = 0.00483 mols)
- Micro (μ) = millionth, milli (m) = thousandth and deci (d) = hundredth
- ppm=μg/g=mg/kg

Approximate conversions

- One litre is approximately equal to 1kg for blood (1.05 kg but this is usually rounded up to 1kg).
- To convert soft tissue dry weight lead concentration to wet weight lead concentration divide by 3.1, and to convert soft tissue wet weight lead concentration to dry weight lead concentration multiply by 3.1.
- To convert bone dry weight lead concentration to wet weight lead concentration divide by 4, and to convert bone wet weight lead concentration to dry weight lead concentration multiply by 4.

2.4 Lead concentrations in livestock considered as elevated from a food safety perspective

The AHVLA reports on potential food safety incidents. Where lead is concerned, an incident is currently recorded and reported where the offal concentrations exceed 0.5 parts per million (ppm) wet weight (ww), muscle lead concentration exceeds 0.1 ppm ww, milk lead concentration exceeds 0.02 ppm or blood lead concentration exceeds 0.48 µmol/l (100µg/l or 0.1ppm). Most incidents occur following animal disease outbreaks i.e. are actual poisoning incidents. However, occasionally as a result of laboratory testing, the AHVLA comes across high blood or tissue lead levels that are not high enough to cause clinical signs but which are still important in terms of food residues and food safety. These are also taken as incidents. The thresholds of toxicity for individual species vary greatly, so that, for instance cattle and poultry will show clinical signs of poisoning at blood and tissue levels lower than those tolerated without signs of clinical disease in horses and sheep (Lonergan & Gould 2002). A clinical disease is detectable by a clinical examination. Animals may be suffering sub-clinical effects but not exhibiting clinical signs. Sub-clinical effects (e.g. reduced ALAD activity in the case of lead) are detectable by clinicopathological tests but not clinical examination.

Over time, analytical methods have improved allowing the detection of lower tissue lead concentrations and increased analytical precision. As a consequence both of this and a wealth of research into the impacts of lead on human health, exposure lead concentrations of concern from a human health perspective have also decreased. Analytical methods like Flame Atomic Absorption Spectroscopy (FAAS) were used decades and are still in use today. FAAS is reliable, but tends to have only moderate detection limits, whereas Inductively coupled Plasma Mass Spectrometry (ICP-MS) has excellent detection limits. However, these methods are comparable across the range of concentrations with which we are concerned for the purposes of this risk assessment. In presenting the results from primary literature below we present both the authors interpretation of their results at the time of publication, and also whether their results would be considered to be of potential concern from a food safety perspective today.

3 The primary evidence

3.1 The extent of lead deposition from ammunition on livestock feeding areas

Within the original PEL for this dRA3 there is no primary data for the deposition of lead from shooting on pastureland or crops cultivated primarily as livestock feeding areas but also used for recreational, occasional or rough shooting or shot over for vermin control.

Sneddon et al (2009) describe one area from which they collected data as a “rough shooting field’ but it is clear that this area was part of an intensive pheasant shoot on a typical sporting estate that had been used for the driven shooting of reared game birds for some 200 years. The area was mowed twice per annum and not grazed. Several PEL sources (Clements 1997, Chrastny et al 2010, Hartikainen & Kerko 2009, Mellor & McCartney 1994, Rantailainen et al 2006, Sneddon et al 2009, Stansley & Roscoe 1996, Stansley et al 1992) investigate contamination by lead shot on shooting ranges, pheasant shoots, clay pigeon shoots or areas used primarily for shooting. Frape & Pringle (1984) and Rice at al (1987) describe poisoning of cattle by conserved forage [haylage and silage] harvested from clay pigeon shooting sites.

Other sources provide few further data for the extent of lead deposition from shooting on livestock feeding areas. Bjorn et al (1982) sampled cattle grazing on Vaerneengene (a coastal wetland habitat), which is described as the area where bird shooting is probably more intense than anywhere else in Denmark, but give no quantitative data for the amounts or density of pellets deposited.

There are few published primary data of lead shot densities on European hunting estates (other than wetlands). Ferrandis et al (2008) provides details of lead shot densities on a Spanish arable estate where wild partridges (*A. rufa*) were shot between 2004 and 2006. This may be of some relevance since the ground was not intensively shot, with a maximum of only two days shooting per season in 2004 and 2006 with no shooting in 2005 because of poor wild bird recruitment. On shooting days there was a maximum of 16 guns set at 40 m intervals. Partridges had been shot on the land since the 1950s. Shot density was 7.4 shot per m2 in the top 1cm of soil, in front of the shooting lines, which equated to 8.1 kg lead per hectare of No 7 shot. This may be similar to the level of shooting activity over English rough-shooting farmland where cattle or sheep graze, but such inferences are speculative only and cannot be considered as primary evidence. The paucity of data for non-wetland hunting areas in Europe, in contrast to intensive shooting areas, is confirmed by Mateo (2008) and Bana (2004).

There is more information about shot density and lead deposition on ground used primarily for shooting, such as clay pigeon shooting ranges, intensively reared game bird shooting estates or target ranges. Within the original PEL there are six sources of primary data that fall within this category.

Clements (1997) is an unpublished undergraduate thesis for a BSc in Environmental Science supervised by Dr Paul Ramsay at Plymouth University in 1997. The student studied lead concentrations in soil and ryegrass on a dairy farm where organised clay pigeon shoots had been undertaken for 10 years in southern Worcestershire. No assessment was made of accumulated pellet density on the pasture, but fall-out pellets were collected in buckets during two shoots. Fifty soil samples and fifty ryegrass samples were assayed for lead content using nitric acid extraction and flame atomic absorption techniques. The grass samples were not washed and pellets were not removed from the soil.

The results showed that soil lead levels were high in the zone between 100 and 175 metres from the shooting stands, where the maximum detected was measured at 8172.42 μg/g and the mean level was 3038 μg/g lead. Non-shooting areas used as controls yielded up to 72 μg/g of lead. The high lead zone coincided with a disused railway track. Forage sample assay revealed undetectable

lead content in 49 of the samples. One sample returned a level of 121.75 μg/g lead from within the zone of highest lead in the soil. Milk samples from the cattle grazing the land were no different from those from a control farm, with all milk samples having levels below the limit of detection using Inductively Coupled Plasma-Mass Spectrophotometry [ICP-MS], although this limit is not specified.

Mellor & McCartney (1994) measured soil and crop (oilseed rape) lead levels at a clay pigeon shooting range near Bolton in Lancashire where shooting had been undertaken for 20 years. Eleven soil samples and a pooled three-plant crop sample were assayed using both nitric acid and acetic acid extraction techniques [to distinguish between total lead and "plant available" lead] measured by atomic absorption spectrophotometry. Pellets were not removed from the soil. The plant material was washed to remove soil before assay. Control samples were taken from a distant site on the same farm.

The results revealed high total lead levels in the soil between 80 and 140 metres from the shooting stands where they ranged from 5000 to 10,600 mg/kg. This equated to some 1000 to 4100 mg/kg of "plant available" lead. This zone of high lead contamination corresponded with the results of pellet counting (up to 257 per soil core sample obtained by using a 10cm diameter corer and taking the top 15 cm soil). Outside the zone of maximum pellet deposition the levels fell rapidly. The "plant available" lead levels within the pooled sample of oilseed rape plants taken at 100 m from the shooting stands were high, with 420 mg/kg in the roots, 62 mg/kg in the stems and 148 mg/kg in the seeds.

Rantalainen et al (2006) reported on the contamination of a disused "shot gun shooting range" although there are few details of the nature of the shooting activities that occurred in the 23 years of usage. The range was divided into uncontaminated (clean), medium and highly contaminated areas based upon previous pellet counting. Organic soil (humus and fermentation layers), enchytraeid worms and microarthropods were assayed for lead using nitric acid extraction and atomic absorption spectrophotometry. Pellets were manually removed from the soil. Tree growth and litter production was measured.

The results indicated that pH rose slightly but significantly with increasing lead contamination. Mean lead level in the control (uncontaminated) area was 75 mg/kg, rising to 8,700mg/kg in the medium and 18,800 mg/kg in the highly contaminated areas. There was a greater bacterial biomass and more enchytraeid worms in the uncontaminated organic soil but biomasses of microarthropods and nematode worms were unaffected by increasing lead levels. There were no differences in tree growth rates between the areas but pine needle litter production was inversely linked to levels of lead contamination. There were subtle effects of increasing lead upon nitrogen and soil respiration rates.

Sneddon et al (2009) collected samples of soil, pore water, plants, small mammal hair and earthworms from a shooting ground in Cheshire that had been used for game shooting for 200 years and for intensive reared pheasant shooting for 20 years. Their hypothesis was that source-pathway-receptor transfer of lead derived from shotgun pellets could be traced to higher trophic levels. Samples were taken from a woodland copse, a shot-over meadow and distant control sites and were assayed using nitric acid extraction and plasma-mass spectrometry. Soil was sieved through a 2mm mesh but pellets were not extracted. Plants were washed until the washing water was shown to be trace element free. Small mammal hair was a mixture of washed and unwashed material. No small mammal samples were obtained from the shot-over meadow. Earthworms were washed and retained until their bowel was empty before assaying.

The results showed elevated mean total soil lead levels in the wood (160 mg/kg) and the shooting field (68.3 mg/kg) compared with the control non-shooting grassland (43.9 mg/kg) and the control woodland (60.25 mg/kg). A distant non-shooting wood had soil lead levels of 120.2 mg/kg attributable to an adjacent former steel works. Ryegrass (L perenne) was the plant species most abundant across the sampling and control field areas and showed elevated lead levels in the above-ground portions sampled from shooting field (38.4 mg/kg) in compared with the non shooting field (0.89 mg/kg). The washed hair of small mammals from the control field had higher

lead content (115 mg/kg) than the hair from small mammals in the shooting woodland (31.3 mg/kg). Mixed washed and unwashed small mammal hair showed no significant variations in lead levels across the sampling sites. Earthworm tissues from the shooting woodland were significantly higher in lead (111.79 mg/kg) than from the control woodland (5.49 mg/kg).

Stansley & Roscoe (1992) examined lead shot densities on eight shotgun [trap and skeet] shooting ranges in the USA where the fall-out areas included wetlands and measured the lead in the surface water, streams and downstream lake. Shot densities ranged from 4.15 X 106 to 3.7 X 109 pellets per hectare. In an acid marsh environment total water lead was as high as 1,270 μg/L and filterable lead was 83 μg/L. They found negligible off-site transport of lead when water pH was 7 or above, but some evidence of lead mobilisation when water pH fell below 7. The shooting range with the highest pellet density in this study was the subject of a further investigation (Stansley et al. 1996) in which lead take-up by small mammals and amphibians within the shot-fall area was studied as an indicator of bioavailability. White footed mice (*Peromyscus leucopus*), short tail shrews (*Blarinia brevicauda*) and green frogs (*Rana clamitans*) were trapped and subject to detailed clinical chemistry investigation. Mice, shrews, frogs and soil were collected from an unspecified control site control site.

Total soil lead assay after removal of pellets showed mean values of 74 μg/g from the control site and 75,000 μg/g from the shot fall area of the shooting range, representing a 1000-fold increase. The authors calculate that there is likely to have been some 266,000kg of lead in the form of pellets distributed within the soil of the shot-fall area of this shooting range at the time of sampling. There was evidence that the lead was being taken up by the mice, which had tissue lead levels many times higher than the controls, with liver levels up to 38.6 μg/g DM (mean 4.98) compared with control mice liver levels of up to 3.93 μg/g (mean 0.98). Only one shrew was trapped at the range, but it appeared also to have very high tissue levels. The combined lead content of the liver and kidney in both small mammals and frogs was only approximately 2% of the lead content of their bones. Clinical chemistry revealed depressed delta-aminolevulinic acid dehydrogenase (ALAD) enzyme levels in the mice and the frogs, which is a recognised indicator of subclinical lead toxicosis in mammals (Loneragan & Gould 2002). Other haematological components appeared to be subtly but not statistically significantly affected by the lead levels to which the mice ands shrews were exposed.

Rooney & McClaren (2000) describe the contamination of three clay pigeon shooting sites in Canterbury, New Zealand, which had been in used for 7, 21 and 51 years. Soil samples were assayed by both EDTA and nitric acid extraction techniques after manual removal of visible pellets and subject to flame atomic absorption spectrophotometry. Lead levels were reported for total lead (nitric acid extraction) because of unacceptable variability of the EDTA extracted results. The analyses showed that the range used for 7 years had maximum lead soil levels of 719 mg/kg whilst the similar range that had been used for 51 years had levels up 55,958 mg/kg. Both ranges were on the Canterbury plain in areas of similar climate and geology. The study also showed that lead was concentrated in the top 15-20 cm of soil and fell to the accepted natural background level of 30 mg/kg at levels deeper than this. The soils were not disturbed by agricultural activities.

In a report to a Local Authority in Northern Ireland, White Green Young Environmental (2006) reported lead deposition from clay pigeon shooting on an acid peat bog in Northern Ireland. The control sampling area of bog, over which no shooting had occurred, returned a background lead level of 67.4 mg/kg in the top 25cm of peat with less than 12 mg/kg in peat deeper than 25cm. The highest level of contamination in the fall-out zone of the shooting range was 15,700 mg/kg with a mean value across the zone of 305.6 mg/kg. Levels above 500 mg/kg were detected down to levels deeper than 1m below ground surface. There was no indication that this peat bog was used for grazing animals. In a similar study Solenen et al (2012) studied the decay and distribution of lead at two woodland clay pigeon shooting grounds. One site had been used for twenty years and then abandoned for twenty years; the adjacent site had been used for the twenty years leading up to the study. They found that over a twenty year period up to 4kg lead per square metre accumulated on the ground, resulting in total lead levels of up to 50,000 mg/kg. After one of the shooting ranges had been abandoned for twenty years lead levels in the organic topsoil had

reduced, but lead levels in water leaching from the site had increased, compared with a shooting range still in use, reflecting increased bioavailability over long time periods associated with oxidization of the lead metal.

Sorlie Heier et al (2009) showed that trout in cages in a stream in Scandinavia subject to run-off from a shooting range showed elevated lead levels within three weeks. The lead was isotopically traced to the lead pellets used on the range.

For both intensively shot over sites and occasionally shot-over pastures, overall densities of lead shot in the soil will increase each year as lead ammunition degrades (chemically and physically) only very slowly under most conditions. However, shot will generally sink slowly through the soil; rates of sinking are affected by soil density and other characteristics. Hartikainen & Kerko (2009) found that on the coarse stony soil of a shooting range in southern Finland the lead shot migrated downwards at a rate of some 2-3mm per year. In contrast Flint & Schamber (2010) found that on tundra wetlands only about 10% of pellets remained within 6 cm of the surface after 10 years, with >50% remaining within 10 cm. The lead may become less available when redistributed by cultivation or some farming practices could hypothetically make lead shot deposited decades ago more available (Chrastny et al 2010, Rooney & McLaren 2000, Stansley & Roscoe 1992, Green White Environmental 2006).

It is clear that elevated lead levels associated with clay pigeon shooting are not limited to the areas of pellet fall-out. Chrastny et al (2010) detected lead levels up to 694 mg/kg topsoil in an arable field adjacent to a clay shooting range and was able to show that spring barley (Hordeum vulgare) grown in the field was contaminated mainly by lead deposition on the foliage. Hartikainen & Kerko (2009) discovered elevated lead levels in the topsoil chosen as a control sample when measuring soil contamination on a clay pigeon shooting site in use for thirty years. The control sample was taken 300 m from the edge of the fall-out zone of pellets and revealed lead levels of up to 2010 mg/kg in the top 7cm of soil. The authors concluded that this contamination was the result of downwind deposition of lead dust and powder generated by impact of lead pellets with the clays in the air.

Secondary sources offer little further information about lead deposition on grazing land in Europe. Bana (2004) was tasked by the Council of Europe with assessing the ecological effects of lead-shot on terrestrial habitats and on the accumulation of lead in wild birds other than waterfowl. He summarised the primary information upon which his submission was based and for non wetland, non waterfowl data he relied upon studies of lead accumulation in american woodcock (Scolopax minor), northern bobwhites (Colinus virginianus) and mourning doves (Zenaida macroura) in Canada and the USA in habitats that he describes only as “upland”.

3.2 The uptake of lead by plants from contaminated soils

The original PEL provides only three primary sources of data that give evidence of plant uptake of lead. Clements (1997) found one ryegrass sample from the fall-out area of the clay pigeon range on the dairy farm studied that contained elevated (121.75 μg/g) lead levels, but the plants appear not to have been properly washed. The remaining 49 ryegrass samples had lead levels below the limits of detection.

Mellor & McCartney (1994) showed that oilseed rape within the fall-out area of the clay-pigeon shooting range contained elevated lead levels, with the highest levels in the roots. According to this report the levels were 23.5ppm in the roots, 3.1ppm in the stems and 7.4ppm in the seeds, specifying that the ppm values are for dry matter. They also give the findings in mg/kg with results increased by a factor of 20 but without further explanation. The differences cannot be explained on a wet matter: dry matter basis since ppm is equivalent to mg/kg and dry matter levels are expected to be higher than those in fresh plant tissue, which abounds in water.

Sneddon et al (2009) found levels of lead up to 38.4mg/kg in carefully washed ryegrass from the shooting field in their study compared with 0.89 mg/kg in the control grassland.

Rooney et al (1999) took soil samples from a heavily contaminated clay pigeon shooting range and cultivated a range of plants in the soil to measure phytoavailability. The clay target shooting ground was alternated between arable crops and pasture although no details of rotational intervals were given. Soil lead levels were measured by both EDTA extractable and nitric acid solvent techniques after sieving through a 2mm sieve and comparisons of these methods showed that the proportion of total soil lead extracted by EDTA increased with increasing lead levels in the soil. At 23 mg/kg lead only 23% was EDTA extractable, whilst at 6174 mg/kg of apparent total lead measured by nitric acid dissolution, EDTA was able to extract over 8,000 mg/kg. This anomaly was explained by reference to the uneven distribution of elemental lead particles of shot less than 2mm in diameter.

The soils used in the cultivation trials varied from an EDTA extractable lead content of 11 mg/kg (equivalent to a natural background level) to 5998 mg/kg. The plants cultivated were barley (*Hordeum vulgare*), lettuce (*Lactuca sativa*), perennial ryegrass (*Lolium perenne*), radish (*Raphanus sativus*) and white clover (*Trifolium repens*). Plants were grown for 120 days and then harvested, washed and separated into roots and leaves.

The full results of the cultivation trial are as follows:

Soil lead (mg/kg)	Mean plant lead concentration in mg/kgDM									
	Barley		Lettuce		Ryegrass		Radish		Clover	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
11	0.2	4.6	0.2	36.1	1.2	3.3	2.7	14.8	3.1	5.1
103	4.3	16.2	4.8	43.8	5.1	3.3	10.4	48.5	6.1	353.7
813	9.1	136.0	11.4	287.0	7.5	84.7	4.0	525.0	10.1	343.6
1391	11.4	264.5	5.0	251.0	24.0	466.6	17.8	556.0	11.7	254.4
4204	46.6	1361.7	7.0	1388.1	51.4	666.7	124.2	2695.0	133.7	2705.1
5998	34.4	2040.2	9.6	1968.5	43.4	1347.2	49.0	3825.0	93.7	2167.7

All plants showed increasing lead tissue with increasing lead soil levels and, with the exception of ryegrass in low lead soil, all plants accumulated more lead in the roots than in the leaves. The leaves of barley and ryegrass showed virtually linear relationships between soil lead and leaf tissue lead levels; both were maximal at a soil lead level of 4204 mg/kg (46.6 mg/kg lead in barley leaf and 51.4 mg/kg in ryegrass leaf). At increasing lead soil levels both the lead content of the leaf tissue and the productivity (growth) of the plants declined, suggesting some lead toxicity at very high soil levels, although lead in root tissue continued to increase. Assays of titanium were used to eliminate the possibility that root lead levels were ascribable to soil contamination.

Nwachukwu & Pulford (2009) grew perennial ryegrass (*L perenne*) on soils contaminated with heavy metals and demonstrated significant uptake of lead, copper and zinc. The soil came from Scottish mining waste and contained 2281 mg/kg lead. Ryegrass grown in this soil contained mean lead levels of 55 mg/kg. The cultivation trial was designed to test the effects of soil amendments (the addition of organic materials) to the lead levels in the soil and in the plants. The addition of organic modifiers in the form of green compost waste, shredded bark or coir all significantly reduced ryegrass leaf lead levels and levels of EDTA extractable lead in the soil. At a level of 1% w/w added organic material to soil the ryegrass leaf lead level declined to 40 mg/kg, at 10% it declined to 25 mg/kg and at 20% organic additive the ryegrass lead levels fell to 20 mg/kg.

Gzyl (1995) measured lead levels in soils contaminated by lead smelting and in plants grown in those soils. The total soil lead levels ranged from 128.9 to 1996.4 mg/kg and celery and parsley leaves from plants grown in these soils yielded lead levels of 7.3 – 46.2 mg/kg, based upon the subsurface tissues to eliminate the effects of atmospheric deposition.

3.3 The intake of lead from forage, water and soil by livestock

There is no primary evidence within the original PEL of the palatability or indeed of the consumption by grazing livestock of pasture or feed crops in situ contaminated by lead from shooting, neither is there any primary evidence in respect of livestock of drinking water contaminated with lead from shooting.

There are two primary sources in the PEL that give information about clinical lead poisoning of cattle by consumption of conserved forage. Frape & Pringle (1984) reported the poisoning of dairy cows by consumption of haylage with a pH of 3.9 - 4.4 that had been harvested from a field where clay pigeon shooting had occurred before harvest. No details of the size of the field were given, but the authors calculated that in excess of 3.5 tonnes of lead might have been deposited on the field and that each cow could have ingested 1kg of soluble lead over a four-week period. Pellets and clay fragments were visible in the haylage.

Rice et al (1987) reported upon two dead yearling cattle that had died after eating silage harvested from a field where clay pigeon shooting had been practiced for a number of years, although no other details are given of the extent of lead deposition on the pasture. Kidney tissue and blood were submitted from the dead cattle to the laboratory. Lead shot was found in the silage, which contained “0.23% mean soluble lead” after removal of the visible lead pellets. The authors report that this was equivalent to 18g oral lead per day if the steers were consuming the expected 8kg DM of the silage. Kidney tissue lead levels (13 & 18 mg/kg) were not considered indicative of acute lead toxicosis for the cattle. The authors do not specify whether the results were for wet or dry matter tissue, but irrespective of this the levels would be well above the current 0.5 mg/kg threshold of concern for human consumption. Blood lead levels from the dead cattle and the remaining animals in the group ranged from 1.4 to 3 μmol/L (29-62 μg/dl), which the authors considered barely above their normal range at the time, although today these levels would be considered to be three to six times above levels of concern reportable as an incident by AVHLA (0.48 μmol/L). Blood ALAD was, however, significantly depressed and the diagnosis of lead poisoning was based upon this parameter and upon the exclusion of thiamine deficiency (cerebrocortical necrosis) from the differential diagnosis.

The authors reported that hay had been harvested from the field without previous evidence of lead intoxication for several seasons. They suggested that the method of harvesting silage (by which wilted grass is collected by powerful vacuum-assisted forage harvesters) would also pick up pellets whereas hay-making (in which grass is continuously turned until dry and then lifted by tines into the baler) will leave pellets on the ground. They also suggested that the acidic environment of silage fermentation dissolves the elemental lead into lead salts that are both more bioavailable than elemental lead and more toxic.

Braun et al (1997, abstract only reviewed) reported the fatal lead poisoning of calves of seven to nine months old turned out to graze on the target (backstop) area of a military (rifle) shooting range where the soil contained 3,900 mg/kg lead and the dry plant material up to 29,500 mg/kg (extraction methods unclear). The range had been used for many years, with some 20,000 rifle rounds fired each year into the soil in the area where the calves were grazing. This abstract indicates that liver lead levels were 38mg/kg and kidney 30mg/kg wet weight with blood lead at 940 μg/L in one of the dead calves.

Bjorn et al (1982) recorded no difference between blood lead levels of cattle grazing the heavily shot-over Vaarneengene and those of compared controls, even in the dry summer of 1981 when grass levels were very low. Interpretation of the significance of both the Rice et al (1987) and the

Braun (1982) findings for this dRA must be made in the light of subsequent improvements in analytical methods and the reduction in acceptable blood lead levels in humans and livestock.

Factors other than consumption of grass and herbage must be included when the possible intake of lead by livestock from contaminated pastures is considered as part of the receptor-pathway-receptor model in this dRA. Strojan & Philips (2002) showed that cattle avoid the consumption of grass contaminated by lead in the form of lead acetate at levels down to 60mg/kg lead/grass fresh weight, but that this avoidance decreases with time. Other forms or compounds of lead were not tested. They also showed that superficial contamination of the grass with lead acetate at these levels reduces the efficiency of rumenal digestion.

Cattle and sheep deliberately consume soil as a proportion of their dry matter intake. This may average 10% for dairy cattle at pasture (Sharpe & Livesey 2006) and may be up to 30% in sheep under some circumstances, with even good clean summer swards providing sheep with 2-3% of their dry matter intake as soil (Payne & Livesey 2010). Ingestion of soil is considered the most significant source of lead for grazing animals by the EFSA (2004) review and Stansley et al (1996) believed that the source of lead that was detected in the mice, shrews and frogs in their study of the contaminated shooting ground probably originated from the soil, not the plants.

Galey et al (1990) reported lead poisoning in 100 out of 300 dairy cattle fed silage made from Sudan grass that had been stored on soil containing up to 77,000 mg/kg lead (assayed by nitric acid extraction for total lead). The soil beneath the silage clamp had been scraped up with the silage as it was loaded into the feeding wagons. Twenty five animals died. Tissue and blood lead levels were elevated in the fatally poisoned cattle: livers contained 2.7-10.8 mg/kg wet weight total lead, kidneys 3.7-9.0 mg/kg wet weight. Blood lead is reported as 0.142 - 0.532 mg/L in the dead animals.

In a review of domestic livestock and poultry poisoning in Europe (Belgium, France, Italy, Greece and Spain) Guitart et al (2010) cite discarded lead acid vehicle batteries as the most common source of lead in episodes of cattle poisoning. Payne & Livesey (2010) review the evidence for the UK, where 454 incidents of lead poisoning of cattle or sheep were investigated by AHVLA between 1998 and 2008. Where the source was established, 133 were associated with lead paint, 117 with lead-acid vehicle batteries and 112 with geochemical contamination. 31 cases (6.8%) were found to be the result of ingestion of metallic lead, which included lead shot from clay pigeon shooting, lead from flashings and pipes and metallic lead from other unspecified sources. The data for metallic lead ingestion is not further subdivided.

3.4 The intake of lead pellets by food producing birds

Birds are different from mammals in both their tendency to ingest lead as they seek out grit sized particles for their gizzard and in their sensitivity to lead; younger birds are more susceptible to lead poisoning than older ones (Trampel et al 2003). The quarterly reports of the Animal Health Veterinary Laboratories Agency of DEFRA provide data on lead poisoning in the UK in birds by means of both the Avian Quarterly Reports and the Chemical Food Safety Reports. The former has indicated that at least one chemical food safety incident is diagnosed in poultry per quarter in the UK, with exposure to lead being the most common (AHVLA 2010, 2012) and examination of the reports yields interesting information about the source of the lead exposure. Of the thirteen incidents reported between October 2007 and December 2012 six appear to be ammunition related, with chickens, ducks and geese ingesting lead shotgun pellets or airgun pellets. One incident records the poisoning of a flock of 2,000 free-range hens that were farmed on land adjacent to a clay pigeon shoot. Representative post mortems were performed to investigate the poor egg productivity and revealed lead pellets in the gizzards of the hens; productivity was reduced but no signs of clinical lead poisoning were reported in individual live birds.

Another reported incident revealed that particles of lead were found in the gizzards of poisoned ducks. These were traced to lead fragments falling into the duck pen from lead roof flashing that

was being chewed by squirrels. There is no indication of whether or not the squirrels were poisoned.

In the quarter ending December 2012 there is a report of a serious outbreak of lead poisoning in a group of 400 adult and immature laying ducks. The birds were reported to show non specific signs including ruffled feathers, decreased egg production, loss of weight and heads tucked into their bodies. Around 150 to 200 birds died over a two week period. Five birds were examined post mortem. There was no evidence of any gross pathology. Blood lead results of 0.66, 1.12 and 110.4 μmol/l (background lead concentrations < 0.2 μmol/l) were obtained for three birds which were submitted alive. The extensive duck enterprise was newly established on land that was previously used to graze horses, with no reported problems. However there was a clay pigeon shoot on the neighbouring land which had been there for the past 10 years. The source of lead ingested by the ducks was considered to be lead shot from the shoot (the land is reported to be very soft and wet which will have facilitated uptake of lead shot by the ducks due to their feeding habit). The producer was immediately advised not to sell eggs to the public nor slaughter the ducks for human consumption. Due to the extent of the exposure, a decision was made to cull all the ducks.

Butler et al (2005) found a low incidence of ingested lead pellets in the gizzards of ring necked pheasants (*Phasianus colchicus*) from shooting estates in lowland England; some 3% of 437 birds examined between 1996 and 2002 had ingested pellets. In the 1997 cohort of 101 female pheasants, two birds had pellets in the gizzard; this cohort was subject to wing bone lead assay by atomic absorbtion spectrophotometry. Between 1947 and 1992 the Game Conservancy Trust conducted over 1,300 post mortem examinations of grey partridges (*Perdix perdix*) from across the UK. The overall incidence of ingested lead pellets in the birds was 4.5% for adult and 6.9% for chicks (Potts 2005).

3.5 The pathophysiology of lead in food producing livestock and the potential for lead passing from animal tissues into animal-derived human food

The original PEL contains three sources that give some primary evidence of the metabolism, distribution or storage of lead in the tissues or secretions of food producing animals exposed to lead. Clements (1997) assayed the milk from cattle grazing the fall-out zone of the clay pigeon shoot and compared the lead levels with those in milk from a control farm where no shooting occurred. There was no indication of any evidence of lead toxicity in the cattle grazing the clay pigeon shoot. All the milk samples from the shot-over grassland had lead levels lower than the lower limit of detection (ie undetectable levels), whilst two out of seven samples from the control farm cattle had detectable levels although well below the permitted EU thresholds of concern.

Frape & Pringle (1984) assayed blood samples of several cows five months after exposure to toxic levels of lead in haylage (up to 3800ppm DM). They reported that with the exception of marginally low haemoglobin, other parameters were normal, although they do not specifically give results for lead levels. One cow slaughtered four months after exposure had kidney and bone lead levels of 5.7 and 2.4 mg/kg of lead in the tissues, which was considered at the upper range of the normal levels for that laboratory at that time.

Rice et al (1987) reported normal kidney lead levels in two acutely poisoned yearling cattle, quoting 30 mg/kg lead in kidney as the level at which they would have diagnosed lead poisoning in cattle in their laboratory and reporting only 13 and 18 mg/kg in the kidneys of the two poisoned steers. The dose of lead received by the cattle was calculated to have been some 18g oral lead per day.

EFSA (2004) and Quy (2010) describe the accepted information about the absorption, distribution and toxicity of lead in the mammalian body, which is repeated in other secondary and tertiary texts such as Loneragan & Gould (2002), Payne & Livesey (2010), Sharpe & Livesey (2006) and will not be described in detail in this dRA. Briefly, the efficiency of absorption of lead varies greatly

according to the form in which the lead is ingested, with lead salts such as the acetate being more readily absorbed than elemental lead. Lead poisoning in animals may be acute, when large doses are ingested over a short period, or chronic when smaller but excessive doses are ingested over a longer period. After absorption into the bloodstream lead is distributed around the body entering soft tissues and eventually sequesters in bone, where the half-life may be several years. Lead also accumulates in the liver and kidneys because it binds with metallothionein. Half-lives of excretion from soft tissues [the time taken to decrease by 50%] are variable because the true half-life after a single toxic dose is complicated by the leaching of lead deposited in the bone back into the circulation and thence back into kidney and liver. However, half-lives tend to be shortest in blood, followed by kidney and liver, and longest in bone. The ratio of blood lead to milk lead levels in cattle and sheep is variable, but increased lead is secreted in the milk if the animal is challenged by other illness. The levels at which lead is toxic to livestock and farm animals is not consistent across species. Cattle are the most sensitive to lead, with single toxic doses of 600 mg/kg body weight in adult cattle and clinical signs of chronic poisoning appearing with more than 6 mg/kg per day. Toxic doses for milk fed calves are much lower (Payne & Livesey 2010). Cattle are also more prone to lead exposure because they have a greater tendency to lick and chew on objects in the yard or field compared with sheep, goats or horses (Sharpe and Livesey 2006, Loneragan & Gould 2002). Clinical signs in horses are both different in character and take longer to manifest than is the case in ruminants (Loneragan & Gould 2002).

Other primary sources give further relevant information. There was no difference in the detectable lead in the blood samples of the cattle grazing the heavily shot meadows of the Vaarneengene compared with those grazing meadows where no shooting occurred (Bjorn 1982).

Galey et al (1990) reported on blood and milk lead levels in dairy cattle seven months after an episode of acute lead poisoning in which the cattle had consumed a mixture of silage and heavily contaminated soil. The source of lead in the soil was not determined; the silage had been stored on the contaminated soil, which had been mixed with the silage when it was shoveled up for feeding. Of 300 heifers, 100 developed clinical signs and 25 died. Mean blood lead level was below the limit of detection of 0.055 mg/L (0.00027 mol/L) in the animals that had recovered from the lead poisoning naturally and in control cattle that had not been exposed. In heifers that had been so severely poisoned that intensive treatment (intravenous EDTA chelation therapy) had been necessary, blood lead levels were still elevated (mean 0.092 mg/L). All milk samples returned lead levels below the lower limit of detection. Blood lead levels in the worst affected and therefore treated cattle rose slightly at calving to a mean of 0.12 mg/L, but there was still no evidence of transfer of lead to milk.

Waldner et al (2002) reported three episodes of lead poisoning in cattle on separate farms. All three events were traced to discarded lead acid vehicle batteries in the paddocks. All premises recorded fatalities associated with the exposure to lead, with mortality rates of 11% (8 out of 68), 4% (5 out of 128) and 18% (4 out of 22). Serial blood samples for surviving or apparently unaffected cattle on the first farm at day 23 post-exposure ranged from 1.05 ppm to 0.10 ppm. By day 92 post-exposure some cattle still had levels of lead up to 1.12 ppm in their blood, although these were not necessarily those with the highest initial levels. By day 188 post-exposure a number of the surviving and clinically normal cattle still had blood levels up to 0.41 ppm, which was considered toxic by the laboratory reporting the assay (normal for this laboratory = 0 – 0.35ppm). Similar findings were reported for the other farms. Seventeen animals had persistently raised blood lead levels for several (unspecified) months, which the authors attributed to lead in the rumen or reticulum. No calves born to cows with elevated blood lead levels showed toxic effects or had elevated blood lead levels themselves. Overall the authors considered that between 4 and 12% of the exposed but asymptomatic cattle had blood levels in the ranges considered toxic for cattle (above 0.35ppm or 1.69 μmol/L) and that the half-life for blood lead in cattle is at least 90 days. Current blood lead levels of concern from a food safety perspective are more than three times lower than this threshold however (at 0.48 μmol/L) and so it is likely that a far higher proportion of cattle would have blood lead concentrations of concern from a food safety perspective, while not necessarily classified as toxic to the animal.

Miranda et al (2006) monitored the blood lead levels of four heifers that survived an episode of lead poisoning in which six out of ten animals died after licking a discarded broken car battery at pasture. From initial blood lead levels of up to 0.758 mg/L the decline was variable, with half life of up to 266 days. The surviving affected heifers initially showed noticeable clinical signs, but all serum indicators of hepatic and renal function remained normal. The authors concluded that cattle surviving acute severe lead poisoning may remain unsuitable for human consumption for several months, although they do not indicate which food products gave them cause for concern.

Phillips et al (2003) experimentally added lead to the diet of pigs at levels of 5,10 and 25 mg/kg of feed using lead acetate. The prescribed upper limit for lead in manufactured complete animal feedstuffs in the EU is 5mg/kg and for green fodder is 40 mg/kg at 12% moisture content (EFSA 2004). Combined doses with cadmium were also trialed to determine whether one of these heavy metals potentiated the toxic effects of the other. This proved not to be the case. Fattening pigs were slaughtered after 137 days of feeding the experimental diets. Samples of tissue were analysed using nitric acid extraction and atomic absorption spectrophotometry of lead and cadmium.

The lead levels in all sampled tissues of the fattening pigs increased in a linear pattern relative to the increasing doses of lead in the feed, although distribution among tissues was not uniform. Of the internal tissues, the greatest lead levels were recorded in liver and kidney (60 μg/kg in both tissues at 10mg/kg added to feed) with much lower levels in the heart muscle, lungs and ribs. Skeletal muscle [meat] was not sampled. At the highest level of lead addition (25mg/kg food) heart muscle contained up to 80 μg/kg, liver 90 μg/kg and the kidneys 200 μg/kg lead, which did not exceed EU maximum permitted levels in offal (SCOOP 2004).

The EFSA (2004) review reports that at intakes of less than 100 mg/kgDM there are no changes in lead levels in muscle (meat) in cattle or sheep, but that there may be increases in lead levels in offal.

Trampel et al (2003) examined chickens that had been exposed to lead paint chips peeling from a farm building. No details were given of the absolute doses of lead but the paint chips were analysed by atomic absorption spectrophotometry and were found to contain 38.5% lead. The laboratory had been alerted by the death of one fowl from lead poisoning with a blood lead of 1,500 ppb. The mean lead level of the 20 surviving hens at admission to the laboratory was 453 ppb. They were then monitored for up to 10 days to assay the relationship between blood lead, egg lead and chicken meat (breast muscle) lead. A positive linear relationship was found between blood lead and lead in liver, ovarian tissue and egg yolk, but there was no consistent relationship between blood lead and muscle [meat] lead content. At day 9 of the investigation the lead in blood ranged from 150 - 720 ppb and in egg yolks 180 - 340 ppb on a wet weight basis. The chicken meat levels at the same stage were 200-400 ppb wet weight, which exceeded current EU maximum permissible levels in meat (SCOOP 2004). Waegeneers et al (2009) report excessive lead and dioxins in home-kept chickens with free ranging access to contaminated soils and suggest seven husbandry measures to reduce contamination in eggs from these hens.

In the AHVLA Quarterly Avian Reports (AHVLA 2010) there is further evidence of the transfer of lead to eggs produced by lead poisoned chickens. In the episode in 2008 in which a free-range flock was poisoned by exposure to lead shot fall-out from a clay pigeon shooting range, analysis of eggs revealed lead levels up to 2.55ppm in the yolks and albumin. The potential risk to human health was recognised and the whole flock was slaughtered.

The Quarterly Food Safety Report of AHVLA for September 2011 (AHVLA 2011) gives details of the poisoning of ducks reared on a pond adjacent to a clay pigeon shoot. Numerous lead pellets were found in the gizzards of affected ducks. In the affected group 3-5% of birds suffered clinical signs, with lead levels typically at 112ppm WM in the livers and 52ppm WM in the kidneys. The affected group of ducks was condemned and not consigned to the human food chain because of the risk to human health.

The analysis by Butler et al (2005) of the bones of pheasants sampled in 1999 revealed lead levels ranging from 7-445 ppm; the median of the whole cohort was 15.5ppm. The two birds with lead pellets in the gizzard had levels of 377.7 and 220.3 ppm, suggesting that lead was passing from the gizzards into the blood and bones of the birds. The authors make the point that lead pellets in the gizzard do not persist for long, eroding and disappearing within 3 weeks and that the finding of pellets in the gizzards of the birds was taken to represent very recent and current exposure. The elevated bone lead levels in pheasants with no pellets must therefore represent previous exposure to environmental lead or the historical presence of lead pellets in the gizzards. The overall incidence of lead pellets in the gizzards of pheasants (2.5% of 437 birds) was much higher than the corresponding incidence in red legged partridges (*A. rufa*) in Great Britain (0.16% of 637 adult birds), (Butler 2005) compared with an incidence of 4.5% in a cohort of 1300 adult grey partridges (*P. perdix*) (Potts 2005).

Although they are not usually considered as livestock, deer are grazing and browsing ruminants that subsist within the same landscape habitats as cattle and sheep in the UK; meat products from deer are consumed as venison. There is limited evidence to suggest that deer differ from cattle and sheep in the metabolism of lead. In the central Netherlands Wolkers et al (1994) discovered very high levels of lead and cadmium in the livers and kidneys of red deer, whilst the comparable tissues of cattle from the same area had only normal levels. Roe deer appear to accumulate heavy metals such as lead, cadmium and mercury, with levels far in excess of those in domestic stock grazing the same pastures, but the deer tissue levels do not appear to represent simple environmental exposure, since lead levels in roe deer tissues from heavily industrialised mining and smelting areas of Slovenia were found to be significantly lower than those in deer from uncontaminated rural habitats (Pokorny 2000). Hermosa de Mendosa et al (2011) found high lead levels in the livers kidneys and muscles of roe deer in Spain that appeared linked to age, whilst levels of cadmium and zinc in the same animals reflected only background environmental levels irrespective of age. This suggested that roe deer accumulated lead over time. The accumulation was not sex linked.

3.6 The hazards to human health

We are unable to find primary evidence that establishes a hazard to human health from lead shot deposition on ruminant or pig feeding areas used for low level or occasional shooting.

The primary evidence indicates that where livestock are exposed through ingestion to elevated levels of lead from a variety of sources including ammunition concentrations of lead in blood, offal and meat may be elevated. They may exceed, often by many times, current exposure levels of concern from a food safety perspective used by the AHVLA. The relationship between lead intake and blood or tissue levels is however not straightforward and is complicated by species, the form of lead ingested and other dietary and physiological factors (Clements 1997, Frape & Pringle 1994, Rice et al 1987, Miranda 1996). Cattle and chickens may have blood levels of lead higher than current thresholds of concern for human health as a result of ingestion but remain free of clinical signs of lead toxicosis (Trampel et al 2003, Galey et al 1990, Waldner et al 2002). Payne & Livesey (2010) report that when elemental lead such as lead shot or fragments of lead acid battery plates are eaten by cattle they may remain in the forestomach and give rise to contaminated milk and offal without the cattle showing any clinical signs. When cattle suffer clinical lead poisoning there is a risk that tissues and milk may remain high in lead for many weeks after recovery (Miranda 1996), but in some cases, for instance the episode of cattle lead poisoning reported by Galey *et al.* (1990), milk remained unaffected. This is confirmed by EFSA (2004)

In pigs fed high diets high in lead acetate (two and five times the recommended level for manufactured feed but well below the level permitted for green fodder which is eight times higher than for manufactured feed) the permitted thresholds of lead in meat and offal were not exceeded although rising lead tissue levels were in linear relationship with intake (Phillips et al 2003).

There is evidence that intensive clay-pigeon shooting or rifle shooting may deposit sufficient lead on pasture to increase lead intake to toxic levels for livestock if the grass is harvested for silage or haylage (Frape & Pringle 1994, Rice et al 1987, Rooney & McLaren 2000) or even if the rifle shooting range target areas are grazed by cattle (Braun 1997, Payne & Livesey 2010).

In the case of poultry there is evidence that chickens kept on high lead soils, ingesting lead paint or exposed to lead shot on the ground may have tissue and egg levels of lead in excess of EU maximum permitted foodstuff levels (Waegeneers et al 2009, AHVLA 2010, AHVLA 2011). This appears also to be the case for other gallinaceous birds such as pheasants, since the bone lead levels reported by Butler et al (2005) of up to 445ppm in pheasants strongly suggest that offal, eggs and possibly meat would also have returned higher than acceptable lead levels if they had been tested, based upon the reported findings in chickens (Trampel et al 2003) and red legged partridges (Alectoris rufa) in which Ferrandis et al (2008) found up to 87 μg/g [ppm] in the bones of partridges exposed to lead shot and found lead liver levels of up 42.83μg/g (ppm DW, equivalent to 13.8 ppm WW) in birds with lead shot currently in their gizzard. These levels exceed the current EU maximum level thresholds for poultry offal of 0.5 mg/kg (ppm) WW (EFSA 2010). No EU maximum levels of lead in any tissues have been set for game birds.

4 Discussion

When considering the primary evidence for assessing the risk of humans being exposed to harmful levels of lead derived from deposition of ammunition on livestock feeding areas several factors must be considered. Lead is very persistent in the environment and a proportion of the elemental lead is gradually converted to more available salts over time (Hartikailen & Kerko 2009). The extent and rate of this conversion depends upon a range of physical and chemical factors relating to factors such as soil type, PH, organic matter and soil structure. The relationship between the total amount of lead in the soil in an area and its availability to plants and animals is therefore not straightforward. Both elemental lead and lead salts can be absorbed by animals following ingestion, but it is likely that a higher proportion of the lead may be available for absorption if the lead is in the form of certain lead salts rather than as particulate elemental lead from shot. While the total lead content of soil gives a good indication of the potential risk to animals exposed to it, researchers have tended to measure the relative availability of lead using a variety of extraction techniques rather than absolute concentrations of different lead salts.(Chrastny et al 2010, Selonen et al 2012, Rooney et al 1999). Rooney & McLaren (2000) showed that total soil lead levels on similar New Zealand clay shooting ranges appeared to be proportional to the number of years for which the ranges had been used.

While many of the results described above (e.g. for livestock tissue concentrations) are directly comparable, as we are interested in lead concentrations that result in disease rather than the very low lead concentrations that modern analytical techniques can detected more precisely, some of the variations in lead extraction and assay techniques also give rise to problems of comparability. This is especially true for soil. True comparisons between primary evidence sources can only be made when such techniques are standardised, a point emphasised by Kimlisch (1997). The primary evidence necessary for this dRA includes environmental lead levels assayed following various extraction methods including the use of nitric acid, EDTA and acetic acid extraction. The different results obtained by these methods when used for soil are documented by Rooney et al (1999).

Comparisons in soil lead can be also confounded by differences in sampling techniques and sample handling, such as the depth at which the soil samples were taken (Mellor & McCartney 1994, Rooney & McClaren 2000, Stansley & Roscoe 1992, White Young Green 2006), whether or not the samples were sieved and whether visible pellets were removed from the samples before lead assay was undertaken (compare Sneddon et al 2009 with Stansley & Roscoe 1992). Terms such as “plant available lead” (Mellor & McCartney 1994) are imprecisely defined. Hartikaiene & Kerco discovered that in a currently used clay pigeon shooting range the difference in lead levels between the organic (top) layer of soil and the deeper mineral levels varied from 160-230 fold. After a similar range had been abandoned for twenty yeas the difference between the two layers was only 18-34 fold because of downward pellet migration and pellet corrosion into soluble salts.

Notwithstanding these difficulties it is possible to make an informed assessment of the risk under consideration. Using the source-pathway-receptor model for this dRA it is helpful to consider the livestock (cattle, sheep, pigs, poultry) as an intermediate receptor of the hazard (lead) and humans as the final receptor.

It is established from the primary evidence that elemental lead is deposited on terrestrial habitats by shooting, that this lead persists for many years and that it is gradually converted into soluble lead compounds that are more readily absorbed by mammals including humans than the pure element (Clements 1997, Mellor & McCartney 1994, Rantailainen et al 2006, Rooney & McClaren 2000, Sneddon et al 2009, Stansley at al 1992, Stansley & Roscoe 1996, White Green Young Environmental 2006, Green & Pain 2012). The source of the potential hazard is therefore irrefutable.

When the pathway to the intermediate receptor is considered, there is evidence that monocotyledonous plants (barley and ryegrass]) and dicotyledenous plants (lettuce, radish, clover, oil seed rape, celery, parsley) will take up lead into their tissues (Gzyl 1995, Mellor & McCartney

1994, Nwachukwu & Pulford 2009, Rooney et al 1999, Sneddon et al 1999). At natural background soil levels this take up is very low but there is evidence that in the case of the monocotyledonous plants (ryegrass and barley) this take-up is linear in nature and may rise to levels in the leaves that exceed the maximum permitted EU lead levels in green fodder when soil lead levels approach 1300 mg/kg of EDTA extractable lead (EFSA 2004, Rooney et al 1999). A potential pathway for the source to the intermediate receptor is therefore established, but the risk of this pathway being significant is proportional to the degree of contamination of the soil attributable to lead shot deposition. This evidence must be considered in the light of the evidence that physical and chemical conditions in the soil influence the availability of lead, e.g. increasing organic material in the soil significantly reduces the bioavailability and uptake of lead from contaminated soil (Nwachukwu & Pulford 2009) and that cattle may avoid lead contaminated pasture if they can (Strojan & Phillips 2002). The soil lead levels reported by the Scandinavian and Czech workers (Chrastny et al 2010, Hartikainen & Kerko 2009, Selonen 2012) were all from forest sites with low soil pH, which the researchers point out would have increased lead corrosion and bioavailability.

Further potential pathways to the intermediate receptor are the ingestion of the soil itself and of metallic lead in the form of pellets on the ground. Primary and secondary evidence indicates that livestock may consume a significant proportion of dry matter dietary intake as soil and that if this soil is contaminated with lead the animals may suffer lead toxicosis (EFSA 2004, Galey et al 1990, Payne & Livesey 2010, Sharpe & Livesey 2006). The uptake of lead into small mammals and frogs at a lead-contaminated site was considered to be a soil origin by Stansley et al (1996). Metallic lead in the form of pellets may be consumed by livestock and poultry when contamination of the ground is excessive, for instance on or adjacent to clay pigeon shooting ranges (Payne & Livesey 2010, Braun et al 1997, AHVLA 2010, AHVLA 2011).

By all potential pathways (consumption of forage, ingestion of soil and ingestion of pellets) it is therefore credible that the intermediate receptor (food producing livestock) could be exposed to levels of lead intake that exceed maximum permitted and recommended levels for livestock feed in the EU (EFSA 2004).

For the general agricultural environment (i.e. pastoral or arable land that is not regularly and/or heavily shot over and not immediately adjacent to clay pigeon shooting ranges) there is currently no primary evidence available to suggest deposition of spent ammunition in the UK at levels that might realise these pathways. For such habitats this risk assessment need not proceed any further and the risk to the final receptor can be considered negligible or non-existent.

The situation is different in areas that are heavily and/or regularly shot over (or are adjacent to and within the fallout range of such areas), such as clay pigeon shooting sites, rifle ranges and driven pheasant shoots where guns are repeatedly sited at the same stands and therefore pellet fall-out is over the same areas and where considerable quantities of gunshot are discharged (Braun et al 1997, Ferandis et al 2008, Hartikainen & Kerko 2009, Selonen 2012) At such sites there is evidence that levels of soil contamination by lead shot may pose a risk of lead toxicosis in livestock, particularly in cattle both through consumption of conserved silage or haylage from such sites and by soil ingestion (Frape & Pringle 1984, Rice et al 1987, Clements 1997, Mellor & McCartney 1994, Rantailainen et al 2006, Rooney & McClaren 2000, Sneddon et al 2009, Stansley at al 1992, Stansley & Roscoe 1996, White Green Young Environmental 2006 Hartikainen & Kerco 2009, Chastny et al 2010). The limited information available suggests that this does not appear to be the case for hay making from such sites (Rice et al 1994, Bjorn et al 1992). Poultry exposed to such sites are at particular risk and there is evidence that birds may be poisoned by ingestion of lead pellets from shooting (AHVLA 2010, 2011, 2012, Butler et al 2005). For such intensively shot-over sites the risk of the pathway from the habitat to the intermediate receptor is therefore credible and established.

Between the two extremes (land with no shooting or very limited shooting on the one hand and long term clay pigeon shooting sites on the other) the extent of lead shot deposition will be proportional to the intensity, frequency and longevity of the shooting activity.

When a non-negligible risk has been identified, the risk assessment should proceed to consider the final step of the potential pathway of source to receptor, which in this case is the transfer by human consumption of foodstuffs derived from the intermediate receptor. Cattle are the domestic species most sensitive to lead toxicosis and manifest clinical signs at lower acute doses and earlier in chronic intakes than other livestock species (EFSA 2004, Loneragan & Gould 2002, Payne & Livesey 2010, Sharpe & Livesey 2006). Livestock exposed to lead of gunshot origin have been shown to have tissue (liver, kidney and blood) lead concentrations in excess of levels that would be now considered to be of potential food safety risk but Galey et al (1990) found no evidence of transfer of lead to milk, even in cattle with persistently elevated blood lead levels after an episode of acute lead toxicosis. Waldner et al (2002) showed that there was no detectable placental transfer of lead to the calves of chronically poisoned cows. Although Miranda (1996) showed persistently elevated blood lead levels in heifers recovering from lead poisoning, there was no testing of meat or milk products from these animals. Pigs fed at five times the maximum EU permitted levels of lead in pigmeal had lead levels in the offal that were below the EU maximum permitted human foodstuff lead level for offal (EFSA 2004, SCOOP 2004, Phillips et al 2003).

Lead-poisoned poultry may be free of clinical signs but have lead levels in eggs and meat that exceed EU permitted levels for up to 10 days after the dietary source of lead is removed. The levels of the dietary lead intake for such effects to be seen are high (EFSA 2004, SCOOP 2004, Trampel et al 2003) but such high levels of intake have been reported in chickens farmed adjacent to a clay pigeon shooting range. Waegeeners et al (2009) considered the risks of lead from contaminated soil being transferred to humans through hens and reported increased lead in chickens foraging on contaminated urban soils.

The implications for food safety of lead poisoning in cattle in the UK were considered by Sharpe and Livesey (2006) who analysed the episodes of recorded bovine lead toxicosis on the Veterinary Laboratory Agency's database between 1990 and 2003 and identified the sources where possible. Some 40% of cases arose from lead paint, 18% from discarded batteries and 17% from geochemical sources. The authors advise caution in committing recovering cattle to the human food chain on the basis of the work of Waldner et al (2002) who found persistent elevated blood lead in recovering cattle, but overall they concluded that accidentally lead-poisoned cattle have little long-term effect on the human food chain.

It must be emphasised that the consideration of this final potential pathway of the hazard from source to final receptor has been based upon primary evidence arising either from reports of clinically poisoned animals following exposure to lead from ammunition sources or of animals experimentally given high doses of lead. There is no evidence that ruminants or pigs grazing agricultural environments where occasional and/or light shooting occurs suffer lead intoxication attributable to the ammunition. In the few reported cases where cattle suffered lead toxicosis from silage and haylage contaminated with very high levels of lead from considerable numbers of pellets, the conserved forage came from clay pigeon ranges. In these cases the cattle had kidney lead concentrations between 8 and 36 times current EU permitted lead levels of 0.5 mg/kg wet weight (depending upon whether wet weight or dry weight value are considered EU 1881/2006).

There are no primary data that show cases of transfer of lead derived from shooting through animals grazing areas of lead shot deposition to humans from consumption of animal products, although the evidence indicates that feeding of silage or haylage harvested from these areas may do so. Control measures are in place to stop such transfer where cases of lead poisoning are identified (Sharpe & Livesey 2006).

By reference to the published information it is possible to provide a rough estimate of the upper possible limit of exposure of humans to lead derived from milk, red meat and offal attributable to ammunition. SCOOP (2004) indicates that the surveillance of food products in the UK between 1993 and 1997 revealed that 0.001 (0.1%) of milk samples, 0.006 (0.6%) of meat samples and 0.09 (9%) of offal samples were found to be at or above the maximum EU levels for lead. Payne & Livesey (2010) indicate that approximately 7% of lead poisoning episodes in cattle and sheep in the UK arise from ingestion of metallic lead, including fall-out from shooting.

From these statistics it is possible to estimate that the upper possibility of the frequency with which shooting-derived lead occurs in red meat is 0.006 X 0.07, equivalent 0.00042 (0.042%) of red meat consumed. The corresponding frequency for milk is therefore 0.00007 (0.007%) and for red meat offal 0.0063 (0.63%). The data provided by Payne & Livesey (2010) for lead poisoning of cattle and sheep combines ingestion of shooting-derived metallic lead with lead pipes, lead flashings and other exposures to the elemental metal; the figures given above represent an estimate of the risk of human exposure if all the metallic lead poisoning incidents were shooting derived. The true risk of human exposure from this source must therefore be lower than these figures, but is impossible to compute this risk from the available data.

In the case of poultry however, there is primary evidence that human food products (eggs and chicken meat) may acquire hazardous levels of lead when chickens range over land heavily contaminated with the fall-out from intensive shooting and it is likely that pheasants and partridges may affected similarly (AHVLA 2010, Butler et al 2005, Ferrandis et al 2008, Guitart et al 2010 b, Potts 2005), although the incidence of ingested lead pellets in pheasants and grey partridges on UK sporting estates appears from the small number of studies conducted to be higher than the corresponding incidence in red -legged partridges (Butler et al 2005, Butler 2005, Potts 2005) .

5 Summary and conclusions

In conclusion, this risk assessment establishes that the risk to human health via the livestock food chain from the deposition of lead from ammunition in England and Wales should be considered in the light of two levels of shooting intensities. These two levels represent opposite ends of a continuum of shooting activity and therefore of lead deposition.

A. For the general agricultural pastoral environment (i.e. areas over which shooting is of low and infrequent intensity)

   This risk assessment establishes that although a potential source-pathway-receptor model of lead from shooting adversely affecting human health in the UK through ruminants, pigs and poultry is credible, we are unable to find evidence that such human exposure occurs. For this intensity of shooting the proposed pathway fails for the following reasons:

   i. We could find no data on the level of contamination of the general agricultural environment or of grazing land by lead from lead ammunition deposition and therefore of plant or soil contamination at levels considered significant.

   ii. There is evidence that on non-acidic soils high organic material content of the soil significantly reduces bioavailability of lead; this is likely to be the case on permanent grassland and productive leys in the UK.

   iii. We could find no data on lead derived from ammunition deposited on agricultural livestock feeding areas being taken up by foraging livestock.

   iv. There is evidence from AHVLA and FSA surveys of lead in livestock derived foodstuffs that lead is rarely taken up form all livestock food sources at levels of potential concern to human health.

In the general agricultural pastoral environment of the UK where little or infrequent shooting occurs the risk to human health from ammunition derived lead in meat eggs or dairy products is considered to be negligible.

B. For areas frequently and intensively shot over

   This level of shooting intensity is epitomised by the clay pigeon shooting ground in use for many years. Such shooting ranges have been widely and objectively investigated for evidence of lead pollution and the results reported in the peer- reviewed literature.

   There is evidence that continuous or repeated use of the same areas for discharging large quantities of lead ammunition, such as many clay pigeon shooting grounds and some long established game shoots (and the target areas of rifle ranges) are likely to give rise to levels of lead deposition that would adversely affect the health of livestock grazing or foraging the areas of pellet fallout and the immediate vicinity. Whether or not similar levels of lead shot discharge and deposition occur in the context of game shooting will be function of the intensity and longevity of the shooting activity in any one localised area.

   Poultry, including chickens, ducks, partridges and pheasants appear to be especially at risk from feeding or foraging in such areas.

   Harvesting silage or haylage from such areas is likely to give rise to toxic effects upon livestock fed on the conserved forage.

   Although we could find no evidence of onward transfer of toxic levels of lead to humans from ruminants or pigs under such circumstances, such potential transfer is plausible and the risk must therefore be assessed as very low but not negligible.

When poultry, including chickens, pheasants and ducks, forage over land that is repeatedly or continuously used for shooting or shot over and on which deposition of lead is high, there is primary evidence of transmission from source to the end stages of one of the potential pathways (eggs) and strong circumstantial evidence for a second pathway (poultry meat). The risk to human health from poultry ranging over these areas must therefore be considered to be present but low.

6 Information gaps in respect of this risk assessment

The primary evidence used in this risk assessment would be strengthened by further reliable data concerning:

- The levels of gunshot-derived lead on agricultural pastures in the UK where rough shooting and occasional shooting occurs.
- The levels of gunshot-derived lead in the vicinity of repeatedly used shooting stands and the extent to which the fall-out includes livestock pasture.
- The effect of soil type and management on the mobilisation of ammunition derived lead.
- The plant lead levels on pasture where occasional shooting occurs compared with appropriate controls.
- The investigation of the physical and chemical dynamics and bioaccessibility of ammunition derived lead in pastures.
- The patho-physiological distribution of lead into skeletal muscle [meat] and eggs in livestock and poultry consuming varying levels of lead.
- The transfer of lead into milk and meat in chronically subclinically poisoned cattle.
- The transfer of lead from pellets ingested by game birds and chickens to meat and eggs in these birds.
- The uptake and patho-physiology of lead in deer.

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KR = Klimisch reliability score.
NPR = Not published in a peer reviewed journal or medium.

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Appendix 3.
Lead Ammunition and Wildlife in England (UK)

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health”
by the Lead Ammunition Group, 2 June 2015.

Risk assessment prepared by Dr John Harradine[1] and Dr Alastair Leake[2] on behalf of the Primary Evidence and Risk Assessment Subgroup for the Lead Ammunition Group.

July 2013

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Abstract

The Defra/FSA Lead Ammunition Group tasked its Primary Evidence and Risk Assessment Subgroup with preparing a science-based assessment of the risks to wildlife (*inter alia*) in England from ingested lead from ammunition, to include welfare considerations, individual and population risks. The PERASG prepared a primary evidence list (PEL) to be used for the wildlife risk assessment (dWRA).

A review of risk assessment models was undertaken, appropriate to a potential environmental contaminant such as ammunition lead. The one adopted was that recommended by Defra and Cranfield University entitled "*Green Leaves III*" (Gormley *et al*. 2011). This identifies key stages in the process: problem formulation, hazard identification, magnitude of consequences, probability of consequences and assessment of risk. The risk assessment was qualitative rather than quantitative, given the lack of appropriate information needed for quantitative assessment.

For use in the dWRA, relevant sources from the PEL were assessed as to their relevance and reliability, guided by Klimisch *et al*. (1997) and Gormley (2011), in order to provide an evidence-based assessment of published information on exposure to spent ammunition lead and its consequences on wildlife in England (UK). Other information sources, particularly recently produced, and from other countries, were used where they appeared helpful.

The dWRA was addressed through six main Source-Pathway-Receptor models:

- direct ingestion by birds and animals of spent gunshot pellets with food or grit;
- ingestion of spent gunshot by predators and scavengers contained within their prey/food;
- ingestion of rifle bullets by predators and scavengers within their prey/food;
- movement of soluble ammunition lead through plants into their consumers;
- movement of soluble ammunition lead through soil organisms/invertebrates into their consumers; and
- movement of soluble ammunition lead from embedded shot or bullet into body tissues/organs.

The main types of wildlife included gamebirds, waterfowl, birds of prey and scavengers, other birds, small mammals, soil biota and invertebrates.

The focus of the dWRA is England (UK) but where evidence helpful to assessing risks to UK wildlife was available from other countries this is also presented.

Finally, UK bird population trends, drawn mainly from the periodic publications *Birds of Conservation Concern, Wetlands Birds Survey and Breeding Bird Survey,* jointly produced by UK statutory and non-statutory conservation agencies, were used to help assess potential population impacts of ammunition lead on certain of the UK bird species covered by the dWRA.

The risk assessment's null hypothesis was that lead ammunition use for sporting and target shooting, pest control and deer management should not cause unreasonable risk of widespread mortality in England (UK) wildlife receptor species, reduced reproductive success or significant welfare issues for affected individuals. The information needed to test it adequately proved limited, even for key wildlife receptors.

Overall, the evidence to date, as available on the PEL and other recent literature, is not indicative of significant impacts at the population level. No single study adequately demonstrates a pathway

between spent lead ammunition and adverse effects on any England (UK) receptor (waterfowl currently excepted) at the population level. Nor do other studies indicate that such impacts are occurring or are likely to be occurring. At the individual level, the evidence does indicate adverse effects, including death, of lead ingestion. It is likely that welfare costs are incurred whenever lead is absorbed.

A small percentage of gamebirds are directly exposed to spent lead shot, from which it is likely that some will die, but there are no indications of any population impact.

Exposure and ingestion continue in waterfowl, perhaps with impacts at the population level, notwithstanding the legislation in place, but this is a matter for the LAG and Defra to address in terms of the effectiveness of, and compliance with, the legislation.

There is some potential for seed-eating passerines to ingest spent lead pellets, but no attention has been given this in this country. Similarly there are no indications that woodpigeons are being poisoned through ingested lead shot.

There is evidence that British birds of prey are exposed to lead in their environments. Hunter-shot birds and animals can be a source of ammunition lead for predators and scavengers feeding on wounded birds and animals, un-retrieved carcases or discarded viscera. Spent lead shot and/or bullets in their prey or other food can be a contributory source and it has been shown in one species, red kite, and circumstantially indicated for buzzard and peregrine falcon. Some species showing elevated tissue lead levels do not normally eat birds or animals subject to shooting. It is likely that, given the continuing, illegal, use of lead shot for, at least, inland duck shooting, exposure of birds of prey and scavengers to lead through eating lead-shot birds continues to some extent. UK raptor populations are not showing evidence of being impacted by such poisoning.

The crow family (corvids) is one group of scavenging birds exposed to lead-shot carcases or discarded viscera but there is no evidence in the UK for either corvids or mammalian predators/scavengers being at risk from such sources.

High concentrations of lead are typically recorded in the soils of shooting grounds. Elevated lead levels in plants and/or animals occupying such sites indicate some mobility of lead, although many variables are involved. Few clear source-pathway-receptor links have been demonstrated between the ammunition lead and receptors, including micro-arthropods, invertebrates, small mammals or frogs. Uptake through inadvertent soil ingestion as well as with food, are suggested pathways. Earthworms appear to accumulate body lead through their consumption of lead-enriched soil.

The evidence indicates potential for some exposure of various wildlife types inhabiting such sites to lead from spent shot, with possible adverse consequences for affected individuals. The population of each species potentially exposed to lead at the site, though, may be small compared to the whole population in the area, region or country in which the shooting site occurs.

In view of elevated lead levels in earthworms, it is possible that woodcock are exposed to lead in their earthworm diet, at least in areas subject to high concentrations of spent lead shot.
The limited evidence does not indicate consistent negative impact of embedded lead pellets in shot waterfowl nor that poisoning occurs as a result.

The sub-clinical, behavioural, developmental and reproductive impacts which can follow ingestion of spent lead ammunition are likely to be associated with negative impacts on ‘quality of life’, including pain, discomfort, reduced performance or viability. In the absence of definitive evidence, and on the basis of lead’s toxicity and general lack of a lower threshold for adverse effects, it is reasonable to assume adverse welfare impacts from lead levels at least above those widely regarded as background for the tissue(s)/organ(s) in question.

A number of information gaps to better inform future risk assessments have been identified.

1 Introduction

Spent lead shot and the impact on waterfowl that ingest it have been the subject of study and legislation in many countries, for many years. More recently exposure of other wildlife to spent shot has been receiving increasing attention. This particularly includes gamebirds and other terrestrial bird species, through their ingestion of spent shot as food or grit, and predatory and scavenging birds, through eating prey that has been shot and is carrying spent shot embedded in its body. In addition concerns have risen over secondary poisoning of predators and scavengers from whole and fragmented lead bullets in rifle-shot game.

These matters were the focus of a Peregrine Fund conference, Idaho, USA in 2008, ("Ingestion of lead from spent ammunition – implications for wildlife and humans" (Watson *et al*. 2009), which has since raised the profile of the issues and generated new activities to address them.

A number of studies, particularly in America but also Europe, have sought to increase understanding of the extent of both primary and secondary poisoning in wildlife by such ammunition, and its consequences. Primary poisoning refers to poisoning caused to an individual bird or animal from direct exposure (by ingestion) to spent lead ammunition, while secondary poisoning refers to poisoning of a bird or animal through the consumption of another bird or animal, or other food, itself carrying lead from ammunition. Many have recorded lead, both metallic and tissue-bound, in a variety of wildlife (receptor) species.

Sometimes the source of the lead has been identified to lead ammunition. Where it is not evident the conclusion is frequently drawn that it is lead ammunition of one sort or another, based on knowledge of the receptor's feeding habits, the overlap in distribution between its habitats and areas subject to shooting activity, or lack of other obvious sources of lead exposure. From tissue levels and clinico-pathological signs, and reference to previous studies, interpretations are typically made in terms of the effects of the ingested lead on the individual. A few studies only have attempted to assess impacts of ingested lead on populations of receptor species. Most studies have concluded, though some with insufficient evidence, that lead ammunition is the likely cause of observed lead poisoning, that it is an unacceptable or unwise consequence of using lead ammunition, and that, not least given the availability of non-lead ammunition, its use should be ended in favour of non-lead types.

In response to specific calls by concerned parties to government environment and health departments during 2008/09, the Department for Environment, Food and Rural Affairs (Defra), in March 2010, set up a Lead Ammunition Group (LAG) to address the issues and advise accordingly. Partner to this initiative was its Food Standards Agency (FSA) as concerns related particularly to human health from the consumption of lead-contaminated game meat.

The LAG's terms of reference state:

The purpose of the Lead Ammunition Group (the Group) is to bring together relevant stakeholders and experts to advise Defra and the FSA on:

a. *the key risks to wildlife from lead ammunition, the respective levels of those risks and to explore possible solutions to any significant risks.*

b. *possible options for managing the risk to human health from the increased exposure to lead as a result of using lead ammunition.*

The scope will be limited to England (though relevant research and evidence may be drawn from anywhere) and focused on safety aspects for human food, impacts on wildlife and issues surrounding possible alternatives to lead. The Group will be established for an initial 12-month period, after which progress will be reviewed by Defra & FSA.*

Aims

- *To advise Defra/FSA on what the significant risks to wildlife from the use of lead ammunition are and what levels of risk these pose in the short, medium and long term. Also any perceived risks which the evidence indicates are not significant.*
- *To advise Defra/FSA on possible options for managing the risk to human health from increased exposure to lead resulting from the use of lead ammunition notably in terms of food safety (including game shot with lead ammunition and spent lead shot deposited on agricultural land).*
- *To advise Defra/FSA of any significant knowledge gaps that may hinder the identification or assessment of risks, the development of technical solutions or the development of government policy.*
- *To advise Defra/FSA on any communication issues, and possible solutions, concerning the relaying of balanced information on issues surrounding the use of lead ammunition to the media, general public and stakeholders.*
- *To advise DEFRA/FSA of any significant impacts of possible advice or solutions on shooting activity and associated recreational, wildlife management, economic and employment impacts.*

The LAG comprises representatives of the range of shooting, deer management, welfare and conservation bodies in the UK, epitomised by: the British Association for Shooting and Conservation (BASC), Country Land and Business Association (CLA), Gun Trade Association (GTA), National Game Dealers Association (NGDA), Universities Federation of Animal Welfare (UFAW), Countryside Alliance (CA), Game & Wildlife Conservation Trust (GWCT), Institute of Environment and Health (IEH), Royal Society for the Protection of Birds (RSPB) and Wildfowl & Wetlands Trust (WWT).

The LAG, in turn, set up the Primary Evidence and Risk Assessment Subgroup (PERASG), with the following terms of reference:

a. *To gather and list sources of evidence for assessing the risks of lead in ammunition under the categories outlined below.*

b. *To advise on the quality, applicability and therefore inclusion of such evidence for risk assessment.*

c. *To propose a risk assessment method.*

d. *To use the proposed evidence sources to prepare an initial risk assessment under the categories outlined below:*

    1. *Risks to wildlife from ingested lead from ammunition. This will include welfare considerations, individual and population level risks.*

    2. *Risks to human health from the ingestion of lead from ammunition. This will include both risks associated with the ingestion of lead gunshot/bullets or fragments thereof in game animals, and the ingestion of animals that have themselves ingested and assimilated lead from ammunition. (It may also include any other perceived risks arising from lead ammunition).*

    3. *Risks to human health through livestock feeding in areas of lead shot deposition. This will include risks from lead deposited through inland shooting, including clay-pigeon and other target shooting.*

First preparation of each of the three initial risk assessments was tasked to different members of the PERASG, drawing to some extent on available knowledge of, and expertise in, the respective subject areas. Risks from lead ammunition to wildlife in England (given Defra's England-only remit but noting FSA's UK-wide remit) are addressed here. Risks to human health under categories (2) and (3) above have been allocated to other members of the PERASG. It was required that only draft risk assessments that had full support of PERASG members, or if that were not achievable then accompanied by minority report(s), would be submitted to the LAG in furtherance of its terms of reference.

2. Preparation of wildlife risk assessment

2.1 Selection of methodology

There are many forms of risk assessment, developed for different purposes and circumstances, and some better suited to specific needs than others. The basic framework adopted by the PERASG for its risk assessments, as applied to this wildlife risk assessment (dWRA) and common to many approaches, comprises:

1. Hazard identification – identifying the harmful effect(s) in wildlife arising from its exposure to lead from ammunition.

2. Hazard characterisation – evaluating the harmful effect(s) caused by the lead – including increasing severity of effect(s) with increasing amount of lead (dose-effect), numbers of animals/birds affected at a known level of lead (dose-response), mechanisms of action, species differences, and any threshold below which no harm occurs (No observable adverse effect level (NOAEL)).

3. Exposure assessment – measuring, estimating or predicting the intake of, or exposure to, lead in terms of its magnitude, duration and frequency, as applied to wildlife populations, sub-populations or individuals.

4. Risk characterisation (assessment) – integrating the hazard identification, hazard characterisation and exposure assessment to enable prediction of:

    - Whether or not harmful effects are likely to occur in wildlife.

    - Nature and severity of harmful effects in wildlife populations, sub-populations or individuals.

    - Proportion of each wildlife population/sub-population that may be affected.

    - Any particularly vulnerable wildlife populations/sub-populations/individuals.

The UK Environment Agency, Institute for Environment and Health and Defra's forerunner, Department of Environment, Transport and Regions (DETR), in 2000, produced (up-dated) guidelines for environmental risk assessment and management (DETR, 2000). Given that "It is…the aim that the principles set down…should be adopted in all routine decisions concerning environmental policy, management and protection.", this lead ammunition/wildlife risk assessment was prepared accordingly. The guidelines reflected governmental thinking within the UK and also had much in common with previous approaches to environmental/ecological risk assessments (some elements of which are also used, as appropriate). Emphasis was on the DETR risk assessment guidelines rather than management responses to any risks identified as the latter are out with the remit of PERASG.

In 2011, however, revised departmental guidance – "Green Leaves III" - was published by Defra and Cranfield University (Collaborative Centre of Excellence in Understanding and Managing Natural and Environmental Risks) (Gormley *et al*. 2011). As the updated guidelines are expected to be consulted widely by practitioners providing risk advice to Government so this lead ammunition/wildlife risk assessment has been revised as appropriate.

Problem formulation begins the process, including a conceptual model to identify actual or potential sources of lead, pathways, receptors and impacts. The whole process requires the hazard(s) to be identified, consequences of exposure to them determined, magnitude of the consequences quantified, their probability estimated and the significance of the risk(s) evaluated. Consideration of

the importance of risks identified by the process and of any management responses was not part of the PERASG task, its being undertaken in due course by the LAG.

The risk assessment was to be based on primary evidence as revealed by published literature, with no new data collection. The literature was first collated into the Primary Evidence List but this later expanded to include relevant published material that became available. The approach was a qualitative rather than quantitative assessment, given the lack of data for quantitative exposure and impact assessments for many UK wildlife species and lack of resources to undertake appropriate modelling.

2.1.1 Problem formulation

A prerequisite to problem formulation is definition of intention. This is for LAG to define but it might take the form:

To enable the LAG to understand the key risks to wildlife in England (UK) from ingested lead from lead ammunition, including welfare considerations, individual and population level risks, and to explore possible solutions to any significant risks.

In turn, such an intention has several facets: baseline, components, process and forecast.

Baseline

This refers to the state of the environment in the locale of the hazard(s) arising from the use of lead ammunition and over the area where harm may be expected. It also requires knowledge of relevant pre-existing hazards that may affect the outcome of the dWRA.

The quantity and availability of lead ammunition deposited into the environment will determine the hazard posed to wildlife and non-target organisms. Whilst atmospheric deposition accounts for the greatest input of lead into the wider environment, and this form is more bio-available than lead from ammunition, these levels have declined with the removal of lead additives from automotive fuels (Ratner *et al.* ????).

Many environmental issues requiring risk assessment are localised and of the nature of point sources of environmental contaminants. Lead ammunition in shooting, however, is geographical in scale, being deposited wherever shooting takes place, and over two thirds of rural land in the UK (PACEC, 2006) – see section 2.1.5. Most of the pellets in each discharged cartridge are dispersed in the environment. Clay target shooting typically produces the densest concentrations of spent gunshot particularly at the many fixed sites around the country, mainly through the frequency and intensity of clay shooting. Some driven game shooting also produces relatively dense shot concentrations, particularly long-established shoots, but generally at a lower level. Individuals practising “rough shooting” and pest control disperse small quantities of shot over wide areas. Deer managers, and some pest controllers using rifles, discharge relatively small numbers of single projectiles, many of which remain in the shot animal.

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It is noted that, notwithstanding the introduction of legislation (England 1999/Wales 2002) prohibiting the use of lead shot over the foreshore, designated wetland sites important for wintering waterfowl and for all waterfowl shooting, non-compliance in (primarily) inland duck shooting continues to deposit spent shot in some wetlands (Cromie *et al*. 2010).

Lead bullets are used in deer management in much of the upland and afforested parts of the country, and for pest mammal control (including fox and rabbit) widely through lowland and upland habitats.

Deposition of spent shot varies from year-round in much clay target and pest control shooting, to autumn/winter only in game/rough shooting. Use of lead bullets in deer management and other mammal control is broadly year-round.

Little information is to hand on the state of the diverse environments or other hazards also present in all the places lead ammunition is used. Broadly, though, game and similar shooting takes place over agricultural land, over moorland and in woodland, with the intrinsic nature, uses and inputs commonly associated with such land. Clay target shooting does so too but many larger grounds are dedicated to that purpose and managed accordingly, with or without arable use of the land. Other clay grounds occur on marginal land, in old quarries and other unused sites formerly used for commercial purposes. Such sites may have histories of contamination by former uses.

Components

The key requirement is to connect the source (S) of the hazard (=lead ammunition), via a pathway (P), to the receptor (R), in this case wildlife, and its impact on wildlife. It is important that connectivity or potential connectivity between the components in each S-P-R model is shown. If any of the components is missing the risk assessment need go no further (DETR, 2000).

Process

Each of the components can relate to other components and can be affected by other factors which may affect the risk. The resulting complexity presents challenges in obtaining the necessary information for thorough wildlife risk assessments.

Forecast

This reflects the need to define what may happen as a consequence of the intention. In the context of the dWRA, if no significant risks to wildlife are identified then no remedial action would likely be appropriate, but spent lead ammunition can be expected to continue accumulating in parts of the environment and creating exposure for wildlife. If significant risks are identified then appropriate remedial measures should reduce the likelihood of this happening. This is a matter for the LAG.

Conceptual model

The main sources and potential pathways of lead from ammunition into wildlife include:

a. Whole gunshot pellets ingested directly from the ground surface or surface of underwater sediments, typically by seed-eating, vegetation-eating and invertebrate-eating birds, in mistake for food or as grit to assist digestion of food – gameshooting/rough shooting/pest control/wildfowling/clay target shooting/clay target shooting.

b. Whole gunshot pellets, parts of pellets or metal residues from pellets, ingested by avian and mammalian predators and scavengers from their food which comprises shot but un-

retrieved birds or animals, either alive (especially wounded or behaviourally-affected) or dead, or, occasionally, their discarded viscera. The pellets, parts of pellets or metal residues are contained in the flesh/tissues of prey items, resulting from pellets penetrating the bird/animal - gameshooting/rough shooting/pest control/wildfowling.

c. Whole gunshot pellets or remains of whole pellets within the digestive system of prey animals that have themselves ingested them as in (a), then ingested in turn by predators/scavengers without their having been shot - gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

d. Lead compounds absorbed into prey animal tissues following ingestion and digestion of pellets, as in (a), then ingested by predators/scavengers, whether themselves shot or not - gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

e. Lead compounds absorbed by plants from soil/water containing soluble lead derived from lead pellets deposited by shooting, then ingested by herbivorous wildlife - gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

f. Lead compounds absorbed by soil organisms/invertebrates from soil/water containing soluble lead derived from lead pellets deposited by shooting, then ingested by carnivorous/omnivorous wildlife - gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

g. Whole lead rifle bullets, parts of bullets or metal residues from bullets, ingested by avian and mammalian predators and scavengers from their food which comprises shot but un-retrieved mammals, either alive (wounded) or dead. The bullets, parts of bullets or metal residues are contained in the flesh/tissues of food animals, resulting from the bullets penetrating the animal. They may also be within the ‘gralloch’ (intestines) of deer/other mammals discarded in the field by deer stalkers/pest controllers after removing the shot deer/other mammal carcase – deer management/pest control.

h. Whole pellets or bullets, or parts of same, embedded in tissue of shot wildlife potentially releasing soluble lead into the affected bird/animal – gameshooting/rough shooting/pest control/wildfowling/deer management.

A conceptual model is given in Figure 1, indicating predicted relationships, both pathway and impact, between bird/animal receptors and spent lead from ammunition sources to which they may be exposed.

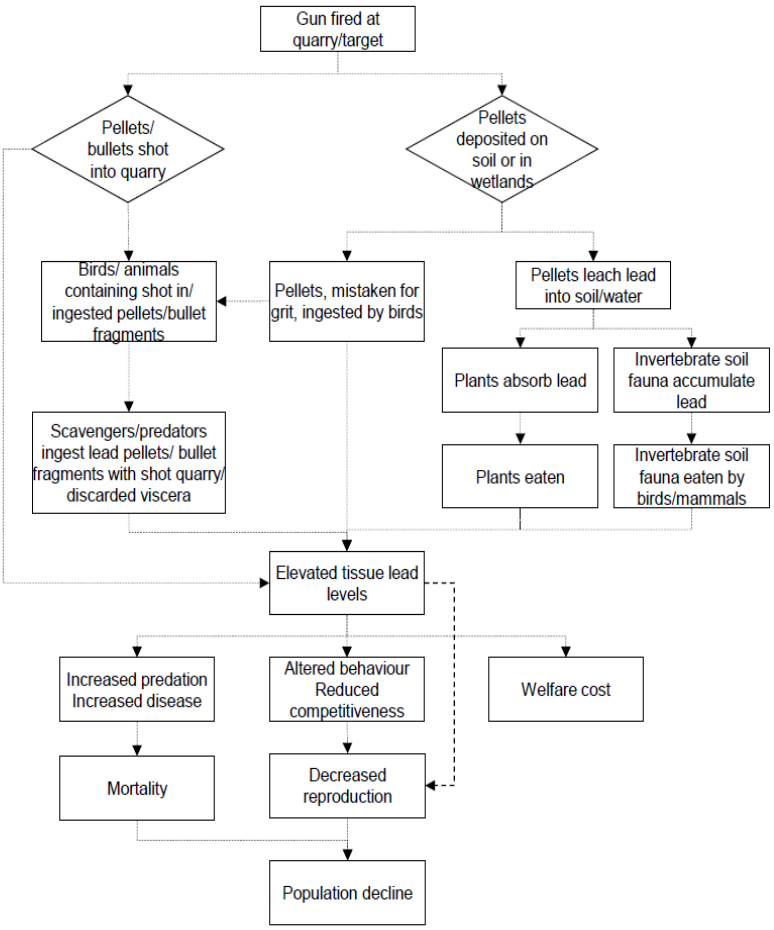

Figure 1. Conceptual model for risks to wildlife in England (UK) from lead from spent ammunition.

The species/type of wildlife that have been included in UK studies involving spent lead ammunition, as revealed by the PEL and other recent literature, are listed below. They are grouped broadly into "Direct", those exposed/potentially exposed to lead poisoning through ingestion of spent ammunition (gunshot or bullet, whole or part), and "Indirect", those exposed/potentially exposed to lead poisoning through consumption of previously-shot prey/food containing spent lead ammunition or its by-products or other contamination by lead. The PEL and other literature include studies involving soil micro-organisms, earthworms and plants/crops as pathways through which lead from ammunition could move through ecosystems into higher forms of wildlife.

Direct

Bird	Gamebird*	pheasant, grey partridge, red-legged partridge, red grouse
	Waterfowl*	duck – mallard, teal, pintail, shoveler, pochard, gadwall, tufted duck, shelduck
		goose – greylag, pink-footed, Canada
		swan – mute, whooper, Bewick's

Indirect

Bird	Raptor	buzzard, golden eagle, white-tailed sea eagle, goshawk, hen harrier, marsh harrier, montague harrier, hobby, kestrel, merlin, peregrine, red kite, sparrowhawk
	Owl	little, long-eared, short-eared
Mammal	Rodent*	wood mouse, field vole
Invertebrate		earthworm*

NB * indicates species potentially containing embedded (if previously shot) or ingested spent gunshot, or other spent lead ammunition by-products, potentially available to predators/scavengers/other consumers through food chains.

The types of wildlife that have been included in studies outside the UK involving spent lead ammunition, as revealed by the PEL and other recent literature, are listed below. They are similarly and broadly grouped into “Direct” and “Indirect” types, and whether (*) they also potentially contribute to onward transmission of ammunition lead through food chains. They are included in this dWRA to help inform on the risks to England (UK) wildlife from spent lead ammunition, on the basis of known or possible similarities in source-pathway-receptor risks and impacts reported in wildlife elsewhere.

Direct

Gamebird*	pheasant, partridge spp., grouse, quail, wild turkey, mourning dove
Passerine*	sparrow, junco, cowbird, pigeon, robin, flycatcher

Indirect

Raptor/scavenger	hawk spp., eagle spp., vulture spp., condor, harrier, owl spp., raven
Waterbird*	woodcock, coot, moorhen, gull, tern
Small mammal*	mouse spp., shrew spp., vole, rabbit
Large mammal	fox, deer
Amphibian*	frog
Fish	bass, sunfish
Invertebrate*	earthworm
Soil biota	microarthropod spp.

2.1.2 Hazard identification

Hazard to wildlife from lead in spent ammunition arises from, firstly, the fact that lead is a toxic metal with no known biological value, and, secondly, from scientific literature showing adverse consequences for wildlife following exposure to such lead (e.g. see reviews in Watson *et al.* 2009, including Pain *et al.* 2009, and Mateo 2009, and Kendall *et al.* 2006).

2.1.3 Identification of consequences

The potential consequences (impacts) of exposure to lead, in either metallic or organic form, are many, variable and dependent on many factors, including quantity, frequency of exposure, form of lead ingested, diet, absorption rate into blood, transfer to other organs, and biochemical processes within the receptor body. Organs and processes affected include the circulatory system (particularly haemoglobin synthesis), nervous system (including brain/cognitive development), kidney function and reproduction. See the abovementioned reviews for details. The effects vary from non-observable (sub-clinical) through sub-lethal to death. Individual birds/animals may be affected in one or several of these ways. They may or may not recover and there may be welfare consequences. If enough of a given population either die directly or indirectly through other factors reducing individual survival (such as increased predation, accidents or disease risk), and/or if reproductive output is adversely affected (such as by reduced fitness), then there may be an impact on population size and/or distribution.

The measures most often used to evidence exposure to lead include:

Potential exposure

- Ammunition lead in feeding areas/food – spent gunshot on or elevated levels in the soil/sediment and/or prey/food items.

Actual exposure

- Ammunition lead in intestine – presence of pellets or bullet/bullet fragments in bird crop or gizzard, or animal stomach.
- Clinical pathology – symptoms associated with (but not necessarily caused by) lead poisoning.
- Depressed d-ALAD (delta-aminolevulinic acid dehydratase – blood enzyme required for haemoglobin production) – sensitive to low levels of lead.
- Elevated blood ammunition lead – blood lead level above background or known non-ammunition source(s) – the main indicator of recent exposure in live birds/animals.
- Elevated soft-tissue ammunition lead – liver and/or kidney levels above background - also indicates recent exposure especially post-mortem.
- Elevated bone ammunition lead – above background but source(s) difficult to identify – indicates long-term exposure as main deposition organ of ingested lead.

NB: Lead levels deemed to be “background” for different tissues/organs vary somewhat in the literature.

As outlined above, the primary hazards that can be identified relate to direct ingestion by birds or animals of spent ammunition lead (gunshot or bullet, whole or part) typically with food or grit, and indirect ingestion, through consumption by predators and scavengers of previously-shot prey/food containing spent lead ammunition or tissue-incorporated lead. In the context of this risk

assessment, secondary hazards include exposure to lead by individual birds or animals through tissue-embedded lead ammunition following their being shot but not killed; exposure of herbivorous species to soluble lead compounds within their vegetable food; and contact with particulate ammunition lead in their home environment. Grazing livestock exposed to spent ammunition lead in or on its herbage food or to soluble lead within the herbage is not covered as that is subject to the third specific risk assessment by the PERASG for the LAG.

The risk assessment endpoints are: a) negative population status of any species of England (UK) bird, mammal or other taxon exposed to lead from lead ammunition, and shown to be or considered likely to be caused by the exposure to ammunition lead b) negative impacts on individual bird or animal health, including development, behaviour, reproduction, welfare and survival, shown to be or considered to be caused by exposure to ammunition lead.

2.1.4 Magnitude of consequences

This requires several component factors to be addressed including spatial and temporal scales of the consequences, the time to onset of the consequences, as well as estimates of the magnitude of the consequences.

Spatial scale

The distribution of spent ammunition lead is determined by the location of historical shooting activity, subject to the deposited lead still being available for ingestion or uptake, and by current shooting for recreational (‘live’), target, deer management and pest control purposes.

Environmental impact from lead shot deposited by shooting activities may not remain confined to the boundaries or geographical locality of the activities. Spent gunshot on the ground surface will remain largely in situ but birds, primarily, picking it up or birds shot but not killed by it have the potential to transfer some outside the immediate area, thereby widening the extent of exposure to predatory/scavenger wildlife. Lead from spent bullets from target shooting is most likely to remain in the area of use (and largely unavailable), while that from ‘live’ shooting can be more widely exposed to predators/scavengers through their eating shot animals or their discarded viscera. Potentially, deposited lead from ammunition can be carried elsewhere by water passing through, or over, the shooting site.

Temporal scale

Potential exposure of wildlife to spent gunshot lead will remain as long as lead gunshot is used and then as long thereafter as the shot remains accessible on the ground or bottom of wetlands, and does not sink inaccessibly into soil or sediment or get buried by farming operations or vegetation growth. Exposure of predatory/scavenging wildlife to lead from ammunition will remain as long as lead gunshot and bullets are used for shooting their bird/animal prey or they are exposed to legacy ammunition lead after its use ends.

Time to onset

This is not relevant as the dWRA relates to an on-going scenario whereby lead ammunition continues to be used in much shooting in England (UK), as it has been for several hundred years.

Magnitude of consequences

There are several indications that an individual bird or animal has been adversely affected by lead poisoning, with evident or presumed associated welfare impacts, to the point that death follows sooner or later, either directly from the condition or as result of predation or accident facilitated by its condition.

There are difficulties in estimating the magnitude of consequences to populations from exposure of individual members to spent lead from ammunition, and particularly so for impacts on communities or ecosystems. The best known is probably the Californian condor population impacted by the birds' ingestion of spent bullets/bullet fragments from shot but un-retrieved deer carcases or discarded viscera (see Watson *et al*. 2009). Considerable resources were needed to elucidate the cause and effect relationships and other possible contributing factors, in a relatively simple scavenger/food/environment system. Relatively little comparable work has been done in Europe, with the white-tailed sea eagle in Germany being the best example (section 3.2.2). Modelling such impacts on England (UK) species is currently not an option for the dWRA as insufficient information is available.

As a result of the likely high level of uncertainty over estimated magnitude of consequences, judgement of the consequences may prove necessary. DETR (2000) suggests an *ad hoc* scaling process to assist in these circumstances:

Negligible	Sub-lethal effects in individuals not causing change in population size or structure.
Mild-moderate	Effects at population level and non-significant effects at ecosystem level.
Severe	Local extinctions of species and local dysfunction of communities and ecosystems.
Very severe	Global extinctions of species and widespread dysfunction of communities and ecosystems.
Extremely severe	Impacts on global ecosystem functioning.

It may not be entirely appropriate, not least as it addresses community and ecosystem impacts and functioning even at its lower scaling levels, which are not and cannot be addressed through this dWRA. At the same time, it has some value as an indicator, along with others, of lead poisoning impacts at individual and population levels, particularly if the second scaling level of "Mild-Moderate" is split into "Mild" – for "sub-lethal and lethal effects in individuals not causing changes in population size or structure" – and "Moderate" – for those "individual effects which result in effects at population level and non-significant effects at ecosystem level". This takes into account that individual impacts of ammunition lead poisoning may occur, including death, but not at a level to impact negatively population size, structure or distribution, through such mechanisms as compensatory mortality.

2.1.5 Probability of consequences

While it is reasonable to expect adverse effects from exposure to lead from ammunition, given its nature and the published literature, there still has to be an assessment of the probability of the hazard occurring, of England (UK) wildlife receptors being exposed to the lead, and of the harm resulting from that exposure.

Probability of the hazard occurring

This is potentially high wherever lead ammunition is used, and remains in the environment in forms available to wildlife.

The quantity of gunshot deposited in the England (UK) environment each year is difficult to estimate. No official estimates of shooting participants, numbers of birds/animals shot, or cartridges fired are available. Using some published and unpublished sources (including estimates of cartridge sales, given commercial sensitivities over such information) allows some indication of possible quantities to be given.

Game and other bird shooting (pheasant, partridge and woodpigeon) are estimated to take place over some 2 million hectares in the UK (PACEC 2006). Approximately 19 million gamebirds and wildfowl are shot a year and around 3.6 million pigeons, accounting for approximately 72 million cartridges fired, on the basis of around three cartridges per bird bagged. Using a standard 32g load per cartridge this equates to some 2,300 tonnes of shot deposited per annum in the pursuit of avian quarry, or the approximate equivalent of 1kg/ha/yr. The proportion of this total which is non-lead in nature is not known. Similarly, the number of lead cartridges fired at bird pests and mammalian quarry is not known.

The number of cartridges used in clay shooting in the UK has been estimated at some 109 million per annum. On the basis of a mean shot load 28g per cartridge, this indicates some 3,050 tons of lead shot fired at clay targets. There are no statistics on the area over which the lead from these cartridges is deposited.

It has been estimated that some 15,000 stalkers use approximately 30 rounds of ammunition each a year. Based on an average projectile weight of 7.5g, this would contribute a further 3.4 tonnes of fired metallic bullet ammunition a year, with, again, an unknown non-lead component. Industry figures indicate that up to 4.5 million rounds of .22 rimfire bullets are purchased for quarry/pest/target shooting each year, which would contribute a further 170 tonnes.

Overall, these estimates suggest some 5,000 plus tons/yr of lead ammunition are deposited each year in this country. It is difficult to calculate the area over which this is deposited with any reliability as different shooting activities disperse spent ammunition in different ways.

Probability of wildlife exposure

Exposure will be influenced by the type of shooting and the history of shooting over a given area of land or water, land type and land use, as well as distribution and feeding activities of relevant wildlife species.

Fixed shooting positions typically produce concentrations of spent shot more or less confined to their localities, particularly in clay target shooting and driven types of game shooting, although their locations can change over time. The volume of shooting will then influence the actual concentrations of spent shot in those areas. In less formal types of shooting, including rough shooting, wildfowling and pest control, shooting positions are much less fixed, and lower quantities of shot are dispersed, over a wider area. In all shooting situations the subsequent availability of spent shot to wildlife will be influenced by the type of ground, its vegetation cover, its use for agriculture and other activities, and the extent to which different wildlife species use the areas of shot deposition for feeding or other purposes.

In rifle shooting, distribution of spent bullets in the general environment depends on the type of shooting. Target shooting typically results in the localisation of bullets in berms or bullet traps, from which they often are periodically recovered while in live shooting, bullets remain largely in the target animal. Relatively few miss the target, but some pass through. In both scenarios most spent bullets are likely to be unavailable to wildlife by virtue of being buried in the ground or vegetation.

Those that remain in the target potentially become available to predators or scavengers if the shot animal is not retrieved or if the carcase is removed but the viscera left behind. Exposure to wildlife then depends on the extent to which predator/scavenger feeding areas overlap with the areas of such shooting.

The key requirement for a pathway between lead ammunition, as the source, and one or more wildlife receptors is addressed below by reference to the published literature. Where a clear or potential source-receptor pathway and impact is evident then the risk assessment will proceed; otherwise there is likely to be little benefit in taking it further. Where such a pathway is evident the degree of exposure via that pathway should be quantified. Reference to the PEL and other literature will guide the extent to which that is achievable, subject to this dWRA being essentially a qualitative rather than quantitative risk assessment (section 2.1).

Probability of harm

The likelihood of harm to a receptor from a given level of exposure to lead is variable. This is due to the varying susceptibilities of individual birds and animals to lead exposure and the many factors which affect it. The PEL and the other literature should indicate the extent to which responses to lead exposure reveal dose-response relationships in wildlife, although it is noted that, increasingly, the medical view is that in humans there is no NOAEL (No Observed Adverse Effect Level) with lead. It is widely believed that any exposure to lead is likely to result in negative effects on the individual exposed.

Characterisation of risk

Based on the literature used, and its relevance and reliability, a largely qualitative assessment is made of the likelihood of occurrence of both known and potential adverse effects of ammunition lead on wildlife in England (UK) under exposure conditions which appear likely in this country. Uncertainties and assumptions are highlighted as appropriate. There are some quantitative guideline values, for example, of tissue lead levels generally used to indicate likely elevation of lead above background levels. These are used to aid interpretation and assessment.

2.1.6 Addressing risks

Once the dWRA is completed it is for the LAG to address the significance of any risks identified for wildlife from lead in ammunition, to put them into context both in terms of other risks encountered by wildlife and the costs and benefits of any remedial measures that might be considered appropriate.

2.2 Use of primary evidence literature

The data needed for a wildlife risk assessment are from scientifically-sound studies in or closely relevant to England(UK), which establish the source, or the likelihood of the source, of any identified effects as being spent lead ammunition, its pathway into any bird or animal species (receptor) and its adverse impact on the individual and/or its population. Initially, a key set of published sources was established – the Primary Evidence List (PEL) - approved by the LAG and posted on the LAG website (www.leadammunitiongroup.co.uk). This was to be the basis of this and the other draft risk assessments. As work progressed it was acknowledged that other potentially-pertinent literature and evidence emerged through literature searches, new publications and re-assessment of primary (and some secondary) sources, and should be used as appropriate, to be added to the PEL in due course. Several new sources relevant to the dWRA were found and have been used.

The actual use of the PEL literature has not been straightforward. It had been previously established (PERASG terms of reference) that:

> *From the many papers on lead risks and impacts, we will aim to list those that provide comprehensive coverage of the key issues and the most current, relevant, science-based information and reviews available. Where authoritative reviews are used we will provide an opinion on the soundness of the authors' interpretations. Additional references may be added if deemed appropriate at any time.*
>
> *The primary evidence gathered will cover the risks from lead in ammunition and not comparative risks of other materials used for non-lead ammunition types.*
>
> *We will cover the following types of information:*
>
> 1. *Published in independently peer-reviewed established journals [Peer review is a process used for checking that research methodology and conclusions are sound, before being published, typically undertaken by other specialists in the field of study].*
> 2. *Published in independently peer-reviewed other literature (*e.g. *proceedings of conferences) or published reports written by 'accredited' expert specialist groups (which may have an ISBN number or be freely available online).*
> 3. *Other reports,* e.g. *commissioned by government, academic institutions and NGOs - not published, or published but not peer-reviewed.*
> 4. *Other literature considered to be of sufficiently high quality in total or with some information useful to the Sub-Group.*
>
> *We may consider it necessary to send literature for independent or further independent peer-review.*
>
> *References will also be tagged as having the following applicability: UK, EU (Europe), INT (International). This is to assist judgment about their relevance to the UK and reflects the fact that, while not necessarily carried out in the UK, they may contain information of relevance to the LAG's purposes.*

To assist in the use of the primary evidence literature the PERASG chairman recommended members be guided by an approach developed by Klimisch *et al.* (1997), designed to provide a systematic evaluation of the quality of data and their use in hazard and risk assessments, albeit particularly in relation to experimental toxicological and ecotoxicological data. It rates the reliability, relevance and adequacy of information being used for risk assessment purposes. Essentially it guides the use of reported studies which meet all appropriate science-based standards, as being fully reliable, through those with various shortcomings to those to be regarded as not reliable.

While the Klimisch evaluation may not necessarily be the most appropriate for the current purpose, it has the merit of subjecting key literature sources to close scrutiny for sound science-base and subsequent transparency in the use of its findings for this dWRA. Furthermore, it broadly accords with a more detailed approach to quality indicators for scientific evidence for use in risk assessments recommended in Green Leaves III by Defra and Cranfield University (section 2.1). Accordingly, where appropriate, each key paper or report gathered for this dWRA has been assessed in terms of relevance and reliability, in terms of the evidence provided on exposure of

wildlife to ammunition lead, and the evidence for individual and population impacts of that exposure.

To assist in characterising main components of exposure to and/or impacts of ammunition lead on receptor wildlife species, and applying the above quality control approach to the evidence reported in each paper, the following system was applied:

Evidence of exposure

Potential exposure

Ammunition lead in feeding areas/food

Actual exposure

Ammunition lead in intestine
Elevated blood ammunition lead
Elevated soft tissue ammunition lead
Elevated bone ammunition lead
Insufficient evidence
Evidence of no exposure
Not addressed

Evidence of impact

Individual

Sub-clinical (e.g. blood-enzyme inhibition)
Behavioural (e.g. hazard avoidance/social)
Developmental (growth/health)
Reproductive (productivity)
Welfare (‘quality of life’)
Reduced survival
Death
Insufficient evidence
Evidence of no impact
Not addressed

Population

Decline in size
Decline in range
Inhibition of growth rate
Inhibition of range expansion
Insufficient evidence
Evidence of no impact
Not addressed

A problem arose over published reviews of effects of spent lead ammunition on wildlife (of which a few occur on the PEL). They may be conducted by knowledgeable and competent scientists, and, if published in respected journals, enjoy the imprimatur of authoritative peer review. If prepared for organisations or government departments they may be published but not subjected to peer review, and thereby some form of scientific quality control. If they result from conferences or meetings any peer-review process they have been through may be by organisers of such meetings rather than independent specialists in the appropriate field. Furthermore, it is unlikely that any peer-review process will check each of the referenced literature sources in turn to verify the validity and science-base of the original work and then the interpretation and presentation of that work by the review author(s). Also unlikely is the checking that the literature sources that are referenced truly

represent all the published literature on the subject and have not been selected, consciously or otherwise, in such a way as to influence the conclusions of the review paper.

Klimisch *et al.* (1997) recognise these issues and generally class review papers as “Not assignable”. A co-author of the Klimisch paper (Dr Mattaus Andreae) was contacted and he suggested that review papers could be re-classified on the following grounds, but it is always a case-by-case decision:

a. Secondary reviewer able to access raw data upon which primary review based.

b. All papers being reviewed had been subjected to peer review and review itself also peer reviewed (this being personal opinion).

c. Evaluation should be carried out by person or persons with expertise to do so.

d. Applicability, not just methodologies used, is important.

e. Reviewer(s) should rely only on studies falling into highest reliability categories.

f. Those lower can potentially be re-assigned following further investigation.

Where possible for this draft risk assessment, such checking of review papers has been done to try to ensure the information and interpretations in them are soundly evidence-based.

2.3 Bird population status

A particular exception to the reliance on PEL and other relevant literature is the use of published bird population status information by the main UK and international statutory and non-statutory conservation bodies. This was done to enable an assessment be made as to whether British bird populations appeared to show evidence of declines, possibly as a response to ingested lead ammunition impacts. This provides supplementary background evidence.

3 Assessment of evidence

The emphasis of the risk assessment process required of the PERASG was that it be soundly evidence-based, using a list of key sources initially identified as likely to be of sufficient quality for the purpose, plus others subsequently considered appropriate. This major section focuses on the evidence used for the risk assessment, in some detail where that appeared appropriate, because of its relevance and contribution to the process. It also highlights, where appropriate, shortcomings which the reader needs to understand in relation to the interpretations and summaries of the evidence and the conclusions drawn from it. The overall assessments and conclusions are presented in section 4.

The assessment of evidence is made in relation to each of the main Source-Pathway-Receptor (S-P-R) links identified in the conceptual model (section 2.1.1). The evidence is presented firstly from England (UK), as available, and then from other countries to the extent that it may inform on any of the components of possible risk to wildlife in England (UK) from exposure to ammunition lead.

Various units are used in the literature to report measured lead concentrations:

Soil/sediments – µg/g (=mg/kg=ppm)
Water – µg/l
Blood – µg/l – µg/dl (=(µg/l)/10) – µmol/l (approx=(µg/dl)/21)
Bone – ppm - µg/g
Human consumption limits – mg/kg

Concentrations of lead are also variously expressed in terms of dry weight (dwt) or wet weight (wwt), and, sometimes, not clearly which of the two. Approximate conversions are:

Soft tissue – 3.1
Bone – 4

3.1 S-P-R: A Direct ingestion of spent lead gunshot

S-P-R: A **Whole gunshot pellets ingested directly from the ground surface or surface of underwater sediments, typically by seed-eating, vegetation-eating and invertebrate-eating birds, in mistake for food or as grit to assist digestion of food.**
Gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

3.1.1 Primary poisoning of gamebirds through direct ingestion of spent lead shot – England (UK)

Butler *et al.* (2005) reported on spent lead shot exposure of common pheasant (*Phasianus colchicus*) on 32 shooting estates in 11 counties in England over 1996-2002. Gizzard contents from 437 birds, half being shot under licence outside the shooting season and half being shot during the season, were examined for presence of ingested shot. Wing bone lead and body condition were also measured in 98 females. Cloacal fat, breast muscle and total body weights were used as indicators of body condition.

Overall, 3% of gizzards (13/437) contained one or more lead shot pellets. The 98 female pheasants contained bone lead levels from 7-445ppm, median 15.5ppm, mean 48.8ppm dry weight (dwt). Two birds with gizzard shot had high concentrations of bone lead. No evidence was found of a link between bone lead levels and body condition. The authors noted other studies showing effects on body condition and that gamebird dosing studies showed considerable variation in effects between and within species.

The authors reported that the wing bone lead levels, which reflect long-term exposure to lead as ingested lead accumulates in bones, were similar to those recorded in British mallard (*Anas platyrhynchos*) in an earlier study of waterfowl exposure to spent lead shot (Mudge 1983). They indicated further research is needed on the relationship between gizzard lead shot and bone lead levels in pheasant, but noted other studies showing strong correlations in waterfowl. They also reported other studies showing that such wing bone accumulation of lead reflects exposure to all sources, not just lead ammunition.

The authors recognised the relatively small samples used in the study prevented them from drawing more detailed conclusions on relationships between exposure to spent lead shot and its effects in pheasants.

Summary

The paper addresses lead shot exposure to pheasant and impacts on individual pheasant but with some shortcomings. It is a competent study but conclusions based on small samples. Limited data presented. Lead pellets found in some gizzards and elevated wing-bone lead levels indicate exposure of some birds to spent gunshot. Evidence of no impact on body condition. Population impact not addressed.

Two studies on partridge are available. Potts (2005) assessed the incidence of ingested lead shot being deemed a cause of death in post-mortem reports of wild grey partridge (*Perdix perdix*) found dead in south-east England from 1947 to 1992. Data provided and data treatments are confusing but from 1963-1992, 20 out of 446 adult birds (4.5%) were recorded as containing shot in their gizzard, and 15 (3.4% of total) were deemed to have died from lead poisoning. Two out of 29 chicks (6.9%) were found with gizzard shot. Death was indicated in a third of birds with 1-3 gizzard pellets and up to 100% with four or more pellets, but sample sizes were small.

Potts estimated 1.2% of living partridges contained ingested shot at any one time and cited a DPhil study at Oxford finding no ingested shot in 77 grey partridges, from 20 sites across England, killed by raptors during 2000-2003 (Watson 2004), as further evidence of low incidence of ingestion in healthy birds.

There are several weaknesses of Potts' study, including its reliance on found-dead birds (which bear an unknown relationship with the wild-living population and can show higher shot ingestion rates than in birds collected by other methods – see Pain 1992 and Pain *et al.* 1995); different pathologists conducting the post-mortems over the years; the post-mortems not being designed to detect all ingested shot; small samples; unsupported assumptions (relating to ingested pellet sizes, rates of gizzard erosion of pellets, and comparative pellet retention times between partridge and waterfowl); and incorporation of data from other studies/reports (each reporting one bird only found dead) and from Denmark.

Potts considered that sampling birds for determining the incidence of ingested shot (considerably) under-estimates annual exposure of partridges to spent shot (because of (presumed) short gut-retention time of ingested shot) but did not quantify the under-estimation. He concluded that the overall incidence in grey partridge was low, and half that reported in ducks and geese.

Summary

Addresses lead ammunition exposure to and impact on grey partridge with some shortcomings. Generally acceptable but some small samples and several uncertainties. Lead pellets found in small percentage of gizzards indicate exposure of some birds to spent gunshot. High likelihood of death indicated following multiple-pellet ingestion. Estimated 3.4% mortality but population significance not addressed.

Butler (2005) used a similar approach, examining post-mortem records of found-dead red-legged partridge (*Alectoris rufa*) from across the UK from 1933 to 1992, for incidence of lead shot ingestion. This was recorded by the Game Conservancy Trust's pathology unit but by four different pathologists over that period. Additionally, 144 shot birds were collected randomly from 10 estates in the Midlands, East Anglia and southern England during the 2001/02 winter, to investigate the then-current incidence of ingested shot.

One of the 637 post-mortem records (0.2%) and two of the 144 shot birds (1.4%) revealed ingested shot.

The study suffered from several of the shortcomings of Potts' grey partridge study above (Potts, 2005), including reliance on found-dead birds; historical records obtained from post-mortem studies, and differing pathologists, in studies not specifically looking for all lead shot, with the 2001/02 study specifically searching for ingested shot.

The author noted the low level of ingested shot found is consistent with low levels reported in similar gamebird species in other countries (grey partridge in Denmark, two quail species in USA and, notwithstanding the shortcomings of the historical data, concluded that the incidence of ingested shot in red-legged partridge in Great Britain was low.

Summary

Addresses lead shot exposure to red-legged partridge with several shortcomings. Not peer-reviewed. Methodological uncertainties but may be indicative. Lead pellets found in (very) small percentage of gizzards indicate exposure of some birds to spent gunshot. Individual and population impacts not addressed.

Thomas *et al.* (2009) studied the lead content of hunter-shot red grouse leg and foot bones on two Scottish estates and one Yorkshire estate, as an indicator of lead exposure. Using lead isotope ratios they attempted to identify the source(s) of the measured lead. The sample size, collected in September 2003, was 234.

In the birds from the two Scottish estates, respectively 3.5% of 85 and 5.4% of 111 birds were judged to have highly-elevated bone lead levels (>20µg/g dwt) and in these birds lead isotope ratios ($^{206}Pb/^{207}Pb$ and $^{207}Pb/^{208}Pb$) were deemed to be consistent with ingestion of lead gunshot available in Europe. Of the 38 birds from the Yorkshire estate 84% were recorded with elevated bone lead levels, as defined, but isotope analysis indicated considerable exposure to galena from lead mining sources such that the authors surmised that 18% of the birds ingested gunshot. They concluded that, for grouse with highly-elevated bone lead levels, having lead isotope ratios inconsistent with environmental lead from mining and smelting, lead shot ingestion was the most likely source of the lead exposure.

The paper has several shortcomings. While lead was measured in grouse bones, evidence for its origins was drawn primarily from the literature and lead isotope ratios. Spent lead shot in the moors and ingestion by grouse were not demonstrated. Reliance on hunter-shot birds raises uncertainties over their representativeness of the living grouse population, and since in a population subject to ingested lead shot poisoning affected individuals have been found more susceptible to being shot (Belrose 1958 and Heitmeyer *et al*. 1993, both in Kendall *et al.* 1996).

There are methodological uncertainties, including use of European gunshot lead isotope ratios (see below) without verifying their applicability on the studied moors, and reliance on published lead isotope ratios, from up to 20 years previously, for gasoline and environmental inputs (rainwater, atmospheric particulates, sphagnum moss, soil, coal and galena) from sites in northern England to northeast Scotland.

Small samples were often involved, either in measured data or those drawn from the literature, not least in the lead isotope ratio analysis where n frequently was under 5.

Inappropriate and/or unreliable literature was used – e.g. Butler *et al*. (2005) to evidence "substantial incidence of lead shot ingestion and toxicosis among upland game bird species" (see above), and similarity of feeding habits among such species, when Butler *et al* studied lowland pheasant with different feeding habits from grouse; references based on single examples; and use of an unpublished Canadian MSc thesis for European gunshot lead isotope ratios for comparison with the measured leg bone ratios. Finally there is much speculation and assumption made without supporting evidence.

The authors acknowledged that bone lead concentrations, in the absence of other data, cannot be used to diagnose lead poisoning in birds. However, on the basis of their findings and the literature, they hypothesized that elevated lead in red grouse could impair grouse immune system functions and increase susceptibility to infection.

Summary

Addresses lead shot exposure in red grouse. Generally acceptable but with several shortcomings. Exposure to lead shown by elevated bone lead levels but significant contribution from anthropogenic lead sources in some areas. Lead isotope ratios indicative of underlying spent shot ingestion but no direct evidence provided. Sub-clinical impacts/reduced survival surmised from literature. Population impact not addressed.

Assessment

The England (UK) evidence, drawn from three studies, indicates that a small percentage (up to some 3%) of British pheasant and both species of partridge ingests spent lead shot each year, generally lower than in ducks and geese. The one study on pheasants showed no adverse effects of poisoning on (hen) body condition. Impacts of ingested lead shot in these gamebirds, either on individual birds or their populations, have not been studied in detail.

From one study, red grouse also are exposed to spent lead shot, perhaps to a higher level than the other gamebird species, but, again, no information is available on individual and population impacts.

3.1.2 Primary poisoning of gamebirds through direct ingestion of spent lead shot – Non-UK

a) Europe

Two studies from Europe are relevant. Ferrandis *et al.* (2008) studied exposure of red-legged partridge (*Alectoris rufa*) to ingested lead shot on a Spanish shooting estate that had specialised in partridge shooting since the 1950s. This moderately-thorough study measured spent shot densities in the soil, ingested shot in gizzards of hunter-shot partridge, and the latter's liver and femur lead levels. Sample sizes, though, were relatively small: 10 birds from 2004 and 54 birds plus 12 gizzards from 2006.

In the study areas around the driven shooting positions, shot densities varied from 59,400-96,000/ha. In 2004, two out of 10 birds (20%) contained ingested shot, and in 2006 one bird out of 66 (1.5%) contained shot, an overall ingestion rate of 3.9%.

The eight shot-free birds in 2004 averaged 2.17ppm dwt (range: non-detectable–10.09ppm) liver lead while the two shot-containing birds contained 0.19ppm and 42.83ppm liver lead. In 2006, 65

birds with no ingested shot averaged 0.58ppm liver lead (range: non-detectable-5.73ppm), and one bird with ingested shot had 30.73ppm.

The corresponding femur lead levels were: 2004 – shot-free average 14.21ppm dwt (range 0.87-87.90ppm), and shot-containing birds 0.70ppm and 2.21ppm; 2006 – shot-free average 0.84ppm (non-detectable-14.62ppm), and one bird with shot 3.47ppm.

No effect of lead prevalence on body condition, weight of liver and spleen or abdominal fat was detected. The authors suggested that partridge and other galliforms may be more resistant to lead toxicosis than other species, subject to more study.

Summary

Demonstrates both potential and actual spent shot exposure, through shot densities in feeding areas, gizzard contents, and elevated liver and bone lead levels. No individual impacts shown. Provides evidence of no impacts in developmental terms (growth/health). Population impacts not addressed.

Mateo's (2009) review "Lead poisoning in wild birds in Europe and the regulations adopted by different countries" deals primarily with waterfowl (see section 3.1.3) but gives some attention to gamebirds. It comprises a compilation of published lead ammunition exposure data, from peer-reviewed and non-peer reviewed sources. It is incorrect and inaccurate in substantive parts relating to waterfowl and lead poisoning (see section 3.1.3), casting doubt on other material in the paper.

It evidences availability of spent shot on a few game-hunting estates in Hungary and Spain. Ingested lead shot in gamebirds is reported from some countries, including UK, Denmark, Spain and Hungary, typically at a prevalence of a few percent in partridges and pheasant. Some elevated tissue lead levels, and deaths have been reported, but no estimates of population impacts made.

b) North America

Kerr *et al*. (2010) investigated lead pellet retention time and associated toxicity in captive northern bobwhite quail (*Colinus virginianus*) in Georgia, USA. After being orally dosed with 1, 5 or 10 lead 2.0mm pellets, batches of six birds were sampled for blood lead, d-ALAD activity (enzyme needed for haem synthesis) and histopathology for up to 28 days. Birds dosed with five or 10 pellets became moribund and lost body weight after two weeks, and were euthanized. At that time 7% of original pellets remained in the ventriculus (21% at week one), with subsequent evidence of reducing size. d-ALAD activity was markedly depressed in all birds, implying possible impaired haematological function. Birds dosed with one pellet showed no changes in red blood cell numbers, packed cell volume, mean corpuscular volume, total blood protein, morphology or percentages of leucocytes, or in histology of tissues examined. Blood lead in these birds increased markedly over the first week but then declined over weeks two to four as pellets were eliminated. The authors concluded that further studies were needed to assess the impacts of one ingested pellet on body functions such as reproductive, neurological or other systems.

In Canada, Kreager *et al.* (2008) examined 123 gizzards and liver lead levels from chukar partridge (*Alectoris chukar*), common pheasant (*Phasianus colchicus*), and other gamebird species, harvested by hunters in southern Ontario. Of the 76 chukar partridge gizzards 8% contained shot, while 34% of 47 pheasant gizzards contained shot, with some containing more than 10 pellets. Of the 129 liver lead analyses from wild turkey (*Meleagris gallopavo*), Hungarian partridge (*Perdix perdix*) and pheasant, 13% exceeded 6µg/g wwt, the lead poisoning diagnostic threshold. The authors concluded there were risks to human health from consuming such lead-contaminated game meat. The study, however, was conducted on a site heavily hunted and shot for clay targets for many years (recognised by the authors as atypical), and the methodology subject to several

shortcomings, including reliance on hunter-shot birds and the contamination of liver lead levels by metallic lead particles.

Assessment

Spanish red-legged partridges appear similarly exposed to spent lead shot as the British birds, and, as for the British pheasant, with no evident impact on individuals. The authors suggested that galliform birds may be more resistant to lead toxicosis than other birds.

The North American study on captive northern bobwhite quail was more comprehensive and demonstrated vulnerability of multi-pellet ingestion birds to physiological effects (and presumed death) but birds with single ingested pellets showed little response. The Canadian study showed potential exposure of several species of gamebirds at least when spent shot is abundantly available.

3.1.3 Primary poisoning of waterfowl through direct ingestion of spent lead shot – England (UK)

The terms of reference established by the LAG for the PERASG are to assess "the risks to wildlife [in England (UK)] from ingested lead from ammunition" (*section 1*). At the time it was recognised that risks of lead poisoning to waterfowl would not be included as, in effect, the UK Government had previously decided that there was an unacceptable risk to waterfowl and had put in place mitigation measures in the form of the lead shot regulations in England. Since then similar regulations have been introduced into Wales, Scotland and Northern Ireland. The LAG, at its September 2010 meeting, agreed that "re-assessing all the literature that had given rise to the lead shot regulations would not be a good use of time".

In turn, the Primary Evidence List, compiled to provide the main evidence base for the (three) risk assessments being prepared by PERASG, contained only one specific reference and one more-general reference to waterfowl lead poisoning: "We have not included the extensive literature covering the long-established risks to wildfowl from ingesting spent lead gunshot…and in the UK…legislation has been introduced restricting the use of lead gunshot over wetlands and/or for shooting wildfowl….".

The issue of compliance with the lead shot regulations, and its implications for continued waterfowl exposure to spent shot, were recognised at the time of finalising the PEL by noting that a report on the recent Defra assessment of compliance was imminent and would be included in the evaluation of on-going risks to waterfowl.

At its April 2012 meeting, the LAG, given the level of non-compliance as revealed by then-published Defra report (Cromie *et al.* 2010), suggested the level of continued risk to waterfowl and wetlands be assessed by summarising recent scientific reviews on the subject. The LAG further agreed that the PERASG should decide on how best to proceed and, again, advised against a full risk assessment of all existing literature.

The PEL paper of Mateo (2009) presented an overview of waterfowl poisoning by spent lead shot in Europe, to the USA Peregrine Fund conference in 2008 (Watson *et al.* 2009). It has been referenced for use elsewhere in the draft wildlife risk assessment (gamebird lead poisoning – section 3.1.2 - and birds of prey secondary poisoning – section 3.2.2), and found wanting in several respects. It comprises a compilation of lead shot deposition data and waterfowl lead exposure data. It shows evidence of bias, inaccurate referencing, and unsound and incorrect information handling and interpretation, and contains much old, unverifiable, and non-peer reviewed, reference material. In terms of its relevance to this risk assessment, the paper addresses waterfowl lead poisoning in Europe and member states' various regulations, and does

include some material from the UK. It attempts to assess waterfowl population impacts of ingested lead shot poisoning in Europe as a whole but the assessment is unreliable.
By way of evidencing shortcomings: Table 1 does not support the statements that "lead shot densities in Europe have been >100 shot/m2 and between 10 and 50 shot/m2 in most wetlands from the UK". Table 2a, purporting to reflect current levels of ingested lead shot, is based on data from 1957 to 1994 on then-reported lead shot ingestion across northern Europe, all before restricted use of lead shot was introduced in several countries. Similarly, Table 3, estimating current waterfowl mortality in Europe uses ingestion data mainly from the 1970s and 80s, before lead shot restrictions, and mortality rates for captive mallard (*A platyrhynchos*) dosed with lead shot in the USA in the 1950s then applied to all species in Europe. The interpretation of Figure 4, that of a "significant relationship between wintering population trend (of commonly hunted duck species in Europe) and prevalence of lead shot ingestion in them", is unsound since the removal of one species (white-headed duck (*Oxyura leucocephala*), which is not hunted)) removes the significance of the relationship. Re-analysis, following the same methodology for deriving the data points, shows no relationship at all. The process used to estimate waterfowl mortality in Europe from ingested lead shot poisoning is unreliable and likely to overestimate its level(s).

Data on ingestion rates of spent shot in the UK are provided for 15 waterfowl species. These are compiled from five different studies, some relating to only one site or species, and with highly variable sample sizes. They all relate to the period 1957-81. Their reliability today, not least in light of reduced deposition of spent lead shot since the 1999 - 2004 lead shot regulations, is not known.

Reported estimates of mortality in British waterfowl include 2.3% ("might die…") for the national population of mallard in 1983, and 1.0-2.8% of shoveler (*Anas clypeata*) and 5.1-8.3% of pintail (*A acuta*), with respect to the Ouse Washes, in 1975. Various studies of lead poisoning in swans are also reported but those relating to mute swans (*Cygnus olor*) are complicated by the swans' parallel exposure to lead fishing weights up to (and probably beyond) the late 1980s. Ingested lead shot was often also present, and particularly in (small samples of) Bewick's (*C columbianus bewickii*) and whooper swans (*C cygnus*), but their impact on swan health or mortality is not reported.

Summary

Generally addresses exposure to lead shot and its effects in waterfowl but with many shortcomings. Poor reliability with much material drawn from old and limited, secondary and non-peer-reviewed literature, plus significant shortcomings in data handling and interpretation. Evidences potential exposure (spent lead shot in feeding areas) and actual exposure (ingested shot and elevated liver and blood lead levels) in many waterbird species in Europe. Death reported and indicated in many species. Population mortality rates for many species estimated but based on old, unreliable data. Population declines linked with lead poisoning but not well-evidenced except for, e.g.*, white-headed duck (Oxyura leucocephala), globally endangered, and possibly marbled teal (Marmaronetta angustirostris).*

O'Connell *et al.* (2008) reported specifically on whooper swans (*C cygnus*) in Iceland and the UK. Blood sampling from 363 wintering swans in Britain and Ireland, and moulting sites in Iceland, during 2001-05, revealed between 43% and 70% from three sites with lead levels regarded as above background levels and indicative of lead ingestion. The threshold used was 1.21µmol/l (equivalent to 25µg/dl), which, in turn, was more than a third lower than previous studies (1.93µmol/l) but without explanation for the change. Comparison of the measured lead levels with reported blood lead levels from swans in both countries 20 years previously (but by different authors) indicated marked decreases from earlier levels. Throughout the paper ingested lead shot is presumed to be the source of the blood lead but no evidence is provided. No assessment is made of the impact of the measured blood lead levels.

Summary

Addresses changes in lead exposure in swans over time. Generally sound but some questionable interpretation and presumption of ingested lead shot poisoning. Study based on contemporary and historical blood lead levels, indicative of lead exposure, but no evidence of source. Individual and populations impacts not addressed.

The PEL refers to the then-imminent Defra study on compliance in England with the lead shot regulations introduced in 1999. Its report was published in 2010 (Cromie *et al.* 2010). The methodology comprised analysing shot ducks bought from game dealers and other retail sources for evidence of lead shot used in their shooting; a postal questionnaire survey of Country Land and Business Association (CLA) members, as providers of shooting, to assess their knowledge of the nature and practice of the regulations in England; and a similar survey of BASC members, as participants in waterfowl shooting affected by the regulations.

The three studies were not without their complications and shortcomings but, individually and collectively, they revealed, across the country, a substantial lack of willingness or perceived need to practise or implement the regulations. This was evidenced particularly by some 70% of the purchased shot duck (93% mallard) having been shot with lead. The authors considered these birds were mainly from inland game and/or duck shooting activities.

This study essentially was a test of the efficacy of the measures put in place by the government in 1999 in response to its assessment that action was needed to reduce the exposure of British waterfowl to ingested lead shot poisoning. The findings indicated that the mitigation measures had not greatly reduced that exposure, at least in inland shooting.

Newth *et al.* (2012), in a recent paper, sought to provide further information on current and historical trends in lead poisoning in British waterbirds "to help inform the development of UK Government policy on the risks to wildlife health from exposure to lead ammunition."

The methodologies used were: blood lead levels in caught waterfowl during the 2010/11 winter; incidence of lead-poisoning-induced mortality recorded in birds found dead between 1971-2010; and variation in that mortality in England between 1971-1987, 1988-1999 and 2000-2010 in relation to legislation restricting lead angling weights (1986) and lead gunshot (1999).
Sample sizes were: 285 blood lead levels, from (177) whooper swan (*C cygnus*), (39) Bewick's swan (*C. columbianus bewickii*), (29) pochard (*Aythya ferina*) and (40) pintail (*Anas acuta*), from (at or near) four Wildfowl and Wetland Trust centres (Gloucestershire, Norfolk, Dumfriesshire and Lancashire); 2,365 birds with cause of death determined over 1971-2010 and from eight WWT centres across Britain (West Sussex, Carmarthenshire, London, Tyne and Wear, Gloucestershire, Norfolk, Dumfriesshire and Lancashire), and some from other sites, for mute swan (*C. olor*) whooper swan, Bewick's swan, Canada goose (*Branta canadensis*), greylag goose (*Anser anser*), pink-footed goose (*A. brachyrhynchus*), mallard (*Anas platyrhynchos*), pintail, gadwall (*A. strepera*), teal (*A. crecca*), shoveler (*A. clypeata*), tufted duck (*Aythya fuligula*), pochard and shelduck (*T. tadorna*).

The paper's conclusions include: 34% of waterbirds tested in the 2010/11 winter (primarily whooper swans) having elevated blood lead; at least 10.6% mortality of waterbirds recovered across Britain from lead poisoning over 1971-2010, and 8.1% mortality between 2000 and 2010; lead gunshot the most likely source of poisoning; and no change in lead-related mortality during 2000-2010, when lead shot regulations were introduced, compared with the preceding test periods. Many uncertainties affect the conclusions, including: unknown reliability/consistency of post-mortems by different personnel at different WWT centres over 40 years (including distinguishing gunshot from anglers' shot); influence of "found dead" birds on estimates of population exposure especially as main source was WWT centres (one of which (Slimbridge) has a history of ingested lead shot poisoning from legacy shot exposed in sediments by pond creation/management); incorrect application of diagnostic test (defined pathological symptoms *and* lead shot in

gizzard/intestine +/- elevated kidney lead (no kidney data provided)) – as 188 birds had 'lead shot' in gizzard (out of 251 "lead poisoned") so estimated mortality is lower than the claimed 10.6% of (recovered) waterbirds, and other such estimates (by time period and for 2010/11); lead poisoning mortality expressed as percentage of total mortality minus infectious causes of death – no information given on extent of infectious disease mortality – resulting in overestimates of lead poisoning mortality; uncertain origin of lead source(s) from within UK or external to UK – of 285 birds with elevated blood lead 17 had been in UK for at least 40 days (indicating likely exposure within that time); unprovided capture dates in relation to measurement and interpretation of blood lead levels and likely UK/non-UK sources of lead; as well as poor evidencing of some statements and claims. There is also no reference made to previous estimates of lead poisoning mortality in the UK, and why the current one is substantially higher.

Summary

Addresses lead gunshot exposure to and impacts on waterfowl over 1971-2010 with shortcomings. Generally indicative but many shortcomings. Provides evidence of exposure in many species through gunshot in intestines, elevated lead in blood, (historical) pathology records, and pathological determination of lead-induced death. Individual impacts revealed by pathology records, likely welfare impacts, and recorded death. (Unreliable) estimates of lead-related mortality given for 13 species of swan, goose and duck, but impact on populations not addressed.

Assessment

These studies provide little new information on ingested lead shot poisoning in British (plus Irish) waterfowl, being largely historical in nature and addressed by the introduction of legislation by the UK government(s) from 1999. The indications of continued exposure to spent lead shot, primarily of mallard at inland sites, through the findings of Defra's compliance study in England, are supported by the most recent estimates of continuing lead shot poisoning in four species of waterfowl (including two migratory swan species). They show that some waterfowl species in his country are still being impacted, physiologically and through likely death, by ingested lead shot, although that exposure would be expected to be less than it was prior to the regulations being introduced. The level of current lead-poisoning mortality by species is not known, nor its population impacts. It may be expected to decline with current renewed efforts by shooting organisations to increase compliance with the regulations.

This appears to be a matter of effective implementation of the current regulations, rather than further risk assessment, and for the LAG and Defra to address in due course.

3.1.4 Primary poisoning of other birds through direct ingestion of spent lead shot – Non-UK

a) North America

The mourning dove (*Zenaida macroura*) is an important quarry species in the United States, shot in large numbers, particularly over arable fields managed to attract the doves for hunting. Schultz *et al*. (2002, 2006, 2007) investigated the exposure of and effects on mourning dove following ingestion of spent lead shot from such fields in Missouri, USA.

They estimated (2002) availability and ingestion of spent shot at the Eagle Bluffs Conservation Area (EBCA, hunted with non-toxic shot) and the James A. Reed Memorial Wildlife Area (JARWA, hunted with lead shot), collecting soil samples one or two weeks prior to the hunting season (pre-hunt) and after four days of dove hunting (post-hunt). Dove carcasses were collected on both areas. At EBCA, 60 hunters deposited nearly 65,000 pellets/ha of non-toxic shot on or around the managed field. At JARWA, nearly 1,100,000 lead pellets/ha were deposited by 728 hunters. Post-hunt estimates of spent-shot availability from soil sampling were 0 pellets/ha for EBCA and 6,342

pellets/ha for JARWA. The authors suggested that existing soil sampling protocols may not provide accurate estimates of spent-shot availability in managed dove-shooting fields.

During 1998/99, 4.8% of 310 mourning doves collected from EBCA had ingested non-toxic shot. Of those 15 birds, 40% contained seven or more pellets. In comparison, 0.3% of 574 doves collected from JARWA had ingested lead shot. These findings suggested that doves feeding in fields hunted with lead shot succumbed to acute lead toxicosis and so became unavailable to harvest, resulting in underestimates of ingestion rates. This, the authors concluded, would help explain previous studies that have shown few doves with ingested lead shot despite their feeding on areas with high shot availability.

They tested that hypothesis (2006) by administering to 157 captive mourning doves 2 - 24 lead pellets, monitoring pellet retention and short-term survival, and measuring related physiological parameters. During the 19- to 21-day post-treatment period, 66% of birds that received lead pellets died; the 22 birds control birds survived. Within 24 hours of treatment, blood lead levels increased nearly twice as fast in those that died compared to survivors. During the first week, heterophil:lymphocyte (H:L) ratios increased twice as fast in those birds than in survivors. Post-treatment survival ranged from 0.57 in doves that retained two pellets for two days compared to 0.08 for those retaining 13-19 pellets over the same period. After controlling for dove pre-treatment body mass, each additional ingested lead pellet increased the hazard of death by 18.0% and 25.7% for males and females, respectively. For each 1g increase in pre-treatment body mass, the hazard of death decreased 2.5% for males and 3.8% for females. The dying birds had the highest lead levels in liver (mean 49.20ppm dwt) and kidney (mean 258.16ppm dwt) tissues, compared with 0.08ppm and 0.17ppm, respectively, in the controls. Doves dosed with pellets showed simultaneous increases in blood-lead levels and H:L ratios, and decreases in packed-cell volume. The authors concluded their results supported an acute lead toxicosis hypothesis, although further research was necessary to investigate the magnitude of lead shot ingestion and toxicosis in mourning doves.

The authors then investigated (2007) whether mourning doves held in captivity freely ingested spent lead shotgun pellets, the relationship between pellet density and ingestion, and the physiological impacts of ingested pellets. They conducted two trials with 60 doves per trial, randomly assigning 10 doves to one of six groups per trial of 10, 25, 50, 100 or 200 pellets mixed with food, with a control group with no added pellets. Ingestion was monitored by x-raying birds one day post-treatment and effects of lead ingestion by measuring heterophil:lymphocyte (H:L) ratios, packed-cell volume (PCV), blood lead, liver lead and kidney lead.

Pooled data showed 5.1% of 717 doves ingested lead pellets with two ingesting multiple pellets in each of the treatments containing mixtures of 25, 100 and 200 lead pellets with food. Doves ingesting lead pellets had higher blood lead levels than before treatment. Post-treatment H:L ratios were not different from pre-treatment values. Overall PCV values were not lower than pre-treatment values. Liver and kidney lead levels for doves ingesting pellets were higher than those without ingested pellets. The lead pellet ingestion rates were similar to previously-reported ingestion rates from hunter-killed doves. The authors concluded that the physiological measurements confirmed earlier reports of rapid and acute lead toxicosis.

Summary

Thorough studies demonstrating many aspects of exposure to and consequences of mourning dove ingestion of spent lead shot. The enhanced exposure to particularly high densities of shot needs to be considered in extending findings to other species.

Franson *et al.* (2009) extended the mourning dove studies by recording 4,884 hunter-shot doves from seven US states for ingested shot in their gizzards. Overall, 2.5% of gizzards contained lead shot where lead shot was permitted, and 2.4% contained steel shot where lead shot was not permitted. Yearling birds contained more shot than adults, either because they ingested more or

because they were preferentially harvested. In doves without ingested lead shot, bone lead levels were lower on an area where lead shot was not permitted than on areas where it was permitted.

Kendall *et al.* (1996) carried out an ecological risk assessment of spent lead shot exposure of upland game birds and raptors in the USA. The paper concentrates on a risk assessment approach, developed at a workshop held for the purpose by the US Environmental Protection Agency. It was one of the first ecological risk assessments to emerge in the 1990s. The EPA approach was developed with particular reference to mourning dove (*Zenaida macroura*) exposure to spent lead shot, enhanced by the common practice of cultivating fields specifically to attract the doves for hunting. For the purpose reliance was made on published information to help estimate amounts of lead shot deposited and available to mourning doves and ingested by them, as well as the likely individual and population effects that might follow. A similar approach was used to estimate possible secondary poisoning of predators and scavengers eating doves with both ingested and embedded shot in them. No primary data were collected.

The risk assessment itself did "not clearly define a significant risk of lead shot exposure to upland game birds", even under the particular circumstances apparently favouring ingestion of spent shot by mourning doves. It did indicate substantial risks of widespread and repeated instances of mortality in mourning doves exposed to high densities of spent lead shot in habitats subject to concentrated hunting or shooting. While hunting and shooting ranges occur throughout North America the extent of risk to this species and other upland bird species and raptors could not be ascertained from the information available. The need for further studies was identified.

It is relevant as it addresses avian exposure to spent lead shot, but is of rather low reliability, not least because of its reliance on secondary literature, sometimes uncritically used. Furthermore, it may be somewhat dated in light of the subsequent attention given by Shultz and other (section 3.1.4 above) to exposure and impacts of ingested lead shot by mourning doves, albeit under the traditional method of dove hunting.

Summary

Addresses exposure of and impacts on upland (American) gamebirds (primarily mourning dove) to spent lead shot but several shortcomings, including focus on artificial system maximising likelihood of receptor ingesting spent lead shot and raptors feeding on it. Uncertain reliability being based on review of 108 papers all of which cannot be verified and several of which appear unsound. Indicates exposure around 3%, comparable to other published data of about 3% elevated liver lead levels. Literature sources indicate developmental, reproductive, reduced survival and death impacts on individual doves. Unable to demonstrate population level impacts at state, regional or national level. Findings may be influenced by subsequent detailed studies of mourning dove exposure to spent lead shot.

Vyas *et al.* (2000) investigated lead poisoning of ground-foraging passerines at a clay target shooting range in Maryland, USA. They sought to determine the lead contamination of the woodland habitat, lead availability to local songbirds, and any lead poisoning of those birds. They measured spent shot content of the soil, lead shot concentrations in the soil and its earthworms, and shot ingestion and blood lead levels of both caged and free-living songbirds juncos (*Junco* spp). The aviary birds (six white-throated sparrows (*Zonotrichia albicollis*) and three brown-headed cowbirds (*Molothrus atar*) were sited over the ground subject to shot fallout.

Spent lead shot was found in varying and often considerable quantities over the site, typically in the top 3cm of the soil, indicating potential availability to foraging birds (to be ingested in place of seed or grit). Varying and locally high lead concentrations were found in the range soil, presumed from dissolution of the metallic lead shot (no lead concentrations were determined from soil not affected by spent shot fallout).

Range soil lead levels were reported as comparable with other shooting range soils (i.e. elevated). No ingested shot was found in sparrows/juncos (sample size not clear but small) from the study site. Protoporphyrin, involved in the production of haemoglobin, and (sometimes) used as an indicator of blood lead elevation, in three remaining aviary sparrows (three other birds having died or escaped) was significantly higher at the end of the study than at the beginning. In 18 free-living juncos sampled at the range their protoporphyrin levels were significantly higher (x 2.6) than those of 12 birds from a control site. A small earthworm sample (number not stated but weight = 16.6g, from one range location) contained a mean of some 750ppm lead. One sparrow and one cowbird carcase from the aviary had “body burdens” of 37ppm and 38ppm dwt lead, respectively.

The study was relatively thorough in its approach but interpretation of the findings is limited by the one study site used, and inadequate sampling and analysis. No evidence of ingested shot was found in passerines using the site but elevated lead was found in some birds and some earthworms. There was considerable speculation and use of the literature over the possible impacts of the measured lead levels on passerines.

Summary

Addresses exposure of earthworms and passerines to spent gunshot lead in clay target range soil. Limited scope of study including (very) small sample sizes and inadequate control measurements. Indicative of some uptake of lead from site by passerines but source and pathways not demonstrated. Indicates some sub-clinical impacts on some birds and possible resulting death but no population impact assessment.

Vyas *et al.* (2001) followed this study with a small-scale investigation into whether songbirds at shooting ranges were exposed to spent shot ingestion, contaminated soil or contaminated diet. They trapped brown-headed cowbirds and fed some birds a single no. 7.5 lead pellet. The first group (10 treated + 10 control) received new pellets and a commercial diet for songbirds. In the second group, with five controls, five birds received new pellets and five received weathered pellets (recovered from a shooting range and matched for weight with the new pellets), with commercial wildbird seed mix, corn and grit added to the basic diet. Three days post-treatment, blood was sampled, and livers both pre-treatment and after death of any birds were analysed for lead.

No mortality or signs of lead poisoning occurred in the first group. In the second group one bird on new pellets and two on weathered pellets died one day after treatment. Surviving birds showed pathological and behavioural signs associated with lead poisoning but no further mortality. Group one birds excreted all but one of the dosed pellets within 24 hours, as did the surviving birds of group two. Erosion of excreted new pellets was less than of excreted weathered pellets (0.8% compared with 3.0%) and less than that of the weathered pellets from the birds that died (4.9%). Blood lead concentrations of both treated groups of birds were not significantly different but higher than the controls. Liver lead in the controls was below detection limits and from 71-137ppm wwt in the birds that died.

The very small sample sizes, and several uncontrolled variables, preclude generalisations but the findings, under the test conditions, indicated the potential for ingested lead shot to cause lead poisoning and death in some cowbirds.

Bannon *et al.* (2011) investigated exposure of captive pigeons (*Columba livia*) to lead-enriched small-arms range soils, in Maryland, USA, on the basis of possible exposure to grit, soil intake from preening or ingestion of contaminated food. Batches of five captive-reared pigeons were orally dosed with 150mg of soil containing (originally containing 18,000ppm lead) 2,700µg lead or 300mg containing 5,400µg lead for 14 days. Copper was added to some treatments as a range soil contaminant that could interact with intestinal uptake of lead. After euthanasia blood lead, erythrocyte protoporphyrin, routine haematology and serum clinical chemistry were assessed. Tail

feathers were analysed for lead to compare with pre-treatment levels and organs were examined for gross pathology.

There were no mortalities or moribund birds during the study, no body weight loss or visible signs of toxicity, and generally normal behaviour. Blood lead and protoporphyrin levels were elevated, compared with the controls (89µg/dl blood and 371µg/dl protoporphyrin, respectively, at the higher dose level), and showing dose-response effects. There were no clinical, haematology or serum clinical chemistry effects. Lead concentrations in organs were: kidney>liver>brain>heart, with all organs showing dose-response effects. Kidney levels averaged 47ppm dwt at the higher lead/soil dose and 22ppm at the lower dose. Feather lead levels were also dose-respondent, with most being in feathers growing during the test period. There were indications that copper interacted in some way and reduced lead absorption and/or tissue retention.

The increased blood and protoporphyrin levels were interpreted as indicating incipient lead poisoning and found to be within the range of values in other studies where lead shot ingestion had caused elevated blood lead levels. The authors noted that from this and other studies pigeons appeared to be more resilient to lead poisoning than other birds but that such increased body burdens of lead created exposure to lead for birds of prey feeding on them.

Assessment

There is no information on the exposure to or impacts of ingested lead shot on non-gamebird and non-waterfowl species in England (UK).

Woodpigeons are widely distributed in arable areas and widely shot for crop protection. The American findings of spent shot ingestion and subsequent lead toxicosis in mourning doves suggest that British pigeons are likely to be susceptible to such poisoning. On the other hand, mourning doves are exposed to concentrated spent shot on the hunting fields to which they are attracted for the purpose of hunting. That situation does not occur in the UK. There could be a low level of shot ingestion from other more widely dispersed gunshot pellets and consequent loss of birds through poisoning, particularly those ingesting several pellets. If so its effect at the population level is not evident, given the size and status of the woodpigeon in England (UK), because lead-induced mortality is negligible, masked by compensatory mortality factors, or less important than other factors determining the population's size.

Other pathways from the lead-contaminated shooting range into Columba livia (same species as the UK rock dove/feral pigeon), namely grit intake, soil intake via preening, or ingestion of contaminated food, were not found.

The early risk assessment focusing particularly on the mourning dove, while finding risks of mortality in areas of high densities of spent shot, was unable to show population impacts at the larger geographical scale.

With respect to British seed-eating passerines, from the American work there appears to be some potential to ingest spent lead pellets during feeding, particularly in areas with high shot densities such as clay target grounds, with some predictable poisoning consequences. There has been no investigation of such ingestion in this country.

3.2 S-P-R: B Ingestion of spent lead gunshot and bullet by predators/ scavengers contained within their prey/food

S-P-R: B (a) **Whole gunshot pellets, parts of pellets or metal residues from pellets, ingested by avian and mammalian predators and scavengers from their food which comprises shot but un-retrieved birds or animals, either alive (especially wounded or behaviourally-affected) or dead, or, occasionally, their discarded viscera. The pellets, parts of pellets or metal residues are contained in the flesh/tissues of prey items, resulting from pellets penetrating into the bird/animal.**
Gameshooting/rough shooting/pest control/wildfowling.

S-P-R: B (b) **Whole gunshot pellets or remains of whole pellets within the digestive system of prey animals that have themselves ingested them as in (a), then ingested in turn by predators/scavengers, without their having been shot.**
Gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

S-P-R: B (c) **Lead compounds absorbed into prey animal tissues following ingestion and digestion of pellets, then ingested by predators/scavengers, whether or not themselves being shot.**
Gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

S-P-R: B (d) **Whole lead rifle bullets, parts of bullets or metal residues from bullets, ingested by avian and mammalian predators and scavengers from their food which comprises shot but un-retrieved mammals, either alive (wounded) or dead. The bullets, parts of bullets or metal residues are contained in the flesh/tissues of food animals, resulting from the bullets penetrating the animal. They may also be within the 'gralloch' (intestines) of deer/other mammals discarded in the field by deer stalkers/pest controllers after removing the shot deer/other mammal carcase.**
Deer management/pest control.

3.2.1 Secondary poisoning of predators/scavengers through consumption of lead-shot (gunshot and bullet) prey – England (UK)

The Peregrine Fund conference (Watson *et al.* 2009) focused attention on predators and scavengers, primarily avian, being exposed to lead poisoning through consumption of prey containing lead shot or bullets, or fragments and residues thereof, after having been shot. Four papers address this topic in the UK.

Pain *et al.* (1995) reported on the lead concentrations in the livers of 424 raptors from 16 different species (including hawks, harriers, eagles, buzzard, red kite and owls) found dead from 1981 to 1992 in the UK, and analysed by the Institute of Terrestrial Ecology. It was recognised that found-dead birds may not be representative of the living population with respect to lead levels. Sample sizes varied considerably: eight species averaged 34 carcases each (range 15-63, plus one of 150), and eight species with 1-7 carcases each. In the first group numbers of carcases with elevated lead levels averaged 2.4 (2.5 including the largest sample); in the second it was 0.3. The authors adopted an arbitrary 6ppm dwt for above-background exposure ("abnormally high"), and 20ppm dwt for acute exposure, after reviewing other published criteria, the upper threshold being broadly accepted.

Median liver lead concentrations ranged from undetectable to 2.17ppm dwt and the percentage of individuals with elevated concentrations (>6ppm dwt) averaged 4.7% ranging from 0% to 29%, from variable sample sizes. Highly elevated (acute) lead concentrations in the liver (>20ppm dry wt) were found in one (ex 26) peregrine falcon (*Falco columbarius*) and one (ex 56) buzzard (*Buteo*

buteo), with one other of each of these two species showing 15 and 20ppm dwt, respectively, although one of these was recorded as shot.

The study did not demonstrate that the, or a, source of the measured lead was ammunition, or any other environmental source, although the former was considered likely to be contributing to much of the liver lead levels recorded, based on feeding habits and literature reports, particularly for buzzard and peregrine. Several raptor species which normally do not feed on shot game species or scavenge from carcases showed elevated lead levels, which could not be explained.

Summary

Addresses lead exposure in UK raptors but without showing linkage between receptors and lead ammunition. Acceptable, but based on found-dead birds, variable sample sizes, and unknown consistency in the ITE post-mortems. Shows elevated liver lead in several raptor species which could be due, in part, to ammunition lead but no source or pathway demonstrated. Individual and population impacts not addressed.

A specific study of exposure of the red kite (*Milvus milvus*) to lead poisoning in the UK was conducted by Pain *et .al*. (2007). They reported on the levels of blood lead in captive red kites prior to being released (under re-introduction programmes), exposure to spent lead shot in a wild population by examining the shot content of regurgitated food pellets, and the levels of liver and bone lead found in dead individuals recovered from the wild over an eight-year period. Lead isotope analysis was used to identify the lead sources. Additionally four shot rabbits were radiographed for the presence of bullet fragments.

The captive red kites (n=125), aged between 4-6 weeks, were obtained from the wild (Spain and England), and fed carcasses of a range of prey species but mostly rabbits and grey squirrels. Efforts were made to remove lead shot from their food. The kites' blood lead levels were measured prior to release.

Elevated blood lead was found in 37% of the pre-release birds, and it was concluded likely that lead from ammunition in their food had been the cause. The authors adopted Franson's (1996) "suggested" blood lead thresholds of <200µg/l for normal environmental exposure; 200-1,000µg/l to indicate sub-clinical effects; 1,000-5,000µg/l as toxic and possibly causing death; and >5,000µg/l as probably causing death, as being generally indicative of such consequences in the face of variable responses to ingested lead from many influencing factors. The basis for Franson's criteria is not explained.

Radiographs indicated that 11% of 264 regurgitated food pellets from a roost site in the English midlands contained radio-dense material, not verified but presumed to be mainly shot or shot fragments. Sixteen pellets were dissected and in six of them objects regarded as lead shot were found, with up to three such objects in each pellet. Some of the objects contained mostly lead and were deemed to be lead shot. The authors estimated that a minimum of 4 to 6 food pellets (some 2% of the total) contained shot. The relationship between the 'shot'-containing pellets and the number of birds producing them is not addressed.

Of the 87 red kites found dead (from 1995 – 2003), post mortem and subsequent analyses were able to establish significant diagnosis in only 21 birds and one of these was attributable to lead poisoning. Four other birds showed pathological symptoms consistent with lead poisoning.

Liver lead analysis revealed 16% of 44 of these birds with levels regarded as elevated over background (>6ppm dwt), six of which (14%) had more than 15ppm dwt, indicative, according to the authors, of potentially lethal lead levels. This threshold is lower than that (20ppm dwt) used by Pain *et al*. (1995) to indicate acute liver lead levels in raptors.

Bone lead analysis of 86 of the found-dead birds showed 18 (21%) above 20ppm dwt and 11 (13%) of these above 30ppm dwt. The authors used Franson's (1996) suggested criteria of 20-30ppm dwt to separate background from elevated bone levels.

Lead isotope analysis of the shot removed from the kite pellets overlapped with the ratios found in the liver and bone samples, suggesting that spent lead shot was a source of the tissue lead, in addition to other sources. Furthermore, the ranges measured lay outside data from other UK sources of lead (petrol, mining waste, coal).

Finally, the radiographs of the rifle-shot rabbits revealed many bullet fragments along the wound channels in three of the four animals.

The authors concluded that lead poisoning was a likely cause of death of 9% of the 44 red kites found dead for which liver lead levels were determined, and caused serious disease in three out of the five birds kept in captivity. From the lead levels found in both the captive birds and in those from the wild, the spent 'shot' in the kite pellets, and the overlapping lead isotope ratios, they concluded lead ammunition a likely source of the lead poisoning. They concluded that there was unlikely to be an impact on the conservation status of the red kite population or the success of further reintroductions, but that the use of lead ammunition created welfare costs for the birds and unnecessary mortality.

This study comprised a comprehensive approach to assessing exposure to, and some consequences from, spent lead ammunition in the (English) red kite population. A number of uncertainties apply to the approach and the findings including: use of birds from two countries with unknown blood lead levels in pre-capture birds; different analytical laboratories, with results lacking complete compatibility; unknown lead levels in the wild population obtaining food by scavenging, including from rubbish tips; inaccurate and selective use of comparative lead-isotope ratio data for petrol, coal, mining waste and urban aerosols from the literature (including use of data from Edinburgh environmental lead studies and from atmospheric aerosol lead studies in northern France), which actually show much greater overlap with the measured tissue and gunshot ratios than is reported; no assessment of the effect of regurgitation and disposal by kites of ingested shot/bullet fragments in their pellets; and much speculation. It did reveal exposure of the English population of red kites to spent lead ammunition, however, and some of its impacts on individuals, including death, but assessed as unlikely they would threaten the conservation status of the red kite or future re-introductions.

Summary

Comprehensive study of lead exposure in red kites with some shortcomings. Generally reliable with some shortcomings. Demonstrates potential exposure (ammunition lead in food), and actual exposure through ammunition lead in intestine, elevated blood, liver and bone lead levels, and indications through isotopic analysis that lead shot was contributing to measured body lead levels. Inferred developmental (growth/health), welfare, reduced survival and death impacts. Considered impact on conservation status unlikely.

Pain *et al.* (2010) reported on lead shot and tissue lead levels in six species of hunter-killed UK gamebirds, for a study of human exposure to lead-shot game. The study was thorough with minor shortcomings. The species were pheasant, partridge, red grouse, woodcock, woodpigeon (*Columba palumbus*) and mallard, with sample size for each ranging from 16 to 26.

Overall, 65% of birds contained embedded lead shot or evident shot material, this percentage between species varying from 50% to 85%. In all shot-containing birds an average 2.2 embedded pellets were found, varying from 1.0 to 3.3 per bird across the species, and, individually, varying from 1 to 18 per bird. Most of the shot was confirmed as lead, with a few pellets comprising non-lead materials. In 76% of birds radio-dense particles were found, ranging from 65% to 85% by species, and presumed, but not shown, to be metallic fragments from shot, sometimes associated

with the pellet wound channels. In total, 87% of birds contained visible whole shot or (presumed) fragments of shot and 60% of birds not visibly containing shot contained small radio-dense particles. These were presumed to result from shot passing through the bird. Tissue lead concentrations were measured only in birds that had been prepared and cooked for human consumption - that is, after removal of visible shot or fragments. Birds containing five or more pellets had high tissue lead concentrations, as did some birds containing fewer or no visible pellets.

The study provided reliable evidence of lead shot presence, both in whole and fragmented form, and elevated tissue lead levels, in a high percentage of lead-shot gamebirds.

Assessment

There is evidence that many British birds of prey are exposed to lead in their environments, with the main pathway presumed to be food. While the likelihood is that lead shot and/or bullets in their food are a contributory source, that has not been confirmed for most species, and only circumstantially indicated for buzzard and peregrine falcon. Some species showing elevated tissue lead levels do not normally eat birds or animals subject to shooting.

The reporting of some two-thirds of shot gamebirds (pheasant, partridge, grouse, woodcock, woodpigeon and mallard) containing embedded shot, or evident shot material following pellet fragmentation, demonstrates the potential source and pathway of spent lead shot into birds of prey through eating shot birds that have either not been killed or not retrieved.

In the case of the red kite, source, pathway and impacts are demonstrated, with lead shot in their prey food clearly being a contributor to measured body lead levels, and causing adverse physiological effects and death in some birds. The authors concluded, in 2007, there was unlikely to be an impact on the conservation status of the red kite population or the success of further reintroductions, but that the use of lead ammunition created welfare costs for the birds and unnecessary mortality.

Knott *et al.* (2010) investigated the potential for secondary poisoning of birds of prey or scavengers in the UK through consumption of deer shot with lead rifle bullets.

Ten red deer (*Cervus elaphus*) and two roe deer (*Capreolus capreolus*) were shot (in Scotland) using standard .270 ammunition (Norma 130gr copper-jacket lead-core bullet), with a single shot to the thorax. Standard gralloching (evisceration) followed before two radiographs, one from each side, were taken of the thorax and a separate one of the associated viscera of each carcase. The types, numbers and weights of all revealed bullet fragments were identified or estimated by analysis of the radiographs and by reference to test firings of the same bullets into other media. On average, the number of fragments recorded was up to some 412 in the carcase and 180 in the viscera. While some of the fragments may have been from the copper jacket of the bullets, the authors considered it likely that not all fragments had been detected. The numbers of fragments equated to some 1.48g and 0.21g of metal in the carcase and viscera, respectively, those in the latter typically being smaller than in the carcase.

The results indicated potential for scavenging wildlife, particularly birds of prey, corvids and gulls, to ingest lead bullet fragments while eating remains of shot deer. The paper raised this but did not address it other than by reference to other published studies showing lead exposure in and mortality of birds of prey. In the UK the authors identified buzzard (*Buteo buteo*) and golden eagle *(Aquila chrysaetos*) as most likely to feed on deer viscera (after the gralloch is removed from the carcase and left behind), rather than deer carcases, as the latter normally are removed for human consumption. Pain *et .al.* (1995) reported 5% of buzzards (found dead) had elevated lead levels in their livers (but without identifying their source) and five golden eagles, of unknown origin, showing no elevation of lead levels.

Summary

Addresses spent lead bullet exposure to predators/scavengers feeding on deer carcases/viscera. Sound but restricted scope of study. Demonstrates potential exposure of scavengers/predators through consumption of shot deer viscera/carcases. Individual and population impacts not addressed.

Assessment

The study shows a potential source and pathway for the poisoning of predatory and scavenging wildlife, particularly birds of prey, corvids and gulls, through ingesting lead bullets or their fragments from the remains of shot deer. The risk of exposure will depend on the frequency and extent of such carcases, or just their viscera (gralloch), being left in the birds' feeding environment.

3.2.2 Secondary poisoning of predators/scavengers through consumption of lead-shot (gunshot and bullet) prey – Non-UK

Studies in various countries have investigated poisoning of birds of prey and scavengers through the use of lead ammunition, both lead bullets and lead gunshot. In addition, several papers on the PEL address exposure of humans to lead from ammunition used to hunt both large and small game. These are assessed in that context within the PERASG human health risk assessment. Where they provide evidence informing on exposure or possible exposure of wildlife to the same ammunition-derived lead they are included here under the dWRA. Being used in this way here they do not necessarily lend themselves to the full assessment applied to papers specifically addressing wildlife lead ammunition or exposure or impacts but commentary on each study's relevance and reliability is included as appropriate.

A number of studies have focused on specific birds of prey and their exposure to secondary lead poisoning.

a) Europe

In France, Pain *et al.* (1993) compared blood lead levels in eight captive marsh harriers (*Circus aeruginosus*) with 94 wild-captured birds over two winters in two parts of southern France. The control birds had 5.3-10.8µg/dl compared with 31% of the wild birds showing >30µg/dl and 14% with >60µg/dl, indicative of clinical poisoning. Regurgitated lead shot in harrier pellets increased between October and December. The authors concluded the elevated lead concentrations in the Camargue were likely to result primarily from ingestion of embedded shot in shot-but-unretrieved waterfowl and from the similarly-shot mammals in Charente-Maritime.

In Germany, Krone *et al.* (2009) reported on previously-published post-mortem examinations beginning in 1996 of 390 found-dead white-tailed sea eagles (*Haliaeetus albicilla*), implicating lead as the most important cause of death in 23% of cases, based on measured lead levels in their liver and kidneys. The authors sought the sources of lead poisoning by x-raying three species of wild-caught geese and shot game animals from many parts of Germany including three deer species, chamois and wild boar. Twenty one percent of the geese (n=154) contained between one and seven embedded shotgun pellets. Radiographs of game shot with semi-jacketed (that is, lead-cored) bullets (n is not indicated but total radiographs = 315) "typically" revealed much fragmentation of bullet material throughout the wound channel and its surroundings. They also conducted feeding trials with some eagles to determine the uptake of differently-sized bullet fragments during feeding on rifle-shot game. This showed the birds selectively avoided ingested fragments of increasing size.

Discarded hunter-shot animal gut piles were also examined (n=14) and found to contain bullet fragments, with from 2 to 600 fragments in each of the samples.

The paper is in the Peregrine Fund conference proceedings (Watson *et al.* 2009).

Summary

Thorough investigation of exposure to lead ammunition sources. Demonstrates availability to sea eagles of embedded gunshot in waterfowl and fragmented lead bullet remains in shot mammalian game if eaten as prey or carrion (carcases and discarded viscera). Individual and population impacts not addressed.

In Poland, Monkiewicz and Jaczewski (1990 - available only in English abstract) appear to have found over 2,000ppm lead around bullet wound channels in shot wild boar (*Sus scofa*), but muscle lead levels generally only slightly exceeding current (unstated) food level limits. A study by Zmudzki and Michalska (1992 - English only abstract) involving wild boar and roe deer (*Capreolus capreolus*) in Poland appeared to find tissue lead levels from 0.06 to 1,494ppm up to 30+cm from the wound channel. Dobrowolska and Melosik (2008) reported on bullet-derived lead in 10 hunter-killed wild boar and 10 red deer (*Cervus elaphus*). Lead levels in and around the wound channels varied from some 60 to 1,096ppm wwt and were measurable at up to 30cm radially from the wound channel. Samples were small and the bullets used unknown, and several interpretations inadequately evidenced.

In Sweden, Helander *et al.* (2009 – abstract only) analysed kidney and liver samples from 118 white-tailed sea eagles (*Haliaeetus albicilla*) from 1981-2004. Twenty-two percent of eagles had lead levels >6µg/g dwt, indicating lead exposure, and 14% had kidney or liver levels diagnostic of lethal lead poisoning (>20µg/g dwt). Lead concentrations were reported several times higher than those in Baltic fish. Reference is made to lead ammunition but no evidence provided of its being the source.

Mateo *et al*. (2009) reviewed exposure and impacts of ingested lead from ammunition reported in 14 species of European birds of prey including four species of vulture and three of eagle, as revealed by elevated blood, liver or bone lead, or lead shot in the pellets regurgitated by birds of prey. Several species addressed in the review have been included in this draft WRA with respect to England (UK) (see section 3.2.1). As with sections in the review paper relating to waterfowl (section 3.1.3) so uncertainties occur over birds of prey, with, for example, text not being consistent with Table 5).

b) North America

The exposure of the Californian condor (*Gymnogyps californicanus*) to spent lead ammunition, and the latter's impact on both individuals and populations, are well documented. Cade (2007) summarised the issues and concluded that current levels of lead exposure were too high to allow reintroduced condors to develop self-sustaining populations in the wild in Arizona and, by inference, in Califomia, USA. More recent publications indicate these impacts continue, notwithstanding efforts to reverse the condor's population status, including re-introduction programmes and use of non-lead ammunition within its range (e.g. Green *et al.* 2008, Parish *et al.* 2008, Global Raptor Information Network 2013, Species account: California condor *Gymnogyps californianus.* Download from http://www.globalraptors.org).

The evidence for lead poisoning and its source came (in 2007) from the following: a) 18 clinical necropsies revealing high levels of lead in body tissues and/or presence of lead shotgun pellets and bullet fragments in digestive tracts b) moribund condors showing crop paralysis and impending starvation with toxic levels of lead in their blood c) widespread lead exposure among free-flying condors, many with clinically exposed or acute levels d) temporal and spatial correlations between

big game hunting seasons and elevated lead levels in condors and e) lead isotope ratios from exposed condors showing close similarity to isotope ratios of ammunition lead but isotope ratios in less exposed condors being similar to environmental background sources which are different from ammunition lead. Simple population models revealed harmful demographic impacts of mortality from lead on population trajectories of reintroduced condors.

Clark and Scheuhammer (2003) examined 184 raptors of 16 species, primarily red-tailed hawks (*Buteo jamaicensis*), great horned owl (*Bubo virginianus*) and golden eagle (*Aquila chrysaetos*), found dead across Canada. Most had very low tissue lead levels, but 3-4% of total mortality in these species was attributed to lead poisoning. It was assumed that lead ammunition in their hunted gamebird and mammal prey/food was contributing to the poisoning.

Several studies have focused on the potential transfer of ammunition lead from the hunted bird or animal to its predatory or scavenging consumer.

From across Canada between 1988 and 1995, Scheuhammer *et al.* (1998) collected nearly 4,000 lead-shot birds from 44 species, primarily waterfowl but also ptarmigan, grouse, woodcock and seabirds. The main purpose was to determine tissue lead levels rather than fragmentation of lead gunshot, although some muscle samples were radiographed to investigate pellet fragmentation. The samples (right pectoral muscle) were pooled (827 in total) and, of these, 92 pooled samples contained lead, averaging around 40ppm dwt (SD±125). Forty individual birds ranged from 5.5 to 3,910ppm dwt. Large differences between individuals and between left and right side pectoral samples were recorded. Among these birds numerous pellet fragments were detected by radiography in the muscle.

The study was generally sound and comprehensive with large samples. No confirmation of the sources of the lead, or its pathways were undertaken, but it is clear that embedded gunshot, especially after fragmentation, contributed to the measured tissue lead levels.

In the USA, Hunt *et al.* (2009) analysed 30 hunter-killed eviscerated white-tailed deer (*Odocoileus virginianus*), shot with one brand of lead-cored 7mm rifle bullet in Wyoming, for evidence (and eventual fate) of bullet fragmentation in the carcases. They recorded from 15 to 409 fragments (median 136) per carcase, with individual fragments within a carcase up to 45cm apart. Results from this experimental procedure, based on one bullet type, cannot be assumed to apply to other types of bullet as different bullets fragment to different extents.

Hunt *et al.* (2009) studied 38 mainly white-tailed, hunter-killed, deer (*O virginianus*) in Wyoming and California, between 2002-04 to investigate the availability to predators/scavengers of spent lead bullets in the carcases. The hunters used a variety of rifles and lead-cored ammunition, as well as some copper bullets. The samples varied from whole carcases (9), partly- and wholly-eviscerated carcases (14) and hunter-discarded intestines (gut piles) (15).

Most radio-graphs revealed many metal fragments generally related to the wound channels. From the lead-killed deer 18/20 gut piles showed fragments averaging 160, ranging from 2 to 521. In five whole carcases an average of 551 (416-783) fragments was recorded with smaller numbers in the partially-eviscerated carcases (25-472). In nine eviscerated carcases the mean was 181 (38-544). Clusters of fragments radiated up to 15cm away from wound channels.

Some uncertainties were introduced by the relatively small samples and the non-distinction between lead and copper fragments in the radiographs.

Summary

Addresses exposure to raptors of lead ammunition in shot deer, with some shortcomings. Basically sound study, with relatively small samples and some uncertainties over radiographic distinction of lead from copper fragments. Demonstrates hunter-shot deer as potential source of lead bullet fragments to feeding predators/scavengers. Individual and population impacts not addressed.

Tsuji *et al.* (2009) reported on lead and bullet fragment levels in small samples (<10) of hunter-killed Canadian white-tailed deer and caribou (*Rangifera tarandus*). Deer liver (5/9) and muscle (3/4) samples showed elevated lead levels, with three and one, respectively, of those samples showing bullet fragmentation. Of the seven caribou muscle samples all showed elevated lead levels and five showed bullet fragments. Some muscle samples were taken from wound channels. Bullet types were unknown.

In Ottawa, Canada, Knopper *et al.* (2006) identified risks to raptors and other scavengers from eating rifle-shot Richardson's ground squirrels (*Spermophilus richardsonii*) which contain bullet fragments. They x-rayed 15 shot ground squirrels for prevalence of fragments of lead 0.22 bullets and measured tissue lead levels in the muscle affected by the bullets. Based on several assumptions about metallic lead erosion, regurgitation, uptake, lead susceptibilities and raptor feeding behaviours, they estimated two species of Canadian hawks' possible lead exposure if feeding on scavenged carcases. They concluded that shot ground squirrel carcases (and similarly-shot prairie dogs (*Cynomys ludovicianus*) appeared to be an appreciable source of lead that would likely result in sub-lethal impacts as well as fatalities in scavenging hawks (and other avian and mammalian scavengers of such carcases).

Most studies of potential or actual secondary poisoning of predators and scavengers have focused on birds of prey. A few studies have extended the scope to other species.

Craighead and Bedrosian (2007) examined 302 blood samples from common raven (*Corvus corax*) scavenging on hunter-killed large ungulates and their offal piles, over two hunting seasons in the Greater Yellowstone Ecosystem, Wyoming, USA. Elevated lead (>10µg/dl) was found in 2% of birds in the non-hunting season and 47% during the hunting season. The authors concluded that the ravens were likely exposed to lead from rifle-shot big-game offal piles.

In a study of large carnivore exposure to lead ammunition in the same area, Rogers *et al.* (2012) assessed blood and tissue samples (n=155) and scat samples (n=423) from bears, wolves and cougars. In no samples was evidence of lead poisoning or lead ammunition found that could be linked with hunting.

Assessment

These various studies add to the evidence that hunter-shot birds and animals can be a source of ammunition lead for predators and scavengers feeding on wounded animals, un-retrieved carcases or discarded viscera. In a few species it has been shown to have significant impact, such as the Californian condor, and the white-tailed sea eagle in Germany.

Actual risks of exposure in any given area will depend on the prevalence of avian predators and scavengers in the areas subject to hunting, availability of shot birds or animals within the feeding ranges of such predators and scavengers, and the prevailing hunting practice, not least the extent to which shot carcases are left behind and viscera discarded, or left purposefully to feed wildlife, as has been the practice in North America.

It is clear that corvids are one group of scavenging birds exposed to lead-shot carcases or discarded viscera. From the one small study it may be that large mammalian carnivores are not at risk but more study is needed.

3.3 S-P-R: C Wildlife ingestion of lead absorbed by plants from spent gunshot

S-P-R: C **Lead compounds absorbed by plants from soil/water containing soluble lead derived from metallic lead pellets deposited by shooting, then ingested by herbivorous wildlife.**
Gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

3.3.1 Lead transfer through plants – England (UK)

Some studies were conducted in the UK from 1989 to 1991, of the distribution and fate of spent lead gunshot from clay target and driven game shooting. They were commissioned by BASC and partner shooting/countryside organisations, and conducted by an environmental consultancy. Reports were produced for each study but they were not published (RPS Environmental Sciences Ltd 1989, RPS Clouston 1991). They are summarised here to add to information on spent lead gunshot in this country.

Clay target grounds

The study examined, firstly, the gunshot lead present at eight long-established clay target shooting grounds in the UK, the relationships between the amount of lead shot and the amount of lead transformation products in the soil, and the associated vegetation lead content. An opportunity arose to incorporate samples from four shooting grounds in Sweden in the same study.

To determine the fall-out pattern from each shooting position, soil samples were taken using a 10cm diameter auger head at 10m intervals along two transects perpendicular to the firing position out to a distance of 250m. Very little shot was recorded under 60m and beyond 140m with the peak fall-out between 100 and 30m. Shot was identified from three profile depths 0.1 - 10cm, 10.1 - 20cm and 20.1 - 40cm. Part of the area 75m from each position and beyond was cultivated prior to sampling. This left 6% of shot on the surface with over 90% still retained in the top 10cm.

Samples taken from the eight UK shooting grounds showed wide variations depending on the length of time the ground had been operational and the level of activity. The highest levels of lead recorded were 70,000 pellets/m² representing 5.7kg lead and 9g/kg of corrosion products elevating soil lead levels to 200 times background levels. The lowest recorded densities were 700 pellets/m² representing 0.03 kg lead with 425mg/kg of soil equivalent to six times background levels.

The samples taken at three Swedish sites produced shot densities of between 15,000 and 17,000 pellets square metre, representing between 0.6 and 1.2 kg lead square meter. The concentration of lead corrosion products in these three soils was between 0.8 and 3g per kg/soil representing an increase of between 40 and 245 times background levels.

A single Swedish site produced the highest pellet count of all the sites measures of 114,000 pellets per square metre, equating to 9kg of lead. 29g of corrosion products per kilo of soil were recorded elevating levels to 380 times background.

Grass samples taken from six of the British sites showed elevated tissue lead levels of between 18 and 95mg/kg dwt against control values of between 4 - 9mg/kg. Single crops of potatoes and beans showed no elevation against controls, whilst heather at four sites showed levels of 8 - 35mg/kg against background levels of 8 - 18mg/kg.

The greatest increase in tissue levels were recorded at the five sites growing cereals, although highly variable, between 9 and 160mg/kg against background levels of 5 - 10mg/kg. The single site showing the highest elevated level was considered to be of concern.

The study concluded that the area of greatest shot deposition is located between 120 and 140m in front of the shooting position with concentrations of total lead residue in soil elevated significantly above normal levels. Maximum concentrations in the soil exceed those considered then by statutory authorities as being suitable for agricultural purposes. Analysis of agricultural crops growing in such conditions indicated that tissue concentrations in the areas of high shot outfall can be elevated and have the potential to exceed the limits for foodstuffs for human consumption.

Intensive game shooting

A similar study focused on the distribution and fate of lead shot from intensive game shoots. The shoots had been operating from 15 to over 100 years, with 10 to 20 drives per stand per year, resulting in the potential for high concentrations of spent lead shot to accumulate in front of the pegs. The survey was augmented by some additional measurements of the environmental availability of gunshot lead in the soil and the availability of lead for uptake by vegetation. At one pheasant shoot and one grouse shoot a soil surface visual inspection survey was conducted at 10m intervals from the peg or butt to a distance of 150m, repeated across three transects, with five metres around each 10m transect point being surveyed in each case.

Seven estates were selected throughout England which included three pheasant drives, three partridge drives, and two grouse shoots. The principle direction of shooting was established and a transect parallel to this direction taken to define the fall-out zone and three sampling locations selected along the transect. Two soil samples were taken at points 80m to 130m from the stand using a 10cm soil auger and to a depth of 40cm and the samples split into an upper and lower profile. At each shoot a control sample was taken from an area that was believed to be outside the shot outfall area. This enabled comparison of lead levels within the outfall zone and local background levels within the environment.

The number of pellets recovered from each soil auger sampling point averaged between zero and 0.2. The highest levels of lead were consistently found in the upper profile of the core, varying between 13 - 565mg/kg. The lower profile samples varied from 8 - 52mg/kg. Background levels varied from 13 - 284mg/kg. Availability was closely related to soil pH with acidic upland soils associated with grouse moors showing 78% and 85% available lead.

The authors reported that the sampling method adopted was not adequate to give an accurate estimate for the quantity of metallic lead in the soil, but considered the sampling programme gave an accurate measurement of the concentration of lead corrosion products within the soil. The soil surface transect surveys produced very low levels of mean pellet recovery of between zero and 0.2 pellets per transect, with maximum levels per 5m observation point of 2 pellets.

The levels measured fell below the (then) acceptable concentrations for lead levels in public open spaces given by the Inter-departmental Committee for the Redevelopment of Contaminated Land, (ICRCL) of 2,000mg/kg. The current levels in agricultural land are set by the Sludge (Use in Agriculture) Regulations, 1990 at 300mg/kg. None of the agricultural soils tested exceeded this level, the range being between 8 - 93 mg/kg.

Informal shooting grounds

Finally, the studies were extended to two informal shooting grounds, each having been operational for a shorter period than the game shoots, namely 12 and 15 years. The shooting frequency for one site was 24 days per year and the other 30 days per year. Samples were taken in transects from 50 - 140m from the shooting positions.

At site 1 no pellets were observed through the visual surface assessment. Upper soil core mean lead levels varied between 18 - 25mg/kg in the upper core and 21 - 25mg/kg in the lower core. Background levels outside the shot-over area were measured at 28mg/kg. At site 2 an average of

1.0 pellet was recovered for the visual surface assessment (range 0 - 5) and upper core lead total averaged 88mg/kg against a background of 19mg/kg. At a second range at this site soil samples recovered an average of 38 pellets per sample point with total lead averaging 36mg/kg.

Overall, soil lead levels were elevated above background levels and appeared to lie within the range of driven game shoots.

Vegetation lead levels

The quantity of available lead absorbed by vegetation within the fallout zones of the game and informal clay shoots was investigated. At each site samples of vegetation were taken at the same time as the soil samples. The vegetation sampled included grasses from permanent and ley pastures and heather from grouse moorland. Levels in pasture soil ranged from 29 - 94mg/kg while the vegetation ranged from 31 - 360mg/kg. Lead in moorland soil ranged from 142 – 1,188mg/kg whilst the levels measured in heather samples were 18 and 19mg/kg, respectively.

There are a number of shortcomings in these studies, the main ones being some inconsistencies in methods and data presentation.

Two papers on the PEL relate to effects of lead shot from clay target shooting in England(UK) on soils and vegetation, including crops (and cow milk). They are addressed fully in the livestock/human health risk assessment but to the extent that they may inform the risk assessment with respect to wildlife in this country, through ingestion of lead-contaminated food from spent lead shot, they are included in this dWRA.

Mellor and McCartney (1994) investigated the movement of lead from spent shot into the soil and oilseed rape crop growing in it, in northern England. In a sound study, other than rather limited sampling, they measured greatly elevated levels, relative to the controls, in the roots (up to 470ppm dwt compared with 10ppm), stem (62ppm dwt compared with 4ppm) and seeds (148ppm dwt compared with below-detection levels) of the plants growing in the shot fallout area.

In a small-scale university honours project, Clements (1997) measured lead levels in the soil and growing ryegrass in the shot-fallout zone of a small farm clay shoot in Worcestershire. Soil lead varied greatly across the sampled site, in part reflecting the concentration of spent shot from the shooting stands, ranging from 48 to 8,172ppm dwt, compared with 32-72ppm in the unshot control area. Lead levels in 49/50 grass samples were below limits of detection, with one, from the main shotfall zone, recording 122ppm, possibly reflecting contamination of the sample.

Assessment

These studies show that lead can be taken up by arable plants from soil containing spent lead shot, particularly by their roots, which then might be eaten by grazing or herbivorous animals or birds. They do not address the many variables likely to influence the behaviour of metallic lead in soil and subsequent plant uptake of soluble forms of the lead.

3.3.2 Lead transfer through plants – Non-UK

a) Europe

In Finland, Manninen and Tanskanen (1993) reported on the transfer of lead from shotgun pellets to humus and plants in a shooting range. Total humus lead varied between 4,700mg/kg and 54,000mg/kg, compared to 240mg/kg in the reference area. Leaves from *Betula pendula* (14-70mg/kg dwt), *Equisetum sylvaticum* (12-31mg/kg) and *Tussilago farfara* (29-50mg/kg) was about one thousandth that in the humus. Lingonberries had a lead content of 0.3mg/kg.

b) New Zealand

Rooney *et al*. (1999) investigated the distribution and phyto-availability of lead in soil from a clay target shooting ground.

Concentrations of EDTA-extractable lead were greatest at 100-160m from the trap houses and ranged from 4,000 to 8,300mg/kg of soil, falling to 500mg/kg at 180m and background levels of <25mk/kg beyond some 220m. The findings also revealed that standard sieving of soil through 2mm mesh, to remove spent lead pellets, can underestimate total lead concentrations depending on the extractant used and the prevalence of whole pellets or fragmented pellets.

A glasshouse study used pots containing soil collected from the shotfall area to provide lead levels from 10mg/kg to 6,000mg/kg dwt. Five plant species (barley (*Hordeum vulgare*), lettuce (*Lactuca sativa*), ryegrass (*Lolium perenne*), radish (*Raphanus sativus*) and white clover (*Trifolium repens*) were grown for 120 days, with nutrient supplements. At harvest, roots and shoots were analysed separately. Lead concentrations in the roots and leaves of all species increased significantly with increasing soil lead levels. Generally higher levels were measured in the roots than the leaves, and generally soil lead and root lead levels were closely related. Relatively small reductions in plant yield with increasing soil lead were recorded and not consistently for all species. The findings were broadly consistent with other studies. The authors concluded there were risks of poisoning to consumers of the vegetation.

Assessment

These studies also show that lead uptake by plants from lead-shot-contaminated soil can occur, sometimes to the point of impacting production, but, again, do not address the factors affecting such relationships. Risks to herbivores consuming the herbage have not been assessed.

3.4 S-P-R: D Wildlife ingestion of lead taken up by soil organisms/ invertebrates from spent gunshot

S-P-R: D **Lead compounds absorbed by soil organisms/invertebrates from soil/water containing soluble lead derived from metallic lead pellets deposited by shooting, then ingested by carnivorous/omnivorous wildlife.**
Gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

3.4.1 Lead transfer through lower animals – England (UK)

A recent paper reports on studies of earthworm accumulation of lead from a clay pigeon shooting site in Norfolk (Reid and Watson 2005). Body burdens of worms from the site averaged 6.1mg/g dry weight while those from the control site contained nearly 1000 times lower levels (7.1µg/g). Body condition observations on worms exposed to lead suggested a tolerance of ingested lead levels.

Sneddon *et al.* (2009) reported on the fate of trace elements derived from shotgun lead pellets discharged in pasture and woodland ecosystems over some 200 years and for some 30 years managed for driven pheasant shooting, with year-round pigeon shooting, in Cheshire, England. They examined a source-receptor pathway from soil to soil pore water and then growing plants, earthworm and worm gut content, and small mammal hair.

Soil samples and soil pore water were collected from the shooting sites and their control sites, and analysed for pH and trace elements. Plants growing at each site were sampled and analysed for trace elements. Earthworms were collected and their bodies and gut contents separately analysed

for trace elements. Wood mice (*Apodemus sylvaticus*) and field voles (*Microtus arvensis*) were trapped on both study and control sites and samples of their hair removed and analysed as for the soil, plant and worm material. Their hair was used as a non-lethal indicator of trace element exposure.

Lead in the shot-over woodland (160.0ppm) and grassland (68.3ppm) soil (which was acid, pH 4.7-6.1) was significantly higher than in the control sites (60.3 and 43.9ppm, respectively). Lead in the earthworms (both body levels and gut contents where measured) were generally but not always significantly higher in the shot-over woodland and grassland than in the control sites. Lead levels in rye grass (*Lolium perenne*) growing on both shot and unshot sites were higher in the former, but only slightly above "normal" levels, with six other grass species showing no difference, as was one moss lead level from the shot-over woodland, as judged by both the results and published data. Lead levels in the rodent hair were variable and showed no consistent pattern.

The results were based on relatively small sample sizes and one location. They indicated that bioavailability of lead in soils from the shot and non-shot sites studied was low but higher in the shooting woodland and shooting field than in the un-shot sites. They indicated limited mobility to higher trophic levels (some plants and earthworms) but not into small mammals. Some of the elevated levels found in the plants and on small mammals, the authors suggested, were caused by atmospheric deposition of lead particulates, but this was not demonstrated. The authors concluded that managed game shooting presents a minimal environmental risk in terms of transfer of lead to soils and their associated biota in both shooting woodlands and pastures.

Summary

Addresses potential wildlife exposure through movement of soluble lead from game shooting through lower trophic levels up to small mammals. Thorough investigation but limited by one study site, possible non-ammunition lead sources, and low-level worm sampling. Small mammals not shown to be exposed to shot-over woodland/grassland where soil, some plant and earthworm lead levels were elevated. No linkage to spent shot source shown. Individual and population impacts not addressed.

Assessment

Accumulation of lead by earthworms from lead-enriched soil, in the context of possible exposure of woodcock, is addressed below (section 3.4.2).

The findings from the woodland and grassland system are the only ones for this country but their wider application is limited by the scale of the study and its shortcomings. They do indicate some plant take up of mobile lead and some ingestion by earthworms but no great uptake by plants in general nor by small mammals.

3.4.2 Lead transfer through lower animals – Non-UK

A number of studies have been conducted in several countries looking variously at the effects of spent lead ammunition deposition mainly at clay target shooting grounds with some at small arms ranges.

a) Europe

In Finland, Rantalainen *et al.* (2006) conducted a holistic study of lead contamination of an old clay target shooting ground in boreal forest, addressing soil chemistry and biology up to ecosystem level. Soil samples were taken from the shotfall and control areas as well as samples of enchytraeid worms and microarthropods. Soil moisture, organic matter, pH, and ammonium and

nitrate content were determined. Basal respiration and microbial biomass were also determined as was community composition of soil microbes, numbers and biomass of the worms, and numbers of nematodes and microarthropods (mites and collembolans). Finally, tree growth and litter production were measured.

The authors reported soil lead levels averaging 75ppm dwt (range 37-210ppm) in the control area, 8,700ppm (range 2,200-21,200ppm) in the medium contaminated area and 18,800ppm (range 2,500-49,700ppm) in the heavily contaminated area. A number of apparent soil changes were recorded, including increasing pH with lead concentration, higher nitrogen levels in the control area, and varying soil respiration rates and microbial biomass differences between control and shotfall areas. Microbe, bacterial, fungal, and enchytraeid worm biomass decreased with lead concentration but nematode abundance did not differ. Microarthropod abundance, in total and by type and taxa, and community composition, did not differ across the study areas. No differences were found in pine growth patterns except an indicative but statistically untestable higher litter production by the pines in the control area.

There were very variable and somewhat inconsistent results among the many parameters measured. The authors provisionally concluded that the lead contamination may be disturbing decomposition processes and nutrient mineralisation, through correlative rather than causative relationships. Important soil organisms (microbes and enchytraeid worms), and possibly pine litter production, appeared to be adversely affected. At the same time, pine growth and soil-dwelling nematodes and microarthropods were not apparently affected. The forest ecosystem appeared to be healthy and the authors concluded that it appeared to resist the contamination despite the negative effects of lead on many (sic) of its components. They hypothesised that such resistance results from low (bio) availability of lead, avoidance of the most contaminated soil horizons and microsites by soil organisms, and functional redundancy and development of lead-tolerant populations among the organisms studied.

Summary

Addresses lower trophic/ecosystem impacts of spent gunshot in boreal forest. Study subject to much speculation and reliance on other literature, with sampling limitations and no measures of tissue lead in target soil fauna. Variation in results may indicate inadequate data, ecosystem compensatory/adaptation processes or no impact on forest ecosystem. Potential exposure demonstrated but no measures of lead intake in soil fauna or trees. Soil microbes and enchytraeid worms were negatively associated with high soil lead concentration but no linkage with lead demonstrated. Nematodes, micro-arthropods and pine tree growth/health not different. Forest ecosystem considered healthy, able to cope with lead contamination.

A detailed study of metal contamination of soil arthropods at a shooting range in Italy is reported by Migliorini *et al*. (2004). Soil lead values varied from 212-1,898µg/g. Collembolan, Proturan and Dipluran species were positively correlated with the major detected contaminants (lead and antimony), while Symphylan species showed negative correlation. The authors concluded that bioaccumulation of soluble lead in a saprophagous (feeding on organic matter) Isopod and its Coleopteran predator showed a significant proportion of metallic lead from spent pellets is bioavailable and can be accumulated by edaphic (soil- living) organisms, entering the soil trophic network but without biomagnification.

Small mammal exposure to spent lead shot was studied by Ma (1989) in an abandoned clay target shooting range on sandy, acidic soil, in the Netherlands. Total lead concentrations in the top 5cm of soil varied between 360-70,000µg/g dwt, compared with the (geometric) mean of 0.03µg/g in the adjoining control site. Wood mouse (*Apodemus sylvaticus*), bank vole (*Clethrionomys glareolus*) and common shrew (*Sorex araneus*) were trapped from both areas. Body weight, liver and kidney weights, and paired femur weight were measured for all animals, and lead levels determined for each organ and bone sample. Species sample sizes varied from 22 to 45.

Tissue lead levels were highest in shrews, and lowest in wood mice and all were higher than in the control area. The shrews and bank voles showed significantly increased kidney:body weight ratios, considered indicative of lead poisoning, both species exceeding the 25µg/g dwt diagnostic lead value for kidney (geometric mean 270µg/g).

The author interpreted the findings in terms of enhanced lead bioavailability from spent shot in the acid, sandy soil, reaching these small mammals via their food chains. No food item analysis was conducted, however, to establish source-pathway linkages. Reference to the author's own previous work showing earthworms, a key component of shrew diets, have high potential to accumulate lead, led to the conclusion that earthworms likely contributed to the lead levels in the shrews in this study.

b) North America

Several studies are reported from North America. A limited undergraduate-based study of lead movement from spent bullets and gunshot into soil, water and biota (including worms, snails, tadpoles, fish, shrimps and insects) at a small-arms firing range in West Point Academy, New York, USA is reported by Labare *et al*. (2004). Samples of soil, water from streams running through the site, and plant material (15 species) were analysed for lead. No sample sizes or sampling protocols are given, and few controls were used.

Soil lead levels were recorded up to 11,000µg/g wwt and in stream sediment 340µg/g. Worms contained between 27-90 times, and tadpoles 16-20 times, the lead concentration of controls. Controls were not measured for the other vertebrates and invertebrates. Plant lead levels varied considerably, with two species reported (*Phalaris* spp and *Carex* spp) showing 22-55 times their control levels.

The authors concluded that spent ammunition lead can be mobilised and enter water, biota and plants and, thereby, create risk to their consumers at higher trophic levels.
Hui (2002 - abstract only) reported on lead shot concentrations and distributions in an abandoned cordgrass (*Spartina* spp) marsh clay target range in southern California, USA. Soil lead reached 16,200ppm dwt, with varying levels found in marsh plants. Horn snails showed mean 1,987ppm tissue lead, over 100 times the plant species with the highest mean tissue concentration (18.1ppm). The author speculated on the risk to birds from consuming such gastropods as well as incidentally ingesting lead-contaminated soil or spent shot.

A limited investigation of wildlife poisoning at a firearms shooting range in Georgia, USA, by Lewis *et al.* (2001), concluded significant lead exposure of local wild bird and mammal communities via bullets and fragments in and on the soil surface. Livers and kidneys from captured or shot 72 animals, comprising 37 mammals of seven species, and 35 birds of 13 species, were tested for a range of metals, including lead, and compared with five mammals and five birds from outside the range. Highly variable concentrations, low diagnostic tissue levels used to interpret results, sample sizes ranging from one to 10 per species, and many uncontrolled variables, preclude generalisations. Some findings may be indicative of exposure of various avian or mammalian species to spent lead bullet ammunition but no pathways were investigated.

Bennett *et al*. (2007) undertook sophisticated ecological risk assessments of lead contamination at two rifle and pistol ranges in Ontario, Canada. The procedures indicated American robin (*Turdus migratorius*) and short-tailed shrew (*Blarina brevicauda*) were most at risk from lead poisoning through eating earthworms which accumulate lead. Eastern cottontail rabbit (*Sylvilagus floridanus*) was deemed also at risk from eating plants which accumulated lead in their tissues. In neither case were individual or population impacts addressed.

Stansley *et al.* (1992) investigated lead contamination of open water and wetland areas subject to shot fallout within clay target shooting ranges or to movement of lead off-site into unshot waterbodies. Spent lead shot concentrations were measured in the fallout zones of eight ranges in

New Jersey, USA, and total lead measured in water samples from each range and nearby control waters. Marshes, ponds, streams and seasonal pools were included. Water samples were also taken at the range with the highest spent shot density, from a stream running through the site into a lake, and from the lake (outside the shotfall zone) and a nearby control lake. Sediment and fish samples (largemouth bass (*Micropterus salmoides*) and pumpkinseed sunfish (*Lepomis gibbosus*)) were analysed from each lake.

Shot densities in the soil and sediments of the water-covered areas in the shotfall zones were high, ranging from $4.2x10^6$ to $3.7x10^9$ per hectare, and surface water total lead concentrations at all shotfall sites exceeded those in the associated control waters by 2 to 194 times, the values ranging from 12 to 581µg/l. Lead concentrations downstream from the shotfall zone was similar to those in the control lake. It was presumed much of the lead was in the form of suspended low-solubility lead carbonate and hydrocarbonate, which appeared to settle out of the water column relatively quickly. Levels in the off-site control lake water were below detection limits and no accumulation was recorded in its sediments. No enhanced uptake of lead was recorded in any of the fish sampled. Sample sizes were low (n=3) for each species from each of the two lakes.

Summary

Investigated exposure of fish to soluble ammunition lead and movement of lead off-site into waterbodies. Generally sound but small samples. Demonstrates potential fish exposure through sediment/water lead levels. No uptake recorded (small samples). Alkaline conditions only assessed. Evidence of no individual impacts. Population impact not addressed.

Stansley and Roscoe (1996) reported lead levels in white-footed mice (*Peromyscus leucopus*), shorttail shrews (*Blarina brevicauda*), and green frog (*Rana clamitans*) collected from a New Jersey, USA, 30-year old clay target shooting site and a “nearby” but undescribed control site. Blood, liver, kidney, and leg bone samples were collected from both sites. Lead shot density in the upper 7.5cm of the shotfall zone (comprising both woodland soil and wetland sediments) was $3.7x10^9$ pellet/ha, and lead concentrations of 75,000µg/g and 74µg/g were measured in the shotfall and control soils/sediments, respectively.

The 22 white-footed mice from the shotfall site had significantly elevated (5 to 64 times higher) liver, kidney and femur lead levels compared with the control site. Two of the mice with elevated kidney lead levels showed abnormal kidney histopathology (including intracellular inclusion bodies); none of the 12 control mice showed kidney abnormalities. There was also evidence for some changes in haematology typically associated with lead: 48% depression in blood ALAD (an enzyme involved in haemoglobin synthesis) activity; 2.5 times elevated ALAD ratio (ratio of activated:unactivated ALAD); and 6.7% depression in haemoglobin concentrations in shotfall mice compared with the controls. Hematocrit (blood cell volume) did not differ between the groups. There was evidence of a disease, unrelated to lead, in the control mice which may have affected other haematological measures.

The one shorttail shrew collected from the shotfall site showed highly elevated liver, kidney and femur lead levels compared to the four shrews from the control site.

The study revealed that some portion of the lead in the shotfall site was bioavailable. The authors hypothesised, based on a modelling process with “considerable uncertainties”, that the small mammal body burdens of lead were more likely the result of ingesting lead-contaminated soil inadvertently when feeding than through contaminated dietary plants or prey animals. They regarded the kidney abnormalities as indicative of lead-induced renal changes but present no information on likely consequences for the affected individuals. They noted that depressed blood ALAD activity indicates recent lead exposure but not necessarily toxic exposure since ALAD is not the rate limiting enzyme in haem synthesis. That haem synthesis was affected to some extent was indicated by the reduced haemoglobin concentrations in mice from the shotfall area.

The green frogs were analysed for lead from the same shotfall site, and in a similar way, as the mice and shrews. Average femur lead levels in 11 frogs from the shotfall site were nearly 1,000 times higher than that of 12 frogs from the control site (1,728µg/g and 1.79µg/g, respectively), and the kidney lead in four frogs from the shotfall area was 67 times higher than that from five control site frogs (96.2µg/g and 1.44µg/g, respectively). Kidney histopathology and erythrocytes appeared to be unaffected. Blood and liver ALAD levels were depressed in frogs from the shotfall site.

Broadly similar conclusions as for the small mammals were drawn, including the presumed pathway of lead-contaminated soil, and low potential food-chain uptake of lead from frogs when much ingested lead is deposited in bones and bones are regurgitated by raptors.

The paper demonstrates a likely link between spent lead shot in the shotfall environment and elevated lead levels in the small mammals and frogs studied, although the source was not explicitly identified as lead shot. Biochemical/histopathological effects were identified but their consequences for the affected individual animals were not examined. The authors also concluded that as body lead concentrates primarily in bone, and that predators, especially raptors, tend to regurgitate such material from their food intake, the potential for food chain uptake of lead is low.

Summary

Addresses exposure of small mammals to spent gunshot lead from clay target shooting. Quite thorough study but small samples and other uncertainties. Demonstrates exposure, and elevated soft tissue and bone lead levels in small mammals; hypothesised pathway is inadvertent ingestion of lead-contaminated soil rather than lead-contaminated food items. Noted possible low food-chain exposure of lead to predators, especially raptors that regurgitate undigestible material. Demonstrates sub-clinical and possible developmental impacts (histopathological kidney lesions) on some mice. Population impact not addressed.

Assessment

The scope, quality, reliability and applicability of the findings from these studies are so variable that generalisations are difficult. There are many factors operating at each site of clay target or intensive game shooting which affect the fate of the spent ammunition and its influence on the plants and animals of the site, and few studies have been able to control for more than a few of them, including other sources of lead.

As expected, high concentrations of lead are recorded in the soils of shooting grounds. Several studies have recorded elevated levels of lead in plants and/or animals occupying such sites, some indicating some mobility of the lead and movement through lower trophic levels. Few clear source-pathway-receptor links have been demonstrated between the ammunition lead and receptors, whether they are micro-arthropods, invertebrates, small mammals or frogs. Where elevated body levels have been found in small mammals and frog, the source has been suggested as uptake through soil ingestion as well as with food. One study demonstrated movement of lead offsite in waters was limited and fish were not affected.

c) Woodcock

Some linkages have been found in North America between the American woodcock (*Scolopax minor*) and lead ammunition.

Scheuhammer *et al.* (1999) examined the lead levels of 1,596 wing bones from hunter-killed woodcock in 1995/96 from south-eastern Canada. They contained (geometric) means of 11.9µg/g dwt (range 0.7-280µg/g) for immature birds and 20.2µg/g (range 1.26-346µg/g) for adult birds. Lead gunshot was suggested a possible source for this contamination, from both ingestion of spent

shot mistaken for grit, and ingestion of lead via the soil content of earthworms from contaminated land. The paper did not assess the impacts of the elevated wing bone lead levels.

Scheuhammer *et al.* (2003) examined lead isotope ratios in earthworms and woodcock to attempt to ascertain the source of the lead contamination. Four hundred and twenty woodcock wings were analysed and found to average lead levels of 20µg/g dwt (range 1.4-280µg/g). Wing bones with low levels of lead (<20µg/g) had lead isotope ratios ($^{206}Pb/^{207}Pb$) similar to those in the soil and earthworms eating the same soil drawn from the habitats of the woodcock studied. Those above 20µg/g showed a mean ratio of 1.18, as did lead shot samples from various North American manufacturers. The authors regarded this as indicative that lead shot was contributing to the elevated bone lead levels (although were not able to confirm the source of lead). In a sample of 228 woodcock intestines (examined fluoroscopically) they found none containing ingested lead shot. They suggested that spent shot is ingested but rapidly passed through the digestive system.

Hiller and Barclay (2011) found similar levels of lead in woodcock wing bones from eastern North America. They also found that woodcock harvested with lead shot had significantly higher pectoral lead levels than those harvested with steel shot. It is not clear, however, if lead fragments were removed from the pectoral muscle prior to analysis.

Assessment

These papers suggest American woodcock are exposed to lead and that lead from spent shot could be contributing to elevated tissue levels. They present no evidence that the body lead levels have significant individual or population level effects.

In view of the other studies previously listed which found elevated lead levels in earthworms, including those in the UK, it is possible that woodcock in this country are also exposed to lead in their diet, at least in those areas subject to high concentrations of spent lead shot. Any population level impacts cannot be addressed until individual effects are better understood.

d) Risk assessment

Peddicord and LaKind (2000) carried out the first reported ecological and human health risk assessment at an outdoor recreational shooting range in New York State, USA. The assessment of ecological impacts of spent lead shot and bullets only is included here.

This was a detailed study focused on one shooting range (shotgun, rifle and pistol shooting) and appeared to follow established (but undefined) procedure for ecological risk assessment as developed in the USA in the 1990s (see Kendall *et al*. 1996, section 3.1.4). It identified a range of wildlife species receptors potentially at risk from exposure to spent lead shot and bullets on the site and selected specific representatives of the main potential exposure routes according to habitats occupied and feeding habits. The risk assessment was then conducted for each of the representative wildlife groups, based on relevant information from the literature or obtained by specific studies for the purpose, quantifying or estimating the likely exposure of each of the defined receptors to spent lead ammunition and its effects on them, relying on documented indications of adverse effects. White-tailed deer (*Odocoileus virginianus*) represented primary consumers or herbivorous feeders; ruffed grouse (*Bonasa umbellus*) the ground-feeding birds potentially able to ingest spent shot with their food; American robin (*Turdus migratorius*) the ground-feeding invertebrate eaters, also potentially exposed to spent shot; Eastern phoebe (flycatcher) (*Sayornis phoebe*) the avian invertebrate feeders in flight; red-tailed hawk (*Buteo jamaicensis*) representing raptors at the top of the ecological food web, feeding primarily on rodents; red fox (*Vulpes fulva*) the carnivorous and omnivorous consumer of foods potentially contaminated by spent lead ammunition; and mice and rabbits as the food of higher trophic level consumers and as representatives of important feeding guilds at the site.

Spent lead shot was found not to pose a risk to hawks and other raptors, foxes or deer, since either they did not ingest spent lead shot directly or any prey items containing lead shot formed a small part of their diet drawn from a larger area than the shooting range. Individual grit-ingesting birds (ruffed grouse) were at risk from incidental ingestion of spent shot with their food in the shotfall area of the clay target shooting. Lead posed a small risk to individual birds not ingesting grit (phoebe and robin). In dietary items it posed only minor potential for risk to individual small mammals (mice and rabbits). Evidence for their ingestion of shot was lacking and the likelihood of their doing so was considered small.

The authors concluded that risks may occur at the level of individual organisms at the shooting range but not at the population, community or ecosystem level. This was partly because the population of each of the main wildlife species studied potentially exposed to lead at the shooting range was likely to be relatively small compared with its whole population in that part of the state, and since density-dependent compensatory mechanisms would likely offset any site-related loss of individuals that might occur on the range itself.

Summary

Investigated exposure of wildlife to spent lead ammunition on rifle/shotgun shooting range. Thorough study but modelling procedures cannot be verified, and one study area only. Much dependence on other studies for lead toxicity input values. Undefined sampling methodologies. No control area/information. Many untested assumptions. Some unexplained results. Approach predicated on any/all wildlife species studied having some lower tolerable limit of lead. Demonstrates potential exposure through elevated soil, sediment/water lead levels. Evidences gunshot in intestine of ruffed grouse only, and hypothesises ingested lead through inadvertent soil intake in the flycatcher and American robin, and through prey/food containing lead in raptors, fox, rabbit and mice. Predicted local (but undefined) individual impacts but ecological impacts not expected at population, community or ecosystem levels as density-dependent compensation mechanisms expected to offset site-related losses of individuals.

Assessment

One risk assessment, based on one site, cannot be applied generally, not least as it suffered from a number of limitations in its own purpose. It is relevant, however, as it helps to put findings from individual shooting grounds or more intensive types of game shooting into perspective. Exposure to and impacts from spent lead shot may occur for individual birds or animals at the shooting site, not least resulting from the considerable accumulations of spent shot that typically occur. The population of each of the species potentially exposed to lead at the site, though, may be small compared to the whole population in the area, region or country in which the shooting site occurs. Losses in and around the site may not impact the population overall on account of their small number or through some compensatory mortality mechanisms operating at population level. Necessarily, such shooting sites and their possible relatively local impacts cannot be viewed in isolation. The number, distribution and impacts of other such shooting sites would need to be assessed in terms of their collective impact on wildlife populations in the whole relevant area.

3.5 S-P-R: E Poisoning of wildlife by embedded spent lead ammunition

S-P-R: E **Whole pellets or bullets, or parts of same, embedded in-tissue of shot wildlife potentially releasing soluble lead into the affected bird/animal.**
Game shooting/rough shooting/pest control/wildfowling/deer management.

In a Danish study of pink-footed geese (*Anser brachyrhynchus*), Madsen and Noer (1996) reported indications of reduced survival in birds with embedded gunshot, as revealed by capturing and x-raying, marking and releasing, and then re-sighting birds that had embedded pellets. They were not able to quantify the impact or identify its direct causes.

Merkel *et al*. (2006) investigated the effect of embedded lead shot on body condition of eider ducks (*Somateria mollissima*) shot in Greenland. No long-term effects were found for immature or adult birds but juvenile birds carried less fat than un-wounded birds. The authors considered this likely to add to other causes of mortality but were not able to assess impact at the population level.
In France Tavecchia *et al*. (2001) reported on effects of lead exposure on survival of mallard (*Anas platyrhynchos*) in the Camargue. They analysed recovery data for 2,740 mallard captured during 1960-71 and x-rayed to determine the prevalence of lead pellets both ingested and embedded after being shot, using innovative modelling and statistical procedures. They reported a 19% relative decrease in survival of adult birds from the combined effect of ingested lead shot (two or more pellets) and tissue-embedded pellets, the separate effects being additive.

Guillemain *et al*. (2007) similarly analysed 39,000 x-rays of teal (*Anas crecca*), trapped in the French Camargue from 1957 to 1978, to assess, by modelling and statistical procedures, the long-term consequences on survival of both ingested and embedded lead shot, in relation to their known ringing/release date. They found no effect on survival from embedded pellets, but a negative (unquantified) impact on survival from one or more ingested pellets.

Assessment

The limited evidence does not indicate consistent negative impact of embedded lead pellets in shot waterfowl. Where it is reported it is not known whether it was caused by physical/physiological impacts on the damaged tissues/organs or mobilisation of lead from the pellets into the blood system, causing or contributing to any lead poisoning from other lead exposure.

3.6 Welfare impacts/effects of lead

Pain *et al.* (2010) expressed concerns about the impacts of ingested lead ammunition poisoning on the welfare of affected birds. Sub-lethal physiological and behavioural effects of lead, including impaired blood synthesis, immune function and reproduction (evidence for these being drawn from the literature), are presumed to increase susceptibility to disease, starvation, predation and death from other sources: “Lead poisoning severely compromises the welfare of large numbers of wild birds, both terrestrial and aquatic”. The authors referenced Sainsbury *et al.* (1995), who investigated the scale and severity of welfare issues in wild European birds and mammals, including the effects of ingested lead shot, apparently finding that lead poisoning through shot ingestion to be among the most significant human activities that severely compromise the welfare of large numbers of animals.

The Sainsbury *et al.* paper is the only PEL source specifically addressing welfare aspects of ingested lead ammunition poisoning. The authors sought to quantify the relative scale and severity of welfare problems in European wildlife caused by a wide range of human activities affecting wildlife (including rodenticides, myxomatosis, lead shot ingestion, oiling of seabirds, road traffic accidents, cat predation and many others). They used literature review and questionnaire survey on human/wildlife interactions which had adversely affected the welfare of free-living birds and animals from 1982-1992. They sought descriptions of causes of welfare problems; species involved; description of pathological effect or stress produced; judgement of likely levels of stress, fear or pain caused; and estimates of numbers affected and duration of harm. They recognised the difficulties involved in obtaining the required information and estimates, these being both published and unpublished, as well as being speculative, and, they believed, conservative.

Ingested lead shot poisoning welfare issues related only to waterfowl and a list of clinically-recorded effects was drawn from veterinary publications. They include lethargy, anorexia, upper digestive tract paralysis, vomiting, diarrhoea, ataxia, limb paralysis, convulsion, anaemia, emaciation, muscular atrophy, degeneration of liver and kidney, oedema, and nervous system lesions. The lesions and signs were deemed “likely to cause severe pain and distress”.

Summary

Addresses ingested lead shot effects at individual level only and on waterfowl, with no guidance on their applicability to non-waterfowl. Based on secondary literature, opinion and unverifiable information from questionnaire surveys. Exposure not addressed. Based on literature sources, sub-clinical, developmental and welfare impacts are associated with individual ingestion of lead shot.

Other effects of lead ingestion

A literature search revealed additional recent studies addressing welfare and other impacts of lead ingestion more specifically than reported in many studies of lead poisoning in birds or animals.

a) Europe

Plouzeau *et al*. (2011) reported on the effect of one ingested lead shot pellet on the growth of juvenile (game farm) mallard (n=51) in France. All dosed birds showed high blood lead levels and some other blood chemistry changes but none developed symptoms of plumbism (except light diarrhoea) and all survived at day 40. No biometric measures nor flight capacity of post-juvenile mallard were affected. The authors recognised that effects on younger birds and of larger exposure to ingested shot needed to be investigated. The applicability of studies on game-farm ducks to wild ducks would also need to be assessed.

Martinez-Haro *et al*. (2011) studied blood lead levels, and blood and enzyme biochemistry, in Spanish waterfowl (mallard (*Anas platyrhynchos*), pochard (*Aythya ferina*), coot (*Fulica atra*) and moorhen (*Gallinula chloropus*)) exposed to environmental lead, presumed to be derived from high concentrations of spent lead shot in their lagoon habitats. They reported, apparently for the first time, on indicators of oxidative stress related to relatively low levels of lead exposure in two of the species studied (mallard and coot), although the responses differed between the species. They also appeared to find reduced ALAD activity in mallard at lower blood lead levels than previously. On the basis of these and other findings they suggested that the widely-used 20µg/dl to identify birds with blood lead levels above background levels (i.e. with potentially sub-clinical effects) be revised downwards.

Notwithstanding much speculation about causes, effects and inter-relationships of the various blood components deemed to have been affected by the ingestion of spent lead shot, the findings suggest the appropriateness of using the 20µg/dl threshold needs to be reviewed. Furthermore, as different waterfowl species apparently respond differently, care is needed in applying the threshold uncritically across waterfowl species, as well as presuming the same level applies to non-waterfowl (e.g. for birds of prey as in section 3.2 above). Such an assessment might alter the interpretation of PEL and other studies relying on blood lead levels in birds exposed to spent lead from ammunition sources.

Gangoso *et al*. (2009) investigated long-term effects of lead poisoning on bone mineralisation of Egyptian vultures (*Neophron percnopterus*). They compared bones from two separate populations showing differing blood lead levels: Canary Islands, 5.10-1,780µg/l, n=137, where blood levels peaked during the hunting season, and Iberian Peninsular, 5.60-217.30µg/l, n=32. Bone lead concentration increased with age, and bone mineralisation decreased as lead concentration increased. The authors suggested such long-term effects of lead poisoning may be important in declines of long-lived raptors exposed to lead poisoning.

Bilandzic *et al*. (2010) reported finding elevated kidney and muscle lead levels in free-living wild boar (*Sus scofa*) across Croatia. They exceeded permitted values (0.1ppm muscle and 0.5ppm kidney), on average, in 13.6% and 8.9% of samples for muscle and kidney, respectively. The tested animals (169) were hunter-shot. Muscle samples were taken from the upper hind leg. No

mention is made of, or efforts to control for, any muscle or kidney contamination from the hunting ammunition used.

Their findings suggest that studies of tissue lead levels in relation to the use of lead ammunition in hunting, at least in wild boar, need to be aware of background tissue lead levels of such animals when interpreting findings.

b) North America

In the USA Burger *et al.* (2000 – abstract only) investigated behavioural effects of low levels of lead in two gull species (herring gull (*Larus argentatus)* and common tern (*Sterna hirundo*)) over 15 years. Birds were injected with lead acetate which produced feather lead concentrations similar to those found in some wild-living birds.

Low-level lead affected growth, locomotion, balance, food begging, feeding, thermoregulation, depth perception, and individual recognition, in both laboratory and wild-living birds. The accuracy of individual recognition was most affected by lead exposure from two to six days of age; exposure at 12 days did not affect accuracy, but it delayed response time significantly. Behavioural deficits observed in lead-injected young in the wild were similar to those observed in the laboratory, except that recovery was more complete by fledging than it was in laboratory-raised chicks. Parents in the wild were able to provision lead-exposed chicks sufficiently so that they fledged at similar weights as control chicks.

The authors believed that lead treatment altered the expression of cell adhesion molecules (CAM), important in the formation and deployment of neurons in the developing brain. “The timing and sequencing of CAM expression is critical to normal development, and the different consequences of lead exposure at different ages may be related to interference at different points in the sequence.” They concluded that lead-induced behavioural deficits observed in the laboratory and in the wild were sufficient to affect growth and survival in wild herring gulls.

From the abstract it is not possible to assess the reliability of the findings and conclusions. Nor can it be determined if intra-peritoneal injections of lead acetate can be regarded as a proxy for the subsequent effects of ingested lead gunshot.

Assessment

There is much published literature reporting various effects of lead poisoning on individual birds and animals, including the effects of ammunition lead. These include sub-clinical, behavioural, developmental and reproductive impacts (section 2.2.1), which are relatively tangible and measurable in their expression. Less easy to determine are welfare impacts resulting from such effects, which might be regarded as those affecting the human notion of ‘quality of life’, including pain, suffering, discomfort, depression, reduced performance or viability. In the absence of definitive evidence, and on the basis of lead’s toxicity and general lack of a lower threshold for adverse effects, it is probably reasonable to assume adverse welfare impacts from lead levels at least above those widely regarded as background, for the tissue(s)/organ(s) in question.

3.7 Other references on the Primary Evidence List

Pain *et al.* (2009) reviewed evidence of lead poisoning of terrestrial birds from ammunition sources at the global level. The review was published in the Peregrine Fund conference proceedings (Watson *et al.* 2009). It is included here for its wider context and relevance to the UK.

There are several features of this review which reinforce the approach that Klimisch advises (section 2.2). The extensive literature reference list includes non-peer reviewed studies, reports

and books, magazine articles and conference proceedings, and "personal communication", among the otherwise peer-reviewed material. The list of reported occurrences of lead shot ingestion and poisoning give no indication of the extent of reported poisoning for the listed species. Some are known to relate to one case only. Some appear not to reflect the findings of the original study. For example, against Vyas *et al.* (2000) are listed three passerine species poisoned by "ingestion (of shot)". Review of Vyas *et al*. (2000) for this risk assessment (section 3.1.4) shows no ingested shot were found in the sampled birds, and that the (three) white-throated sparrows studied were caged on the site rather than free-living.

Another example relates to the referenced Butler *et al.* (2005) study of lead exposure in British pheasants. The reviewers note a single pheasant with ingested lead shot reported in *The Field* in 1876 and then, from the Butler *et al.* (2005) study, state "an overall lead shot ingestion rate in these pheasants of 3% with correspondingly elevated bone lead levels", concluding that evidence for lead poisoning in pheasant spans 129 years in the UK. Review of the Butler *et al.* (2005) paper – the first reported reference to pheasant lead shot poisoning in the UK since 1876 - for this risk assessment (section 3.1.1), however, shows that while 3% of gizzards contained one or more lead shot pellets (13/437), only two birds with gizzard shot had high concentrations of bone lead. Particularly pertinent to this wildlife risk assessment, but not acknowledged by Pain *et al.*, is the Butler *et al.* reported finding of no evidence of a link between bone lead levels and pheasant body condition. Their study has its own shortcomings but it gives a different view of lead poisoning in British pheasant than implied in the Pain *et al.* (2009) review.

A third example relates to UK red kites and the Pain *et al*. reference to an earlier study (Pain *et al*. 2007), which "found lead isotope ratios in the livers of red kites in the UK to be consistent with ratios in lead pellets". The 2007 study has been reviewed for this risk assessment (section 3.2.1) and uncertainties found in the section reporting on the lead isotope studies. Again, therefore, while not incorrect in itself, the reference to the 2007 findings gives a different impression than the evidence appears to warrant.

Notwithstanding these shortcomings, the review paper claims that, globally, some 33 free-living raptor and 30 other terrestrial species have been recorded as showing lead shot ingestion and/or poisoning. The non-raptor species include Gruiformes (coot, crane, rail etc), Galliformes (pheasant, grouse, quail, partridge etc), Columbiformes (pigeon, dove), passerines and gulls. For the UK the list comprises: pheasant, grey partridge (but not red-legged partridge – see section 3.1.1), red kite, common buzzard and peregrine falcon. The two Galliformes are potentially exposed to lead poisoning through ingestion of spent shot with food or grit. The birds of prey potentially are exposed through eating prey animals or scavenging shot animals or birds containing lead shot (or lead bullet) material. The studies including these species have been reviewed for the current risk assessment as follows: pheasant and grey partridge – section 3.1.1; red kite – section 3.2.1; common buzzard and peregrine falcon (based on dead birds) – section 3.2.1.

Summary

Addresses lead shot exposure to many species and indicates some impacts but with many shortcomings. Many uncertainties including uncritical use of secondary literature. Evidences exposure of many species but with no measure of extent or frequency, through published reports. Some individual impacts reported. No population impacts addressed.

Quy's (2010) report to Defra "Review of evidence concerning the contamination of wildlife and the environment arising from the use of lead ammunition" reports on lead ammunition impacts on wildlife, in several countries, including the UK, but it is essentially an uncritical summary and collation of some of the published sources on the subject. While it includes many of the PEL sources these have been subject to much fuller assessment in this draft wildlife risk assessment.

It reports exposure of waterfowl and gamebirds to spent lead shot, through spent shot densities in their habitats and ingested shot in gizzards; sub-clinical effects on enzyme and blood haem synthesis; elevated blood, soft tissue and bone lead levels; and some pathology resulting from ingestion. It also reports on predator and scavenger exposure to embedded gunshot and bullets/bullet fragments in their lead-shot prey/food. Some of the evidence relates to the UK but most is from other countries.

The Coburn *et al.* (2003) report to Defra's Food Standards Agency "Hazards and risks from wild game: a qualitative risk assessment" contains brief reference to lead ammunition in relation to wildlife, focusing mainly on biological risks. It is incomplete, based on limited data and few published sources, and contains inaccuracies. It does not effectively address exposure to or impacts on England (UK) wildlife from lead ammunition.

The PEL also includes three links to government agency websites reporting on differing monitoring schemes for, *inter alia*, lead ultimately finding its way into the human food chain: Food Standards Agency, Veterinary Medicines Directorate, and Animal Health and Veterinary Laboratories Agency. Currently (July 2013) these websites are not revealing findings of lead in England (UK) wildlife which has or may have an origin in shooting.

3.8 Impacts at population level

The risk assessment endpoints (section 2.1.3) are: a) negative population status of any species of England (UK) bird, mammal or other taxon exposed to lead from lead ammunition, and shown to be or considered likely to be caused by the exposure to ammunition lead and b) negative impacts on individual bird or animal health, including development, behaviour, reproduction, welfare and survival, shown to be or considered to be caused by exposure to ammunition lead.

When sufficient individual members of a population suffer mortality, with or without reduced population breeding success then, after a point which will vary from species to species, and perhaps population to population in different geographical areas, the status of the population will be affected. This may be manifested as a decline in a population previously stable, a reduced rate of increase in an expanding population or an increased rate of decline in one already declining. It may also be revealed by changes in population distribution.

There are several difficulties involved in assessing possible adverse impacts of spent lead ammunition on the wildlife populations in England (UK) subject to this risk assessment.

There is no definitive source of population data for each species. Different monitoring schemes are in place, over differing lengths of time and with differing levels of accuracy and reliability in their monitoring data. Many factors typically are involved in the dynamics of any given population, some known and others not known. Detailed study would likely be needed to tease out the relative contribution of lead poisoning from other factors in a population clearly showing a decline in size (or distribution). In situations where even a decline was not entirely clear the difficulties in assigning any contribution from lead would be greater. Finally, lead ammunition has been in use for hundreds of years in the UK. It is unlikely, therefore, that any change in rate of population increase or decline could be linked with ingested lead poisoning from ammunition unless it were linked with a combination of factors which, in turn, were the cause of a change in population dynamics. A population increasing evidently is not being unduly affected by lead poisoning (other than a possible reduced rate of increase). A stable population could be showing impacts of lead poisoning in preventing increase. A declining population could be showing impacts of lead poisoning.

Information drawn from UK avian population monitoring schemes has been used to determine if there is any basis in population monitoring for concern, possibly or evidently linked to exposure to spent lead ammunition, over any of the target species in the UK.

The most relevant and recent indicator for most of the species covered by this dWRA is the *Birds of Conservation Concern* (Eaton *et al.* 2009), a periodic assessment conducted jointly by most of the UK statutory and non-statutory conservation bodies. Populations are assessed against objective criteria and classed as *Green* – "No significant concern", or *Amber* or *Red,* depending on the level of concern attached to declines in either their range and/or numbers as revealed by the monitoring schemes in place. *Amber,* amongst other things, indicates moderate decline (25-49%) in breeding population, non-breeding population, or breeding range in the last 25 years (measured by numbers of 10km squares occupied by breeding birds). *Red* indicates severe (>50%) decline in breeding population over similar time scales and can include European measures of conservation concern.

Population trends for named species are presented, where available, from WeBS (Wetlands Birds Survey) and BBS (Breeding Birds Survey).

Of the 13 species for which BoCC assessments are available six are currently *Green* – pheasant (*Phasianus colchicus*), red-legged partridge (*Perdix rufa*), common buzzard (*Buteo buteo*), sparrowhawk (*Accipiter nisus*), peregrine falcon (*Falco peregrinus*) and woodpigeon (*Columba palumbus*). As these species are classified as having "No conservation concern" it appears unlikely any exposure to lead from lead ammunition is having an adverse effect at the population level.

Five species are Amber – red grouse (*Lagopus lagopus scoticus*), kestrel (*Falco tinnunculus*), red kite (*Milvius milvius*), mallard (*Anas platyrhynchos*)(Red in Wales), and woodcock (*Scolopax rusticola*).

Two species are *Red* – grey partridge (*Perdix perdix*), white-tailed sea eagle (*Haliaeetus albicilla*).

With respect to the *Amber* species:

Red grouse (breeding population decline of 25-49% for greater than 25 years) - Population change and long-term decline, at least in Scotland and less so in northern England, has been comprehensively studied and its causes linked strongly to habitat condition, disease and probably predation. Lead poisoning has been investigated and there is some exposure and possible individual impacts indicated but no evidence presented that lead ammunition has contributed to the population decline (section 3.1.1). See Figure 2.

Kestrel (also categorised as a Species of European Conservation Concern (SPEC)) – No evidence is available to show that kestrels are at particular risk from ingested lead shot/bullet poisoning (See section 3.2.1 (Pain *et al.* 1995), and Figure 2).

Red kite (also SPEC) – The red kite population, contrary to its BoCC status, is increasing and spreading rapidly, following recent successful re-introduction programmes. See Figure 2. Pain *et al.* (2007) showed some exposure to ingested lead shot poisoning and mortality actually or likely caused by spent lead ammunition in red kite prey/food. The authors did not consider the impacts threatened its conservation status (section 3.2.1). The rapid growth in the population would appear to confirm this (although growth is enhanced by reintroduction programmes).

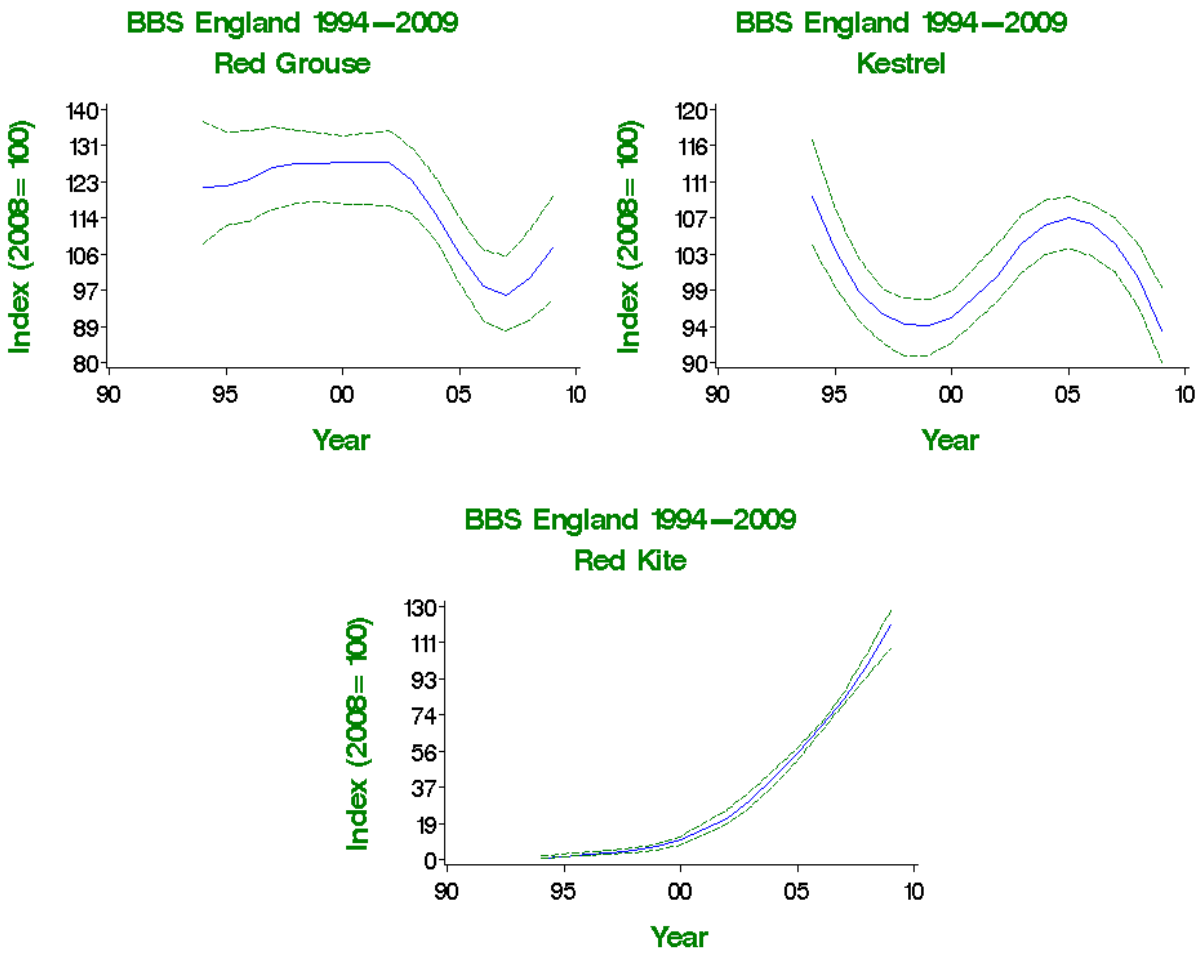

Figure 2. Population trends, from the British Breeding Bird Survey (BBS) for amber-listed species.

Mallard (non-breeding population decline of 25-49% for greater than 25 years) – There is currently no explanation for the apparent long-term decline in counted wintering mallard in this country (Figure 3) (Holt *et al.* 2011). Climate change causing 'short-stopping' - i.e. warmer northwest European winters no longer requiring migratory birds to move as far west/southwest in Europe for overwintering – is widely considered likely to be a factor. Mallard are exposed to ingested lead shot poisoning (section 3.1.3) but no evidence or suggestion that lead shot poisoning is now adversely affecting the species is known.

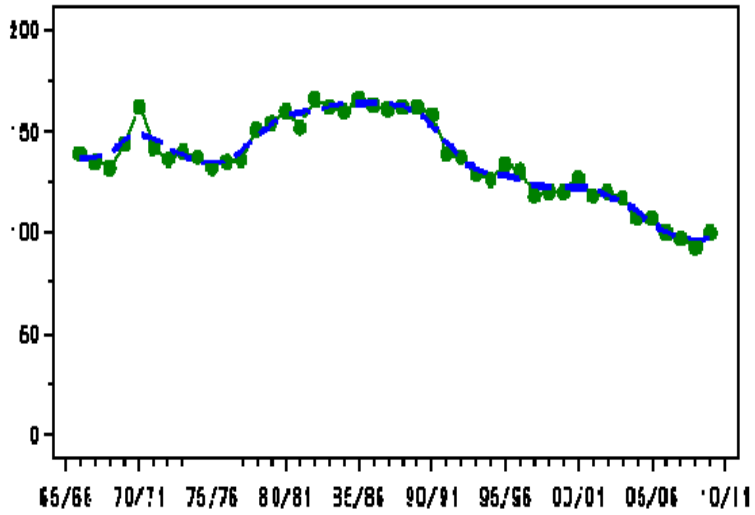

Figure 3. UK Mallard wintering population trend from Wetland Birds Survey (WeBS)

Woodcock (also SPEC) – uncertainty continues over its population size and status, not least because of difficulties in population counting and monitoring the crepuscular species. No concerns are known that lead poisoning might be affecting the population.

With respect to the *Red* species:

Grey partridge (breeding population decline of greater than 50% for more than 25 years, and SPEC) - In England (UK) its long-term decline (Figure 4) has been strongly linked to changes in its lowland arable habitat through farming and other influences. There is no evidence that ingested lead shot poisoning is contributing to the decline (see Potts (2005) section 3.1.1). On estates with a long history of shooting and associated management, grey partridges continue to thrive (Aebischer & Ewald 2010).

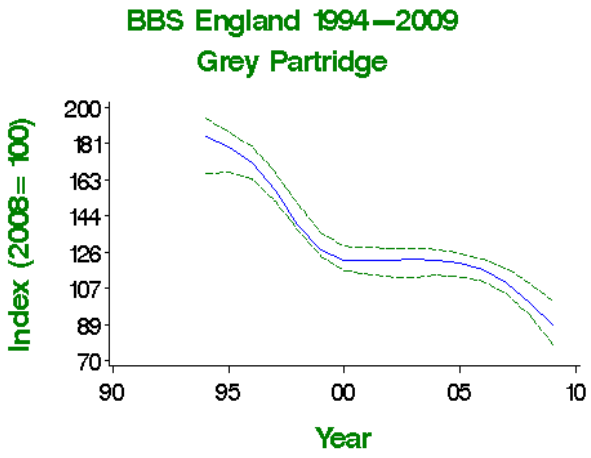

Figure 4. Grey partridge population trend, from UK/British Breeding Bird Survey (BBS).

White-tailed sea eagle (severe decline between 1880 and 1995, currently also SPEC) – The white-tailed sea eagle alert, based on the historical decline to extinction in the UK, does not yet reflect the results of recent re-introduction programmes.

No evidence is known in this country for any impact of lead from lead ammunition ingested in the eagle's prey. From the studies in Germany (section 3.2.2), however, it must be a possibility that English (UK) eagles are exposed, or will become exposed, to some extent to spent lead in their food but it can be only speculation as to any future impact at the population level. There are currently no BBS data on UK white-tailed sea eagles.

4 Discussion

4.1 Requirements of risk assessment

The key steps involved in the wildlife risk assessment, based on the Defra/Cranfield University (2011) *Green Leaves III* guidelines, include problem formulation, with a conceptual model (section 2.1.1) to identify actual or potential sources of lead from spent ammunition, pathways for that lead to reach England (UK) wildlife receptors, and demonstrable or likely negative impacts at individual and/or population level. The process requires the hazard to be identified – spent lead from lead ammunition, either gunshot or bullet - and consequences of wildlife exposure to it to be determined. The magnitude of the consequences needs to be assessed, their probability estimated and risks characterised.

It is reasonable to expect adverse effects on wildlife from exposure to lead from ammunition, given its nature and the published literature, but they are variable and not always demonstrable. The probability of the hazard occurring is high, however, wherever lead ammunition is used and remains in the environment potentially available to wildlife. The hazard is increased where shot fallout is high such as in clay target shooting grounds.

The probability of wildlife exposure to that available lead is determined by, *inter alia*, a pathway between the lead and one or more wildlife receptors. As guided by *Green Leaves III,* clear or potential pathways, identified by the evidence available, are a pre-requisite for the risk assessment to proceed, and efforts made to quantify the exposure via that pathway. Many factors influence the likelihood of harm from exposure to ammunition lead at the individual receptor level. The occurrence, nature and extent of harmful effects on individual wildlife receptors need to be determined, estimates made of the numbers of individuals affected and thence proportion of their population affected. Finally, the impact of that part of the population being adversely affected on the well-being, size and distribution of the population as a whole needs to be determined before the true impact of the stressor (lead) can be assessed, and decisions taken over any remedial measures to manage that impact.

Few risk assessments of the impact of spent lead ammunition on wildlife have been conducted, and none is known in the UK. That carried out by Kendall *et al.* (1996) in the USA on mourning doves and their predators on areas of high spent shot density (section 3.1.4), albeit a developmental ecological risk assessment, suggests some of the data needs and issues involved in a meaningful risk assessment intended to guide management of hunting/shooting activities and the wildlife affected by them.

Based on that study, a starting null-hypothesis for England (UK) might be that lead ammunition use for sporting and target shooting, pest control and deer management should not cause unreasonable risk of widespread mortality in wildlife receptor species, reduced reproductive success or significant welfare issues for affected individuals. To test such a hypothesis requires information on: whether the spent lead ammunition deposited by such shooting activities results in sufficient exposure to lead to make wildlife poisoning possible; whether birds ingest potentially toxic amounts of such ammunition; whether predators and scavengers of dead birds are exposed to lead from ammunition through their consumption of lead-shot birds or animals; whether adverse sub-lethal effects, impaired reproduction or death can be reliably inferred from laboratory and other studies, demonstrating negative effects of such lead on birds; and whether the scale of sub-lethal and/or mortality consequences can be reliably determined such that judgements can be made as to their acceptability, and thence what measures are appropriate to manage them.

Data and measurement endpoints include estimates of wildlife exposure (by species or group), such as gunshot/bullet/ammunition fragment density in their habitats or food; prevalence of its ingestion; lead tissue levels in blood, liver or other appropriate organ(s); and associated likely toxic effects drawn from controlled studies. Toxicological endpoints, related to lead levels in defined organs, need to be linked specifically with their pathological effect such as increased mortality or

reduced reproduction, as well as sub-lethal effects which may bear welfare costs. Ecological endpoints then need to include the percentage of each affected population at (high) risk, evidence of widespread/repeatable mortality or reproductive impacts as a result of that exposure to the ammunition lead, and then evidence of population decline or contraction of range evidentially linked to the aforementioned impacts. Reliable, consistent, representative and science-based information is needed on each of these aspects to enable the assessment process to be adequately conducted.

4.2 Source-Pathway-Receptor evidence for England (UK) wildlife

The components of the conceptual model identify the main sources and potential pathways of ammunition lead into wildlife receptor species/groups for this dWRA. The evidence for exposure to ingested ammunition lead and adverse impacts on England (UK) wildlife is summarised for each in turn:

4.2.1 S-P-R: A Direct ingestion of spent lead gunshot

S-P-R: A **Whole gunshot pellets ingested directly from the ground surface or surface of underwater sediments, typically by seed-eating, vegetation-eating and invertebrate-eating birds, in mistake for food or as grit to assist digestion of food.**
Gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

4.2.1.1 Primary poisoning of gamebirds through direct ingestion of spent lead shot

The England (UK) evidence, drawn from three studies, indicates that a small percentage (up to some 3%) of British pheasant and both species of partridge ingests spent lead shot each year, generally lower than in ducks and geese. The one study on pheasants showed no adverse effects of lead on (hen) body condition. Impacts of ingested lead shot in these gamebirds, either on individual birds or their populations, have not been studied in detail.

One study has indicated that red grouse also are exposed to spent lead shot, perhaps to a higher level than the other gamebird species, but, again, no information is available on individual and population impacts.

Outside of the UK, Spanish red-legged partridges appear similarly exposed to spent lead shot as the British birds, and, as for the British pheasant, with no evident impact on individuals. There is a possibility that galliform birds are more resistant to lead toxicosis than other birds.

The more comprehensive North American study on captive northern bobwhite quail demonstrated vulnerability of multi-pellet ingestion birds to physiological effects (and presumed death) but birds with single ingested pellets showed little response. The Canadian study showed potential exposure of several species of gamebirds at least when spent shot was abundantly available.

Overall, it seems that a small percentage of gamebirds within England (UK) is exposed to spent lead shot, from which it is likely that some will die, but there are no indications of any population impact.

4.2.1.2 Primary poisoning of waterfowl through direct ingestion of spent lead shot

The indications of continued exposure to spent lead shot, primarily of mallard at inland sites, through the findings of Defra's 2010 compliance study in England, are supported by more recent estimates of continuing lead shot poisoning in four species of waterfowl (including two migratory

swan species). They show that some waterfowl species in his country are still being impacted, physiologically, behaviourally and likely death, by ingested lead shot, although that exposure would be expected to be less than it was prior to the regulations being introduced. The level of current lead-poisoning mortality by species is not known, nor are its population impacts. It may be expected to decline with current renewed efforts by shooting organisations to increase compliance with the regulations.

This appears to be a matter of effective implementation of the current regulations, and for the LAG and Defra to address in due course.

4.2.1.3 Primary poisoning of other birds through direct ingestion of spent lead shot

There is no information on the exposure to or impacts of ingested lead shot on non-gamebird and non-waterfowl species in England (UK).

Woodpigeons are abundantly distributed in arable areas and widely shot, through the needs of crop protection. The American findings of ingested lead toxicosis in mourning doves suggest that British pigeons are likely to be susceptible to such poisoning. On the other hand, mourning doves are exposed to concentrated spent shot on fields managed for their hunting. That situation does not occur in the UK. There could be a low level of shot ingestion by woodpigeons from other more widely dispersed gunshot pellets and consequent loss of birds through poisoning, particularly those ingesting several pellets. If so its effect at the population level is not evident, given the size and status of the woodpigeon in England (UK), because lead-induced mortality is negligible, masked by compensatory mortality factors, or less important than other factors determining the population's size.

The American risk assessment focusing particularly on the mourning dove, while finding risks of mortality in areas of high densities of spent shot, was unable to show population impacts at the larger geographical scale.

With respect to British seed-eating passerines, from the American work there appears to be some potential to ingest spent lead pellets during feeding, particularly in areas with high shot densities such as clay target grounds, with some predictable poisoning consequences. There is no evidence of such ingestion in this country.

4.2.2 S-P-R: B Ingestion of spent lead gunshot and bullet by predators/scavengers contained within their prey/food

S-P-R: B (a) Whole gunshot pellets, parts of pellets or metal residues From pellets, ingested by avian and mammalian predators and scavengers from their food which comprises shot but un-retrieved birds or animals, either alive (especially wounded or behaviourally-affected) or dead, or, occasionally, their discarded viscera. The pellets, parts of pellets or metal residues are contained in the flesh/tissues of prey items, resulting from pellets penetrating the bird/animal.
Gameshooting/rough shooting/pest control/wildfowling.

S-P-R: B (b) Whole gunshot pellets or remains of whole pellets within the system of prey animals that have themselves ingested them as in (a), then ingested in turn by predators/scavengers without their having been shot.
Gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

S-P-R: B (c) Lead compounds absorbed into prey animal tissues following ingestion and digestion of pellets, then ingested by predators/scavengers, whether or not

themselves shot.
Gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

S-P-R: B (d) Whole lead rifle bullets, parts of bullets or metal residues from bullets, ingested by avian and mammalian predators and scavengers from their food which comprises shot but un-retrieved mammals, either alive (wounded) or dead. The bullets, parts of bullets or metal residues are contained in the flesh/tissues of food animals, resulting from the bullets penetrating the animal. They may also be within the 'gralloch' (intestines) of deer/other mammals discarded in the field by deer stalkers/pest controllers after removing the shot deer/other mammal carcase.
Deer management/pest control.

The three S-P-R components, B (a) to (c), relating to differing mechanisms for potential poisoning from the presence of lead shot within a bird or animal, in practice are difficult to distinguish and no literature to date has treated them separately. Similarly, distinguishing between gunshot lead and bullet lead, (B(d), is not always clear or possible. In many situations it is clear that gunshot is the only or main source, in gamebirds and waterfowl, particularly. Deer and other large mammals typically, but not universally, are shot with rifle bullets. Many pest species are subject to both type of shooting. Consequently the dWRA addresses the transfer of ammunition lead in prey or other food into its consumer as being through one or more of these mechanisms, identifying sources where possible.

4.2.2.1 Secondary poisoning of predators/scavengers through con of lead-shot (gunshot and bullet) prey

There is evidence that many British birds of prey are exposed to lead in their environments, with the main pathway presumed to be food. While the likelihood is that lead shot and/or bullets in their food are a contributory source, that has not been confirmed for most species, has been shown in one species, red kite, and only circumstantially indicated for buzzard and peregrine falcon. Some species showing elevated tissue lead levels do not normally eat birds or animals subject to shooting (although there is a possibility of their eating species exposed to spent gunshot).
The recent reporting of some two-thirds of shot 'gamebirds' (pheasant, partridge, grouse, woodcock, woodpigeon and mallard) containing embedded and/or fragmented shot demonstrates the potential source and pathway of spent lead shot into predators and scavengers through eating shot birds that have either not been killed or not retrieved.

For the red kite, source, pathway and impacts are evidenced, with lead shot in their prey being a contributor to elevated body lead levels, and causing adverse physiological effects and death in some birds. The authors concluded, in 2007, there was unlikely to be an impact on the conservation status of the red kite population or on the success of further reintroductions, but that the use of lead ammunition created welfare costs for the birds and unnecessary mortality. The population appears to have continued increasing in size and distribution since then.

The study on bullet and bullet fragmentation in deer carcases shows a potential source and pathway for the poisoning of predatory and scavenging wildlife, particularly birds of prey, corvids and gulls, through ingesting bullet lead from the remains of shot deer. The risk of exposure will depend on the frequency and extent of such carcases, or just their viscera (gralloch), being left in the birds' feeding environments.

Many of the similar studies outside the UK add to the evidence that hunter-shot birds and animals can be a source of ammunition lead for predators and scavengers feeding on wounded animals, un-retrieved carcases or discarded viscera. In a few species it has been shown to have significant impact, such as the Californian condor, and the white-tailed sea eagle in Germany.

Actual risks of exposure in any given area will depend on the prevalence of avian predators and scavengers in the areas subject to hunting, availability of shot birds or animals within the feeding ranges of such predators and scavengers, and the prevailing hunting practice, not least the extent to which shot carcases are left behind and intestines discarded for wildlife to consume.

In the UK it has not been the practice, as widely so in the USA, to leave deer gralloch in order specifically to provide food for wildlife. There are on-going debates about the benefits and dis-benefits of leaving grallochs, more driven by animal by-product and waste regulations than concerns about possible secondary poisoning by lead ammunition. At the same time UK raptor populations are not showing evidence of being adversely impacted by such poisoning, and no such concerns are being expressed.

On the basis of the American studies corvids is one group of scavenging birds exposed to lead-shot carcases or discarded viscera, and, from the one small study, it may be that large carnivores are not at risk. There is no evidence in the UK for either corvids or mammalian predators/scavengers being at risk from such sources.

4.2.3 S-P-R: C Wildlife ingestion of lead absorbed by plants from spent gunshot

S-P-R: C **Lead compounds absorbed by plants from soil/water containing soluble lead derived from lead pellets deposited by shooting, then ingested by herbivorous wildlife.**
Gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

4.2.3.1 Lead transfer through plants

The studies from this country show that lead can be taken up by arable plants from soil containing spent lead shot, particularly by their roots, which then might be eaten by grazing or herbivorous animals or birds. There is no information on the extent to which this occurs, or its consequences, to wildlife species as opposed to livestock.

Outside the UK similar studies have also shown that lead uptake by plants from lead-shot-contaminated soil can occur, sometimes to the point of impacting production. None of the studies has addressed the many variable affecting the solubilisation and plant uptake of lead from metallic pellets and so generalisations are not possible.

4.2.4 S-P-R: D Wildlife ingestion of lead taken up by soil organisms/invertebrates from spent gunshot

S-P-R: D **Lead compounds absorbed by soil organisms/invertebrates from soil/water containing soluble lead derived from lead pellets deposited by shooting, then ingested by carnivorous/omnivorous wildlife.**
Gameshooting/rough shooting/pest control/wildfowling/clay target shooting.

4.2.4.1 Lead transfer through lower animals

The findings from the shot-over woodland and grassland system are the only ones for this country but their wider application is limited by the scale of the study and its shortcomings. They do indicate some plant take up of mobile lead and some ingestion by earthworms but no great uptake by plants in general nor by small mammals.

Accumulation of lead by earthworms from lead-enriched soil, in the context of possible exposure of woodcock, is addressed below.

The scope, quality, reliability and applicability of the findings from the various studies in other countries are so variable that generalisations are difficult. There are many factors operating at each site of clay target or intensive game shooting which affect the fate of the spent ammunition and its influence on the plants and animals of the site, and few studies have been able to control for more than a few of them, including other sources of lead.

As expected, high concentrations of lead are recorded in the soils of shooting grounds. Several studies have recorded elevated levels of lead in plants and/or animals occupying such sites, some indicating some mobility of the lead and movement through lower trophic levels. Few clear source-pathway-receptor links have been demonstrated between the ammunition lead and receptors, whether they are micro-arthropods, invertebrates, small mammals or frogs. Where elevated body levels have been found in small mammals and frog, the source has been suggested as uptake through soil ingestion as well as with food. One study demonstrated movement of lead offsite in waters was limited and fish were not affected.

These non-UK studies of spent lead from clay target grounds, all with various limitations, indicate potential for some exposure of various wildlife types inhabiting such sites to lead from spent shot, with possible adverse consequences for affected individuals. Only one study investigated impacts at the population level and it concluded such impacts were unlikely.

Woodcock

Some papers suggest that American woodcock are exposed to lead from spent shot, although they present no evidence that the elevated body lead levels have significant individual or population level effects.

In view of studies, in both this country and elsewhere, finding elevated lead levels in earthworms, it is possible that woodcock in this country are also exposed to the lead in their earthworm diet, at least in those areas subject to high concentrations of spent lead shot.

4.2.5 S-P-R: E Poisoning of wildlife by embedded spent lead ammunition

S-P-R: E **Whole pellets or bullets, or parts of same, embedded in tissue of shot wildlife potentially releasing soluble lead into the affected bird/animal.**
Game shooting/rough shooting/pest Control/wildfowling/deer management.

The limited evidence does not indicate consistent negative impact of embedded lead pellets in shot waterfowl. Where it is reported it is not known whether it was caused by physical/physiological impacts on the damaged tissues/organs or mobilisation of lead from the pellets into the blood system, causing or contributing to any lead poisoning from other lead exposure.

4.3 Welfare impacts/effects of lead

There is much literature reporting various effects of lead poisoning on individual birds and animals, including the effects of ammunition lead. These include sub-clinical, behavioural, developmental and reproductive impacts (section 2.2.1), which are relatively tangible and measurable in their expression. Less easy to determine are welfare impacts resulting from such effects, which might be regarded as those affecting the human notion of ‘quality of life’, including pain, suffering, discomfort, depression, reduced performance or viability. In the absence of definitive evidence, and on the basis of lead’s toxicity and general lack of a lower threshold for adverse effects, it is probably reasonable to assume adverse welfare impacts from lead levels at least above those widely regarded as background, for the tissue(s)/organ(s) in question.

4.4 Impacts at population level

The risk assessment endpoints (section 2.1.3) are: a) negative population status of any species of England (UK) bird, mammal or other taxon exposed to lead from lead ammunition, and shown to be or considered likely to be caused by the exposure to ammunition lead and b) negative impacts on individual bird or animal health, including development, behaviour, reproduction, welfare and survival, shown to be or considered to be caused by exposure to ammunition lead.

Bird populations are generally well monitored in this country. Notwithstanding the difficulties involved and of identifying causes of population decline or reduced rates of increase, there is no evidence of any England (UK) species of bird being negatively impacted by ammunition lead at the population level. Similarly, there are no current, and supported, expressions of concern that this is the case.

Similarly, there is no evidence of any England (UK) species of mammal or other taxon being negatively impacted by ammunition lead at the population level. Similarly, there are no current, and supported, expressions of concern that this is the case. Mammals, and other taxons, however, are much less well monitored than birds.

Excluded from this assessment are waterfowl. There is evidence of on-going ingested lead shot poisoning but this is an issue related to implementation of and compliance with the current lead shot regulations, which were introduced as mitigation measures following previous governmental risk assessment of waterfowl exposure to ingested lead shot, not one of risk assessment in the present context.

4.5 Uncertainty

As with any risk assessment so uncertainty is associated with every component of the dWRA, comprising both epistemic uncertainty – lack of knowledge – and aleatory uncertainty – inherent variability within any natural system (Gormley *et al*. 2011). Particularly relevant for this dWRA are the evidence sources used, as they are the basis for the assessment – no new data were generated for the purpose. Attempts have been made, based on Klimisch *et al*. (1997) and Gormley *et al*. (2011), to rely on primary evidence, screening the literature for relevance and reliability and generally giving less weight to secondary and review literature as it is often not possible to verify the original data collection, analysis and interpretation behind that which is presented in the paper. Where weaknesses of a published source affect or are likely to affect the dWRA reader's understanding and interpretation of the risk assessment, they are identified.

Another source of uncertainty is the technical knowledge of the dWRA authors in some of the laboratory and data handling (modelling and analysis) used in the reported studies, such that it has had to be assumed that correct laboratory and analytical procedures have been followed and outputs interpreted appropriately.

Other sources include use of historical data sets (with likely varying personnel and procedures over time); use of found-dead birds as sources of information on lead poisoning (with unknown relationship with the wild-living population); use of hunter-killed birds for similar purposes (with unknown representation of the wild-living population); differing diagnostic levels, and terminology, for determining elevated tissue/organ lead levels as indicative of poisoning both within and between bird/animal groups; lack of identity of other environmental sources of lead in tissues/organs compared with ammunition lead sources; lack of clarity on reported tissue/organ lead levels as being wet or dry weights and their conversions.

The qualitative nature of this dWRA, rather than being quantitative, does not allow such uncertainties to be quantified and corrections made to estimates of exposure or lead poisoning impacts, or ranges of possible consequences to be produced.

5 Conclusions of risk assessment

Overall, against the requirement for an evidence-based risk assessment of the exposure of wildlife to spent lead ammunition in England (UK), the evidence to date, as available on the PEL and other recent literature, is not indicative of significant impacts at the population level. Neither do individual studies claim such impacts nor do the UK monitoring schemes, where available, reveal population declines linked by any studies to ingestion of ammunition lead.

At the individual level, the evidence does indicate adverse effects, including death, of lead ingestion, not unexpectedly in light of the well-established toxicity of lead in animal systems. It is likely that welfare costs are incurred whenever lead is absorbed, at least above appropriate background levels, as it does not appear to have an NOAEL (No Observed Adverse Effect Level) in animals.

Direct ingestion of spent lead shot is recorded in gamebirds (pheasant, partridges and grouse). A small percentage of gamebirds is exposed to spent lead shot, from which it is likely that some will die, but there are no indications of any population impact.

Direct ingestion also continues in waterfowl, notwithstanding the legislation in place, but this is not a matter for the PERASG, rather for LAG and/or Defra to address in terms of the effectiveness of, and compliance with, the legislation.

There appears to be some potential for seed-eating passerines to ingest spent lead pellets, particularly when feeding in areas with high spent shot densities such as clay target grounds, but there is no evidence of such ingestion in this country.

Similarly there is no evidence that woodpigeons are affected by ingested lead shot poisoning.

There is evidence that many British birds of prey are exposed to lead in their environments, with the main pathway presumed to be food. Evidence indicates that hunter-shot birds and animals can be a source of ammunition lead for predators and scavengers feeding on wounded birds and animals, un-retrieved carcases or discarded viscera. Spent lead shot and/or bullets in their food may be a contributory source, but that has not been confirmed for most species. It has been shown in one species, red kite, and circumstantially indicated for buzzard and peregrine falcon. Some species showing elevated tissue lead levels do not normally eat birds or animals subject to shooting.

It is likely that, given the continuing, illegal, use of lead shot for, at least, inland duck shooting, exposure of birds of prey and scavengers to lead through eating lead-shot birds continues to some extent.

UK raptor populations are not showing evidence of being adversely impacted by such poisoning, and no such concerns are being expressed.

The crow family (corvids) is one group of scavenging birds exposed to lead-shot carcases or discarded intestines. There is no evidence in the UK for either corvids or mammalian predators/scavengers being at risk from such sources.

High concentrations of lead are typically recorded in the soils of shooting grounds. Several studies have recorded elevated levels of lead in plants and/or animals occupying such sites, indicating some mobility of the lead and its movement through lower trophic levels, although many variables are involved. Few clear source-pathway-receptor links have been demonstrated between the ammunition lead and receptors, whether they are micro-arthropods, invertebrates, small mammals or frogs. Where elevated body levels have been found in small mammals (and frog), the source has been suggested as uptake through inadvertent soil ingestion as well as possibly with their food. Earthworms appear to accumulate body lead through their consumption of lead-enriched soil. One study demonstrated movement of lead to waters offsite was limited and fish were not affected.

The evidence indicates potential for some exposure of various wildlife types inhabiting such sites to lead from spent shot, with likely adverse consequences for affected individuals if actually exposed. One study investigated impacts at the population level of a range of birds and animals on a target-shooting site and it concluded such impacts were unlikely. The population of each species potentially exposed to lead at the site, though, may be small compared to the whole population in the area, region or country in which the shooting site occurs. Losses in and around the site may not impact the population overall on account of their small number or through some compensatory mortality mechanisms operating at population level. At the same time, such shooting sites and their possible relatively-local impacts cannot be viewed in isolation. The number, distribution and impacts of other such shooting sites would need to be assessed in terms of their collective impact on wildlife populations in the whole relevant area.

In view of findings of elevated lead levels in earthworms, it is possible that woodcock in this country are exposed to the lead in their earthworm diet, at least in those areas subject to high concentrations of spent lead shot.

The limited evidence does not indicate consistent negative impact of embedded lead pellets in shot waterfowl. The extent of individual lead poisoning caused by such embedded metallic lead is not known.

The sub-clinical, behavioural, developmental and reproductive impacts which can follow ingestion of spent lead ammunition are likely to be associated with negative impacts on ‘quality of life’, including pain, discomfort, reduced performance or viability. In the absence of definitive evidence, and on the basis of lead’s toxicity and general lack of a lower threshold for adverse effects, it is reasonable to assume adverse welfare impacts on individual birds and animals from lead levels at least above those widely regarded as background for the tissue(s)/organ(s) in question.

Overall, it appears that, on the basis of the evidence available for this dWRA and relevant information from elsewhere, and using DETR’s (2000) *ad hoc* scaling procedure, given the limited evidence and its shortcomings, the impacts of spent lead ammunition on wildlife (excluding waterfowl) in England (UK), are consistent with “Mild – for sub-lethal and lethal effects in individuals not causing changes in population size or structure”, rather than with “Moderate” since there is no evidence of effects at population level or non-significant effects at ecosystem level.

6 Information gaps

No single study adequately demonstrates a pathway between spent lead ammunition and adverse effects on any wildlife receptor at the population level in this country. This is not to say that they do not exist rather that research has not been focused in this area.

It is widely assumed that different birds and animals respond to lead (above their normal background levels) in similar ways. It is not known if this is correct or what the main variables are. Similarly it is not clear whether the same background *versus* toxic levels of lead, even in the same tissue/organ, are appropriate across all species.

It is also assumed that elevated tissue/organ lead levels are associated with adverse welfare impacts for individual birds or animals but the extent to which this is true and what is actually incurred by the individual is not known.

The potential for secondary poisoning from consumption of lead-shot birds or animals is not confined to birds of prey yet they are the primary predators and scavengers that have been studied to date. Corvids and gulls also utilise carcases of wildlife and potentially too are exposed to lead from lead-shot game. No information is available on (small) mammalian predators' or scavengers' risk of secondary lead poisoning through this pathway.

There is a possible link between woodcock and spent lead shot mediated through their consumption of lead-accumulating earthworms in heavily-shot areas.

Contemporary environmental risk assessment typically involves modelling to help assess different scenarios of exposure to, and consequences of, an environmental contaminant. None was available for the current dWRA. Provided appropriate and adequate data were available then such modelling would have a place in future studies of wildlife risk arising from spent lead ammunition in this country.

7 Literature

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Appendix 4.
An evaluation of the risks to wildlife in the UK from lead derived from ammunition

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health” by the Lead Ammunition Group, 2 June 2015.

Risk Assessment prepared by Dr. Debbie Pain and Professor Rhys Green of the Primary Evidence and Risk Assessment Subgroup for consideration by the Lead Ammunition Group.

Presented to the LAG on 19 December 2013.

Final version 11 April 2014.

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Background

Lead is a naturally occurring toxic metal that has been used by humans for centuries, and is consequently widely distributed in the environment. Increasing knowledge of the negative health effects of even low levels of exposure to lead has resulted in society taking many actions to reduce emissions. For example, largely as a result of the phase-out of leaded petrol, atmospheric emissions in England declined by 98% between 1990 and 2011, to 41 tonnes in 2011.

There is no biological requirement for lead, *i.e*. it is non-essential. There is currently no defined minimum blood lead level below which the toxic effects of lead cannot be expected to occur in humans and research suggests that this likely applies to other vertebrates.

The Lead Ammunition Group (LAG) established under the auspices of Defra and FSA appointed a sub-committee (the Primary Evidence and Risk Assessment Subgroup (PERASG)) to prepare material for consideration by the LAG. This risk assessment evaluated the evidence for the following question: *"What are the risks to wildlife from ingested lead from ammunition, including welfare considerations, individual and population level risks?".*

Following the "Green Leaves III" approach (Defra 2011), a conceptual model of the issues considered in the risk assessment was developed. This includes the identification of Source-Pathway-Receptor (S-P-R) linkages which describe the route by which the potential hazard from ammunition-derived lead to the population processes and welfare of wildlife might arise. This approach involves evaluation of evidence for each element of the linkage set.

Linkage Set A: Primary poisoning from direct ingestion of lead ammunition from the environment

Lead shot is deposited in the environment where shotgun shooting takes place. This includes a high proportion of the land surface of the UK. At time of writing, precise amounts of lead shot used per year are not known. However, it is estimated (based on numbers of animals killed, number of clay targets shot, and likely numbers of cartridges used) to be in the region of 5,500-12,900 tonnes of lead shot, which equates to *c.*68-154 billion shot, being deposited into the UK's environment year on year. Lead shot densities and environmental contamination from lead shot will usually be greatest where shooting intensity and frequency is greatest.

Pellets of lead shot generally remain relatively intact in the environment for long periods of time (decades or centuries) but do degrade slowly resulting in elevated environmental lead concentrations. Shot generally sink through soils and sediments becoming less available to feeding animals over time depending on substrate type and other environmental factors. While some ingestion of historically deposited shot occurs, most of the shot directly ingested by birds is that most recently deposited.

Numerous field studies confirm that lead shot is consumed by wild birds directly from the environment in both wetlands and terrestrial environments. Wildfowl and other waterbirds are particularly susceptible to shot ingestion. There is strong evidence that English regulations to prevent the shooting of waterfowl with lead shot and its use in wetlands have only resulted in a modest reduction in the use of lead shot for this purpose. Evidence of shot consumption by Anseriformes, Gruiformes, Charadriiformes and Galliformes is provided by UK studies. There is a high probability that any species exposed to lead shot deposited in the environment and susceptible to its ingestion due to their feeding habits will ingest shot to some extent. The extent to which this occurs is related to a species' feeding habits and shot availability and independent of political boundaries.

Consumption of spent lead shot by non-avian wildlife has received little research attention. We cannot therefore evaluate the probability that this occurs to any great extent, but judge that it is likely to be relatively low based upon the feeding habits of non-avian wildlife, perhaps with the exception of wild deer feeding in and around areas of high lead shot usage.

Field studies, supported by laboratory studies, provide strong evidence that where shooting using lead shot occurs, the ingestion of lead shot by waterbirds and terrestrial birds is the primary cause of widespread elevated (above background levels) tissue lead concentrations in birds in the UK and across the world. These studies include the use of stable isotopes of lead that can help identify the source of exposure, and temporal and spatial correlations between elevated tissue (primarily blood, liver, kidney and bone) lead concentrations and exposure patterns to ammunition-derived lead in the environment or food sources (e.g. pre and post lead ammunition bans, in shooting and non-shooting areas). Most other sources of elevated lead exposure are likely to result in relatively local rather than widespread contamination.

From the known pathology and physiology of lead poisoning plus its effect on coordination and mobility, there is high probability of welfare impacts, which may be of a serious nature, in the majority of birds that ingest lead shot and do not eliminate it rapidly.

There is strong evidence, both experimental and from field studies, of mortality and morbidity occurring as a result of direct lead shot ingestion in a wide range of avian species and high probability of this occurring in the UK wherever lead shot usage overlaps with at risk species' feeding areas. There is recent evidence illustrating this in wildfowl in the UK, including an analysis indicating no change in lead-related mortality of wildfowl in England following the introduction of restrictions on use of lead shot. Risks are currently considered to be greatest, in descending order, in: wildfowl (both grazing and non-grazing); other waterbirds including cranes, rails and waders; terrestrial gamebirds; other avian taxa. Were current restrictions on the use of lead gunshot in the UK adhered to, the risks would likely be highest to grazing wildfowl, followed by the other groups mentioned.

Field studies comparing wildfowl that have or have not ingested shot have found reduced survival in birds that have ingested shot. The magnitude of effects is likely to be underestimated due to turnover of lead in the intestine.

As lead can affect both breeding productivity and survival it has the potential to affect population size and growth or decline rates. The extent of lead exposure in some species suggests that it may potentially be having effects upon population size, although the requisite studies have not been done to confirm this. It should be noted, however, that the ban on most sizes of lead fishing weights in 1987 in the UK is thought to have contributed to the increase in population of mute swans *Cygnus olor* in the Thames Valley. The extent to which lead poisoning mortality in wildfowl may be compensated for by other factors affecting survival is unknown. It is estimated that at least hundreds of thousands and possibly more than a million waterbirds alone may die of lead poisoning as a result of shot ingestion every winter in Europe. It would be difficult to obtain more precise estimates as many factors, such as weather and food availability, are likely to influence the level of annual mortality resulting from a range of diseases including lead poisoning.

In areas of high lead bullet usage such as firing ranges, lead bullets, or fragments thereof, will accumulate within the environment. No studies were found detailing the extent to which bullet deposition results from typical UK live quarry shooting, although bullet densities resulting from this activity may generally be low.

There is an absence of studies evaluating the potential for wildlife to consume deposited lead bullets, or fragments thereof, directly from the environment. While there is an evident risk of this occurring in areas of high bullet use, such as firing ranges, there is a low probability that this occurs at any great frequency for any wild animals away from areas of high bullet use, in the UK or elsewhere.

Linkage Set B: Secondary poisoning in predatory and scavenging animals from ingestion of lead ammunition contained within prey or carrion

Whole gunshot pellets and fragments of pellets and bullets are frequently present in the tissues of most game animals killed using lead ammunition. The number and mass of lead fragments can be substantial and widely distributed in the carcass. Substantial numbers of small fragments frequently occur in the abdominal viscera of deer killed using lead bullets; viscera are often discarded and potentially available to scavengers.

Research studies show that a substantial proportion of wildfowl and likely other game animals are wounded but survive and that the lead fragments they contain are a potential source of dietary lead exposure in their predators.

The number of animals, or parts thereof, shot using lead ammunition which are potentially available to predators and scavengers in the UK is uncertain, but it is likely to be at least hundreds of thousands of individuals per year. The true number might be substantially larger.

Knowledge of the natural history and foraging ecology of birds and mammals and field studies suggest that many species are exposed to dietary lead derived from spent ammunition through feeding on prey animals or scavenging at carcasses or on viscera of hunter-killed deer. This includes several bird and mammal species present in the UK.

Post mortem examinations and studies of regurgitated pellets show that predatory and scavenging birds ingest fragments of metal, including remnants of lead ammunition, present in their food and experimental studies show that ingested metallic lead from ammunition is absorbed into the blood and distributed to other tissues. Evidence that ammunition-derived dietary lead is absorbed into the bloodstream by mammals (humans and pigs) is presented in another risk assessment prepared for the LAG ‘An evaluation of the risks to human health in the UK from lead derived from ammunition’.

Studies of the stable isotope signatures of lead from terrestrial predatory and scavenging birds in the USA and Europe, including the UK, provide strong evidence that the provenance of elevated (above background) lead concentrations in the tissues of these birds is primarily lead from ammunition. Other non-ammunition sources of lead contribute but are usually much less important than ammunition-derived lead. Correlations between blood lead dynamics and hunting seasons and movement patterns of individual birds lead to the same conclusion. The isotope and blood lead information both provide strong evidence that ammunition-derived dietary lead from carrion is absorbed by birds and that its effect on blood lead concentrations is substantial.

We found no available evidence of these types for absorption of significant amounts of ammunition-derived lead by scavenging and predatory mammals in the USA and Europe, but fewer studies addressing this topic are available than there are for birds.

As for waterbirds, there is strong experimental evidence that absorption of lead from ammunition can cause negative impacts on biological functioning and death in predatory and scavenging birds. Although there is individual and interspecific variation, approximate tissue lead thresholds can be identified at which serious health impacts and death becomes progressively more likely.

Studies based on tissue lead concentrations and *post mortem* examinations of predatory and scavenging birds in the USA, Canada and Europe report proportions of deaths from lead-poisoning as 3% to 35% of deaths in species likely to be at risk of ingesting ammunition-derived lead. In Europe 14 – 28% of deaths in white-tailed sea eagles *Haliaeetus albicilla* have been attributed to lead-poisoning. Lead poisoning mortality has been reported in red kites *Milvus milvus* in England. Several other raptors in Britain have been reported with tissue lead concentrations consistent with lead poisoning mortality but some of the species most likely to be susceptible have not been investigated. Insufficient studies have been conducted to draw reliable conclusions about the proportion of predatory or scavenging birds dying from lead poisoning in the UK.

Much less is known about potential impacts of lead on predatory and scavenging mammals, but the few studies available do not indicate large effects.

Quantitative assessment of the impact of lead poisoning on population trends of predatory and scavenging animals has only been attempted for a small number of bird species. In the California condor there is strong evidence that mortality from lead poisoning is sufficient to prevent population recovery and would lead to extinction of the wild populations if remedial measures and releases of captive-bred birds were to cease. In Japan, the Hokkaido population of Steller's sea eagles, was predicted to decline due to adult lead poisoning-related mortality without intervention. In Germany the white-tailed sea eagle population trend is likely to remain positive at recent levels of lead-poisoning mortality but would increase more rapidly in the absence of lead poisoning.

The proportion of all deaths attributed to lead poisoning is the most readily available measure of additional mortality caused by lead contamination. Results of this kind from North America and Europe indicate substantial effects on population processes in some cases but, except in the case of the California condor, the upper limit of additional mortality caused by lead which would lead to extinction was not exceeded. In the UK, the proportions of deaths of scavenging and predatory birds attributed to lead poisoning measured so far do not approach the upper limit. However, it should be noted that the data available are sparse or missing for several of the species in the UK most likely to be affected and that which is available does not adequately cover regions where ingestion of ammunition-derived lead is most likely.

The risk assessment found that this Linkage Set was connected to all the other Linkage Sets as raptors and scavengers are exposed to ammunition-derived lead which has found its way into their prey or carrion by a range of pathways.

Linkage Set C: Poisoning of wildlife from ammunition-derived lead that has degraded and entered soil and water and/or become incorporated into biota

Numerous studies show that where shooting occurs regularly and/or at high intensity, both in and possibly close to the shot fallout areas, soil lead levels will generally be elevated, slightly to thousands of times, above those in control soils. The form of the transformed lead and its mobility and distribution through the soil profile will depend largely upon soil chemistry. The extent of soil contamination will increase as shooting with lead shot continues over time.

Fewer studies are available either measuring lead in water from sites contaminated with ammunition-derived lead, or lead in biota exposed to water contaminated by lead from ammunition.

Those studies available provide evidence that in some areas of high shot deposition water lead concentrations may be elevated above those at control sites. The extent to which such contamination is likely to affect sites downstream of shooting areas is unknown. It seems likely that the majority of water contamination will be relatively local but there may be a potential groundwater pollution risk in areas of high lead ammunition deposition.

The available studies provide strong evidence that some of the lead from deposited ammunition is available for plant uptake and in areas of high deposition there is a high probability that plant lead levels will be elevated, from slightly to many hundreds of times, above those in plants from control soils. In addition to lead shot densities, the degree of elevation may vary with the part of the plant analysed, the plant species, the soil type, soil pH and other factors.

Similarly, where levels of soil, water and/or biota are elevated as a result of the degradation of lead from ammunition, there is good evidence that there is likely to be uptake of lead by certain invertebrates and vertebrates. The extent to which lead uptake results from the ingestion of, or exposure to, contaminated soil and water, or to the ingestion of contaminated plants or lower animals, is not generally known. Lead from ammunition can bioaccumulate/bioconcentrate in aquatic and terrestrial foodchains, but with no evidence of biomagnification.

Where invertebrate and vertebrate animals are exposed to (and absorb) elevated levels of lead of ammunition origin in soil, water or biota, there is a high probability that lead will exert sub-lethal negative effects on the physiology (i.e. both welfare and individual survival) of many species, and in some animals may cause mortality. The literature also suggests the possibility that some invertebrates may potentially develop a high level of tolerance to lead, and one study suggested that while lead may affect the functioning of many components of a forest ecosystem, the ecosystem itself was very resilient.

The studies also provide strong evidence that local populations of certain invertebrates can be negatively affected by exposure to elevated levels of lead from ammunition. There is potential for contaminated wildlife (e.g. from earthworms to small mammals and passerines) to be consumed by scavengers and predators, including raptors, and for this to present a local risk to these groups that is additional to those described in Linkage Set B.

Linkage Set D: Primary poisoning from direct absorption of lead from embedded lead ammunition

There is strong evidence that lead ammunition becomes embedded in the tissues of animals that have been shot but not killed, with uncertainty about whether this causes increases in tissue lead levels (studies from humans provide limited evidence that this may happen but their transferability to many species of wildlife is uncertain). It is also likely that embedded lead ammunition affects survival, although studies do not discriminate between the impacts of the wounding itself or the lead.

Table 1 summarises these overall conclusions and the strength of evidence for different links in the Source-Pathway-Receptor linkage sets identified, and the strength of evidence for effects on welfare, survival, morbidity/mortality and population level effects overall. Table 1 should be considered alongside Figures A.2, B.1, C.1 and D.1, presented at the end of each section on Linkage sets A-D. These figures provide overall evidence and impact scores but specifically take into account the UK context. For example, there is **strong** evidence that ingestion of lead of ammunition origin from scavenged food could result in population level effects in the California condor and several other species across the world – as indicated in Table 1. However, while the evidence for this pathway is established in this risk assessment, and therefore it could potentially apply to species in the UK, there is currently only **limited** evidence to suggest that **some** impacts on population level processes may potentially be occurring to predatory or scavenging species in the UK (as indicated in Figure B.1). This is also identified as an information gap elsewhere in the conclusions. The impact scores in the figures provide a semi-quantitative/semi-qualitative assessment of the effects and numbers of birds/other animals likely to be affected or potentially affected in the UK. The figures also, where appropriate, provide relative impact and evidence scores for different taxa.

Table 1. Overall conclusions for all four Source-Receptor-Pathway Linkage Sets describing risk to wildlife from ammunition-derived lead

Linkage Set Section	Section No.	Nature of Evidence	Evidence	Impact	Comments
Linkage Set A: Primary poisoning from direct ingestion of lead ammunition from the environment					
WILD BIRDS					
Evidence for widespread lead shot deposition in the UK	A.1.1.1 A.1.1.2	Amount of shot used annually Areas of the UK used for shooting	Strong		Two thirds of the UK's rural land is managed by shooting providers for a combination of reasons including shooting; 13% of the UK's rural land specifically managed for shooting activities. An estimated 5,500-12,900 tonnes of shot (68-154 billion individual shot) are deposited into the environment annually (excluding shot used for pest control and mammal shooting e.g. used for killing rabbits). The majority of this is likely to be lead.
Evidence that deposited lead shot in the environment is available to wildlife	A.1.1.3 C.1.1.2	Assessment of shot densities in the environment	Strong		Shot deposited in the environment is relatively stable under most environmental conditions but will slowly degrade as a result of physical and chemical processes. It is assumed that complete degradation may take tens to hundreds of years. Shot settles through soils and sediments over time and it may take several decades for deposited shot to become unavailable to feeding wildlife.
Evidence for direct shot lead ingestion by birds	A.2.1	Shot in the intestines of birds found dead or killed by hunters	Strong		Shot ingestion has been reported in a range of terrestrial and waterbirds, including species from the following taxa: Anseriformes, Gruiformes, Charadriformes, Galliformes, Columbiformes, Pelicaniformes and Phoenicopteriformes.

Linkage Set Section	Section No.	Nature of Evidence	Evidence	Impact	Comments
Evidence to link lead shot ingestion to elevated tissue lead concentrations	A.2.1.3	Experimental studies on captive birds Stable isotope analyses Associations between tissue lead levels & spatial & temporal variation in exposure to lead shot- contaminated food	Strong		Many factors influence lead retention and absorption (A.3) and numerous experimental studies have been undertaken linking shot ingestion to tissue lead concentrations. Field studies indicate that, where shooting occurs, the ingestion of lead shot by waterbirds and terrestrial birds is the primary cause of widespread elevated tissue lead concentrations.
Evidence of welfare impacts resulting from direct lead shot ingestion	A.3.2.1	Experimental studies of sub-lethal impact on captive birds dosed with lead shot and/or other forms of lead Field studies relating blood lead concentrations to physiological effects	Strong	Great	From the known pathology and physiology of the poisoning plus its effect on coordination and mobility, there is high probability of welfare impacts, which may be of a serious nature, in the majority of birds that ingest lead shot and do not eliminate it rapidly. The many sub-lethal effects include those on blood enzymes, productivity, immunology and behaviour. Large numbers of some taxa and species are likely to be affected. For example, 25% of wildfowl trapped in England and Scotland (winter of 2010-11) had blood lead levels elevated to the sub-clinical level (and 9% of birds had higher blood lead levels), with lead shot ingestion the most likely lead source.
Evidence of reduced survival in free living birds resulting from direct lead shot ingestion	A.3.2.1	Field studies relating increased blood lead levels to reduced survival	Strong	Great	Field studies show reduced survival of wildfowl following shot ingestion. Sub-lethal effects may subsequently contribute to reduced survival and premature death from other causes such as flying accidents (collisions), susceptibility to being shot, susceptibility to infectious disease and starvation etc. No studies were found on other avian taxa but there is no reason to suspect that effects on survival will be different in nature, although they may be in extent.

Linkage Set Section	Section No.	Nature of Evidence	Evidence	Impact	Comments
Evidence of morbidity and mortality resulting from direct lead shot ingestion	A.3.2.2	Experimental studies with captive birds dosed with lead shot under varying conditions Field studies where lead poisoning mortality from shot ingestion has been diagnosed	Strong	Great	Large numbers of experimental and field studies conducted over the last 60 years in many avian species. Under some circumstances ingestion of one or two lead shot is sufficient to kill a duck. Lead poisoning was diagnosed as responsible for deaths of 10.6% of 2,365 wildfowl of 14 species found dead in Britain between 1971 and 2010 with no reduction in incidence in England after the introduction of restrictive regulations (likely due to known poor compliance).
Evidence indicating the scale of poisoning and mortality	A.3.2.3	Projected mortality based on levels of shot ingestion (largely in hunter-killed birds) by different wildfowl and probable outcomes (based on experimental studies)	Strong Imprecise estimates	Great	It is estimated that at least hundreds of thousands and possibly more than a million waterbirds alone may die of lead poisoning as a result of shot ingestion every winter in Europe. It would be difficult to obtain more precise estimates as many factors, such as weather and food availability, are likely to influence the level of annual mortality resulting from a range of diseases including lead poisoning. No estimates of magnitude of effect are available for other avian taxa but these are likely to be proportionately lower as levels of shot ingestion tend to be lower. No current UK specific mortality estimates are available, and (given that some of the species affected are protected from shooting) specific methods would have to be employed to obtain estimates for some species.
Evidence of lead shot ingestion affecting population size or growth/decline rates	A.3.2.3	Few studies available	Lack of studies	Some	Lead can affect both breeding productivity and survival thus has the potential to affect population size and growth/ decline rates. The extent of lead exposure in some species (see above) suggests that it may potentially be having effects upon population size, although the requisite studies have not been done to confirm this. The extent to which lead poisoning mortality in wildfowl may be compensated for by other factors affecting survival is unknown. After a ban in 1987 on the importation and sale of lead angler's weights (of 0.06 - 26.5g) in the UK a sharp reduction in most areas in the numbers of mute swans dying or becoming sick from lead poisoning was reported along with a subsequent increase in the species' population.

Linkage Set Section	Section No.	Nature of Evidence	Evidence	Impact	Comments
OTHER WILDLIFE					
	A.2.2 A.3.3		No studies Generally low probability	Unknown	Consumption of spent lead shot by non-avian wildlife has received little research attention. We cannot therefore evaluate the probability that this occurs to any great extent, but judge that it is likely to be relatively low based upon the feeding habits of non-avian wildlife, perhaps with the exception of wild deer feeding in and around areas of high lead shot usage.
Linkage Set B: Secondary poisoning in predatory and scavenging animals from ingestion of lead ammunition contained within prey or carrion					
WILD BIRDS					
Evidence that large numbers of game animals are shot with lead ammunition in the UK	B.1.1	Estimates from shooting and market research organisations of numbers of different game species shot in the UK annually using lead ammunition	Strong		Estimates indicate that tens of millions of animals (c. 30+ million) are shot in the UK annually using lead ammunition.

Linkage Set Section	Section No.	Nature of Evidence	Evidence	Impact	Comments
Evidence of the presence of shot-in projectiles, and fragments thereof, in the tissues of game animals killed or wounded using lead shot and bullets	B.1.2	X-radiography of the carcasses of both large game animals shot with lead bullets and small game animals shot with lead gunshot in the UK and elsewhere X-radiography of live-trapped wild gamebirds	Strong		Both lead bullets and gunshot fragment to some extent on impact. Relatively intact gunshot/bullets and/or fragments from them are present in the tissues of most large and small game animals shot with lead. The number and mass of lead fragments can be substantial and widely distributed in the carcass. Abdominal viscera of deer, often discarded and thus potentially available to scavengers, frequently contain lead fragments. A substantial proportion of wildfowl (from a few percent to several tens of percents in live trapped wildfowl), and likely other game animals are wounded but survive, carry lead shot and/or fragments from shot, providing a potential source of exposure to dietary lead in predators which subsequently kill and eat them. The proportion of animals, or parts thereof, shot using lead ammunition, which are potentially available to predators and scavengers is unknown for some species but may be high for quarry species such as ducks and geese, and likely represents hundreds of thousands of animals potentially contaminated with ammunition-derived lead per year entering the food supply of wild predators and scavengers. The true number may be substantially higher.
Direct evidence concerning the ingestion of ammunition-derived lead projectiles and fragments by scavenging and predatory animals	B.2.1 B.2.2	*Post mortem* examinations Field investigations of presence of lead shot in regurgitated pellets Feeding experiment on captive birds	Strong		Foraging ecology suggests that many species, including some in the UK, could be exposed to dietary lead derived from spent ammunition thorough feeding on prey animals or scavenging at carcasses or on viscera of hunter-killed deer. Field and experimental evidence shows that predatory and scavenging birds ingest fragments of metal, including remnants of lead ammunition, present in their food.

Linkage Set Section	Section No.	Nature of Evidence	Evidence	Impact	Comments
Evidence that lead derived from ingested ammunition sources is absorbed by scavengers and predators	B.2.3.1 B.2.3.2	Experimental studies on captive birds Analyses of stable isotopes of lead Associations between tissue lead levels and temporal variation in exposure to lead shot contaminated food	Strong		Studies of the stable isotope signatures of lead from terrestrial predatory and scavenging birds in the USA and Europe, including UK, indicate that, where tissue lead concentrations are elevated, the provenance of lead is most often lead from ammunition. Other non-ammunition sources of lead contribute but are usually much less important than ammunition-derived lead. Correlations between blood lead dynamics and hunting seasons and movement patterns of individual birds lead to the same conclusion.
Evidence of significant welfare impacts resulting from indirect lead shot ingestion	A.3.1 A.3.2 B.3.1	Experimental studies on captive birds dosed with lead shot and/or other forms of lead	Strong	Great	Experimental studies show that the effects of ingested lead shot are similar in predatory and scavenging birds to those in other wild birds. From the known pathology and physiology of the poisoning plus its effect on coordination and mobility, there is high probability of welfare impacts, which may be of a serious nature, in the majority of birds that ingest lead shot and do not eliminate it rapidly. The many sub-lethal effects include those on blood enzymes, productivity, immunology and behaviour.
Evidence that lead absorption affects functioning and survival in predatory and scavenging birds and that elevated tissue lead concentrations can be used as indicators of effect	B.3.2	Experimental studies of sub-lethal impact on captive birds dosed with lead shot and/or other forms of lead Field studies relating blood lead concentrations to probability of survival	Strong	Great	Although experiments and measurements of tissue lead and *post mortem* examinations of carcasses show that there is variation among species and individuals, approximate tissue concentrations of lead can be identified at which the risk of serious loss of function and death becomes progressively more likely. Two independent studies of free-flying California condors indicate approximate agreement between risk indicators based upon blood lead concentration and observed deaths.

Linkage Set Section	Section No.	Nature of Evidence	Evidence	Impact	Comments
Evidence of morbidity and mortality in predators and scavengers resulting from indirect ingestion of lead from ammunition	B.3.2 B.3.3	Experimental studies with captive birds dosed with lead shot under varying conditions Field studies where lead poisoning mortality from shot ingestion has been diagnosed using tissue concentrations and clinical signs	Strong Insufficient studies in the UK of at-risk species	Great Insufficient studies in the UK of at-risk species	Studies in the USA, Canada and Europe report proportions of deaths caused by lead in species likely to be at risk of ingesting ammunition-derived lead ranging from 3% of deaths to 35% of deaths. In Europe the bird species with the most consistently high proportions of deaths attributed to lead poisoning is the white-tailed sea eagle (14 – 28% of deaths attributed to effects of lead). In the UK there is evidence of death being caused by lead poisoning in several red kites in England, and individual buzzards and peregrines collected in Britain had tissue concentrations of lead consistent with death being caused by lead toxicosis. However, a lack of studies on several species potentially at risk (e.g. white-tailed sea eagle, golden eagle and western marsh harrier) means that no reliable conclusions can be drawn about the proportion of birds dying from lead poisoning in the UK.
Evidence of indirect ingestion of lead from ammunition affecting population size or growth/decline rates in predatory and scavenging birds	B.3.4	Field studies Population modelling	Strong Insufficient studies in the UK of at-risk species	Some Insufficient studies in the UK of at-risk species	Quantitative assessment of the impact of lead poisoning on population trends of predatory and scavenging animals has only been attempted for small number of bird species. In the California condor there is strong evidence that mortality from lead poisoning is sufficient to prevent population recovery and would lead to extinction of the wild populations if remedial measures and releases of captive-bred birds were to cease. Lead poisoning was considered to be major factor in the predicted population decline of Steller's sea eagles in Hokkaido. With the maintenance of recent levels of lead poisoning, the population trend of white-tailed sea eagles in Germany is likely to remain positive but grow more slowly than it would in the absence of lead poisoning. Results suggest substantial effects on population processes in some cases. In the UK, the proportions of deaths of scavenging and predatory birds attributed to lead poisoning measured so far are unlikely to result in population declines and extinction. However the data available are sparse or missing for several species in the UK and that which is available does not adequately cover regions where ingestion of ammunition-derived lead is most likely.

Linkage Set Section	Section No.	Nature of Evidence	Evidence	Impact	Comments
OTHER WILDLIFE					
	B.2.3.2	Limited number of field studies	Few studies No evidence of effects	No evidence of impacts	We found no available evidence for absorption of significant amounts of ammunition-derived lead by scavenging and predatory mammals in the USA and Europe, but far fewer studies addressing this topic are available than there are for birds.
Linkage Set C: Poisoning of wildlife from ammunition-derived lead that has degraded and entered soil and water and/or become incorporated into biota					
Evidence that lead from spent gunshot and bullets degrades resulting in elevated lead concentrations in soil and water.	C.1.1.1 C.1.1.2	Experimental studies Field studies under a variety of different soil and other conditions	Strong for soil Limited studies or evidence for water		Under most environmental conditions lead ammunition degrades slowly. Chemical and physical processes affect degradation rates. A high proportion of lead is generally retained in the upper soil layers. In areas of high deposition of lead ammunition soil lead levels will generally be elevated, slightly to thousands of times, above those in control soils. In some areas of high shot deposition water lead concentrations may be elevated above those at control sites, but the majority of water contamination may be relatively local. Potential groundwater pollution risk in areas of high lead ammunition deposition cannot be discounted but the level of risk is unknown.
Evidence of uptake of lead by plants from water and soils contaminated with lead from ammunition	C.2.1	Experimental studies (using soil from areas of high lead ammunition deposition) Field studies	Strong		Some of the lead from deposited ammunition is generally available for plant uptake and in areas of high deposition there is a high probability that plant lead levels will be elevated, from slightly to many hundreds of times, above those in plants from control soils. The degree of elevation may vary with the part of the plant analysed, the plant species, the soil type, soil pH and other factors. Both roots and leaves can have elevated lead concentrations, but lead levels are generally highest in roots.

Linkage Set Section	Section No.	Nature of Evidence	Evidence	Impact	Comments
Evidence of uptake of lead by animals from soil, water and biota contaminated by lead ammunition	C.2.2	Field studies Experimental field studies (where animals are kept in cages or aviaries in the field)	Strong		Where levels of soil, water and/or biota are elevated as a result of the degradation of lead from ammunition, there is good evidence that there is likely to be uptake of lead by certain invertebrates and vertebrates. The extent to which lead uptake results from the ingestion of or exposure to contaminated soil and water, or to the ingestion of contaminated plants or lower animals, is not generally known. Lead from ammunition can bioaccumulate/bioconcentrate in aquatic and terrestrial foodchains, but with no evidence of biomagnification.
Evidence of wild animals being adversely affected by the hazard and the risks of impacts (welfare, survival, direct mortality)	C.3	Experimental studies Field studies Predictive modelling	Strong	Moderate	Where invertebrate and vertebrate animals are exposed to elevated levels of lead of ammunition origin in soil, water or biota, there is a high probability that lead will exert sub-lethal negative effects on the physiology (i.e. both welfare and individual survival) of many species, and in some animals may cause mortality. There is limited evidence that some invertebrates may develop a high level of tolerance to lead while local populations of others may be negatively affected. Contaminated wildlife (earthworms to small mammals and passerines) may present a potential local risk to scavengers and predators.
Extent of contamination					Significant contamination is most likely in those areas that are heavily or regularly shot over, such as shooting ranges and shooting blinds. In areas that are less frequently or lightly shot over any contamination is likely to be proportionately lower, but will increase over time as lead continues to be deposited, as soil is the environmental sink for lead.

Linkage Set Section	Section No.	Nature of Evidence	Evidence	Impact	Comments
Linkage Set D: Primary poisoning from direct absorption of lead from embedded lead ammunition					
Evidence that lead ammunition becomes embedded in tissues of animals.	D.1 B.1.2	X-radiographic studies	Strong		There is strong evidence that lead ammunition becomes embedded in the tissues of animals that have been shot but not killed.
Evidence of primary poisoning from direct absorption of lead from embedded lead ammunition	D.3	Field studies and survival analyses	Few studies Little evidence	Unknown	There is uncertainty about whether this causes increases in tissue lead levels (studies from humans provide limited evidence that this may happen but their transferability to many species of wildlife is uncertain). It is also likely that embedded lead ammunition affects survival, although studies do not discriminate between the impacts of the wounding itself or the lead.

Introduction

Lead is a naturally occurring toxic metal that has been used by humans for centuries, and is consequently widely distributed in the environment. Increasing knowledge of the negative health effects of even low levels of exposure to lead has resulted in society taking many actions to reduce emissions and exposure such as removal from petrol and paint. For example, air quality pollutants inventories for England estimated emissions of lead at 41 tonnes in 2011. This represented a decline of 98% since 1990, dominated by a 1,799 tonne reduction in transport sources due to the phase-out of leaded petrol (Thistlethwaite *et al.* 2013).

Today in the UK, legislative controls and monitoring of industrial, municipal and agricultural lead emissions are such that cases of clinical lead poisoning from these sources in wildlife are likely to be rare occurrences. Nevertheless, levels of exposure in soils from non-ammunition and fishing weight sources are likely to be higher in urban areas and near some centres of current and historical industrial activity, especially metalliferous mining (contaminated soils from such areas of mineralisation represent approximately 2% of the area of England). However, beyond these, the literature does not currently suggest any geographically widespread and common sources of environmental lead contamination to which wildlife is likely to be exposed, beyond lead angler's weights (which appear particularly to be a problem for mute swan populations) and lead ammunition (Supporting Information 1 reviews the reduction in exposure of wildlife to lead from a variety of sources). The contribution of lead from ammunition and the level of risk that this presents to wildlife are dealt with within this risk assessment.

1 Background to the draft Risk Assessment and the procedure adopted

The Lead Ammunition Group (LAG) established under the auspices of Defra and FSA appointed a sub-committee (the Primary Evidence and Risk Assessment Subgroup (PERASG)) to prepare material for consideration by the LAG. The terms of reference of PERASG, agreed in 2009, are as follows:

a. To gather and list sources of evidence for assessing the risks of lead in ammunition under the categories outlined below.

b. To advise on the quality, applicability and therefore inclusion of such evidence for risk assessment.

c. To propose a risk assessment method.

d. To use the proposed evidence sources to prepare an initial risk assessment under the categories outlined below:

    1. Risks to wildlife from ingested lead from ammunition. This will include welfare considerations, individual and population level risks.

    2. Risks to human health from the ingestion of lead from ammunition. This will include both risks associated with the ingestion of lead gunshot/bullets or fragments thereof in game animals, and the ingestion of animals that have themselves ingested and assimilated lead from ammunition. (It may also include any other perceived risks arising from lead ammunition).

    3. Risks to human health through livestock feeding in areas of lead shot deposition. This will include risks from lead deposited through inland shooting, including clay pigeon and other target shooting.

In preparing this risk assessment use has been made of the publication “Green Leaves III Guidelines for Environmental Risk Assessment and Management” published by Defra and Cranfield University in November 2011 (Defra 2011). The general approach proposed in this document has guided the design of this risk assessment. However, it should be noted that the terms of reference specified by the LAG for PERASG do not include the evaluation of options for reducing risk, which are the subject of chapters 4 - 6 of these Defra guidelines (Defra 2011). Hence, this risk assessment focuses only on the subject matter of chapters 2 and 3 of the guidelines (Defra 2011), which are formulating the problem and assessing the risk.

The approach to risk assessment adopted here requires the evaluation of scientific studies of the numerous steps in the Source-Pathway-Receptor (S-P-R) linkages relevant to the problem. Hence, evaluation of previous studies which have conducted a complete risk assessment for effects of ammunition-derived lead on wildlife health, welfare and populations only forms a small part of the assessment presented herein. Instead, this assessment assembles and examines the evidence available for each of the many steps connecting the use of lead ammunition in shooting to potential risks to wildlife. Only a small part of a particular study might be used in doing this, and several parts of a study might be used in evaluating disparate steps in an S-P-R linkage set. Where evidence is referred to in this assessment, it is considered sufficiently reliable for the use to which it is put. Significant limitations or caveats on the use of a particular piece of evidence for a given purpose are mentioned as they occur. Use of the Klimisch approach (Klimisch *et al.* 1997) to the evaluation of the reliability of toxicological data was considered, but was judged to be inappropriate for general use in this risk assessment. This Klimisch approach involves scoring the reliability of whole studies on a four point scale, but it was not used here because the reliability of an entire study was rarely relevant to this risk assessment. For this reason, Klimisch scores have not been assigned to any of the studies cited. However, the relevance and reliability of the *elements* of the studies cited are examined in the text. All the study elements used to draw conclusions here may be regarded as having Klimisch Reliability Score 1 (reliable without restriction) or Klimisch Reliability Score 2 (reliable well-documented study with acceptable restrictions).

With respect to the breadth of literature evaluated, we note that the toxicity of lead, the relationships between lead concentrations in soil, water and biota and the toxicological effects of lead on biota respect no national, political or other administrative boundaries. Rather, lead concentrations and toxicological risks are related to a range of physical, chemical and biological factors which may vary considerably between, and even within, sites and species. Such factors are described within this risk assessment. Global literature is both relevant to an evaluation of risks from lead from ammunition in the UK and essential in helping understand the breadth of the risks and variables that may affect them. Where relevant factors exist that affect risks in an England or UK specific context, e.g. amounts of shot deposited in the environment and numbers of birds and other animals shot annually, these have been made explicit.

2 Formulation of the problem

2.1 Framing the question

Based upon the categories set out in the terms of reference of the PERASG, the relevant question for this risk assessment is:

"What are the risks to wildlife from ingested lead from ammunition, including welfare considerations, individual and population level risks?"

Quy (2010) recently reviewed the evidence concerning the contamination of wildlife and the environment arising from the use of lead ammunition in a report to Defra. Although the approach taken was different to the approach taken herein, much of the same evidence is reviewed.

2.2 Development of a conceptual model

Following Defra (2011), a conceptual model of the issues considered in the risk assessment was developed. This includes the identification of Source-Pathway-Receptor linkages which describe the route by which the potential hazard from ammunition-derived lead to the population processes and welfare of wildlife might arise. The intention of this is to represent the scope of the problem, clarify assumptions about its underlying mechanisms, and set the boundaries of the risk assessment.

Based upon scientific literature, which is described and evaluated in Sections A, B, C and D of this risk assessment, four plausible pathways by which ammunition-derived lead might affect the population processes and welfare of wildlife were identified:

Linkage Set A: Primary poisoning from direct ingestion of lead ammunition from the environment.

Linkage Set B: Secondary poisoning in predatory and scavenging birds from ingestion of lead ammunition contained within prey or carrion.

Linkage Set C: Poisoning from ingesting lead from ammunition that has degraded and entered soil and water.

Linkage Set D: Primary poisoning from direct absorption of lead from embedded lead ammunition.

These Source-Pathway-Receptor linkage sets are summarised in Figure 1.

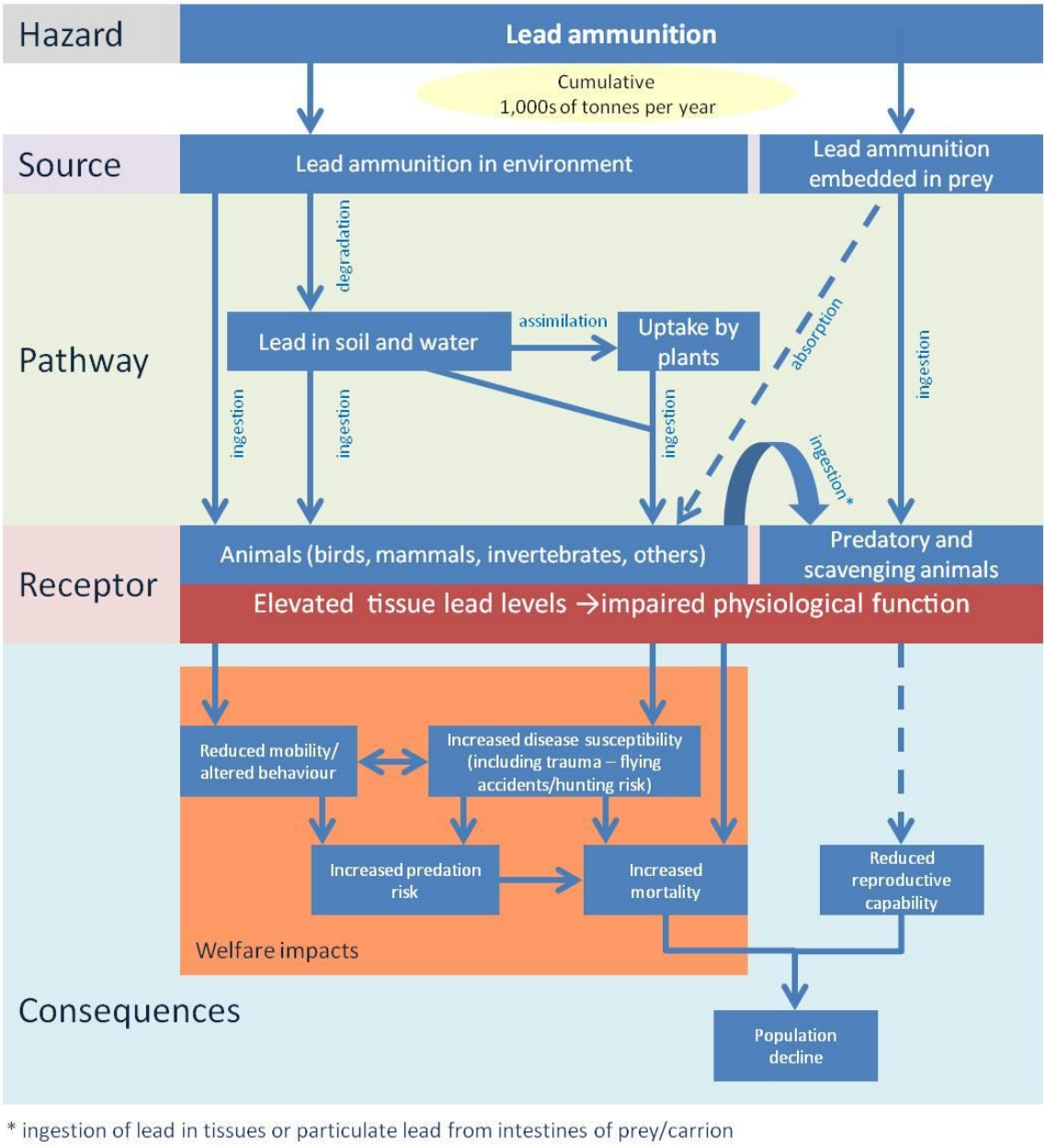


Figure 1. Conceptual model of potential hazard from ammunition-derived lead to wildlife illustrating pathways and consequences with population processes and welfare impacts outlined. This summarises the four main Source-Pathway-Receptor linkage sets.

3 Assessment of the risks

3.1 Approach to the assessment of the S-P-R linkages

Following Defra (2011), the probabilities of the hazard arising, the pathway steps which lead to exposure and the consequences for animal health, welfare and population processes, conditional on exposure, were evaluated at least qualitatively and, wherever possible quantitatively.

The nature and physiological effects of inorganic[98] lead are the same regardless of source. To avoid repetition within each Source-Pathway-Receptor linkage set, these effects of lead on an individual animal are presented in Section A.3.2 and thereafter referred to in subsequent linkage sets. Similarly to avoid repetition, evidence for provenance of lead in cases where elevation of blood or tissue lead concentration is observed is provided in Section A.2.1.3b and referred to thereafter.

[98] See A.3.1 – in this risk assessment the term 'lead' generally refers to inorganic lead except in Supporting Information 1.

Linkage Set A: Primary poisoning from direct ingestion of lead ammunition from the environment

The conceptual model Source-Pathway-Receptor that suggests a hazard to animals from direct ingestion of spent lead ammunition i.e. shot and bullets, and fragments thereof, from the environment is given in Table A.1.

Table A.1. Conceptual model of Source-Pathway-Receptor linkages which might result in lead derived from ammunition being ingested by wildlife directly from the environment and adversely affecting their health or populations.

S-P-R linkage label	Source	Pathway	Receptor
A (i)	Spent lead shot deposited on ground and water	Spent shot is ingested directly from the environment by terrestrial and waterbirds either mistakenly for food items or for grit for those birds with a muscular gizzard.	Adverse effects on the health of the animals from effects of absorbed dietary lead acting on the functioning of various organ systems and potentially leading to reduced welfare, death and impacts on population size.
A (ii)	Spent lead shot deposited on ground and water	Non-avian animals ingest shot from the environment whilst feeding, either intentionally or accidentally along with other foodstuffs such as during grazing by animals such as ungulates.	
A (iii)	Spent lead bullets, or fragments thereof, deposited on ground and water	Any animals ingest bullets, or fragments or particles thereof, directly from the environment either inadvertently or mistakenly for food items or for grit.	

A.1 Source: Evaluating the probability that lead shot and bullets, and fragments thereof, accumulate and are available within the environment

A.1.1 Lead shot densities in the environment and its availability to wildlife

A.1.1.1 Lead shot densities in the environment

Each lead shotgun cartridge may contain between 100 and 600 lead pellets depending on shot size, with a typical 30g load containing approximately 300 individual number 6 pellets. As pellets leave the barrel of the gun they spread out thus even if the target is hit, many pellets will miss. Only a small proportion of the pellets from a single shotgun cartridge may be retrieved within a killed animal (see e.g. Cromie *et al*. 2010, Pain *et al*. 2010 for number of pellets found in shot quarry). Thus, it is clear that the vast majority of lead shot fired from shotguns falls irretrievably into the environment.

The majority of studies of environmental contamination from lead shot focus on shot density on ground used primarily for shooting, such as clay pigeon or target shooting ranges, intensively reared game bird shooting estates or regularly used blinds. The available information supports the

trend that one would reasonably expect in showing that shot densities tend to be highest in these areas of intense and/or regular hunting/shooting pressure.

At a clay pigeon shoot in Lancashire, which had been active for some 20 years, Mellor and McCartney (1994) found up to 257 shot/m^2 in the top 15cm of soil within the highest pellet deposition zone, with pellet numbers dropping off away from this zone. Studies at two Dutch clay shooting ranges found shot densities of 400 and 2195 shot/m^2 (Smit *et al*. 1988).

Petersen and Meltofte (1979) report four Danish shooting ranges located near shallow water which had shot densities ranging from 44-2045 shot/m^2. O'Halloran *et al*. (1988) report shot density in the vicinity of a clay pigeon shooting range in Lough Neagh, County Antrim, containing 2400 shot/m^2 in the upper 5cm of shoreline in front of the range and with shot being retrieved up to 60m out on the lake bed.

Densities of shot resulting from live quarry shooting rather than clay pigeon shooting are, not surprisingly, greatest where shooting is most concentrated (Stansley *et al.* 1992). As well as scale, the method of the activity will determine the density of shot deposited in the local environment (Mateo 2009). In the USA high densities of shot have been reported (e.g. 860,000 per hectare) at the end of the shooting season in areas where mourning doves *Zenaida macroura* are intensively hunted (Franson *et al*. 2009, Kendall *et al*. 1996).

Of the few published studies of lead shot densities on European shooting estates (other than wetlands), Ferrandis *et al*. (2008) reported lead shot densities on a Spanish arable estate where red-legged partridges *Alectoris rufa* had been shot since the 1950s.The ground was only lightly shot over, e.g. there was a maximum of only two days shooting per season in 2004 and 2006 and no shooting in 2005. On shooting days there was a maximum of 16 guns set at 40m intervals. Shot density was 7.4 shot/m^2 in the top 1cm of soil, in front of the shooting lines, which equated to 8.1kg lead/hectare of number 7 shot.

The paucity of data on shot densities for non-wetland live quarry shooting areas in Europe is confirmed by Mateo (2009) and Bana (2004). With respect to live quarry shooting in wetlands, there have been a number of studies of density of lead shot within the environment in European countries (e.g. summarised in Mateo 2009). Some 20 years ago it was estimated that approximately 4000 tonnes of lead shot was deposited into European wetlands annually (Pain 1992). As discussed, both the method and scale of live quarry shooting will determine density of shot deposited within the environment (Mateo 2009). As an example, high densities of shot have been found within southern European wetlands (up to 399 shot/m^2 within the top 30cm) where hunting pressure is high and habitats for waterbirds relatively restricted. In comparison, northern European wetlands may often have lower densities e.g. fewer than 100 shot/m^2 within the top 20cm (Mateo 2009).

Mudge (1984) reported between 10 and 50 shot/m^2 in UK wetlands in the 1980s (see Table A.2 adapted from Mateo 2009). Within the UK, flight ponds, to which ducks are attracted by supplemental feed, are heavily shot over and thus can accumulate high densities of shot (Thomas 1982).

Table A.2 summarises findings of shot densities associated with different types of shooting activity from the above and some other studies. This is not a comprehensive list, but gives an indication of the range of densities one might expect to find. As would be expected, these vary considerably.

Table A.2. Shot densities in the environment originating from clay pigeon shooting and live quarry shooting.

		Country	Area	Depth of soil measured (cm)	Year	Shot/m^2 (range or max)	Reference
Clay pigeon shooting		UK	Lancashire	15	1994	257	Mellor & McCartney (1994)
		Netherlands				400-2195	Smit *et al.* (1988)
		Denmark				44-2045	Peterson & Meltofte (1979)
		Northern Ireland	Lough Neagh, County Antrim	5	1998	2400	O'Halloran *et al.* (1988)
Live quarry shooting	Wetlands	UK	Gloucestershire	15	1981-82	3-30	Mudge (1984)
			Loch of Strathbeg	15	1981-82	2-10	Mudge (1984)
		Denmark	Western Jutland	20	1978	0-36	Peterson & Meltofte (1979)
			Ringkøbing Fjord	20	1978	12-184	Peterson & Meltofte (1979)
		France	Carmargue	15-20	1987	6-200	Pain (1991)
		Spain	Ebro Delta	20	1991-96	6-266	Guitart *et al.* (1994), Mateo *et al.* (1997), Mateo *et al.* (1998)
			Sadiz-Sevilla	10-30	2001-02	12-399	Mateo & Taggart (2007)
			Guadalquivir M.	10-20	1993-2002	7-25	Mateo (1998, 2000), Mateo & Taggart (2007)
	Terrestrial	USA	Eddy Co., New Mexico	1.3	1987	17-86	Best *et al.* (1992)
		Spain	Arable farm, central-southeastern Spain	1	2004-06	59-96	Ferrandis *et al*. (2008)

A.1.1.2 Scale of shooting in the UK and tonnage of shot involved

At the time of writing no official figures exist for tonnage of lead ammunition used annually in the UK.

The shooting of live quarry, clays and other target shooting are popular sports in the UK. To help appreciate the extent of ammunition usage in the UK, of which the majority will be lead, a reasonably recent assessment of the scale of sporting shooting activity within the UK is provided by a report by Public and Corporate Economic Consultants (PACEC 2006). This "PACEC Report" found that some two thirds of the rural land in the UK was managed by shooting providers for a combination of reasons including shooting. Of this 15 million hectares, 2 million (13% of the UK's rural land (8% of the total land area)) is specifically managed for shooting activities. Many areas not managed specifically for shooting activities are nonetheless shot over for sport shooting, subsistence hunting and/or the control of animals (e.g. pigeons and corvids), including farmland and the foreshore.

For target shooting, including clay pigeon shooting, the vast majority of the ammunition used is likely to be lead. This is probably because the International Shooting Sports Federation (ISSF) rules currently prevent the adoption of non-toxic alternatives (Thomas and Guitart 2013). In 1991, it was reported that 220 million clay pigeons were released in the UK with at least one shot fired at each (B Carter, Clay Pigeon Shooting Association, *pers. comm*., cited in Mellor and McCartney 1994). With a 28g load commonly used to shoot clays and a number 8 cartridge (containing approximately 400 shot) this represented a minimum annual release of 6,160 tonnes of lead shot (approximately 88 billion individual shot) at the time, with a predicted rise in the popularity of clay shooting.

The total annual number of cartridges used in clay shooting in the UK has been recently estimated at some 109 million (Harradine and Leake 2013). On the basis of a mean shot load 28g per cartridge, this suggests that some 3,050 tons of lead shot (approximately 44 billion) pellets are fired at clay targets per annum. There are no available data on the area over which the lead from these cartridges is deposited (Harradine and Leake 2013).

For live quarry shooting, the vast majority of the ammunition used is likely to be lead. A small, but unknown proportion will be non-lead (see below). There are no official estimates of numbers of shooting participants, numbers of birds or other animals shot, or cartridges fired. However, broad estimates can be made using some published (e.g. PACEC 2006) and unpublished sources (e.g. shooting media, web articles and social media).

Relatively intensive game and other bird shooting (pheasant *Phasianus colchicus*, partridge and woodpigeon *Columba palumbus*) is likely to take place over some 2 million hectares managed specifically for shooting in the UK, with some shooting over much of the 15 million hectares managed for a range of activities including shooting (PACEC 2006). These authors report approximately 19 million gamebirds and wildfowl being shot a year (approximately 79% being pheasants; this figure excludes pigeons). Aebischer (2013) reports higher numbers and using a conservative scaling of these "PACEC" figures (see Supporting Information 2) it is likely that the estimated UK total for 2011 was 21.3 million gamebirds and wildfowl excluding pigeons. PACEC (2006) report 3.6 million pigeons being shot per year, not as part of a job, in 2004 and that 53% of the total number of pigeons shot were killed not as part of a job. Hence, the total number of pigeons shot is estimated as 6.8 million per year. On the basis of using between three and eight cartridges per bird (based on shooting web articles and social media[99] which, for pheasants i.e. the greatest proportion of this total, ranges from ~4 up to (but unusually) 20 cartridges per bird; pigeon shooting which may be broadly comparable; and wildfowling which will use fewer), this total of 28.1 million birds shot annually would account for 84-225 million cartridges used. Using a conservative

[99] http://forums.shootinguk.co.uk/showthread.php?3058-How-many-cartridges, http://forums.pigeonwatch.co.uk/forums/topic/55518-how-many-cartridges-should-i-take-pigeon-shooting, http://www.fieldsportsmagazine.com/Shooting-Instruction/high-wide-and-impossible.html.

30g load per cartridge (32g typically used for pheasant but 30g may be used for pigeons), this equates to between 2,529-6,744 tonnes of shot deposited per annum in the pursuit of avian quarry, or 25-67 billion individual shot. The proportion of this total which is non-lead in nature is not known but, as previously mentioned, most live quarry shot in the UK is shot using lead ammunition, and it is legal to do so for the majority of shooting.

Most of the non-lead shot used is likely to be used for shooting wildfowl or over wetlands. In wetlands, current legislation in the UK (HMSO 1999, 2002a, 2002b, 2003, 2004, 2009) aims to reduce lead shot contamination. In England, lead shot cannot legally be used for shooting wildfowl or over certain wetlands or below the high watermark. However, compliance with these regulations is known to be poor (Cromie *et al*. 2002, 2010). Some 45% of shooters responding to a questionnaire who were legally obliged to use lead (n=558) admitted to not always complying with the law (including one third of coastal wildfowlers (n=130) i.e. who are likely to be shooting within or near wetlands e.g. below the high watermark) (Cromie *et al*. 2010). The same two studies found ~70% of mallards *Anas platyrhynchos* purchased in England as part of game dealer surveys had been shot illegally with lead. Thus lead was still being (illegally) deposited in certain wetlands or used for shooting wildfowl in England.

PACEC (2006) estimate that 1,017,000 ducks and geese were shot in 2004. If an average of three cartridges was used per duck or goose killed this would result in 91.5 tonnes of ammunition a year being used for wildfowl shooting. Of this, we estimate that approximately 70%, i.e. approximately 64 tonnes representing some 0.6 billion individual shot, is likely to be lead ammunition (as compliance with the Regulations restricting the use of lead for shooting wildfowl or over certain wetlands in England was reported at 30%, Cromie *et al*. 2010).

PACEC (2006) estimate that 47,000 hares *Lepus europaeus* and 590,000 rabbits *Oryctolagus cuniculus* were shot (most likely with shotguns) in 2004, not as part of a job. The numbers shot as part of a job are not known. The number of lead cartridges, and tonnage of lead, used for shooting these mammals, plus those used during pest control activities, is not known but is additional to the above[100].

It should be noted that these figures represent annual deposition into the environment where it will accumulate year on year[101]. Section A.1.1.3 and Section C.1 discuss fate of lead ammunition in the environment.

Excluding shot used for pest control and mammal shooting e.g. used for killing rabbits, in total, we estimate that approximately 5,500-12,900 tonnes of lead shot, which equates to 68-154 billion individual shot, are deposited into the UK's environment year on year. The lower figure assumes three shots per bird killed and the lower estimate for clay target shooting. The higher figure assumes 8 shots per bird killed and that the level of clay pigeon shooting is similar to that estimated for 1991.

[100] Red foxes *Vulpes vulpes* have been included in Section A.1.2 which deals with bullet usage on the assumption that the majority are shot with rifles yet a proportion (unknown) will be shot with shotguns.

[101] As described, we have based our estimates of tonnage of shot deposited annually (with assumptions clearly stated) on that published information that we could readily access and information gleaned from various shooting websites. However, we cannot evaluate the accuracy of this information. The industry estimates that approximately 5000 tonnes of lead shot is deposited annually (J. Batley *pers. comm*. based upon unpublished Association of European Manufacturers of Sporting Ammunition figures). It may therefore be that the true annual tonnage of deposition is near the bottom end of this range, although this would not appear to correspond with the numbers of animals reported as being shot annually. The range we have given is therefore only approximate and for illustrative purposes. It does not directly affect our evaluation of risks to wildlife which are based upon factors like animals exposure to (e.g. ingestion of) lead ammunition rather than amounts deposited *per se*. However, it would affect cumulative tonnage deposited.

This estimate is not precise, but based upon the relatively recent information available we believe gives a reasonable approximation of amounts of shot used. Ammunition used for target shotgun shooting is concentrated in and around target shooting clubs. Ammunition used for live quarry shooting is distributed, to variable degrees, across large tracts of the countryside, although it is difficult to calculate the area or habitats over which lead shot is deposited with any reliability as different shooting activities disperse spent ammunition in different ways.

A.1.1.3 Availability of lead shot in the environment to wildlife

Lead is a relatively stable metal under most conditions (see also Section C.1.1.2) and remains as "shot" for considerable periods of time. It has been used in the UK for over two centuries and, indeed, the potential for a "historical legacy" of shot remaining available to wildlife is an important aspect of the epidemiology of lead poisoning in wildlife. Complete decomposition of particulate lead likely takes tens or hundreds of years under most conditions (Scheuhammer and Norris 1996, Rooney *et al.* 2007). Shot degradation is caused by a combination of physical erosion/abrasion, which is accelerated in course and gritty soils and/or those with lots of soil movement, and chemical processes, which depend upon the pH of the soil and other aspects of soil chemistry (see Section C.1.1.2).

Overall densities of lead shot in the soil will increase over time as lead shot continues to be used. However, shot will generally sink slowly through the soil with rates of sinking affected by soil density and other soil characteristics. Hartikainen and Kerko (2009) found that on the coarse stony soil of a shooting range in southern Finland, lead shot migrated downwards at a rate of some 2-3mm per year. Flint (1998) found in various experimentally seeded wetland types that most shot was still within the top 4cm of sediment three years after deposition.

In the Camargue marshes (southern France), assuming a constant settlement rate, Tavecchia *et al.* (2001) estimated a half-life of shot in the first 0-6cm, thus available to waterfowl, of 46 years, with complete settlement after 66 years. Flint and Schamber (2010) found that 10 years after seeding experimental plots on tundra wetlands with number 4 shot, about 10% remained in the top 6cm and >50% in the top 10cm. These authors predicted that it would probably require >25 years for spent lead pellets to exceed depths at which waterfowl forage. However, one would expect the proportion of pellets available to feeding waterfowl to decrease with time over this period. Lead shot may become less available when redistributed by cultivation (e.g. Thomas *et al*. 2001), and some farming practices could hypothetically make lead shot deposited decades ago more available (Chrastny *et al* 2010, Rooney and McLaren 2000, Stansley *et al*. 1992, White Young Green Environmental 2006).

Conclusion A.1.1 Probability of lead shot being available in the environment to wildlife

The numerous available studies and logical analysis provide **overwhelming evidence** and **certainty** that where shooting with lead shot takes place, pellets will be deposited in the environment wherever this occurs i.e. in both wetland and terrestrial environments. There is **strong evidence** that shooting using lead ammunition takes place over a high proportion of the land surface of the UK, thus deposition of lead pellets will be widespread. There is **strong evidence** that, because of weak compliance with current legislation, shooting with lead ammunition takes place in many wetlands in the UK, thus deposition of lead pellets will be widespread. **Strong evidence** and logical analysis suggest that environmental contamination is greatest where shooting intensity and frequency is greatest.

Given the nature of metallic lead, and numerous studies, there is **strong evidence** and **certainty** that shot generally remain relatively intact for long periods of time (decades or centuries). However, there is a **high probability** that shot may sink and become less available to feeding animals over time depending on substrate type and other environmental factors. This conclusion is supported by the results presented in Sections A.2.1 and C.1.1.2

A.1.2 Tonnage of lead bullets and their densities in the environment, and their availability to wildlife

In areas of intensive lead bullet usage e.g. firing ranges, bullets or fragments thereof, are found deposited within the environment (e.g. Vantelon *et al*. 2005, Lewis *et al*. 2001). Similarly, intensive hunting activities such as organised prairie dog *Cynomys ludovicianus* shoots in north America or similar animal control activities where a large number of rounds of lead ammunition are used in a relatively limited area over a relatively short period of time will likely result in a relatively high density of lead bullets or bullet fragments entering the environment having either missed or passed through the body of their intended target (Pauli and Buskirk 2007, Stephens *et al*. 2009, Stroud and Hunt 2009). Although studies have described extremely elevated surface soil-lead concentrations in firing ranges associated with corrosion of bullets (see Section C.1.1.2), reports of the density of pieces of particulate lead from bullets away from these sites are lacking.

The authors are not aware of UK studies investigating the density of bullets, or fragments thereof, in the environment in areas of lower intensity of usage such as areas in which other more “typical” UK live quarry shooting activities occurs. However, it is probable that bullets that either miss their targets or travel through their targets are deposited within the environment, most likely penetrating whatever substrate by a distance dependent on the density of the substrate they hit, and the velocity and mass of the projectile.

In terms of numbers of mammals killed with rifle and or air-rifle ammunition, PACEC (2006) provides information on the numbers of mammals shot in 2004 which include 120,000 red foxes *Vulpes vulpes*, 170,000 grey squirrels *Sciurus carolinensis*, 120,000 deer and thousands of other animals, not as part of a job. It is uncertain how many more of these mammals were shot as part of a job. In Scotland, about one-third of the red deer *Cervus elaphus* shot per year are killed for sport (which we take to be not as part of a job), with two-thirds being killed as part of deer control measures, which we assume are largely undertaken as part of a job (Callander and MacKenzie 1991).

There is a variety of ways of estimating bullet usage. As stated above, PACEC provide information on deer shot annually for sport not as part of a job (120,000) but also as part of the job or by unpaid guns (40 per shoot provider and 17,000 providers of deer stalking = 680,000). If only one bullet were to be used per deer shot and deer shooting was the only activity using bullets, this would equate to 7.5g per bullet x 800,000 = 6 tonnes of bullets a year with an unknown lead omponent. This would represent a minimum number of bullets used. It has also been estimated that some 15,000 stalkers use approximately 30 rounds of ammunition each year. Based on an average projectile weight of 7.5g, this would contribute 3.4 tonnes of fired metallic bullet ammunition a year, with an unknown non-lead component. Industry figures indicate that up to 4.5 million rounds of .22 rimfire bullets are purchased for quarry/pest/target shooting each year, which would contribute an additional 170 tonnes (Harradine and Leake 2013). The proportion of lead ammunition remaining unretrieved within the environment is not known but will accumulate over time where lead ammunition is used. See Section C.1 for degradation of lead ammunition within the environment.

We are not aware of the quantity of ammunition used in shooting ranges by the military and/or police.

Excluding tonnage of lead used by the police and/or the military, industry figures suggest up to 4.5 million rounds of .22 rimfire bullets are used which equates to 170 tonnes plus between 3 and 6 tonnes for use in deer shooting (sport and control) year on year.

Conclusion A.1.2 Probability of lead bullets, or fragments thereof, being available in the environment to wildlife

There is **evidence** and **high probability** that in areas of high lead bullet usage, such as firing ranges, lead bullets, or fragments thereof, accumulate within the environment.

We have not been able to find studies that measure accumulation within the environment of lead bullets, or fragments thereof, resulting from typical UK live quarry shooting although there is a **high probability** based on logic that this does occur, albeit generally at a likely low density.

A.2 Pathway: Evaluating the probability that wild animals will be directly exposed to lead ammunition from the environment via ingestion

A.2.1 Ingestion of spent lead shot from the environment by wild birds

The first reports of lead shot's toxicity to birds were made in the 1870s with recognition of the ingestion of spent shot being responsible for deaths of wildfowl in the USA and ring-necked pheasants in the UK (e.g. as reported in Friend *et al.* 1999, Beintema 2001, Pokras and Kneeland 2009, Franson and Pain 2011). Since these earliest reports, this pathway of ingestion of lead shot has been well documented and reviewed globally but primarily in North America and Europe, including in the UK (e.g. Bellrose 1959, Bingham *et al.* 2009, Brown *et al.* 1992, Butler *et al.* 2005, Franson and Pain 2011, Hall and Fisher 1985, Kimmel and Tranel 2007, Locke and Friend 1992, Mudge 1983, Newth *et al.* 2012, O'Connell *et al.* 2008, Olney 1960, Owen and Cadbury 1975, Pain 1990a, 1990b, 1991a, 1992, 1996, Parslow *et al.* 1982, Potts 2005, Scheuhammer and Norris 1995, Schulz *et al.* 2009, Sharley *et al.* 1992, Street 1983, Thomas 1975, 1982).

There is an extensive body of literature from across the world, including the UK, demonstrating shot ingestion directly from the environment by birds with a muscular gizzard, primarily wildfowl, other waterbirds and terrestrial birds such as Galliformes (see Table A.3). This literature spans more than a century but is particularly extensive post 1950 and comprises many 100s of scientific articles. The birds ingest the shot, possibly mistakenly for food items, or for grit, which is retained in the muscular gizzard to aid mechanical breakdown of food. Other taxa reported as ingesting lead shot directly from the environment include waders (e.g. Pain *et al*. 2009, Scheuhammer and Norris 1996, Thomas 1975), rails (Thomas 1975), cranes (e.g. Pain *et al* 2009); flamingos (Scheuhammer and Norris 1996); pelicans (Scheuhammer and Norris 1996) and pigeons and doves (e.g. Clausen and Wolstrup 1979, Franson *et al*. 2009) (see Table A.3).

Table A.3 summarises some of the studies which illustrate shot ingestion in a range of non-wildfowl waterbirds and terrestrial birds.

Table A.3. A selection of non-wildfowl avian species reported as ingesting lead shot from the environment

Species	Countries	References
Galliformes		
Chukar (Alectoris chukar)	USA	Hanspeter and Kerry, (2003)
Grey partridge (*Perdix perdix*)	Denmark, UK	Clausen and Wolstrup (1979), Keymer and Stebbings (1987), Potts, (2005)
Red-legged partridge (*Alectoris rufa*)	UK	Butler (2005)
Common pheasant (Phasianus colchicus)	Denmark, UK, USA	Calvert (1876), Elder (1955), Clausen and Wolstrup (1979), NWHL (1985), Dutton and Bolen (2000), Butler *et al.* (2005)
Wild turkey (Meleagris gallopavo)	USA	Stone and Butkas (1978)
Scaled quail (Callipepla squamata)	USA	Campbell (1950)
Northern bobwhite quail (*Colinus virginianus*)	USA	Stoddard (1931), Keel *et al.* (2002)
Ruffed grouse (*Bonasa umbellus*)	Canada	Rodrigue *et al.* (2005)
Guinea hen (Numida meleagris)	Canada	Hunter and Haigh. (1978)
Columbiformes		
Rock pigeon (*Columba livia*)	USA , Belgium	DeMent *et al.* (1987), Tavernier *et al.* (2004).
Mourning dove (*Zenaida macroura*)	USA	Locke and Bagley (1967), Lewis and Legler (1968), Best *et al.* (1992), Schulz *et al.* (2002).
Gruiformes		
Sandhill crane (*Grus canadensis*)	USA	Windingstad *et al.* (1984), NWHL (1985)
Clapper rail (Rallus longirostris)	USA	Jones (1939)
King rail (*R. elegans*)	USA	Jones (1939)
Virginia rail (*R. limicola*)	USA	Jones (1939)
Common moorhen (Gallinula chloropus)	Europe, UK, USA	Jones (1939), Locke and Friend (1992), Thomas (1975)
Common coot (*Fulica atra*)	France	Pain (1990a)
American coot (*F. americana*)	USA	Jones (1939)
Charadriiformes		
American woodcock (*Scolopax minor*)	Canada	Scheuhammer *et al.* (1999, 2003)
Black-necked stilt (*Himantopus mexicanus*)	USA	Hall and Fisher (1985)
Long-billed dowitcher (*Limnodromus scolopaceus*)	USA	Hall and Fisher (1985)
Common snipe (Gallinago gallinago)	France	Beck and Granval (1997)
Jack snipe (Lymnocryptes minimus)	France	Beck and Granval (1997)
Dunlin (Calidris alpina)	Canada	Kaiser *et al.* (1980)
Pelecaniformes		
White-faced ibis (*Plegadis chihi*)	USA	Hall and Fisher (1985)
Phoenicopteriformes		
Caribbean flamingo (Phoenicopeterus ruber ruber)	Mexico	Schmitz *et al.* (1990)

See also Kimmel and Tranel (2007).

A.2.1.1 Ingestion levels of shot from the environment in wild birds

It is worth appreciating the methods used for studying ingestion levels of lead shot as these may have various biases associated with them. Methods include demonstrating shot within the alimentary canal using radiographic studies of living birds or from *post mortem* examination of harvested or found dead birds.

- ***Ingestion levels in hunted birds:*** these will be subject to the inherent biases of hunting e.g. young birds are often over-represented in hunting bags. Also ingestion of lead may remove those individuals from a population (*via* lead-related morbidity and mortality) or conversely lead ingestion may disable birds sufficiently to make them more likely to be harvested (e.g. Bellrose 1959, Heitmeyer *et al.* 1993, Demendi and Petrie 2006). In field experiments Bellrose (1959) found that mallard dosed with one No. 6 pellet and released were 1.5 times as vulnerable to shooting as were the undosed controls; those dosed with two pellets, 1.9 times, and those dosed with four pellets, 2.1 times as vulnerable to being shot.

- ***Ingestion levels in trapped birds:*** Biases may not be as significant as with hunting as trapping methods will not necessarily positively select for weakened birds. However, little information exists.

- ***Ingestion levels in found dead birds:*** these have a number of number of confounding factors also. Firstly, "found dead" studies are biased towards those species most likely to be visible to humans e.g. large, white or close to human habitation. The nature of lead poisoning as a debilitating condition may make individuals more prone to "disappearing" into vegetation and predation (Pain 1991, Sanderson and Bellrose 1986). Moreover, shot may be ground down or dissolved in the bird's alimentary canal and thus unapparent (Stutzenbaker *et al*. 1986, Pain 1991). While ingestion levels from found dead birds will probably not accurately reflect the situation in the wild population, finding shot in found dead birds obviously illustrates the pathway of ingestion.

To investigate ingestion levels of shot in a manner to help eliminate many of the aforementioned biases, ingestion levels of shot would need to be studied in wild populations, across age classes, in countries where non-toxic shot has been used for some period of time. Unfortunately such data, on the whole, do not exist and thus data presented herein must bear these biases in mind, particularly when comparing the results of different methods of data collection. However, comparison between studies with similar data collection methods can provide a relatively robust indicator of trends in ingestion levels.

Mateo (2009) provides a summary of historic prevalence of lead shot ingestion in 19 species of wildfowl from Europe (15 of which are species of swans, geese and ducks from northern Europe). Ingestions levels are both site and species specific with overall combined levels for mallard of 3.6% of over 8,600 birds in northern Europe and 17.9% of nearly 12,000 birds in southern and central European wetlands. For species such as pintail *Anas acuta* and pochard *Aythya ferina*, ingestion levels of 5.4% and 9.3% respectively are reported in northern European wetlands reaching 55% and 26% respectively in southern and central European wetlands (Mateo 2009). The majority of studies summarised by Mateo (2009) appear to relate to hunter shot and sometimes trapped birds.

Flint *et al*. (1997) detected shot, on radiograph, in the gizzards of 11.6% of live-trapped spectacled eiders *Somateria fischeri* in the Yukon-Kuskokwim Delta.

Seventy one percent of 34 globally threatened (Endangered) white-headed duck *Oxyura leucocephala* found dead or moribund in El Hondo, Spain, had ingested shot in their gizzards (Svanberg *et al*. 2006) and lead concentrations were high in the bone and livers of most individuals (Svanberg *et al.* 2006, Taggart *et al.* 2009).

Beck and Gronval (1997) report ingestion levels of spent lead gunshot by common snipe in three areas of north west France similar to ingestion levels of wildfowl over the 1983-1993 hunting seasons. Average ingestion levels of 15.6% (n = 180)(with a year to year variation of 10-27%), 2% of 59 birds and 3% of 36 birds, were found. For jack snipe *Lymnocryptes minimus* from one region ingestion levels were reported as 8.7% of 47 birds.

Kreager *et al.* (2008) found lead shot in the gizzards of 8% of 76 chukars *Alectoris chukar*, and 34% of 47 pheasants killed by hunters at a heavily shot-over site in Ontario, Canada.
Mourning doves have been the subject of a number of lead shot related studies in north America as they are often intensively harvested in shooting fields where the density of lead shot can be subsequently high (Castrale 1989, Best *et al*. 1992). Franson *et al*. (2009) found an overall ingestion level of lead shot of 2.5% (4,229 hunter-shot birds with 'shot in' pellets were discounted) from seven States across the USA. Schulz *et al*. (2007) report an ingestion level of 5.1% from 117 doves offered pellets with food in an experimental ingestion level study.

A number of UK studies illustrate the pathway of ingestion from the environment of lead shot by both domestic and free-living wildfowl, Galliformes and other taxa. Whilst this risk assessment deals with wild animals, the first examples are of recent case reports of lead poisoning in domestic poultry and mallards, and reared mallards for shooting as it illustrates the ingestion pathway:

Payne *et al*. (2013) report lead poisoning following consumption of shot from nearby clay pigeon shooting activities in: a flock of 2000 free-range poultry (which resulted in a recall of eggs and the flock being culled due to the low production of the remaining flock); a new commercial enterprise rearing domesticated mallards which suffered high losses (with up to half of the flock of 400 birds lost over a two week period); and clinical disease in 3-5% of 400 ducks being reared for shooting 150m from a clay pigeon shoot.

Mudge (1983) reported ingested lead shot being found in swans found dead in the UK; seven (22%) of 32 mute swans, two (10%) of 20 Bewick's swans *Cygnus columbianus bewickii* and two (40%) of five whooper swans *C. cygnus*.

More recently, Newth *et al.* (2012) report lead poisoning in wildfowl (between 1971 and 2010) within the UK where the majority of cases of birds dying of lead poisoning (75% of 251) still had lead shot in various stages of dissolution in their gizzards. The *post mortem* data used for this study reveal a small number (13) of lead poisoned birds with >40 pellets within the gizzard, three of which contained more than 100 pellets, including a Canada goose *Branta canadensis* whose gizzard contained 438 pellets. These cases imply a high density of shot available to these birds and such active selection probably implies the birds mistook pellets for grain rather than grit (WWT unpublished). This dataset contains reports of species such as tufted duck *Aythya fuligula* and whooper swan. Given the feeding ecology of these species, it is reasonable to assume that tufted duck would pick up lead shot from wetlands, whereas the whooper swans, plus other grazing species including geese and other swans, would be exposed to lead shot in both wetlands and terrestrial environments such as agricultural land where a large proportion of time is spent feeding (Newth *et al*. 2012). For whooper swans, 86% of the 104 birds diagnosed as lead poisoned contained pellets in the gizzard.

Thomas (1975) reported on ingestion levels in hunter-harvested wildfowl from the Ouse Washes and found 0% of 10 coots *Fulica atra*, 6.3% of 32 moorhen *Gallinula chloropus* and 1.5% of 67 common snipe with ingested lead shot. Of the ducks sampled, those species reported with ingested shot were mallard (9% of 380 birds), pintail (10.1% of 89 birds), shoveler *Anas clypeata* (2% of 102 birds) and teal *Anas crecca* (3.2% of 278 birds).

Potts (2005) reports some 4.5% of 446 adult grey partridge *Perdix perdix* and 6.9% of 29 juvenile birds harvested between 1963 and 1992 as having lead shot within their gizzards.

Butler (2005) describes an overall ingestion level of 3% of 437 harvested pheasants from 32 estates in Britain between 1996 and 2002.

A.2.1.2 Factors affecting exposure to shot and likelihood of ingestion by birds

As well as taxon and feeding ecology, other environmental and anthropogenic factors affect the level of exposure of wild birds to lead shot and their proclivity to ingest it, including:

1. proximity to hunting or other shooting activities where lead ammunition is being used;

2. hunting intensity with lead shot, in general the greater the intensity the greater the potential exposure;

3. time in relation to hunting seasons where exposure towards the end of a hunting season will be greater (e.g. Bellrose 1959, Mudge 1983);

4. habitat over which lead is used and its attractiveness to birds e.g. wetland type;

5. substrate type, water inundation and other local conditions and how these affect sinking/movement of shot over time;

6. land management e.g. ploughing in of shot (Thomas *et al.* 2001);

7. land disruption e.g. temporary inundation of terrestrial shot-over areas may attract dabbling ducks; spates and flooding can erode watercourses and expose historically deposited lead;

8. chemical and physical processes in the environment breaking down the shot over time (see Section C.1.1.2); and

9. legislation (and compliance therewith) relating to the use of lead shot. The work of Anderson *et al*. (2000) and others, clearly illustrates the impact of a ban on the use of lead shot for waterfowl shooting in the USA as the relative proportions of type of shot removed from gizzards of shot birds at *post mortem* examination shifted to the introduced non-lead alternatives. However, this major shift to non-lead shot after a ban on lead shot did not occur in England (Newth *et al.* 2012) where compliance with legislation is apparently low (Cromie *et al.* 2010). While current regulations differ between UK countries, they restrict usage in wetlands; if adhered to, this would reduce risks to waterbirds but to varying degrees. Those species not protected from exposure are those which feed in terrestrial areas i.e. swans (Bewick's and whooper more so than mute), geese and grazing ducks e.g. wigeon *Anas penelope* and dabbling ducks when feeding on newly inundated/flooded fields (see Figure A.1 for further illustration).

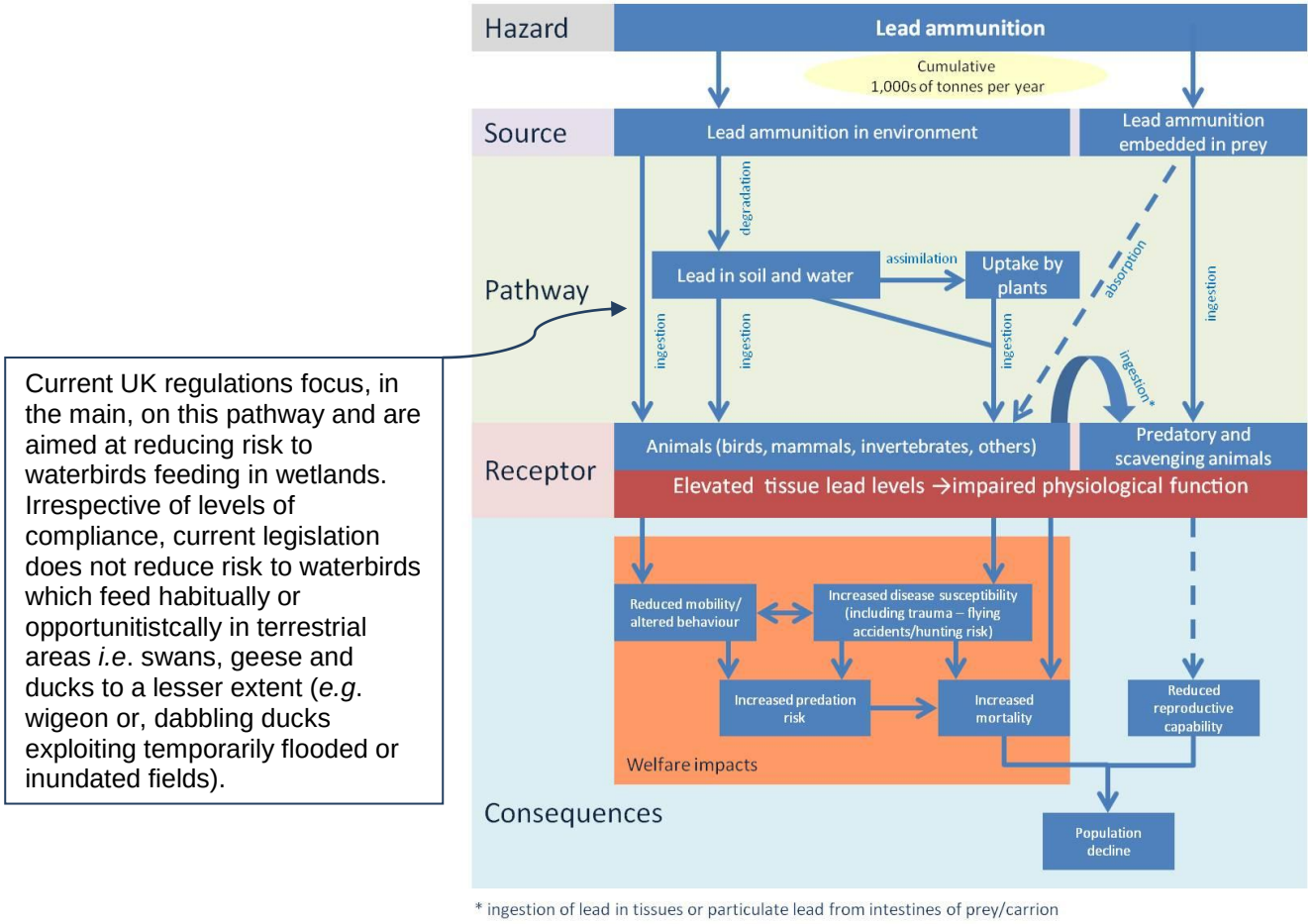


Figure A.1. Conceptual model of potential hazard from ammunition-derived lead to wildlife with details of current lead shot restrictive regulations in UK

As well as availability of shot, consumption of shot may be affected by:

1. the availability of alternative grit, the absence of which increases level of shot ingestion (Mateo *et al*. 2007).

2. diet of the bird e.g. birds with a muscular gizzard eating a diet of mainly hard food such as seeds may ingest relatively high amounts of grit to aid digestion or ingestion may change over time depending on seasonal diets (Rocke *et al.* 1997).

A.2.1.3 Absorption by animals of ammunition-derived dietary lead

The first reports of lead shot ingestion, absorption and toxicity to birds were made in the 1870s with recognition of the ingestion of spent shot being responsible for deaths of wildfowl in the USA and hunted ring necked pheasants in the UK (e.g. as reported in Friend *et al.* 1999, Beintema 2001; Pokras and Kneeland 2009, Franson and Pain 2011). Since these earliest reports, exposure to lead shot, absorption of lead from shot and lead poisoning has been well documented and reviewed globally but primarily in North America and Europe, including in the UK (e.g. Bellrose 1959, Bingham *et al.* 2009, Brown *et al.* 1992, Butler *et al.* 2005, Franson and Pain 2011, Hall and Fisher 1985, Locke and Friend 1992, Mudge 1983, Newth *et al.* 2012, O'Connell *et al.* 2008, Olney 1960, Owen and Cadbury 1975, Pain 1990a, 1990b, 1991a, 1992, 1996, Parslow *et al.* 1982, Potts

2005, Scheuhammer and Norris 1995, Schulz *et al.* 2009, Sharley *et al.* 1992, Street 1983, Thomas 1975, 1982).

In these following Sections A.2.1.3a-b we outline a selection of the evidence for absorption of ammunition-derived dietary lead.

A.2.1.3a Evidence of elevation of blood and tissue lead following experimental exposure to ingested lead shot (see also Section B.2.3.2 for provenance of ammunition-derived lead)

Following ingestion, lead shot may be lost from the body rapidly with little absorption of lead through the intestine wall and into the bloodstream, or retained and partially or completely eroded with correspondingly higher lead absorption. See Section A.3.1 for details of lead absorption and distribution among body tissues – primarily the liver and kidney in the short-medium term and bone as the long-term deposition site. When shot is not voided immediately following ingestion blood lead levels can become elevated within hours. Absorption of lead into the bloodstream following shot ingestion, and associated effects (see Section A.3) has been well established in wildfowl and several other avian taxa (e.g. see B.2.3.2) for many decades, with a wide range of experimental studies conducted in the 1970s and 1980s, and some more recently. For reviews see Eisler (1988) and Franson and Pain (2011) and for a selection of specific studies on a range of species see Buerger *et al.* (1986), Chasko *et al.* (1984), Clemens *et al.* (1975), Dieter and Finley (1978; 1979), Fimreite (1984), Finley and Dieter (1978), Finley *et al.* (1976), Franson and Smith (1999), Kendall and Scanlon (1983; 1984), Longcore *et al.* (1974a; 1974b), Pain and Rattner (1988), Veit *et al.* (1983). The factors that influence lead absorption from shot, and subsequent effects, are dealt with in more detail in Section A.3.

A.2.1.3b Evidence concerning the provenance of lead in cases where elevation of blood or tissue lead concentrations is observed in wild birds (see also Section B.2.3.2)

The diagnosis of large scale and geographically extensive wildfowl mortality from lead poisoning following shot ingestion was first reported in the USA in the 1950s, supported by extensive *post mortem* data, and subsequently confirmed through experimental studies. Since that time, birds with particularly elevated tissue lead levels, and for which there is a well established source and pathway of exposure to lead shot have, in the absence of other obvious sources of exposure to lead, generally been assumed to have been exposed to lead from shot. Numerous reviews of lead in the environment illustrate that, following strict regulatory controls on lead emissions, there are relatively few other exposure sources likely to result in significant and widespread contamination of birds and other wildlife (see Supporting Information 1 and Pattee and Pain 2003) and thus is likely to be a reasonable assumption. However, wildlife can nonetheless be exposed to significant amounts of lead from other sources under certain circumstances. These tend to be localised, and are predominantly in mining areas (where there are ongoing and significant problems for wildfowl at specific locations, e.g. see Supporting Information 1), and sometimes urban areas, but local point sources of contamination can also potentially occur, for example, near old buildings where leaded paint chips may be ingested.

One way of establishing the origin of elevated tissue lead concentrations is through measuring the ratios of stable isotopes of lead found in blood or tissue samples and comparing these with those in lead from ammunition and the other potential sources that exist in the area where the animal lived. There are usually marked differences among the sources in the ratios of different lead isotopes because of their geological origins. If the tissue sample ratios match those of a source and there is no overlap with ratios of this source and the others, then the provenance of the lead in the sample can be establish unambiguously. More often, there is some overlap between sources in their isotope ratio signatures, but in these cases the provenance of the lead in the sample can still be evaluated on a probability basis (see also Section B.2.3.2).

Stable isotope studies have linked shot ingestion with elevated tissue lead concentrations in wild birds in a number of studies. Scheuhammer *et al.* (2003) analysed lead levels and isotope ratios in undamaged wing bones from young-of-the-year American woodcock *Scolopax minor*, soil and earthworms, which are extensively fed upon by these birds. Both total lead and lead isotope concentrations were positively correlated in soil and worms, but most woodcock with elevated bone lead had isotope ratios different to those in soil, worms, historical gasoline or mining wastes, but consistent with ingestion of spent lead shot. Similarly stable lead isotopes signatures in the majority of Canadian waterfowl and loons (divers, *Gavia* spp.) with high lead exposure were consistent with those of lead shot (or angler's weights) and not with petrol, mining or smelter sources (Scheuhammer and Templeton 1998).

Martinez-Haro *et al*. (2011) analysed waterbird faeces for lead isotopic signatures, Pb/Al ratios and biomarkers of toxicological effects, to determine sources of lead contamination at sites in southern Spain. They found that lead shot ingestion was the likely cause of the high lead levels in some samples, and sediment ingestion was linked to lower/background levels. Correspondingly, biomarkers indicating disruption in haem synthesis were higher in faeces with higher lead concentrations.

Svanberg *et al*. (2006) analysed lead isotope ratios and lead levels in the livers and bones of globally threatened white-headed duck and marbled teal *Marmaronetta angustirostris* found dead or moribund in El Hondo, Spain. While the isotopic ratios in soils and shot to some degree overlapped, the ratios supported lead shot ingestion being the principal source of lead exposure, consistent with high tissue lead concentrations and evidence of ingested shot.

Another method that helps attribute the provenance of lead in tissues to a particular source is to measure the degree of association between tissue lead levels and spatial and temporal variation in exposure to food contaminated with ammunition-derived lead and a range of studies of this type have been conducted. Several of these compare tissue lead levels in wildfowl pre and post bans on the use of lead shot. For example, the prevalence of elevated blood lead in American black ducks *Anas rubripes* wintering in Tennessee declined by 44 %, six to eight years after the ban on lead shot (Samuel and Bowers 2000), and average bone lead concentrations in dabbling ducks and American black ducks in Canada decreased significantly following the establishment of a national regulation prohibiting the use of lead shot for waterfowl hunting (Stevenson *et al.* 2005).

In Canada, Scheuhammer and Dickson (1996) investigated the geographical pattern of elevated lead concentrations in several thousand wing bones from young-of-the-year ducks to investigate their relationship with activities known to cause environmental lead contamination, i.e. waterfowl hunting, non-ferrous metal mining/smelting, and urban/industrial development. Proximity to metal mining sites was significantly correlated with lead exposure, accounting for about a quarter of the total area characterised by a high incidence of elevated lead exposure, although results indicated spent lead-shot ingestion as the likely primary source of elevated lead exposure for wild ducks in Canada. In areas of significant waterfowl hunting, a widespread pattern of elevated bone-lead was found, rather than a few small local sites of high lead exposure.

Demendi and Petrie (2006) found that one year after a ban on the use of lead shot for waterfowl hunting in Canada only 0.6% (n=4 of 722) of scaup *Aythya marila* and *A. affinis* contained lead shot and 3.1% contained non-toxic shot, compared with 11% and 8% for *A. marila* and *A. affinis* respectively before the ban. These results suggested that shot may quickly become inaccessible to scaup on the lacustrine areas studied. While this alone only suggests changes in exposure, the low incidence of overall shot ingestion post ban *vs* pre-ban (3.7% *vs* 8-11%) suggests that high pre-ban numbers may have resulted partly from lead shot ingestion affecting the birds (i.e. being absorbed and causing harm) and increasing harvest susceptibility.

Franson *et al.* (2009) investigated levels of shot ingestion in mourning doves from seven States across the USA. 4,229 doves were harvested in areas where the use of lead shot was allowed, and 655 doves from areas where non-toxic shot was required. 106 birds (2.5%) from the areas where lead shot was allowed contained ingested lead shot (and two contained ingested steel and

no lead); 16 birds (2.4%) contained ingested steel shot (and two of these also had ingested lead) in the area where non-toxic shot was required. In doves without ingested lead pellets, bone lead concentrations were lower on an area requiring the use of non-toxic shot than on areas allowing the use of lead shot.
Newth *et al*. (2012) studied the proportion of various species of wildfowl found dead between 1971 and 2010 that had succumbed to lead poisoning and compared these before and after the introduction of regulations restricting lead shot use in England. Having controlled for site, species, age and sex, no significant difference in the proportion of birds diagnosed as having died of lead poisoning was found following the introduction of regulations. These results are, nonetheless, consistent with those above as a large scale study of compliance with the regulations found this to be poor, with 70% of duck purchased in England having been shot illegally with lead ammunition (Cromie *et al*. 2010).

Conclusion A.2.1 Probability of ingestion of spent lead shot from the environment by wild birds and subsequent absorption of lead

There is **strong evidence** from field studies to confirm the pathway of lead shot being consumed by wild birds directly from the environment. Evidence is particularly extensive for ingestion by wildfowl, but **evidence** exists for a wide range of other avian taxa. There is **strong evidence** to suggest that waterbirds are particularly susceptible to shot ingestion, and often have far higher shot ingestion rates than other avian species susceptible to this pathway. Evidence of consumption by anseriformes, gruiformes, charadriiformes and galliformes is provided by UK studies and there is a **high probability** that this occurs.

There is **strong evidence** from field studies illustrating that, where shooting occurs, the ingestion of lead shot by waterbirds and terrestrial birds is the primary cause of widespread elevated tissue lead concentrations.

A.2.2 Ingestion of spent lead shot from the environment by wild non-avian animals

It is known that domestic hoofstock may be exposed to lead shot within silage harvested from areas contaminated with shot (Rice *et al*. 1987). This route has been reported recently in UK cattle feeding on maize silage harvested from a field adjacent to a clay pigeon shoot (Payne *et al*. 2013). The same study reports lead poisoning from cattle grazing directly from a field near a shoot. Thus, there is the potential for wild grazing ungulates such as cervids consuming shot in a similar manner. However, reports of this pathway are lacking.

Conclusion A.2.2 Probability of ingestion of spent lead shot from the environment by wild non-avian animals

The pathway of consumption of spent lead shot by non-avian wildlife has received little research attention. We cannot therefore evaluate the probability that this occurs to any great extent, but judge that it is likely to be relatively **low** based upon the feeding habits of non-avian wildlife, perhaps with the exception of wild deer feeding in and around areas of high lead shot usage *e.g*. clay pigeon shoots.

A.2.3 Ingestion of spent lead bullets, or fragments thereof, from the environment by wild animals

Unlike lead shot, studies on ingestion or ingestion levels of bullets, or fragments thereof, directly from the environment are lacking. Lewis *et al*. (2001) describe cases of lead poisoning in birds and

mammals in the vicinity of a law enforcement firing range facility in Georgia, USA, i.e. suggestive of ingestion of lead. Fatal cases, occurring over two one month time frames, included eight yellow-rumped warblers *Dendroica coronata*, a solitary vireo *Vireo solitaries* and grey squirrel *Sciurus carolinensis*. Further investigations to evaluate exposure to lead were undertaken over a short period of time with some 72 wild animals (37 mammals and 35 birds), comprised of 22 different species, being collected and euthanized from a 24ha area surrounding the shooting range. Five mammals and five birds were collected from areas 1.5–3km outside the shooting range area as a small control group. Some 12 individuals collected nearby the facility (17%) (both birds and mammals) had lead tissue levels of >2.00ppm w.w. which can be classified as sub-clinical lead poisoning including a grey squirrel with levels indicative of severe lead poisoning. Carcasses of one brown-thrasher *Toxostoma rufum* and two white-tailed deer *Odocoileus virginianus* contained lead fragments. None of the 10 control animals had levels above 2.00ppm (although one bird had elevated kidney lead concentration yet below the suggested 2.00ppm cut off point for sub-clinical poisoning).

It is assumed by the authors that the wild animals consumed the bullet-derived lead from the environment. However, it was not known whether this wildlife (other than the animals containing obvious particulate lead) had been exposed to lead that had already been abraded or otherwise released from the rifle bullets and was in the soil component, (which is dealt with in Section C) or had ingested lead fragments - although this would not necessarily affect the outcomes.

No reports are known to the authors of ingestion of fragments of bullets resulting from UK live quarry shooting activities directly from the environment and we can find no records of studies investigating this issue either in the UK or overseas. The penetration of bullets into the substrate will also serve to reduce likelihood of direct ingestion by feeding wildlife.

Conclusion A.2.3 Probability of ingestion of spent lead bullets, or fragments thereof, from the environment by wild animals

There is an apparent **absence of studies** to fully evaluate the potential for wildlife to consume lead bullets, or fragments thereof, directly from the environment. While there is an evident risk of this occurring in areas of high bullet use, such as firing ranges, there is a **low probability** that this occurs at any great frequency for any wild animals away from areas of extremely high bullet use, in the UK or elsewhere.

A.3 Receptor: Evaluating the probability of impacts on the health, welfare and population processes of wildlife consuming ammunition-derived lead directly from the environment

A.3.1 What constitutes lead poisoning?[102]

Lead is a metal that is neither essential nor beneficial to living organisms. It is toxic in most of its chemical forms to all vertebrate taxa. It is an accumulative metabolic poison that is non-specific and affects a wide range of body systems; most notably it affects behaviour and the haematopoietic, vascular, nervous, renal, and reproductive systems (Eisler 1988, Franson and Pain 2011). Organic and inorganic lead differ in the rates at which they enter the body, their distribution and half lives among body tissues, and their toxicity (toxicokinetics and toxicodynamics – see ATSDR 2007 for full details). Tetraethyl and tetramethyl (organic) leads were commonly used as petrol additive to increase octane rating until this was banned in the UK (Supporting Information 1). Lead occurs primarily in inorganic form in the environment and lead in ammunition is in its elemental (inorganic) form. In this paper, the term "lead" generally refers to inorganic lead, other than in Supporting Information 1.

[102] This Section should be referred to from Linkage Sets B, C and D.

The toxic effects of lead on birds and other wildlife are similar to those in all vertebrates. In wild animals these effects are well known from a wealth of experimental and field studies, have been reviewed numerous times (e.g. Eisler 1988), and details will not be repeated here. A brief synthesis of some of the sub-clinical and clinical signs poisoning is given below in Section A.3.2.

Many clinical signs of poisoning are associated with chronic exposure to lead in birds. Chronic exposure is extended exposure – at a level that is not necessarily likely to cause *immediate* failure of biological functioning or death; although death may eventually result from chronic exposure. These include anaemia, lethargy, muscle wasting and loss of fat reserves, green diarrhoea staining the vent, wing droop, lack of balance and coordination and other neurological signs such as leg paralysis or convulsions (e.g. Locke and Thomas 1996, Wobeser 1997, Friend 1999, Eisler 2000, Pattee and Pain 2003). In cases where birds die rapidly following acute exposure to high levels of lead, many of these signs may be absent.

It is currently considered that there are no identified “no observed adverse effect levels” (NOAEL) or predicted no effect concentrations (PNEC) levels for lead in humans (EFSA 2010) and thus likely for other vertebrates. However, given the ubiquitous nature of lead, levels at which significant adverse health consequences can be expected (regardless of source) have to be determined and these are presented within this risk assessment in the following section.

Ingested lead shot, bullets, or fragments thereof, may be mechanically ground down in the gizzards of some bird species, and in birds and other wild animals may be dissolved by the stomach acids with lead salts subsequently absorbed into the bloodstream. Absorbed lead is carried around the body in the bloodstream and deposited rapidly into soft tissues, primarily the liver and kidney, bone, and in birds also in growing feathers. Much of the lead not retained in bone is eliminated in waste, but with continuous or repeated exposure some absorbed lead will continue to be retained and bone lead concentrations will increase. Highest lead concentrations are generally found in bone, followed by kidney and liver, with intermediate concentrations in brain and blood, and lowest concentrations in muscle (Longcore *et al*. 1974a, Johnson *et al*. 1982, Custer *et al*. 1984, Garcia Fernandez *et al*. 1995). Blood lead is a good indicator of recent exposure and usually remains elevated for several weeks to several months following exposure, in relation to the initial amount absorbed and the duration of exposure. Lead in bone is relatively immobile accumulating over an animal’s lifetime, although it can be mobilised under certain conditions, particularly in birds, and especially in female birds.

General conclusions on tissue lead concentrations and associated effects on an individual animal can be made using information on exposure, clinical signs of poisoning and the severity of effects (e.g. Franson 1996, Pain 1996, Franson and Pain 2011). According to one such assessment (Franson and Pain 2011; Table A.4), birds with sub-clinical poisoning are those that suffer physiological effects that are insufficient to severely impair normal biological functioning, resulting in no external signs of poisoning. Birds are also deemed likely to recover (relatively – although they will carry a residual lead burden, as lead is a cumulative poison) should lead exposure stop. Birds with clinical poisoning will suffer from a range of pathological manifestations and physiological effects leading to probable death with the continuation of lead exposure. Birds with severe clinical poisoning are deemed to have their lives directly threatened in the short term. Background concentrations refer to those that are common and widespread in populations not considered to be exposed to specific sources of contamination, and background concentrations result from natural and diffuse anthropogenic lead (Franson and Pain 2011). Concentrations of <10-20 mg/kg d.w. of bone are generally classified as ‘background’ or are reported in birds not exposed to particular sources of lead (Franson *et al.* 2009, Martin *et al.* 2008, Moore and Meredith 1978, Pain *et al.* 1992, Szymczak and Adrian 1978).

Acute toxicity, following the absorption of a large amount of lead, is usually associated with high lead levels in blood, liver and kidney and such levels within these tissues are generally regarded as measures of recent exposure (Table A.4).

Table A.4. Suggested interpretations of tissue lead concentrations in three orders of birds, adapted from Franson and Pain (2011).

Order	Blood µg/dl	Liver mg/kg w.w.	Kidney mg/kg w.w.	Sources
Anseriformes				
Sub-clinical	20<50	2<6	2<6	1,2
Clinical	50-100	42283	42156	2,3,4
Severe	>100	>10	>15	2,3,4,5,6,7,8,9,10,11,12,13,14
Falconiformes				
Sub-clinical	20<50	2<61	2<4	15,16,17
Clinical	50-100	42283	42159	17,18
Severe	>100	>10	>6	17,19,20,21,22,23
Columbiformes				
Sub-clinical	20<200	2<6	2<15	24,25,26,27,28,29
Clinical	200-300	42156	15-30	25,30,31
Severe	>300	>15	>30	25,30,31,32,33,34

(a) Lead concentrations in bone reflect lifetime accumulation and chronic low exposure to lead may result in similar concentrations in bone as acute exposure to higher levels. If evidence of acute exposure exists, we recommend that bone lead concentrations (dry weight basis) of <10 µg/g be considered background, 10–20 µg/g be considered evidence of sub-clinical to clinical poisoning, and >20 µg/g be considered evidence of severe clinical poisoning.

(b) Divide µg/dl by 100 for an approximate conversion to mg/kg.

(c) Although in many species lead concentrations are often higher in kidney than in liver, the reverse has been found in lead exposed and poisoned eagles and some species of hawks/buzzards; thus, for Falconiformes, we have suggested a conservative lead threshold concentration in kidney.

(d) For raptors the tissue lead levels considered to be associated with toxicity vary between studies and some authors consider that liver lead concentrations of 5 mg/kg w.w. to be associated with mortality in raptors e.g. Franson (1996) and Fisher *et al.* (2006).

(e) Care must be taken with sample preparation and interpretation of analytical results from animals shot by hunters using lead ammunition as elevation in soft tissue (liver or kidney) can sometimes be caused small particles of metallic lead being embedded in the tissue (Frank 1986). For example, occasionally in a study on lead poisoning in wild birds a liver lead concentration will be reported that is too high to have resulted from biologically incorporated lead (e.g. 7766 µg/g w.w. in one chukar, see Kreager *et al.* 2008). It has been suggested that the upper limit for lead concentrations in livers of wild or lead-pellet-dosed waterfowl is approximately 283 µg/g dw (Tsuji *et al*. 2002). To minimise this bias organs for analysis from animals shot with lead ammunition should be discarded when there is any obvious damage from the ammunition; duplicate samples from an organ will also help identify anomalies.

References for Table A.4: [1]Dieter and Finley (1979), [2]Degernes (1991), [3]Longcore *et al*. (1974a), [4]Beyer *et al*. (2000), [5]Cook and Trainer (1966), [6]Mautino and Bell (1986), [7]Beyer *et al*. (1998a), [8]Pain and Rattner (1988), [9]Pain (1989), [10]Blus *et al*. (1991), [11]Blus *et al*. (1999), [12]Kelly *et al*. (1998), [13]Nakade *et al*. (2005), [14]Degernes *et al*. (2006), [15]Custer *et al*. (1984), [16]Henny *et al*. (1991), [17]Kramer and Redig (1997), [18]Lumeij *et al*. (1985), [19]Redig *et al*. (1980), [20]Hoffman *et al*. (1981), [21]Pattee *et al*. (1981), [22]Pattee *et al*. (2006), [23]Langelier *et al*. (1991), [24]Ohi *et al*. (1974), [25]Cory-Sletchta *et al*. (1980), [26]Kendall *et al*. (1982), [27]Kendall and Scanlon (1982), [28]DeMent *et al*. (1987), [29]Scheuhammer and Wilson (1990), [30]Anders *et al*. (1982), [31]Boyer *et al*. (1985), [32]Locke and Bagley (1967), [33]Barthalmus *et al*. (1977), [34]Schulz *et al*. (2006).

A.3.1.1 Factors affecting absorption of lead and delivery to body tissues of wild birds

In attempting to understand the impact of ingestion of particulate lead ammunition (bullets or shot) directly from the environment, the physiology of the receptor (host) must be considered. Whilst particulate lead may be ingested, it does not necessarily follow that substantial amounts will be absorbed. Factors that affect absorption of lead include:

Stomach type: The anatomical characteristics of the avian stomach differ between species and can influence the retention and thus to some extent the absorption of lead items (Franson and Pain 2011). Birds that consume grain, vegetation and insects have a more muscular gizzard whereas scavengers, carnivores and piscivorous birds have thin-walled stomachs that are adapted for a relatively soft diet (Denbow 2000, Franson and Pain 2011). Acidic conditions in both types of stomach facilitate the dissolution of lead, but waterbirds are particularly prone to ingesting shot as they mistake them for food particles or the grit that is deliberately ingested to aid grinding and digestion of food within their gizzard (UNEP/AEWA 2011). Abrasion of lead within the gizzard, and in particular in the presence of grit, may accelerate this process.

Retention time of lead in the alimentary canal: The fate of pieces of lead such as shot within the alimentary canal of a bird may vary. Some pieces may be voided quickly, with little absorption from the gut, and some may be retained within the gizzard or stomach until completely eroded, dissolved and absorbed. Stages between these two states are also possible with lead shot for example being partially eroded, dissolved and absorbed before being voided (Franson and Pain 2011, Schulz *et al.* 2007,Kerr *et al*. 2011). Within 20 days of ingestion by wildfowl most lead shot will either have passed through the gastrointestinal tract or been completely eroded (Franson *et al*. 1986, Sanderson and Bellrose 1986). Although many Passeriformes and Columbiformes have a muscular gizzard, experimental work indicates that shot may transit the gut of these taxa relatively quickly (Schulz *et al.* 2007, Vyas *et al.* 2001). Raptors and other carnivorous bird species may regurgitate pieces of lead in their pellets, although the impact on total lead absorption has been found to be variable (Pattee *et al.* 1981)(See Linkage Set B).

Diet: The nutritional, chemical, and physical characteristics of diet affect lead absorption and deposition in tissues (Franson and Pain 2011, Jordan and Bellrose 1951, Koranda *et al.* 1979; Longcore *et al.* 1974, Marn *et al.* 1988, Sanderson and Irwin 1976, Scheuhammer and Norris 1996, Vyas *et al.* 2001). Nutritionally balanced diets high in protein and calcium may mitigate the effects of lead exposure as high levels of both may, to some extent, reduce the absorption of lead and its subsequent delivery to tissues (Franson and Pain 2011, Koranda *et al.* 1979, Sanderson 1992, Scheuhammer and Norris 1996). In addition, certain chemical groups in food bind lead in an insoluble form in the intestine (Morton *et al.* 1985). Experimental work has demonstrated that calcareous grit consumption can reduce lead bioaccessability and thus absorption by reducing acidity within the gizzard (Martinez-Haro *et al*. 2009).

Gender: Medullary bone forms in egg-laying birds and acts as a labile reservoir for the supply of eggshell calcium; when this calcium is mobilised for eggshell formation, intestinal absorption of calcium, and concurrently lead, can increase, resulting in higher bone lead concentrations in similarly exposed female than male birds (Krementz and Ankney 1995, Scheuhammer 1996). A diet deficient in calcium has been shown experimentally to increase lead absorption in female birds (Scheuhammer and Norris 1996).

Age: Over time, as lead accumulates within the bird's body, the likelihood of negative health impacts resulting from chronic low level exposure may increase. In the UK, Newth *et al*. (2012) found that in a sample of wildfowl found dead, adult birds (over two years of age) were significantly more likely to have been diagnosed as dying of lead poisoning than juveniles. Sero-prevalence of lead poisoning is also often found to be higher in adults than juveniles (e.g. Samuel *et al.* 1992).

A.3.2 Impacts of ingestion of spent lead shot from the environment by wild birds

Lead is a metal that is toxic to all vertebrate taxa, acting as a non-specific poison that affects all body systems (see above).

Political boundaries are artificial with respect to ecology and toxicology, and do not affect the nature of the source, pathway and effect links for lead poisoning of wildlife (as illustrated in the numerous conference proceedings and reviews of the literature on this subject, including reports under the African-Eurasian Waterbird Agreement). Whilst political boundaries do not affect the nature of the risk, they may and often do affect the level of risk and ways in which it is managed, which itself can influence the level of risk, and this has been accounted for and described where appropriate.

This section, therefore, includes both UK studies and similar studies from the same or related species with similar genetics, physiology and ecology as they provide relevant evidence for this section of the risk assessment.

The authors are aware of hundreds of scientific articles reporting lead poisoning following ingestion of lead shot by birds directly from the environment, with the vast majority of these surveillance and research reports being undertaken in North America and Europe. The strong source-pathway linkages described above in Section A.1 and A.2 clearly illustrate that in countries where shooting takes place using lead ammunition, environmental contamination with ammunition lead occurs. Whilst wildlife remains exposed to somewhat elevated lead concentrations from non-ammunition sources around mining areas and urban areas, the evidence also shows that lead from ammunition is likely to be the primary source of environmental lead exposure in birds and other wildlife in countries where it is used. We have found no studies that contest this.

In species and situations for which there is a strong source-pathway linkage for lead shot, and in the absence of other obvious sources of lead as candidates for increased blood lead concentrations, a number of studies investigating tissue lead concentrations in live or dead birds (e.g. in blood or bone) have assumed that very elevated concentrations in individuals most likely result from lead shot ingestion. This appears to be the most logical and parsimonious explanation, is consistent with the evidence, and supported by the numerous studies on the pathway linkages outlined in the sections above.

In the next section we examine evidence for (1) a range of sub-lethal effects of lead, (2) lethal effects of lead i.e. mortality, (3) population level effects, and (4) welfare impacts.

A.3.2.1 Sub-lethal effects of lead poisoning from the ingestion of ammunition-derived lead

Lead affects wildlife in a range of ways, and experimental studies, largely on birds but also certain other animal taxa show the following four main areas of sub-lethal effect (which may, depending on effect and/or continuing exposure to lead, become lethal). We focus here on studies on birds as these have the strongest source-pathway linkages and we provide mainly examples of the experimental studies conducted as the literature is extensive.

a. Impacts on the activity of certain blood enzymes

Lead inhibits the activities of several enzymes necessary for the synthesis of haem, e.g. delta-aminolevulinic acid dehydratase (ALAD) and haem synthetase. Inhibition of erythrocyte ALAD activity is considered to be the first measurable biochemical change resulting from lead absorption (Hernberg *et al*. 1970, Tola *et al*. 1973) occurring at very low blood lead concentrations in birds - below 5 µg/dl blood lead level (Martinez-Lopez *et al*. 2004, Pain 1987, 1989). Some reduction in ALAD activity appears to be tolerated in birds without other obvious consequences, especially if this is low level and not sustained for protracted periods. However, acute exposure to lead can result in severe inhibition in ALAD activity and be associated with

haemolytic anaemia (Pain and Rattner 1988, Mateo *et al*. 2003). Holladay *et al*. (2012), in attempting to better simulate the effects of field exposure to lead shot, experimentally fed northern bobwhite quail *Colinus virginianus* with one to three spent number 9 shot with a particular interest in the effects of just one of these small 50mg shot. Some 38 birds (18 males and 20 females) were used in the experiments and a range of physiological and pathological impacts measured. All dosed birds lost weight relative to the control birds, and levels of erythrocyte ALAD were reduced in dosed birds for the duration of the eight week study to levels associated with negative health impacts. The authors conclude that the ingestion of just one number 9 shot is sufficient to cause ill health. Kerr *et al.* (2010) orally dosed northern bobwhite quail with one, five or 10 shot and blood lead, and other toxicity measures were examined along with radiographic studies over time. Quail given 5 or 10 shot exhibited morbidity between weeks one and two and were removed from the study. Radiographs, and high blood lead levels in birds fed one lead shot, indicated that shot dissolved over time. Birds fed one shot showed persistently depressed plasma ALAD activity suggesting possible impaired haematological function, but few other signs of toxicity were seen over the course of the study.

Inhibition of blood ALAD has been exhibited after exposure to lead in many other wildlife species including mammals (see Eisler 1988).

b. Effects on breeding productivity

Scheuhammer (1987) provides a review of the toxic effects of lead including a range of studies on aspects relating to productivity, some of which show no impacts and others which show negative impacts. As examples, the paper reports greater susceptibility of young altricial birds to chronic lead exposure than adults exposed to similar dietary levels, including reduced growth rate of American kestrel *Falco sparverius* nestlings, and lower haematocrits in nesting starlings *Sturnus vulgaris*. The well documented impacts of lead on the developing central nervous system of vertebrate animals would suggest that exposure to lead at an early stage of life is likely to have negative impacts.

Kendall *et al*. (1981) report impacts on spermatogenesis of ringed turtle doves *Streptopelia risoria* experimentally dosed with lead shot.

Flint *et al*. (1997) speculate that exposure to lead of spectacled eiders in a region of Alaska, USA, may lead to reduced fecundity.

c. Immunological effects

Lead may be associated with immunosuppression in birds (e.g. Redig *et al*. 1991, and see Franson and Pain 2011 for review). An effect of lead on antibody-mediated immunity was indicated seven days after the dosage of mallards with one number 4 lead shot (Trust *et al*. 1990) and there was a reduction in cell-mediated immune response in western bluebird *Sialia mexicana* nestlings dosed with three (but not one or two) number 9 lead shot (Fair and Myers 2002). There may also be sex differences in immune response to lead, in relation to storage of lead in bone prior to, and during, breeding by females. Rocke and Samuel (1991) reported an increased immunosuppressive effect of lead in male compared with female mallards during the pre-breeding season while the reverse was found in antibody-mediated immunity in non-breeding zebra finches *Taeniopygia guttata*, but only in birds on a low calcium diet (Snoeijs *et al*. 2005). One experimental study exposing Japanese quail *Coturnix coturnix japonica* to lead acetate found no evidence to support the immunotoxicity of low level lead exposure, and found that it may have instead stimulated the immune system (Nain and Smits 2011); but this was at odds with another study on the same species (Grasman and Scanlon 1995) which found immunosuppressive effects of lead.

d. Behavioural effects

Although a considerable amount is known of the behavioural effects associated with low level lead exposure in humans, relatively little is known about behavioural effects in wildlife. However, in birds there has been a long-term research programme on this issue using herring gulls *Larus argentatus* and common terns *Sterna hirundo* as models (Burger 2005, Burger and Gochfeld 2000). In a number of studies these authors exposed birds in the laboratory and the wild to lead acetate at levels sufficient to result in feather concentrations comparable to those found in some wild birds. They found a range of effects of low-level exposure to lead, including on growth, locomotion, balance, food begging, feeding, thermoregulation, depth perception, and individual recognition in the laboratory and in wild birds. These authors concluded that the lead-induced behavioural impacts were sufficient to affect growth and survival in wild herring gulls. Lead impacts on the developing brain were implicated.

e. Increased susceptibility to mortality from other non-lead related factors

Sub-lethal effects causing loss of coordination and/or partial paralyses may subsequently contribute to reduced survival and premature death from other causes (see above section on immunological effects) such as infectious diseases, parasitism, starvation, predation and flying accidents (Scheuhammer and Norris 1996, Kelly and Kelly 2005, Tavecchia *et al.* 2001).

Kelly and Kelly (2005) found that a significantly larger proportion of birds admitted to RSPCA centres following collision with power lines had moderately elevated blood lead levels (1.21-2µmol/l) [25-41 µg/dl] compared with non-collision birds, and a significantly smaller proportion had high blood lead levels (>5µmol/l) [104 µg/dl]. This suggests an increase risk of collision in swans with moderately elevated blood lead levels, whilst those higher blood lead levels may have a reduced risk of collision, possibly because they are too weak to fly.

Demendi and Petrie (2006) found that one year after a ban on the use of lead shot for waterfowl hunting in Canada, not only did the proportion of hunter-shot birds having ingested lead shot decline sharply, but so did the total proportion of birds having ingested any type of shot (from 8-11% pre-ban to 3.7% post-ban total shot ingestion). This suggests that high pre-ban numbers may have resulted partly from the health effects of lead shot ingestion having affected the birds and increased harvest susceptibility. A similar effect was suggested by Heitmeyer *et al.* (1993) where hunter-killed mallard from a region of Missouri, USA, had a far higher incidence of ingested shot than birds collected by researchers.

Evidence of shot ingestion by birds, and by implication sub-lethal effects, comes from a wide range of studies from the UK and elsewhere around the world; where lead ammunition is commonly used it follows that species with strong source-pathway linkages will be at high risk of ingesting it and will generally be affected by it in some way. A recent study in Britain gives contemporary information on elevated blood lead levels, within the ranges at which the sub-lethal effects described above occur.

A recent study of lead poisoning in Britain found that a third of wintering wildfowl of four species (whooper swans, Bewick's swans, pintail and pochard; n=285) trapped primarily using decoy-type pipes during the winter of 2010/2011 had elevated blood lead levels indicative of lead poisoning (>20µg/dl, Table A.5) (Newth *et al*. 2012). Blood lead levels of >100 µg/dl were reported in some birds. Birds were bled in mid-winter (December – February) i.e. at a time when the majority of birds would have been within UK for over 40 days, to increase the likelihood of measuring the effects of *recent* ingestion of lead i.e. the lead detected would likely have been picked up within the UK. The authors suggested that the birds may have been exposed to some historical lead but that the majority of the lead ingested would probably have been recently deposited lead (see Section A.2.1 above for studies illustrating that most shot ingested is the most recently deposited), some of which may have been illegally deposited in wetlands or legally deposited on terrestrial habitats where swans and geese, in particular, feed.

Table A.5. Blood lead concentrations in wildfowl trapped in the winter of 2010/11 in Britain.

Species	Blood lead concentrations (µg/dl)				
	0 – <20	20 – <50	50 – 100	>100	Total
Bewick's swan	34	4	1	0	39
Whooper swan	101	57	16	3	177
Pochard	23	6	0	0	29
Pintail	30	3	5	2	40
Total	**188 (65.9%)**	**70 (24.6%)**	**22 (7.7%)**	**5 (1.8%)**	**285**

Data from Newth *et al.* (2012)

O'Connell *et al*. (2008) measured blood lead levels of whooper swans in Scotland in the winters of 2003 and 2005, and in England in the winters of 2004 and 2005 and using the equivalent of 25µg/dl as a cut-off to illustrate elevated levels, they found between 38% and 88% of birds to be affected. In general these levels were higher than those found in birds sampled in the summer months in Iceland.

These studies indicate that a substantial proportion of wildfowl still suffer from sub-lethal effects of lead poisoning, at sub-clinical, clinical or severe levels (see Table A4) in England.

A.3.2.2 Lethal effects i.e. mortality caused by lead poisoning from ingestion of lead pellets

The weight of evidence about the problem of lead poisoning and mortality in birds caused by the ingestion of lead shot is great enough to have prompted the formulation and implementation of legislative processes to reduce risks in at least 27 countries including 22/27 European Union member states, including the UK (Avery and Watson 2009, HMSO 1999, 2002a, 2002b, 2003, 2004, 2009, Mateo 2009).

As discussed, both acute and chronic lead poisoning can cause mortality of birds. A large number of experimental studies have shown toxic effects and mortality resulting from the ingestion of lead shot by birds, and similarly extensive literature exists from field studies where dead birds have been diagnosed as dying of lead poisoning following shot ingestion. This literature has been reviewed numerous times and is not reviewed again in detail here (e.g. see the following references and reference lists within: Beintema 2001, Bellrose 1959, Brown *et al.* 1992, Franson *et al.* 2009, Franson and Pain 2011, Friend and Franson 1999, Hall and Fisher 1985, Locke and Friend 1992, Mudge 1983, O'Connell *et al*. 2008, Olney 1960, Owen and Cadbury 1975, Pain, 1990a, 1990b, 1991a, 1992, 1996; Pokras and Kneeland 2009, Potts 2005, Scheuhammer and Norris 1995, Schulz *et al*. 2007, Sharley *et al*. 1992, Thomas 1975). Instead a small number of illustrative reports and issues are discussed in slightly more detail within this section.
Pain and Rattner (1988), Tavecchia *et al.* (2001), Guillemain *et al.* (2007) and Mateo (2009) report ingestion of one or two lead pellets being sufficient under certain circumstances to kill a duck outright or reduce survival. Clinical signs of poisoning may be seen within a few days of lead shot ingestion with death occurring from the toxicosis within as little as 4-6 days to a few weeks, or even months later. Larger doses will hasten mortality (Bellrose 1975, Pain 1990a, Pokras and Kneeland 2009).

Flint and Grand (1997) report high levels of mortality in breeding female spectacled eiders with the toxicosis being responsible for more than half of the mortality in one area of Alaska.

O'Connell *et al*. (2008) report a lead poisoning morbidity and mortality event during December 2005 involving c.100 swans (two thirds whooper and one third mute) on the Wexford Slobs, Republic of Ireland. The authors speculated that the birds had been actively seeking grit in an area

used by hunters with the situation being exacerbated by birds being attracted by bait put out by hunters to attract ducks.

Newth *et al*. (2012) found lead poisoning to be responsible for deaths of 10.6% of 2,365 wildfowl of 14 species found dead in Britain between 1971 and 2010. These included cases in mute swan, whooper swan, Bewick's swan, Canada goose, western greylag goose *Anser anser*, pink-footed goose, mallard, northern pintail, gadwall *Anas strepera*, common teal, European pochard, tufted duck, common shelduck *Tadorna tadorna* and shoveler. The toxicosis was responsible for 27.3% of whooper swan deaths, 23% of Bewick's swan deaths and 16.7% of pochard deaths. More recently (from 2000-2010), i.e. following introduction of regulations to reduce unnecessary lead poisoning mortality due lead shot, this proportion of deaths due to lead poisoning was 8.1% (a non-significant decline).

Schulz *et al*. (2009) found, in a study involving experimentally feeding 157 mourning doves lead pellets (of which 104 died within three weeks), that short-term survival (two days post treatment) of mourning doves experimentally fed lead pellets was inversely related to number of pellets retained, with each additional pellet increasing the hazard of death by 18% for males and 25.7% for females. Potts (2005) describes mortality of grey partridge found dead between 1947 and 1992. Overall 1.4% of 1,318 birds were considered to have died of lead poisoning due to ingestion of lead shot (with prevalence ranging from 0.3% to 4% in different time periods).

As discussed, lead shot ingestion, poisoning and sometimes associated mortality have been described in other taxa including waders (e.g. see Scheuhammer and Norris 1996, Thomas 1975, review by Mateo 2009), rails (Thomas 1975), cranes (e.g. Windingstad 1988); flamingos (Scheuhammer and Norris 1996, Ancora *et al.* 2008); Pelecanifomes (Scheuhammer and Norris 1996) and Columbiformes (e.g. Clausen and Wolstrup 1979, Franson *et al*. 2009).

Cases of lead poisoning in apparently rarely affected species i.e. woodpeckers in Sweden are reported by Morner and Petersson (1999) who speculate that birds ingested shot which had been shot into trees and birds picked them out assuming them to be burrowing insects.

A.3.2.3 Population impacts

Determining impacts at a population level is not straightforward. The only reasonably robust way of doing this is to model and compare alternative population trajectories for a species based upon demographic rates estimated when effects of ammunition-derived lead are present and absent.

Robust surveillance data, current ingestion levels and a sound understanding of population dynamics and factors such as dispersal, philopatry, site fidelity, long-term reproductive success and mortality rates are required for survival analyses and an assessment of population-level effects of contaminants. The long-term and complicated nature of collecting such information means that for most species, an accurate assessment of the extent of mortality, and possible population level effects from lead ingestion, whatever the source, is currently not possible for most species (UNEP/AEWA 2011).

When detailed information on demographic rates is not available, it is legitimate to adopt a comparative approach to the detection of effects of external drivers on population trends (Green 1995). This involves comparing population trends across species or populations with differing levels of exposure to ammunition-derived lead. A negative correlation between population trend and exposure may be suggestive of population-level effects. Mateo (2009) correlated population trends in a set of 15 taxonomically similar European wildfowl species with broadly comparable life-history characteristics with reported prevalence levels of shot ingestion. There was a statistically significant tendency for species with high levels of shot ingestion to have more negative population trends than species with low shot ingestion levels. As was pointed out by Mateo, correlation is not causation and effects of some unidentified factor might have led to a spurious correlation. Nonetheless, this analysis is suggestive of an effect of lead contamination on population trend and

indicates that it is worth looking further at the effects of lead, especially for species with high shot ingestion levels.

Whilst large-scale mortality events occasionally occur which may cause local population impacts (e.g. the aforementioned Wexford Slobs case - O'Connell 2008), mortality from lead poisoning is usually less conspicuous as it may often result in the frequent and largely invisible losses of small numbers of birds that remain undetected (Scheuhammer 1987, Newth *et al.* 2012). Moribund birds often become increasingly reclusive and dead birds may be scavenged before being detected (Sanderson and Bellrose 1986, Stutzenbaker *et al.* 1986, Pain 1991b, Newth *et al.* 2012). This can present risks to scavengers, as outlined in Linkage Set B. Some birds may die from lead poisoning without exhibiting typical pathology, especially in cases of acute exposure, and thus their death may be subsequently attributed to another cause (Beyer *et al.* 1998a, Newth *et al.* 2012).

In 1983, prior to legislation aimed at reducing lead poisoning mortality due to ingestion of lead shot, Mudge provided a conservative estimate that some 2.3% of mallards, about 8000 birds, died annually from lead poisoning in Britain, with an estimate of some 10,000-100,000 wildfowl dying annually within Europe. More recently, in Europe, it has been estimated that approximately a million wildfowl (from 17 species), i.e. 8.7% of the total population may die every winter from lead poisoning caused by ingestion of lead shot (Mateo 2009). Such mortality estimates based upon shot ingestion levels reported for hunter-shot birds, even when taking into account the possible increased susceptibility of lead poisoned birds to being shot (see A.2.1.2), could overestimate mortality if shot ingestion levels have declined significantly over time. However, such mortality estimates will exclude birds that have already succumbed to lead poisoning and so may underestimate the magnitude of mortality, and similarly shot ingestion levels may have increased in some species over time. While Mateo has not claimed his estimate to be precise, direct mortality from shot ingestion by wildfowl is undoubtedly very high and can be reasonably expected to be at least hundreds of thousands of birds a year across Europe, and possibly approaching a million or more. This, like mortality from other diseases, is also likely to vary considerably annually in relation to prevailing environmental conditions such as temperature and food availability, which have the potential to influence survival in diseased birds (e.g. see A.3.1.1. on diet and A.3.2.1. on behavioural impacts and increased susceptibility to mortality from other factors).

The potential exists, due to very high shot ingestion levels, for lead poisoning to be contributing to the declines of certain species of common wildfowl, e.g. pochard and northern pintail (*e.g*. Mateo 2009). Lead is known to be a serious threat to certain globally threatened European wildfowl, e.g. white-headed duck (Mateo *et al.* 2001, Svanberg *et al.* 2006, Mateo 2009, Taggart *et al.* 2009). The impacts of sub-lethal effects will affect many more birds.

As grey partridge are red-listed in the UK in 'Birds of Conservation Concern 3' (Eaton *et al*. 2009), losses caused by lead shot ingestion are worthy of detailed evaluation (Potts 2005).

Prior to the ban on lead shot for waterfowl hunting in the USA, it was estimated that between 1.6 and 2.4 million of some 100 million wildfowl, or 2.5% of the autumn population died from lead poisoning annually (Bellrose 1959, Friend *et al.* 1999). Although birds continued to be poisoned by lead after the ban on lead shot, the mortality rate from lead poisoning declined from 4% estimates for 1938-1954 to around 1.44% estimated for 1996-1997 (after the ban) and an estimated 1.4 million ducks were thought to have been prevented from dying from lead poisoning in 1997 alone (Anderson 2000).

As lead can affect both breeding productivity and survival, it has potential to affect population size. However, the effect of lead on reproductive output has not been studied to any great extent, though several studies have investigated survival in relation to lead burden:

Guillemain *et al*. (2007) analysed approximately 40,000 x-rays of teal, trapped in the Camargue, France, from 1957 to 1978, to assess, by modelling and statistical procedures, the long-term consequences on survival of both ingested and embedded lead shot, in relation to their known

ringing/release date. They found a negative but unquantified impact on survival from one or more ingested pellets but no indication of a negative effect on survival of embedded shot.

Tavecchia *et al.* (2001) analysed recoveries of adult mallard ringed in the Camargue, France, over the period 1960–71, for which the amount and type of lead exposure had been determined by X-radiography when the birds were captured for ringing. Presence of radio-dense shot in the gizzard and embedded in tissues was distinguished. The proportion of gizzard-contaminated birds was 11%, whilst the proportion of birds carrying pellets in body tissues increased linearly from 19% to 29% during the study period. The annual survival rate of mallards containing radio-dense shot was 19% lower than the survival of unaffected birds. Embedded and ingested shot had similar and additive effects on survival. Based upon the proportion of birds with shot in the gizzard and the estimated effect of shot on survival, these authors estimated that 1.5% of wintering mallards could die of lead poisoning from ingested shot every year in the Camargue. Mortality from embedded shot and wounding would be additional to this.

Several factors may make it difficult to detect the effects of lead on survival. For ingested lead, the number of pellets in the gastrointestinal tract may change over time. If so, individuals X-rayed when captured for ringing which are found to have radio-dense pellets in the gizzard may lose them subsequently whilst birds with no pellets may acquire them shortly after examination. These changes will cause any estimate of the effects of ingested lead on survival by comparison among groups of birds with different lead status at one examination to be an underestimate of the true effect, with the degree of underestimation increasing with the rate of turnover of lead in the alimentary tract. Birds that were not contaminated at the time of capture may have been exposed to lead previously (Guitart *et al*. 1994) or subsequently and; trapped mallards may have expelled their pellets soon after release (Hovette 1972). These lend increased significance to the finding that decreased survival was detected in the studies described above and are likely to mean that the estimated proportions of all birds dying as a result of ingested lead are underestimates.

Grand *et al*. (1998) found reduced survival for adult female spectacled eiders exposed to lead prior to hatching their eggs with only 44% annual survival rate compared with 78% annual survival rate for females not exposed to lead. The authors considered that 61% of the mortality of the lead exposed birds could be considered additive to the expected mortality of unexposed birds. However, in wildfowl, the extent to which mortality from lead poisoning might be partially compensated for within hunting mortality (e.g. see Heitmeyer *et al.* (1993), Demendi and Petrie (2006) and Section A.3.2.1) or be additive to other mortality, as suggested above, is unknown. Potential exists for mortality to be additive, and it is likely that this would be related not only to the extent of lead poisoning but also other factors.

Lead poisoning poses a particular problem for long-lived species with relatively low rates of reproduction. In the UK, swans are the taxon at greatest risk of such impacts given these life history traits and high prevalence of elevated blood lead levels (O'Connell *et al*. 2008, Newth *et al*. 2012).

No experiments have been undertaken to determine impacts of lead at a population level. However, it is worth noting that after a ban in 1987 on the importation and sale of lead angler's weights of between 0.06 and 26.5 grams in weight in the UK, and subsequently on their use by the Regional Water Authorities in England and Wales, there was a sharp reduction in most areas in the numbers of mute swans dying or very sick from lead poisoning. This is considered to be a key factor in the subsequent increase in the species' population (Sears and Hunt 1991, Perrins *et al*. 2003). Given the high prevalence of elevated blood lead levels originating from ammunition sources in migratory swans in the UK it is likely that removal of this sub-lethal toxic burden may result in increased survival, and possibly productivity, which may have an impact at a population level.

A.3.2.4 Welfare impacts

The welfare impacts of lead poisoning are varied and as discussed, include mortality with a short or long period of time between ingestion and death. Additionally, the toxicosis can result in a range of pathological changes and clinical conditions which impact welfare. These include: anaemia; lethargy; anorexia, paralysis of the upper alimentary canal leading to food impaction and vomiting, weight loss, muscular atrophy and emaciation; degeneration of liver and kidney; oedema; a range of central nervous signs including reduced muscular coordination, demyelinating lesions in the central nervous system, paralysis of the legs and/or wings (birds losing their ability to walk may drag themselves about causing abrasions on their wings, WWT, unpublished); convulsions; and diarrhoea (Sainsbury *et al.* 1995, Friend *et al.* 1999).

Many of these pathological changes and clinical signs are considered likely to cause severe pain and distress lasting throughout the period of poisoning (Sainsbury *et al*. 1995). Reduced coordination and impacts on mobility are likely to increase fear of predation.

Also see Section A.3.2.1 Sub-lethal impacts. Predisposition to other conditions such as infectious diseases will result in a further range of welfare issues.

Also see Section A.3.2.3 Population impacts for estimation of numbers of animals affected annually and thus the scale of likely suffering.

Conclusion A.3.2 Probability of impacts on health, welfare and populations of wild birds from ingestion of spent lead shot from the environment

There is **strong evidence**, both experimental and from field studies, of mortality and morbidity occurring as a result of direct ingestion in a wide range of avian species and **high probability** of this occurring in the UK wherever lead shot usage overlaps with at risk species' feeding areas. These risks are currently considered to be greatest, in descending order, in: wildfowl (both grazing and non-grazing); other waterbirds including cranes, rails and waders; terrestrial gamebirds; other avian taxa. Were current restrictions on the use of lead gunshot in the UK adhered to, the risks would likely be highest to grazing wildfowl, followed by the other groups mentioned.

From the known pathology and physiology of the poisoning plus its effect on coordination and mobility, there is **high probability** of serious welfare impacts in the majority of individual birds that ingest lead shot.

The extent of lead exposure in some species suggests the potential for effects upon population size, although the detailed studies necessary to establish this have not been undertaken in species found in the UK. The extent to which lead poisoning mortality may be compensated for by other factors affecting survival is unknown. It is estimated that at least hundreds of thousands and possibly more than a million waterbirds alone may die of lead poisoning as a result of shot ingestion every winter in Europe. It would be difficult to obtain more precise estimates as many factorsmay influence the level of annual mortality resulting from lead poisoning.

A.3.3 Impacts of ingestion of spent lead shot from the environment by wild non-avian animals

A.3.3.1 Individual impacts

Regardless of source, particulate lead will affect the host as described in Section A.3.1. There is a theoretical possibility of grazing and other mammals consuming lead shot from the environment, particularly from areas where lead shot usage is high e.g. clay pigeon shoots, with consequent clinical or sub-clinical impacts. The authors are not aware of any studies investigating this Source-Pathway-Receptor linkage set.

A.3.3.2 Population impacts

There is no available evidence to support a population impact on non-avian animals from this Source-Pathway-Receptor linkage set.

Conclusion A.3.3 Probability of impacts on health, welfare and populations of wild non-avian animals from ingestion of spent lead shot from the environment

There is **no available evidence** to support an impact from this source in non-avian animals and a **low probability** that this occurs to any great extent. Any impacts are likely to be restricted to individual animals rather than populations and are more likely to occur in the vicinity of high lead shot usage e.g. clay pigeon shoots.

A.3.4. Impacts of ingestion of spent lead bullets, or fragments thereof, from the environment by wild animals

A.3.4.1 Individual impacts

As for Section A.3.3, regardless of source, particulate lead will affect the host as described in Section A.3.1. As discussed previously, Lewis *et al*. (2001) describe mortality due either to ingestion of bullets, or fragments thereof, and/or lead that had already been abraded or otherwise released from them into the soil component, in a firing range facility in the USA, poisoning passerines and a grey squirrel, i.e. an area of extremely high bullet usage. The authors are not aware of other reports of poisoning from this source in the UK or elsewhere.

A.3.4.2 Population impacts

There is no available evidence regarding population impacts on wild animals from this Source-Pathway-Receptor linkage set.

Conclusion A.3.4 Probability of impacts on health, welfare and populations of wild animals from ingestion of spent lead bullets, or fragments thereof, from the environment

Away from firing ranges, there are **insufficient studies** to support an impact from lead bullets, or fragments thereof, in the environment on wildlife and a **low probability** that this occurs to any great extent. At, or in close proximity to, firing ranges local population level effects in some species could conceivably occur.

Figure A.2 summaries the pathway and effects of direct ingestion of lead ammunition from the environment. Note that most of the risks identified result from exposure to lead from shot rather than bullets. Table 1 provides a synthesis of explanatory text.

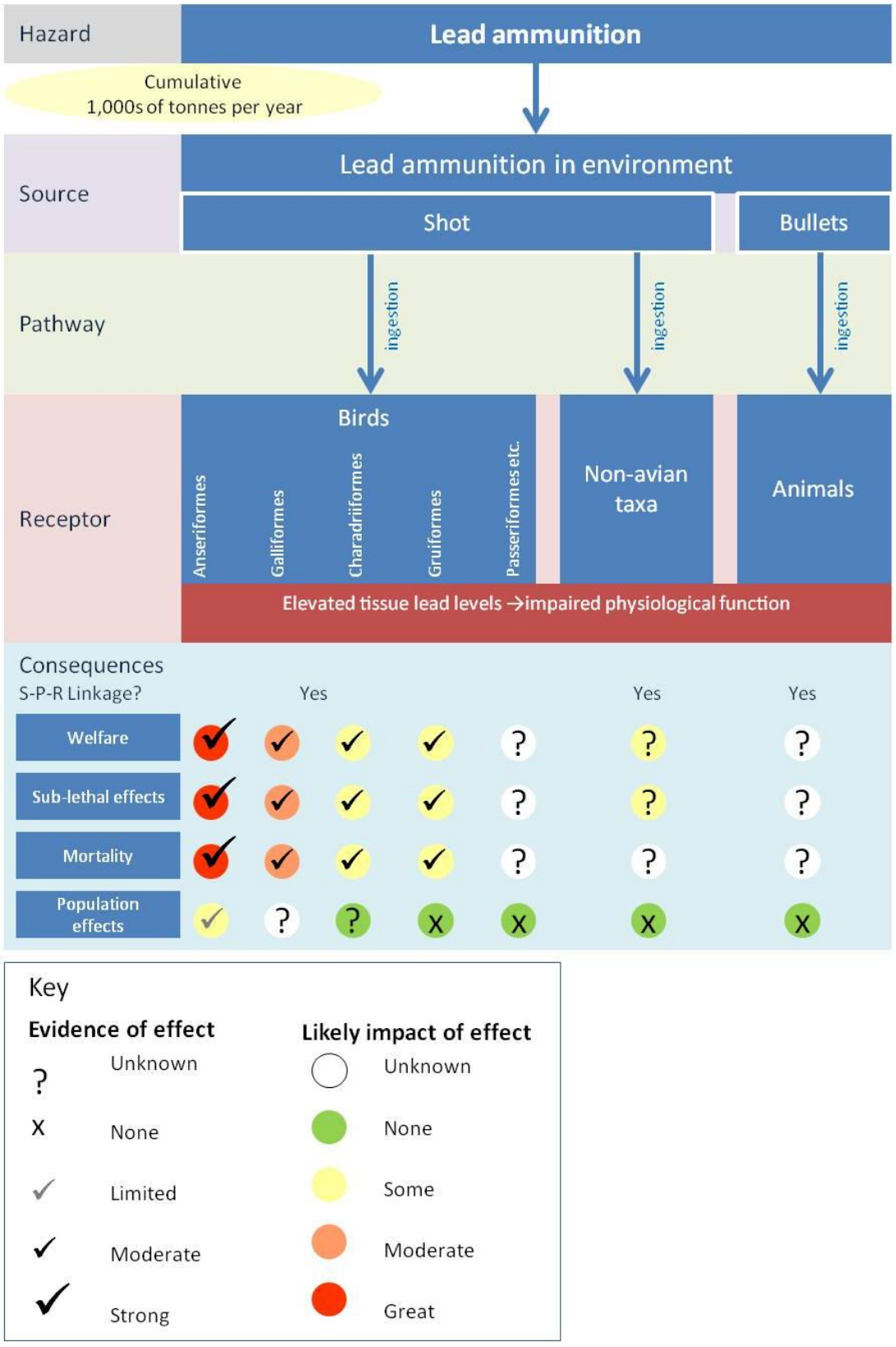


Figure A.2. Linkage Set A illustrating pathways and summary of effects on animals ingesting either lead shot or bullets directly from the environment.

Linkage Set B: Secondary poisoning in predatory and scavenging animals from ingestion of lead ammunition contained within prey or carrion

Linkage Set B represents the risks associated with whole gunshot and shot or bullet fragments being ingested by predators and scavengers (Table B.1).

Table B.1. Conceptual model of Source-Pathway-Receptor linkages which might result in lead derived from ammunition being taken up by predators and scavengers and adversely affecting their health or populations.

S-P-R linkage label	Source	Pathway	Receptor
B (i)	Large and small fragments and particles of ammunition-derived lead from projectiles used to kill game animals and predators are embedded in the animal's tissue. This may include fragments shot into the animal during previous episodes when it was shot but not killed.	• Whole carcasses of animals, including large game, waterfowl, gamebirds, small game mammals and predators, killed using lead may be accidentally or deliberately left in the field and not recovered. • Some of the tissue of game animals contaminated with lead fragments and particles is not removed from the field when the carcass is removed. For example, the viscera of deer may be discarded and left for scavengers. • Some quarry animals are wounded but not killed by the projectiles, but are weakened and selectively taken later by predators. Carcasses and viscera of quarry animals, or live but wounded animals are used for food by scavengers and raptors. Fragments of ammunition or whole projectiles may be ingested along with this food, though some may be detected by the animal and rejected.	Adverse effects on the health of the predatory or scavenging animal from effects of absorbed dietary lead acting on the functioning of various organ systems and potentially leading to reduced welfare, death and impacts on population size.
B (ii)	Shotgun pellets in the environment are ingested by birds (see Linkage Set A) and are then present in the alimentary tract. Elevated lead levels in tissues of lead poisoning prey or carrion (either from ingestion *i.e*. Linkage Sets A and C, or absoption from embedded ammunition *i.e*. Linkage Set D).	(Mainly) birds which have ingested gunshot pellets may suffer adverse effects on their health and be weakened. Predators kill birds which have ingested shotgun pellets and may preferentially take them because some are weakened. Shotgun pellets in the alimentary tract may be ingested when the predator eats the bird's carcass, though some predators may not eat the alimentary tract. When a predator discards the alimentary tract of its prey it may be eaten by scavengers or the bird may die of another cause and be eaten by scavengers. Some pellets may be detected by the predator or scavenger and be rejected. In other cases lead shot or shot fragments in the intestines of predator or scavengers' food is ingested, absorbed in the alimentary tract and enters the bloodstream. Predators and scavengers may also be exposed to lead from ammunition that has been absorbed and biologically incorporated in their prey (see Linkage Sets A,C and D).	
B (iii)	Ammunition-derived lead in water, soil or sediment.	Projectiles from game shooting are deposited in soil, water and mud. They are ingested directly by animals, sometimes after a considerable period during which lead compounds are formed on the surface of the projectile or nearby. Ammunition-derived lead compounds also pass into water and plant and animal foods of animals and are ingested by them, are absorbed and incorporated in tissues (see Linkage Set C). The animal dies or is killed by a predator. Its carcass is eaten by the predator or scavengers.	

B.1 Source: Evaluating the probability of retention and fragmentation of projectiles shot into tissues of game animals

B.1.1 Numbers and species of game animals killed using lead gunshot and bullets

See Section A.1.1.2 and Supporting Information 2 for calculations of amount of shot used annually in UK. In summary, excluding shot used for pest control and mammal shooting e.g. used for killing rabbits, in total, we estimate that approximately 5,500-12,900 tonnes of lead shot, 68-154 billion individual shot, are deposited into the UK's environment annually.

See Section A.1.2 for amount of lead bullets used annually. In total, excluding bullets used by the police and military it is estimates that approximately 200 tonnes of lead bullets are used annually.

Although the total numbers of animals shot in the UK per year using lead ammunition is uncertain (see Section A.1.1.2 and A.1.2), it is clearly tens of millions. An unknown proportion of the carcasses is not recovered and hence potentially available to scavengers. For many of the tens of thousands of red deer shot per year, the viscera are discarded in the field and they and the remnants of lead ammunition within them are potentially available to scavengers. A further additional set of animals are wounded by gunshot and bullets and may carry remnants of lead ammunition in their bodies. These animals may be eaten by predators and perhaps selected as prey because of their weakened condition or die later and be eaten by scavengers.

The proportion of animals, or parts thereof, shot using lead ammunition, which are potentially available to predators and scavengers is unknown for some species but may be high for quarry species such as ducks and geese (see following Section B.1.2), and must represent hundreds of thousands of animals potentially contaminated with ammunition-derived lead per year entering the food supply of wild predators and scavengers. Hence, the quantity and fate of this potential source of lead contamination is worthy of further examination.

Sales of non-lead ammunition in the UK are low. Use of lead ammunition is permitted throughout the UK away from wetlands and for species not associated with wetlands and ammunition composed primarily of lead is used for almost all shooting of game in these habitats. The use of lead ammunition to shoot ducks has not been lawful in England since 1999, but a recent study found that 70% of wild duck carcasses bought from game dealers in England had been shot using lead ammunition (Cromie *et al*. 2010). Hence, it is clear that the vast majority of game animals shot in the UK are killed using lead ammunition.

Conclusion B.1.1 Probability of large numbers and species of game animals being shot with lead ammunition

The number of animals, or parts thereof, shot using lead ammunition which are potentially available to predators and scavengers in the UK is uncertain, but there is **strong evidence** that it is likely to be at least hundreds of thousands of individuals per year. The true number might be substantially larger.

B.1.2 Presence of shot-in projectiles and fragments thereof in the tissues of game animals killed or wounded using lead shot and bullets

The tissues of game animals killed using shotgun pellets usually contain some of the pellets which struck the animal and killed it. Pain *et al.* (2010) performed X-radiography on 121 entire carcasses of wild-shot red grouse *Lagopus lagopus*, red-legged partridge, pheasant, mallard, woodpigeon and woodcock *Scolopax rusticola* obtained from retailers and shoots in the UK (16 – 26 individuals per species). They detected large radio-dense objects, which almost certainly represented whole shotgun pellets or portions of pellets greater than 50% of the initial pellet mass, in 65% of

individuals, with an overall average of 2.17 pellets or large fragments per bird and a maximum of 18. Samples of the large objects identified on the X-rays were recovered and tested to determine which metals they were composed of. Ninety-one percent of the objects were composed principally of lead, with the other objects being non-lead shot. Many of the 35% of birds which had no detectable pellets remaining in the carcass had radio-dense fragments smaller than 50% of initial pellet mass scattered within the birds' tissues, so that 87% of all birds examined had whole pellets, large fragments, small fragments or some combination of the three types.

Substantial fragmentation of lead shot occurs when gamebirds and waterfowl are killed using gunshot. X-radiographic studies show that gamebirds and mammals shot either with lead gunshot or lead bullets often contained lead fragments which were small, numerous and widely dispersed in edible tissues away from the wound canals. In the Pain *et al.* (2010) study, the authors found small fragments on X-ray in 76% of 121 gamebirds and duck of six species examined. Most fragments were less than about a tenth of a shot in size. The small radio-dense particles sometimes appeared to follow the track taken by a shotgun pellet during passage through a bird, were sometimes clustered around bone, but sometimes appeared to be scattered throughout the bird.

Pain *et al.* (2007) obtained X-radiographs of four rabbits shot using 0.22 calibre lead rifle bullets through the thorax or neck. Radio-dense objects were identified in three of the four radiographs, with between one and ten objects per animal. No radio-dense object was visible in the carcass of the fourth animal. The objects were widely distributed in the thorax and abdomen in two animals and one object was located in a forelimb of a third animal. The objects ranged between 0.5 and 4mm in diameter. Whilst the identity of the objects was not established to be bullet fragments, this seems the only plausible explanation for their presence. Hence, this study indicates that remnants of projectiles remain within the edible tissues and that projectile fragmentation occurs for bullet-shot small game animals.

Several examples of reliable evidence exist for substantial fragmentation of lead bullets within the carcasses of large game animals. Dobrowolska and Melosik (2008) measured lead concentrations in samples of muscle tissue from ten wild boar *Sus scrofa* and ten red deer obtained immediately after they had been shot. The samples were collected from around the entry and exit wounds, from around the bullet pathway at different sites along its length, from distances of about 5, 15, 25, and 30cm from the bullet track and also from muscle as far away as possible from the bullet track. The results showed that lead concentration was consistently elevated in tissues close to the bullet track in all 20 animals. All individuals had higher mean concentrations of lead in tissue 5cm and 15cm from the bullet track than in muscle from the same animal distant from the bullet track and the mean lead concentration was also elevated at 25 and 30 cm from the bullet track. The degree of elevation of lead concentration near the bullet track was substantial, with geometric means of the ratio relative to the distant sample of 158 times the distant level at 5cm from the bullet track, 28 times at 15cm, 2.8 times at 25cm and 1.8 times at 30cm. The only plausible interpretation of these large and consistent increases in lead concentration in the vicinity of the bullet track is that fragments and particles of lead were embedded in the tissues at substantial distances from the bullet track.

Knott *et al.* (2010) performed X-radiography on carcasses of ten red deer and two roe deer *Capreolus capreolus* killed by a single shot to the thorax using copper-jacketed lead-cored bullets. The thoracic region of each of the eviscerated carcasses was X-rayed. An average of 356 radio-dense fragments per deer was visible on radiographs of the thoracic region of the carcass. All deer contained some fragments. Tests using known particles of grit indicated that the presence of radio-dense particles on X-rays was not an artefact of the presence of grit on the pelage, so the fragments are likely to have been metallic and predominantly composed of lead. The weight of fragments was estimated by reference to an X-rayed scale of bullet fragments of known weight. The average total weight of radio-dense fragments, in the thoracic portion of the carcass, was estimated to be 1.2g, which is 14% of the weight of the bullet. An average of 180 radio-dense fragments was visible on radiographs of the viscera. The mean fragment size was smaller than for the carcass and the average total weight of radio-dense fragments in the viscera was estimated to be 0.21g.

Grund *et al.* (2010) performed X-radiography on carcasses of wild white-tailed deer killed using a rifle and on carcasses of domestic sheep *Ovis aries* which were euthanized and the carcass shot through the side of the thorax using a rifle to simulate shooting of deer. As the authors pointed out, the experiments on sheep were expected to give lower levels of fragmentation that the shooting of wild deer because the point of entry of the bullet was standardised and avoided the scapula, which was sometimes struck when wild deer were shot. Different types of lead-based bullets from centrefire rifles were compared in the experiment on the sheep carcasses. Centrefire bullets of two types designed to expand rapidly after impact both left large numbers of bullet fragments distributed widely in the carcasses (mean number of fragments detected per carcass, 60 in deer and 141 in sheep for one bullet type, 86 fragments per carcass, tested in sheep only, for the other bullet type). For two types of centrefire bullets designed to have more restricted expansion after impact, one type left a similar mean number of fragments in sheep to one of the rapid expansion types (mean 82 fragments), whilst the other type resulted in far fewer fragments (mean 9 fragments).

Hunt *et al.* (2006) studied remains of white-tailed deer and mule deer *Odocoileus hemonius* killed with centre-fire rifles and lead hunting bullets. X-radiography showed a mean of 551 radio-dense fragments per deer in five whole deer carcasses. There was a mean of 181 fragments per deer in eviscerated carcasses of nine deer and a mean of 144 fragments per offal pile in discarded abdominal viscera from 20 deer. Radio-dense fragments were detected in 90% of the offal piles studied and 25% of piles contained more than 200 detectable fragments. Equivalent studies of carcasses and viscera of deer killed using expanding copper bullets contained few radio-dense fragments (0-2 fragments per carcass or pile).

In a separate study, Hunt *et al.* (2009) X-radiographed the area of the wound channel in eviscerated carcasses of 30 white-tailed deer shot with conventional copper-jacketed lead hunting bullets and found metal fragments in all the deer. The median number of visible fragments was 136 per deer, with a maximum dispersion of 45cm between fragments and a mean extreme distance between fragment clusters of 24cm. Ninety-three percent of small samples of minced meat derived from these deer and selected from packages because a radio-dense metal fragment was visible on an X-radiograph in that part of the flattened package, contained lead at levels above background, indicating that the metal fragments detected by radiography were predominantly composed of lead. The two samples without lead contained copper, which was probably derived from the jacket of the bullet.

Krone *et al.* (2009) analysed X-radiographs of 315 freshly-killed wild-shot game ungulates (deer, wild boar and chamois *Rupicapra rupicapra*) killed by hunters with bullets containing lead. They found means of 89 – 279 radio-dense fragments per carcass, with variation among the types of bullets used and a maximum per carcass of 500 fragments. Discarded viscera from 14 ungulates killed using bullets containing lead all contained some radio-dense fragments, but numbers detected per animal ranged from 2 to 600.

In addition to studies of lead ammunition and fragments in dead animals and animal parts, numerous studies have used X-radiography to investigate proportions of live birds, predominantly wildfowl, carrying embedded shot pellets.

Newth *et al*. 2011 reported on the proportion of embedded pellets in migratory swans trapped and radiographed in the UK. Some 31.2% of 735 Bewick's swans and 13.6% of 397 whooper swans were found to be carrying embedded pellets.

In a Danish study of pink-footed geese, Noer and Madsen (1996) reported 24.6% and 35% of juvenile and adult birds respectively carrying embedded shot.

Tavecchia *et al*. (2001) in their study of 2,740 trapped mallards in the Camargue, France, between 1960 and 1971, found between 19 and 29% to be carrying embedded shot, with the proportion increasing almost linearly over the period of the study, in line with the number of hunters in the Camargue (Tamisier and Dehorter 1999).

Guillemain *et al.* (2007) in their study of nearly 40,000 teal trapped in the Camargue and subjected to X-radiography found that on average 9.6% of adult males, 7.5% of adult females and 4.4% of first year birds had shot embedded in their body tissues.

Krone *et al.* (2009) collected X-radiographs from 154 wild migratory geese captured alive and apparently healthy in Germany. Radio-dense objects which were almost certainly embedded shot, were detected in 21% of birds. It could not be determined whether the shot were composed of lead or of other metals, but the study demonstrates that birds regularly survive being shot with shotgun pellets of some kind and are then available to predators, and to scavengers when they are predated or die of some other cause.

Table B.2 summarises prevalence of embedded shot in a range of bird species.

Table B.2. Summary table of prevalence of embedded shot in mainly wildfowl species.

Species	Country	M*	Embedded shot (%)	Reference
Pink-footed goose (*Anser brachyrhynchus*)	Denmark (1990-92)	T	24.6 (juvs)-36.0 (adults)	Noer & Madsen (1996)
	Denmark (1998-05)	T	9.2-22.2	Noer *et al.* (2007)
Greylag goose (*Anser anser*)	Spain, Doñana	FD	65.3	Mateo *et al.* (2007)
	Spain, Doñana	T	44.4	Mateo *et al.* (2007)
Canada goose (Branta canadensis)	Canada (Maritimes)	T	32	CWS, unpublished data
	USA	T	42	Funk (1951)
Bean goose (*Anser fabalis*)	Sweden		28 (juvs); 62 (adults)	Jönsson *et al.* 1985.
Small Canada goose (Branta canadensis parvipes)	Canada	T	≥25	Macinnes *et al.* (1974)
Brant goose (Branta bernicla)	USA	T	20	Kirby *et al*. (1983)
Barnacle geese (*Branta leucopsis*)	Denmark	T	13	Holm & Madsen (2012)
Mallard (Anas platyrhynchos)	France, Camargue	T	23.4	Tavecchia *et al*. (2001)
	Netherlands	T	1.4-3.4 †	Lumeij & Scholten (1989)
	Netherlands	T	22-68 ††	Lumeij & Scholten (1989)
	Netherlands	S	4.9-6.4 †	Lumeij & Scholten (1989)
	Spain, Doñana	FD	14.2	Mateo *et al.* (2007)
	Canada	T	28	Elder (1950)
	USA	T	13	Funk (1951)
	USA	T	27	Murdy (1952)
Northern pintail (*Anas acuta*)	USA	T	13	Funk (1951)
Northern shoveler (*Anas clypeata*)	Spain, Doñana	FD	0	Mateo *et al.* (2007)
American black duck (*Anas rubripes)*	Canada (Maritimes)	T	12–18	CWS, unpublished data
Common teal (*Anas crecca*)	France, Camargue	T	4.4-9.6	Guillemain *et al*. 2007
Canvasback (*Aythya valisineria*)	USA	T	29	Perry & Geissler (1980)
Lesser scaup (*Aythya affinis*)	USA	T	10	Perry & Geissler (1980)
Redhead (Aythya americana)	USA	T	15	Perry & Geissler (1980)
Ring-necked duck (*Aythya collaris*)	USA	T	21	Perry & Geissler (1980)
King eider (Somateria spectabilis)	Greenland	FD	11.3-20	Falk *et al*. (2006)
Common eider (*Somateria mollissima*)	Greenland	FD/ T	15.1-29	Merkel *et al.* (2006)
	Canada (Maritimes)	T	20-35	CWS, unpublished data
Eurasian coot (*Fulica atra*)	Spain, Doñana	FD	5	Falk *et al*. (2006)
Red-legged partridge (*Alectoris rufa*)	Spain	S	87.5	R. Mateo unpublished data

†Gizzard wall †† Whole body. *M = Method: T = Trapped; FD = Found dead S = Shot.

There are relatively few studies of embedded ammunition in living mammals. Elmeros *et al*. (2012) used X-radiography to monitor prevalence on embedded shot in red foxes, otters *Lutra lutra* and badgers *Meles meles* in Denmark. Prevalence in foxes fell from 24.9% in the late 1990s to 8.5% in the late 2000s. Prevalence was found to be 6.9% in otters and 0% in badgers.

Concentrations of lead in the flesh of game animals shot with lead ammunition can be very elevated (*e.g*. Dobrowolska and Melosik 2008, Tsuji *et al*. 2009, Pain *et al*. 2010, Lindboe *et al*. 2012) and representative concentrations are presented in detail in Section 3.3.3 of 'An evaluation of the risks to human health in the UK from lead derived from ammunition' (PERASG 2013). Details are not repeated here, but the conclusion from that Section reads (accepting that the Regulation mentioned refers to human health not animal health):

"It is concluded that mean lead concentrations in meat from game shot with lead ammunition are often elevated, and frequently considerably elevated, above the levels considered acceptable for meat derived from the muscle tissue of non-game animals under EU Regulation 1881/2006. Mean lead concentrations are likely to be generally higher in game meals made from small game (e.g. gamebirds and waterfowl) shot with lead gunshot than meals made from large game (e.g. deer) shot with lead bullets. However, meat and offal derived from tissues from the region of the body where a large game animal has been shot (such as the thoracic region) may have high lead concentrations".

Conclusion B.1.2 Probability of presence of shot-in projectiles, and fragments thereof, in the tissues of game animals killed or wounded using lead shot and bullets

There is **strong evidence** that whole gunshot pellets and fragments of pellets and bullets are present in the tissues of most gamebirds and waterfowl, in small game mammals such as rabbits and in larger game animals killed using lead ammunition. The number and mass of lead fragments can be substantial and widely distributed in the carcass. This occurs both when small game and deer are shot with lead rifle bullets and when small game, gamebirds and waterfowl are killed using shotgun cartridges containing lead shot. There is **strong evidence** that there are usually substantial numbers of small fragments in the abdominal viscera of deer killed using lead bullets, which are often discarded and hence potentially available to scavengers. Hence, the hypothesis that remnants of lead ammunition is present in tissues of game animals shot with it and the viscera of deer and therefore a potential source of dietary lead to scavengers is strongly supported by evidence.

There is **strong evidence** that a substantial proportion of wildfowl, and likely other game animals are wounded, but survive, and that lead fragments are a potential source of exposure to dietary lead in predators which subsequently kill and eat them.

B.2 Pathway: Evaluating the probability of ingestion of ammunition-derived lead projectiles and fragments by scavengers and predators

B.2.1 Species of scavenging and predatory animals most likely to ingest ammunition-derived lead projectiles and fragments

Widely available information from ornithological and mammalogical reference works summarise observations of the principal food sources of birds and mammals. From these, it is apparent that many bird and mammal species worldwide frequently scavenge tissue from carcasses of dead vertebrates and parts of their bodies discarded by hunters. Among the birds, these include New and Old World vultures, eagles, kites, buzzards, caracaras, gulls, corvids, and egrets. Among the mammals, many species of carnivores scavenge. Predatory birds and mammals include species from the same taxonomic groups as the scavengers, but also include owls, falcons and a wider range of accipitrid raptors. In the UK, red kite *Milvus milvus*, golden eagle *Aquila chrysaetos*, white-tailed sea eagle *Haliaeetus albicilla*, buzzard *Buteo buteo*, raven *Corvus corax*, carrion crow *Corvus corone*, hooded crow *Corvus cornix* and magpie *Pica pica* are the bird species most likely to scavenge from carcasses or discarded viscera of game animals. Badger, red fox and pine marten *Martes martes* are the mammal species in the UK most likely to scavenge from these sources. All species of raptors and owls and carnivorous mammals in UK could potentially kill and feed upon a game animal with fragments of lead ammunition shot into its tissues. Because they frequently prey upon waterfowl which may be contaminated with shot-in lead gunshot, western marsh harriers *Circus aeruginosus* and peregrine falcons *Falco peregrinus* are the raptor species which might be expected to be most exposed to ammunition-derived lead *via* this route.

Conclusion B.2.1 Probability of a range of scavenging and predatory species ingesting ammunition-derived lead projectiles and fragments

Knowledge of the natural history and foraging ecology of birds and mammals suggests **high probability** that many species are exposed to dietary lead derived from spent ammunition through feeding on prey animals or scavenging at carcasses or on viscera of hunter-killed deer. This includes several bird and mammal species present in the UK.

B.2.2 Direct evidence concerning the ingestion of ammunition-derived lead projectiles and fragments by scavenging and predatory animals

Despite this potential exposure to the risk of ingesting fragments of lead, it is conceivable that scavengers and predators identify all or most of the whole projectiles and fragments of lead ammunition as foreign bodies in their food and reject them. In this section, only direct evidence about ingestion of fragments of metallic lead is considered. Indirect evidence, such as that from elevated lead concentrations and stable isotope ratios in tissues and feathers of predators and scavengers is considered in a later section (Section B.2.3.2).

Kenntner *et al.* (2001) found fragments of metallic lead in the stomachs of two out of 61 white-tailed sea eagles found dead or moribund in Germany and Austria, indicating that they had ingested the fragments. X-radiography was used to locate the fragments and electron microprobe analysis showed them to be composed principally of lead. Their macroscopic appearance suggested that they were fragments of ammunition, but this could not be established with certainty.

Helander *et al.* (2009) found radio-dense objects in the gastrointestinal tracts of stomachs of six out of 106 white-tailed sea eagles found dead or moribund in Sweden by X-radiography. The objects were dissected out and found to be lead gunshot pellets (four birds) or lead bullet fragments (two birds), indicating that they had ingested whole shot or fragments of lead.

Saito (2009) reports on deaths from lead poisoning of 37 white-tailed sea eagles, 92 Steller's sea eagles *Haliaeetus pelagicus* and two mountain hawk eagles *Spizaetus nipalensis* in Hokkaido, Japan, during the period 1996-2007. Lead shot and rifle bullet fragments were found within the gastrointestinal tract of (at least) a proportion of the birds: "necropsies and radiographs have revealed pieces of lead from rifle bullets and from shotgun slugs to be present in the digestive tracts of poisoned eagles". The hunting and control of sika deer *Cervus nippon* in the region were assumed to be the source of much of the lead ammunition.

Rideout *et al.* (2012) reported results of *post mortem* examination of 85 carcasses of full-grown, free-flying California condors *Gymnogyps californianus* from the reintroduced populations of this species in the USA and Mexico. Metal fragments in the gastrointestinal tract were identified in eight individuals and these were confirmed to be derived from lead ammunition in five of these cases. One condor was found to have ingested a 22-calibre bullet from the carcass of a donkey it fed upon. In six cases observed since 2007, overlapping with those reported by Rideout and colleagues, the composition of stable isotopes in the lead-containing metal from the fragment was determined. The stable isotope ratio $^{207}Pb/^{206}Pb$ in the metal fragments matched the distribution of $^{207}Pb/^{206}Pb$ ratios determined for samples of lead-based ammunition (Finkelstein *et al*. 2012). Since the stable isotope ratios for ammunition-derived lead and other background environmental sources of lead differ markedly, these findings establish that condors ingest fragments of lead ammunition (Finkelstein *et al*. 2012).

Craig *et al.* (1990) reported on elevated liver lead concentrations in 16 golden eagles found moribund or dead in Idaho, USA. Five eagles were diagnosed as dying of lead poisoning and necropsies were performed on two additional golden eagles that had elevated liver lead concentrations. One contained an apparently ingested lead shot in the lower gastrointestinal tract and the other contained a fragment of copper-jacketed lead bullet in its proventriculus.

Examination of regurgitated pellets of birds provides additional information on the frequency of ingestion of remnants of lead ammunition. X-radiographs of 29 (11%) of 264 regurgitated food pellets from a roost site of red kites in the English Midlands contained radio-dense objects (Pain *et al*. 2007). These were assumed to be mainly shot or shot fragments. Seven pellets contained radio-dense objects that were clearly spherical in shape and therefore likely to be shotgun pellets. Sixteen pellets, including these seven, were dissected and in six of them objects which appeared macroscopically to be lead shot were found, with up to three such objects in each pellet. Eleven of the thirteen spherical objects studied contained over 60% of lead. Of the other 15 objects dissected out and analysed, four had 5 – 50% lead content. It was determined from these data that a minimum of 4 to 6 regurgitated pellets (about 2% of the total) contained lead shot. Since the study area included estates on which partridges and pheasants were shot with lead gunshot, scavenging of unrecovered shot birds or of wounded birds that died later appears to be the most likely route by which the red kites obtained the lead shot.

Similarly, Pain *et al.* (1997) found that the proportion of regurgitated pellets of western marsh harriers collected during three hunting seasons in France which apparently contained shotgun pellets, based upon X-radiography was 12 – 25% in different years compared with 1% outside the hunting season. Although the shotgun pellets were not conclusively identified as such by dissection and testing, the marked difference in their prevalence in regurgitated pellets between seasons makes it highly likely that they were shotgun pellets derived from waterfowl or gamebird shooting.

Helander (1983) found that the presence of lead shot in regurgitated pellets of white-tailed sea eagles collected in Sweden in 1964–1980 correlated with the hunting season. Nine percent of pellets contained shot during the winter hunting season, compared with 0.7% lead shot in pellets collected during the non-hunting season.

Hirano *et al*. (2004) collected 296 pellets of eastern marsh harriers *Circus spilonotus* from two winter roosts sites in Watarase Marsh, Japan, between January 2002 and late February 2004. Eighteen (6%) of these contained lead shot. The authors reported that a significantly higher

frequency of lead shot in marsh harriers pellets was found in January and February, during the open season for game birds.

Donázar *et al*. (2002) found shot in 13 of 424 regurgitated pellets of Egyptian vulture *Neophron percnopterus* collected under roosting places in the Canary Islands during and just after the hunting season in 2000.

Mateo *et al.* (2013) review information on lead shot ingestion and lead poisoning in Spain, and report on lead shot in regurgitated pellets from red kite (in central Spain and Doñana), Egyptian vulture (in the Canary Islands), marsh harrier (from the Ebro delta and Doñana), Spanish imperial eagle *Aquila adalberti* (from central Spain, Castilla-La Mancha and Doñana) and peregrine falcon (in Doñana). These authors report that levels of ingestion of lead shot in the Spanish imperial eagle in Doñana can vary between years in relation to goose hunting pressure, which in turn varies with water levels in the protected areas (see Mateo *et al*. 2007). See also Cerradelo *et al.* 1992, Mateo *et al*. 2001, González and Hiraldo 1988, Castaño López 2005, Mateo *et al.* 1999, Gonzalez 1991, Garcia and Viñuela 1999.

Krone *et al.* (2009) performed experiments on white-tailed sea eagles in which iron nuts of various sizes were inserted into carcasses or discarded viscera which they fed from. The eagles always avoided ingesting nuts of 7.7mm diameter or larger, but ingested some of the nuts smaller than this (2.7 – 6.0mm). For the smallest size of nuts used in the experiment (2.7 mm), 80% of the nuts presented were eaten. It should be noted that these nuts were considerably larger than most of the fragments of ammunition-derived metal seen in X-radiographs of deer carcasses and discarded viscera. Knott *et al.* (2010) found that 83% by weight of the radio-dense fragments they found in deer viscera had a diameter less than 1mm and the largest fragment seen on the radiographs was only slightly larger than the smallest nuts used in the experiment. Hence, this experiment indicates that white-tailed sea eagles almost certainly ingest a high proportion of the metal fragments, including those of ammunition-derived lead present in their food whilst scavenging on the remains of game animals.

We are not aware of direct observations of ingestion of ammunition-derived lead fragments by scavenging or predatory mammals. However, it seems probable from the feeding behaviour of many species, in which large chunks of meat and some bone fragments are swallowed, that some ingestion of remnants of ammunition occurs.

Conclusion B.2.2 Probability, based on direct evidence, of the ingestion of ammunition-derived lead projectiles and fragments by scavenging and predatory animals

There is **strong evidence** from *post mortem* examinations and studies of regurgitated pellets that predatory and scavenging birds ingest fragments of metal, including remnants of lead ammunition, present in their food. Experiments on the extent to which white-tailed sea eagles rejected pieces of metal in their food provide **strong evidence** that, whilst large pieces of metal are discarded, much of the mass of ammunition-derived metallic lead present in discarded deer viscera and carcasses is in small pieces would therefore be eaten.

B.2.3 Absorption of ammunition-derived dietary lead by scavengers and predators

In this section, evidence as to whether lead derived from ingested ammunition is absorbed into the blood of scavengers and predators, and from there is distributed into tissues, is examined. The evidence is presented in two subsections: one concerned with observations of elevated blood and tissue lead in scavengers and predators, and the other concerned with analyses to indicate its probable source.

B.2.3.1 Evidence of elevation of blood and tissue lead following experimentally manipulated exposure to ingested ammunition-derived lead

Pattee *et al.* (2006) performed an experiment in which four captive immature Andean condors *Vultur gryphus* were dosed with 2 – 6 pellets of number 00 lead shot equivalent to 0.575 – 1.92g/kg body weight, whilst two others acted as untreated controls. The mass of lead eroded from the pellets was estimated after the birds had died or been euthanized. It was 126 – 290mg for the two birds given two pellets and 527 – 603mg for the two birds given six pellets. Blood lead concentration tended to increase over time in the dosed condors, but remained low in the control untreated birds. Mean blood lead concentration reached 17.16mg/kg (c. 1,716 µg/dl) in the birds given two shot, and 16.41mg/kg (c. 1,641µg/dl) in the birds given six shot, but never exceeded 0.16mg/kg (c. 16µg/dl) in the untreated controls. The birds dosed with two shot had *post mortem* liver concentrations of lead of 45 – 50mg/kg w.w., whilst the birds given six shot had 59 – 109mg/kg. The *post mortem* lead concentrations in the kidney was 115 – 124mg/kg w.w. for the birds given two shot and 179 – 230mg/kg for the birds given six shot. Despite sample size, this experiment provides unambiguous evidence that lead derived from shotgun pellets in the gastrointestinal tract of condors is absorbed into the bloodstream and distributed to the tissues.

Similar experimental dosing of captive bald eagles *Haliaeetus leucocephalus* resulted in blood lead concentrations rising to 5.4mg/kg w.w. (540µg/dl) 14 days after dosing (Hoffman *et al.* 1985). Dosing of nestling American kestrels resulted in changes in blood parameters indicative of absorption of ingested lead (Hoffman *et al.* 1981).

Conclusion B.2.3.1 Probability of elevation of blood and tissue lead following experimentally manipulated exposure of predators and scavengers to ingested ammunition-derived lead

Experimental studies of predatory and scavenging birds provide **unambiguous evidence** that ingested metallic lead from ammunition is absorbed into the blood and distributed to other tissues. Evidence that ammunition-derived dietary lead is absorbed into the bloodstream by mammals (humans and pigs) is presented in another Risk Assessment (PERASG 2013).

B.2.3.2 Evidence concerning the provenance of lead in cases where elevation of blood or tissue lead concentrations is observed in wild predators and scavengers

See Supporting Information 1 for discussion about sources of lead and relative exposure to wildlife.

A number of studies of predatory and scavenging birds have used stable isotope analysis to assess the provenance of tissue lead. The study which is largest in scale is that of free-living and captive California condors in the USA and Mexico. These studies indicate that the majority of free-living condors have a blood lead isotopic composition consistent with that of lead-based ammunition. For six condors from which a fragment of lead-containing metal was recovered from the gastrointestinal tract, the composition of stable isotopes in the lead-containing metal from the fragment and of the blood of the bird from which it was taken was determined. There was a close match between the isotopic signatures of the blood lead and the fragment lead in all six cases (Finkelstein *et al*. 2012). Since the stable isotope ratios for ammunition-derived lead and other background environmental sources of lead differ markedly, these findings establish that these condors absorbed lead into the bloodstream from the fragments of lead ammunition they had ingested. Condors kept in captivity had lead isotope composition typical of background environmental lead derived from a combination of a range of non-ammunition sources in California (Finkelstein *et al.* 2012). Free-flying condors with low total blood lead concentrations also had this background isotope signature in their blood lead. However, the departure of the blood lead isotope signature from the background pattern increased progressively as the total blood lead concentration increased (Finkelstein *et al.* 2012). There was a strong tendency, across the blood

samples from different birds, for the ratio of isotope ^{207}Pb to that of ^{206}Pb to tend towards the distribution of $^{207}Pb/^{206}Pb$ ratios measured in lead from ammunition and fragments of bullets recovered from lead-poisoned condors as the total blood lead became higher (Church *et al.* 2006, Finkelstein *et al.* 2012). This would be expected if the increase in blood lead concentration was due to acquisition of lead derived from lead ammunition forming a mixture with the background lead derived from various other environmental sources. Of the free-flying condors sampled, 79% had lead isotope ratios indicating that the lead in their blood, in excess of the background level, was principally ammunition-derived lead. Of the remaining birds, 13% had isotope ratios inconsistent with exposure to ammunition-derived lead. For about one-third of these anomalous birds (about 4% of the total), a match was found to lead in peeling lead-based paint from a fire observation tower used by the condors for perching (Finkelstein *et al.* 2012).

The studies of the provenance of blood lead in California condors have been augmented by studies of lead isotope ratios in condor feathers. Lead is deposited in feathers during the period of their growth. On a given day, lead from the bloodstream is incorporated in the narrow band of a growing feather in which the feather structure, largely composed of keratin, is being laid down. After this, the lead within the feather remains there. Since feathers grow slowly from their bases, changes in the concentration and isotopic composition of lead along the long axis of the feather form a permanent record of changes over the period of growth in the concentration and isotopic composition of the lead in the bird's blood. A flight feather of a California condor takes 2 – 4 months to grow. Studies of the lead in sequential feather sections showed that free-flying condors often experienced episodes of high blood lead concentration. An example is Condor 422, studied by Rideout *et al.* (2012), whose feathers contained a record of several exposures to lead within a period of 180 days. The average frequency of these exposures was higher than had previously been supposed, based upon intermittent captures of condors for blood sampling. During these high-exposure periods, the lead isotope ratios in the feather sections deviated from that of background environmental lead, most often towards the ratio typical of lead ammunition (Finkelstein *et al.* 2010). These findings are consistent with regular exposure of free-flying California condors to lead derived from ammunition, followed by its partial removal from the blood into other tissues, including the feathers.

Saito (2009) reports stable isotope analysis of the lead found in Steller's sea eagles and white-tailed sea eagles in Hokkaido, which indicated rifle ammunition as the source of 5/8 lead poisoning cases in the former and 2/3 in the latter (it is unclear from the paper whether stable isotope analyses were conducted for the the other four cases).

Pain *et al.* (2007) measured the relative quantities of the stable lead isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb in samples of bone and liver from carcasses of red kite obtained in England and shot recovered from regurgitated pellets of roosting kites in the English Midlands. The stable isotope signatures of these samples were compared with published data from the UK on the isotope composition of lead from soil, lead ores, coal and atmospheric deposition onto vegetation. The lead isotope signature of the shot removed from kite pellets overlapped with the ratios found in the liver and bone samples from kites, suggesting that lead shot was a source of the tissue lead, in a mixture with low levels of lead from other non-ammunition environmental sources. Liver samples with low total lead concentrations had isotope signatures that indicated greater exposure to background non-ammunition lead rather than lead shot.

Walker *et al.* (2012) reported lead isotope results for liver samples from carcasses of red kites found dead by members of the public in England, and of sparrowhawks *Accipiter nisus* found dead in England, Wales and Scotland. The lead isotope signatures for both species were distinct from that of leaded petrol, and only marginally overlapped that for coal, but substantially overlapped with those for lead ammunition. There was no tendency in either species for the lead isotope composition to be correlated with the total concentration of lead in the liver.

Helander *et al.* (2009) measured the relative quantities of the stable lead isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb in kidney samples from white-tailed sea eagles found dead or moribund in Sweden. Birds with high total concentrations of lead, indicative of lead poisoning, in the tissue samples had

markedly different stable isotope signatures from those with low total lead levels, indicating a source of lead contamination different from background environmental lead. The isotope signatures of the heavily contaminated birds overlapped with those of lead from ammunition and were less consistent with lead from atmospheric deposition. However, the isotope data did not exclude all possible non-ammunition sources of lead.

An additional method for attributing the provenance of lead in tissues to a particular source is to measure the degree of association between blood lead levels and spatial and temporal variation in exposure to food contaminated with ammunition-derived lead. An example of this type of analysis was made possible by exceptionally detailed data on the blood lead concentrations of free-flying California condors in the reintroduced population centred on the Grand Canyon, USA. These birds are recaptured regularly, sometimes several times per year, to determine their blood lead concentration and to begin chelation therapy to reduce the risk of death from lead poisoning, which is otherwise high. The birds are also tagged with VHF radio tags or satellite tags and their roosting places are located regularly; almost daily for much of the year. Green *et al.* (2008) analysed variation among individual condors, and across times of year, in the magnitude and direction of changes in blood lead concentration between successive blood samples from the same individual. They showed that blood lead concentration tended to rise rapidly when a tagged condor spent time during the autumn deer-hunting season in areas with high levels of deer hunting, but that visits to these same areas outside the hunting season, and visits to other areas with low levels of deer hunting at any time of year were not associated with rises in blood lead levels. The mean daily increment in blood lead for condors spending time in two widely separated deer-hunting areas during the deer-hunting season was 11 - 19 times higher than for the same areas during the non-hunting season, whereas hunting and non-hunting season daily blood lead increments were both low and showed little change for three areas without significant deer hunting at any time of year.

Condors were frequently recorded feeding on unrecovered carcasses and discarded viscera from deer killed using lead bullets in the deer hunting areas. Hence, this study of the correlation between the dynamics of blood lead changes and movement patterns provides strong evidence that condors accumulate blood lead rapidly only when they frequent areas in which large numbers of piles of discarded offal from deer killed with lead bullets are being deposited. These findings are virtually impossible to explain except through lead derived from ammunition used to hunt deer being the source of the great majority of the blood lead in these condors.

Several other studies use less detailed information than that available for condors to measure the association between blood lead levels in predatory and scavenging birds and variation over time in exposure to ammunition-derived lead in food, especially that caused by hunting seasons. Craighead and Bedrosian (2008) took blood samples from ravens *Corvus corax* in the USA and measured blood lead levels. Of samples collected during the hunting season for large game animals (mainly deer) 47% of samples had elevated blood lead (>10µg/dl), compared with 2% outside the hunting season.

Similarly, Pain *et al*. (1997) found that geometric mean blood lead levels were 3-4 times higher in free-flying live-trapped western marsh harriers during the hunting season in France than outside the hunting season. Kelly and Johnson (2011) found that the blood lead concentrations of turkey vultures *Cathartes aura* in California were significantly higher during the big game hunting season than outside it. The median blood lead concentration inside the season was double that outside it and the geometric mean concentration was three times higher within the deer hunting season than outside it. Gangoso *et al.* (2009) found that the geometric mean concentration of lead in the blood of Egyptian vultures in the Canary Islands was about four times higher during the hunting season than outside it.

Rogers *et al.* (2012) reported that blood lead levels of grizzly bears *Ursus arctos* in the Greater Yellowstone Ecosystem, USA, were not appreciably higher during the hunting season, despite the presence of carcasses and discarded viscera of deer during the hunting season. In addition, they found that lead concentrations in blood and tissues of wolves *Canis lupus* and mountain lions *Puma concolor* in the region were low. Hence, in this region there was no evidence that ingestion

of lead from hunter-killed carcasses or viscera was leading to the absorption of lead by these mammalian carnivores. Similarly, Millán *et al.* (2008) found relatively low levels of lead in liver, muscle and bone in five species of carnivorous mammals in Spain.

It should be noted that most studies which contrast the blood lead concentration of birds and mammals within and outside the hunting season underestimate the difference in exposure to lead which underlies this difference. The underestimation arises because blood lead remains high for some time, often several weeks, after the ingestion of lead. This is both because the lead is retained in the gastrointestinal tract and because the transfer of lead in the blood to other tissues takes time. The result is that some blood samples obtained in the early part of the non-hunting season will still contain appreciable amounts of lead acquired during the hunting season. Hence, the difference in blood lead underestimates the difference in acquisition of lead from the environment.

Conclusion B.2.3.2 Probability of lead from ammunition sources being responsible for elevated blood or tissue lead concentrations observed in wild predators and scavengers

Studies of the stable isotope signatures of lead from terrestrial predatory and scavenging birds in the USA and Europe including UK provide **strong evidence** that the provenance of lead in the tissues of these birds is most often lead from ammunition in cases where tissue lead concentrations are elevated above background levels. Other non-ammunition sources of lead contribute but are usually much less important than ammunition-derived lead. Correlations between blood lead dynamics and hunting seasons and movement patterns of individual birds lead to the same conclusion. Comparisons between the blood lead concentrations of scavenging birds during hunting and non-hunting seasons in the USA and Europe in areas where carrion is contaminated by remnants of lead ammunition indicate that geometric mean concentrations are consistently several times higher in the hunting season, when potential exposure occurs, than outside the hunting season, when it does not. The isotope and blood lead information both provide **strong evidence** that ammunition-derived dietary lead from carrion is absorbed by birds and that its effect on blood lead concentrations is substantial.

We found **no available evidence** of these types for absorption of significant amounts of ammunition-derived lead by scavenging and predatory mammals in the USA and Europe, but fewer studies addressing this topic are available than there are for birds.

B.2.3.3 Assessment of risks from Linkage Sets A, C and D

Linkage Sets A, C and D are related to Linkage Set B (see Figure 1) in these ways:

- Risks from Linkage Set A are derived from ammunition that is consumed directly from the environment by animals which are subsequently fed upon by predators or scavengers if they die whilst the ammunition is still present in the gastrointestinal tract and/or there are elevated tissue lead levels following ingestion.

- Risks from Linkage Set C involve any animals which have elevated tissue lead levels following ingestion of ammunition-derived lead from soil/water/biota being subsequently fed upon by predators or scavengers.

- Risks from Linkage Set D involve any animals which have elevated tissue lead levels from embedded ammunition being subsequently fed upon by predators and scavengers.

B.3 Receptor: Evaluating the probability of impacts of ammunition-derived lead on the health, welfare and population processes of predators and scavengers

B.3.1 Impacts of absorption of lead on function and survival of predators and scavengers

Lead poisoning in raptors is widely reported, inparticular from North America and Europe (e.g. Fisher *et al*. (2006), Gill and Langelier (1994), Locke *et al*. (1969), Locke and Thomas (1996), Redig *et al*. (1980, 1991); various papers in Watson *et al*. (2009), Wayland and Bollinger (1999), Wayland *et al.* (1999), Wiemeyer *et al.* 1989)). Evidence concerning the effects of absorption of lead on the health, welfare and survival of vertebrates is presented elsewhere in this risk assessment (see A.3.1, A.3.2, Table A.4). However, a brief review of evidence specific to predatory and scavenging species is presented here.

Once lead has been absorbed into the bloodstream, it is distributed among body tissues, primarily the blood, liver, kidney and bone (Franson and Pain, 2011). Exposure to even low levels of lead can result in marked physiological effects. In birds, this includes the inhibition of the enzyme ALAD. This effect has been demonstrated especially clearly in scavenging birds. For example, Finkelstein *et al*. (2012) analysed ALAD assay data for California condors in relation to blood lead concentration measurements from the same individuals and found that ALAD activity declined rapidly and exponentially across the whole range of blood lead concentrations, without any indication of a threshold lead level below which there was no effect of lead on ALAD activity. In this species, ALAD activity at 0.2mg/kg w.w. (20µg/dl) of lead in blood, which is a frequently used risk threshold for the appearance of sub-clinical effects in birds (see below and Table A.4), was already less than one-third of that for birds with no detectable blood lead. Exposure to high concentrations of lead can adversely affect the central nervous system, peripheral nerves, kidney, haematopoietic system, reproduction, juvenile growth and, ultimately, survival.

Conclusion B.3.1 Probability of impacts of absorption of lead on function and survival of predators and scavengers

Absorption of lead, including ammunition-derived lead, causes diverse effects upon the functioning and survival of vertebrates. These impacts also affect scavenging and predatory animals. There is **evidence** of marked effects on some physiological processes occuring at low blood lead concentrations and showing no indication of a threshold, below which no impact is apparent.

B.3.2 Association between elevation of lead concentrations in tissues and impacts upon function and survival

Experiments in which predatory and scavenging birds were fed lead shot have established that death can result after relatively short-term exposure. However, there are large individual variations in the susceptibility to lead poisoning, with concentrations in the liver at death ranging from 24 to 384mg/kg d.w. (Pattee *et al*. 1981, Carpenter *et al*. 2003, Pattee *et al*. 2006). The clinical signs of ill-health and *post mortem* findings in the experimental studies closely match those seen in carcasses of wild raptors with high tissue levels of lead. These include anorexia, lethargy, muscle weakness, green-stained faeces and emaciation. Hence, comparisons between *post mortem* signs and tissue concentrations measured for the same individual have been used to establish approximate diagnostic concentrations for death attributed to lead poisoning. Lead concentrations in liver above 10 mg/kg w.w. have been established as probably lethal for most raptorial bird species (see Table A.4 and Franson and Pain 2011). Based upon a range of studies, Franson and Pain (2011) summarised the blood lead concentrations in raptors associated with different risks as

approximately: exceedance of 20 <50 µg/dl associated with sub-clinical effects on health, 50<100µg/dl associated with clinical poisoning, > 100µg/dl with severe clinical poisoning that could result in death is exposure continues (Table A.4). Franson (1996) and Fisher *et al*. (2006) considered that 500µg/dl blood lead was associated with death.

Green *et al.* (2008) reconstructed the blood lead concentrations of individual free-flying California condors in the Grand Canyon region of the USA using a statistical model based upon observed blood lead changes in relation to foraging area and time of year. They compared these reconstructions for birds that died from lead poisoning with equivalent reconstructions for those that did not die from this cause. Condors that died with clinical signs of lead poisoning had much higher recent mean blood levels over a preceding period of over 100 days than those that did not. The individuals that died had recent mean blood lead concentrations for periods ending on the day of death between 0.5 and 0.8mg/kg (50 and 80µg/dl). Using a completely independent method, Rideout *et al.* (2012) reconstructed the blood lead concentration of a California condor (Condor 422) using lead concentrations measured in growing feathers converted to estimated blood lead concentration using the method of Finkelstein *et al.* (2010). In this way, blood lead concentration was estimated for a 180-day period before the bird died from lead poisoning. The mean blood lead concentration during this period was 0.5mg/kg w.w. (50µg/dl), the blood lead concentration at death was 0.6mg/kg (60µg/dl) and there was a peak of blood lead of 3.0mg/kg (300µg/dl) about 100 days before death. The reconstructed blood lead values for these condors which are known to have died from lead poisoning can be compared with the approximate risk thresholds derived by Franson and Pain (2011) and Franson (1996) and Fisher *et al.* (2006) which suggest exceedance of 1.0mg/kg (100µg/dl) is associated with severe clinical effects and 5.0mg/kg (500µg/dl) with death. The approximate risk thresholds for toxic effects and death match observations for California condors reasonably well.

Using the methods described above, Green *et al.* (2008) reconstructed the blood lead concentrations of individual free-flying California condors in the Grand Canyon region of the USA and examined the relationship between the daily probability of a condor dying from lead intoxication on a given day to its mean blood level over a preceding period of over 100 days. The daily probability of death from lead poisoning rose from zero when blood lead concentration was less than 0.1 mg/kg (10 µg/dl) to 0.02 %/day at 0.5mg/kg (50µg/dl) and was 1.29 %/day at 1.0mg/kg (100µg/dl). At prevailing levels of exposure to ammunition-derived lead in the region, the estimated mean annual probability of a condor dying from lead intoxication was 5.1%.

Conclusion B.3.2 Probability of an association between elevation of lead concentrations in tissues and impacts upon function and survival of predators and scavengers

There is **strong experimental evidence** that elevation of lead levels in the body, due to dosing of captive birds with lead shot, cause negative impacts on function and death in predatory and scavenging birds. Athough these experiments and measurements of tissue lead and *post mortem* examinations of carcasses show that there is variation among species and individuals, approximate tissue concentrations of lead can be identified at which the risk of serious loss of function and death becomes progressively more likely. Two independent studies of free-flying California condors indicate approximate agreement between risk indicators based upon blood lead concentration and observed deaths diagnosed as having been caused by lead poisoning.

Observations of free-flying California condors quantified a strong relationship between their probability of dying from lead intoxication and blood lead concentration inferred from movement patterns and monitoring of blood lead.

B.3.3 Proportions of samples of dead predators and scavengers that were killed by lead poisoning

Rideout *et al.* (2012) analysed *post mortem* and tissue lead results from carcasses of free-flying California condors recovered dead in the western USA and Mexico. They determined a definitive cause of death for 50 juveniles and 15 adults. Twenty-six percent of juvenile deaths and 67% of adult deaths were determined to have been caused by lead toxicosis. Hence, 35% of all determinable deaths of full-grown condors were assigned to lead poisoning. In addition, Finkelstein *et al.* (2012) estimated that 20% of individual condors in the California subpopulation were captured and given chelation therapy in an average year. The blood lead concentration of captured birds was measured and they were retained for chelation therapy if blood lead exceeded 0.45mg/kg w.w. (45µg/dl). If these birds were not tested and treated, it is highly likely that the death rate and proportion of condor deaths from lead toxicosis would be substantially higher than that reported by Rideout *et al.* (2012).

Clark and Scheuhammer (2003) reported analyses of lead in liver, kidney and bone for 184 raptors of 16 species, using carcasses found across Canada. They adopted a threshold of 20mg/kg d.w. of lead in kidney, and/or 30mg/kg d.w. in liver for assigning deaths to having been caused by lead poisoning. Most individuals of most species had very low levels of tissue lead, but 10% of deaths of golden eagles were assigned to lead poisoning (total sample n=21), as were 13% of bald eagle deaths (n=8) and 3% of deaths of red-tailed hawks *Buteo jamaicensis* (n=58). Similarly, Elliott *et al*. (1992) estimated using tissue lead concentrations that of 65 bald eagles found dead between 1988 and 1991 in British Columbia, Canada, 14% had died from lead poisoning. Wayland and Bollinger (1999, Wayland *et al*. 1999) reported that 14% of 86 bald eagle carcasses and 17% of 77 golden eagle carcasses obtained in the prairie provinces of Canada had tissue lead levels indicative of death caused by lead poisoning. They use concentrations of 30mg/kg d.w. in liver and 20mg/kg d.w. in kidney as being diagnostic of death by lead poisoning.

Kenntner *et al.* (2001) reported liver concentrations of lead from 61 free-ranging white-tailed sea eagles found dead or moribund in Germany and Austria between 1993 and 2000. Lead concentrations that they considered to induce lethal lead poisoning (> 0.5mg/kg w.w.) were found in 28% of the birds. Helander *et al.* (2009) reported concentrations of lead in liver and kidney samples from 118 dead white-tailed sea eagles collected between 1981 and 2004 in Sweden. Fourteen percent of the individuals had either liver or kidney lead concentrations diagnostic of lethal lead poisoning. Equivalent proportions of white-tailed sea eagles found dead or moribund with lead concentrations diagnostic of lead poisoning are 17% in Greenland (Krone *et al*. 2004) and 22 % in Finland (Krone *et al*. 2006). Hence, the proportion of European white-tailed sea eagles deaths assessed as being probably caused by lead poisoning is broadly consistent at 14 – 28%.

Pain *et al*. (2007) reported lead concentrations from tissue samples from carcasses of red kites obtained in England between 1995 and 2003. Seven of 44 red kites found dead or that were captured sick and died subsequently had elevated (>6 mg/kg) lead concentrations in the liver and 14% had concentrations of >15mg/kg d.w., compatible with fatal lead poisoning. However, *post mortem* analyses indicated that two of these birds had died from other causes (poisoning by rodenticide and a banned agricultural pesticide). Hence, the remaining four (9%) had probably died from lead poisoning. Walker *et al.* (2012) also reported lead concentrations in liver for another sample of 20 carcasses of red kites collected in England in 2010 and found no cases with concentrations >2mg/kg d.w. and therefore no indication of any birds that died from lead poisoning; similarly none of 18 red kite carcasses collected in 2011 had elevated lead concentrations in the liver (Walker *et al.* 2013).

Pain *et al.* (1995) reported lead concentrations from the livers of 424 individuals of 16 raptor species found dead in Britain and sent for analysis to the Institute of Terrestrial Ecology, Monks Wood, from the early 1980s to the early 1990s. There were eight species for which ten or more carcasses were analysed: short-eared owl *Asio flammeus*, buzzard, little owl *Athene noctua*, kestrel *Falco tinnunculus*, sparrowhawk, peregrine falcon, merlin *Falco columbarius* and long-eared owl *Asio otus*. The other eight species with fewer than ten carcasses included three of the

species most likely on the grounds of diet to consume carrion contaminated with ammunition-derived lead (red kite (6 carcasses), golden eagle (5), white-tailed sea eagle (1)), and one species especially likely to prey upon waterfowl with shot-in or ingested shotgun pellet-derived lead in their tissues (western marsh harrier (1)). Elevated lead concentrations in liver (>20mg/kg d.w.), within the range associated with lead poisoning mortality in raptors, were recorded in one peregrine falcon (4% of species sample) and one buzzard (2% of species sample). Another one each of these species had liver concentrations of 15-20mg/kg d.w. No individuals of any other species had >15mg/kg d.w., although some had elevated liver lead concentrations in the range of 6-15mg/kg d.w.

Walker *et al.* (2012, 2013) reported lead concentrations in liver for a sample of 30 carcasses of sparrowhawks collected in Britain in 2010 and 30 in 2011. One sample had a lead concentration of 12.6mg/kg d.w. which is close to the threshold for clinical effects, but concentrations in all of the others were <2mg/kg.

It should be noted that the geographical distribution within the UK of the locations from which carcasses of scavenging and predatory birds were collected and sent for analysis is likely to be atypical of the distribution of the species as a whole for some of the species with potentially high risks of exposure to ammunition-derived lead. In particular, the collection of carcasses of buzzard, golden eagle and white-tailed sea eagle from areas in which large numbers of red deer are culled and viscera discarded is probably infrequent relative to the proportion of the population of these species in such areas. Carcasses are usually collected by members of the public. Areas with high levels of culling of deer tend to be remote from human populations and hill-walking and other outdoor recreations are discouraged in many of the areas where shooting of deer occurs for reasons of safety.

Studies of predatory and scavenging mammals discussed in detail earlier in the risk assessment tend to indicate lower levels of blood lead than for predatory and scavenging birds (Rogers *et al.* 2012, Millán *et al.* 2008) with few or no individuals appearing to have lethal levels. Rogers *et al.* (2012) found elevated levels of blood lead in grizzly bears in the Greater Yellowstone Ecosystem, USA, but the source of this lead is uncertain and it appears unlikely to be derived from lead ammunition. We are not aware of detailed studies of ammunition-derived lead in predatory and scavenging mammals in the UK.

Conclusion B.3.3 Probability of individual predators and scavengers being killed by lead poisoning

Measurements of lead concentrations in tissue samples from carcasses of dead predatory and scavenging birds have been used, together with *post mortem* examinations, to assign the cause of death to lead poisoning and other causes. Such studies in the USA, Canada and Europe report proportions of deaths caused by lead in species likely to be at risk of ingesting ammunition-derived lead ranging from 3% of deaths to 35% of deaths. In Europe the bird species with the most consistently high proportions of deaths attributed to lead poisoning is the white-tailed sea eagle (14 – 28% of deaths attributed to effects of lead). In the UK there is evidence of death being caused by lead poisoning in 9% of a sample of red kites carcasses collected in England. Two percent of carcasses of buzzards and 4% of carcasses of peregrine falcons collected in Britain had tissue concentrations of lead consistent with death being caused by lead toxicosis. However, the necessary measurements of tissue lead concentration have not been reported from sufficient numbers of carcasses of several species potentially at risk to draw any reliable conclusions about the proportion of birds dying from lead poisoning in the UK. In particular, sufficient observations are lacking for white-tailed sea eagle, golden eagle and western marsh harrier. For some of these and other species, especially buzzard, the geographical distribution of carcass collection for analysis probably does not sufficiently cover areas, such as parts of Scotland, where culling of deer and disposal of discarded viscera containing remnant of lead ammunition are prevalent. Hence, whilst there is **strong evidence** that a sometimes substantial proportion of predatory and scavenging birds die from lead poisoning from studies in North America and Europe, equivalent studies with sufficient statistical power to measure the proportion of birds affected have not yet been conducted in the UK.

Much less is known about potential impacts of lead on predatory and scavenging mammals, but the few studies available do not indicate large effects i.e. probability of significant effects is **low**.

B.3.4 Impacts of lead poisoning on population trends of scavenging and predatory birds

The effects of lead poisoning on population processes of scavenging and predatory animals have only been studied in any detail for birds. We know of no relevant studies of mammals. The most sufficiently robust set of studies from which firm conclusions about effects of lead poisoning on population trend can be drawn is that conducted on the reintroduced population of California condors in the western USA and Mexico. Population models based upon demographic data for this species show convincingly that population size would decline to extinction because of deaths caused by lead poisoning if releases of captive-bred birds, routine chelation treatment of affected birds and voluntary measures to replace lead bullets used for deer hunting with non-lead bullets were to cease (Meretsky *et al*. 2000, Green *et al.* 2008, Finkelstein *et al.* 2012).

Sulawa *et al.* (2010) used population modelling and recent estimates of demographic parameters to assess the future viability of the population of white-tailed sea eagles in Germany. It was found that a future decline to extinction was unlikely given recent population trends and if recent demographic rates continued to apply. These demographic rate estimates included the additional mortality due to lead poisoning described by Kenntner *et al.* (2001), so the study establishes that there is a low risk that lead poisoning at recently observed levels could cause this population to go extinct. However, removal of additional mortality caused by lead poisoning would reduce the risk of future population declines or allow the population to increase more rapidly than would be the case if lead poisoning continues to occur.

Ueta and Masterov (2000) used population modelling to study the effect of lead poisoning on Steller's sea eagles on Hokkaido. The model predicted that the population would slowly decrease and that adult mortality had the strongest affect on this population trend. Given the level of lead

poisoning it was considered that the toxicosis was a major factor in the predicted population diecrease thus prompting lead ammunition restrictions in the region.

We are not aware of population modelling studies like those described above for any other species of scavenging or predatory birds affected by lead poisoning. However, Niel and Lebreton (2005) developed the Demographic Invariants Method (DIM) to estimate an approximate maximum level of additional mortality which might be imposed upon a bird population whilst allowing it to maintain its numbers using much more readily available information. This method can be used, with caution, to make an approximate assessment of whether additional deaths caused by lead poisoning are occurring too frequently for there to be a real prospect that the population will compensate for them by density-dependent increases in demographic rates.

Additional mortality can be compensated for in a bird population if the birds' demographic rates are able to respond positively by a density-dependent mechanism to the additional losses. Additional mortality is intended to represent here deaths caused directly or indirectly by humans, such as hunting offtake, which are beyond those that occur naturally. Here we assume that the additional cause of death is lead poisoning. Niel and Lebreton's method does not allow the actual realised compensatory response to be estimated, but it sets an upper boundary to the maximum additional losses the population might sustain without declining. The estimation requires the mean annual adult survival rate and mean age at first breeding in growing populations of the species of interest. Information on the life history parameters of predatory and scavenging raptors has been reviewed and summarized by Newton (1979, 1998). As adapted by Dillingham and Fletcher (2008), the DIM method requires that a value of the recovery factor f is selected. Various recommendations have been made about f based upon theoretical considerations and opinion. Most of the recommendations have been based upon experience with populations of marine mammals, not birds (e.g. Wade 1998). The extreme maximum value suggested by Niel and Lebreton (2005) is f = 1, but most authors suggest using a value of f = 0.5 for stable populations (Wade, 1998, Taylor *et al* 2000, Hunter and Caswell, 2005). We therefore use the DIM method with f = 0.5 for two hypothetical scavenging or predatory raptor species with life histories selected to encompass those of most species of this kind. Based upon Newton (1979, 1998), we consider a long-lived species with annual adult survival of 0.95 and mean age of first breeding of 5 years at one extreme and a shorter-lived species with annual adult survival of 0.8 and mean age of first breeding of 2 years at the other extreme. Using equation 17 of Niel and Lebreton (2005) and the extreme values of survival and age of first breeding we calculate that the long-lived raptor species might be capable of compensating for additional annual mortality of 2.2% per year, whereas the short-lived species might be capable of compensating for additional annual mortality of 8.2% per year. We converted these into the proportion of all adult deaths caused by the abnormal cause, because the absolute value of additional annual mortality is rarely known, but the proportion of dead birds killed by it can be estimated from *post mortem* data. The additional mortality values translate into about 30% of all adult deaths being due to the additional, abnormal cause of death for both the long-lived and short-lived species.

The results of these simple calculations can be compared with the findings reported in Section B.3.2. At prevailing levels of exposure to ammunition-derived lead in the Grand Canyon region of the USA, the estimated mean annual probability of a condor dying from lead intoxication was 5.1% (Green *et al.* 2008). In the absence of additional mortality caused by lead poisoning, California condors are long-lived birds maturing late in life, so they match the assumptions used for the long-lived species in the DIM calculation described above. The DIM calculation for long-lived species indicates that additional mortality caused by lead poisoning of 2.2% per year is likely to the maximum which could potentially be compensated for without causing the population to decline. This is exceeded by the estimated level of additional mortality in the population due to lead. Hence, the simple DIM calculation reaches the same conclusion as the detailed modelling studies of condor populations that the recent levels of mortality caused lead poisoning cannot be sustained by the population without continued intervention. Only rarely is the absolute annual death rate caused by lead poisoning known. More frequently the proportion of recorded deaths due to lead poisoning has been estimated from studies of carcasses. The DIM calculation indicates that 30% of adult deaths caused by lead poisoning is likely to the maximum which the California condor

population could potentially compensated for without declining. Assignment of causes of death of California condors found that 67% of adults died from lead poisoning (Rideout *et al.* 2012). The simple DIM calculation again reaches the same conclusion as the detailed modelling studies of condor populations that the recent levels of mortality caused lead poisoning cannot be sustained by the population without continued intervention.

Comparison of the range of proportions of deaths assigned to lead poisoning in Section B.3.3 with the DIM calculation that 30% of all deaths indicates an upper limit to the level of additional mortality due to lead that could be compensated for, indicates that whilst the observed proportions for species other than California condor are below the upper limit, some approach it. For results obtained so far in the UK, the proportions of deaths of scavenging and predatory birds attributed to lead poisoning do not approach the DIM upper limit. However, it should be noted that the data available are sparse or missing for several species and does not adequately cover regions where ingestion of ammunition-derived lead is most likely.

Conclusion B.3.4 Probability of impacts of lead poisoning on population trends of scavenging and predatory birds

Quantitative assessment of the impact of lead poisoning on population trends of predatory and scavenging animals has only been attempted for a small number of bird species. In the California condor there is **strong evidence** that mortality from lead poisoning is sufficient to prevent population recovery and would lead to extinction of the wild populations if remedial measures and releases of captive-bred birds were to cease. In the Hokkaido population of Steller's sea eagles, population modelling implicated lead poisoning in their predicted population decline due to adult mortality. In the German population of white-tailed sea eagles the population trend is likely to remain positive and extinction is unlikely with recent levels of mortality due to lead poisoning. However, the population would increase more rapidly if lead poisoning was prevented. The proportion of all deaths attributed to lead poisoning is the most readily available measure of additional mortality caused by lead contamination. Results of this kind from North America and Europe indicate substantial effects on population processes for in some cases but, except in the case of the California condor, the upper limit of additional mortality caused by lead which would lead to extinction was not exceeded. In the UK, the proportions of deaths of scavenging and predatory birds attributed to lead poisoning measured so far do not approach the upper limit. However, it should be noted that the **data available are sparse** or missing for several of the species most likely to be affected in the UK and that which is available does not adequately cover regions where ingestion of ammunition-derived lead is most likely.

Figure B.1 summaries the pathway and effects of lead ammunition on raptors and scavenging birds. Table 1 provides a synthesis of explanatory text.

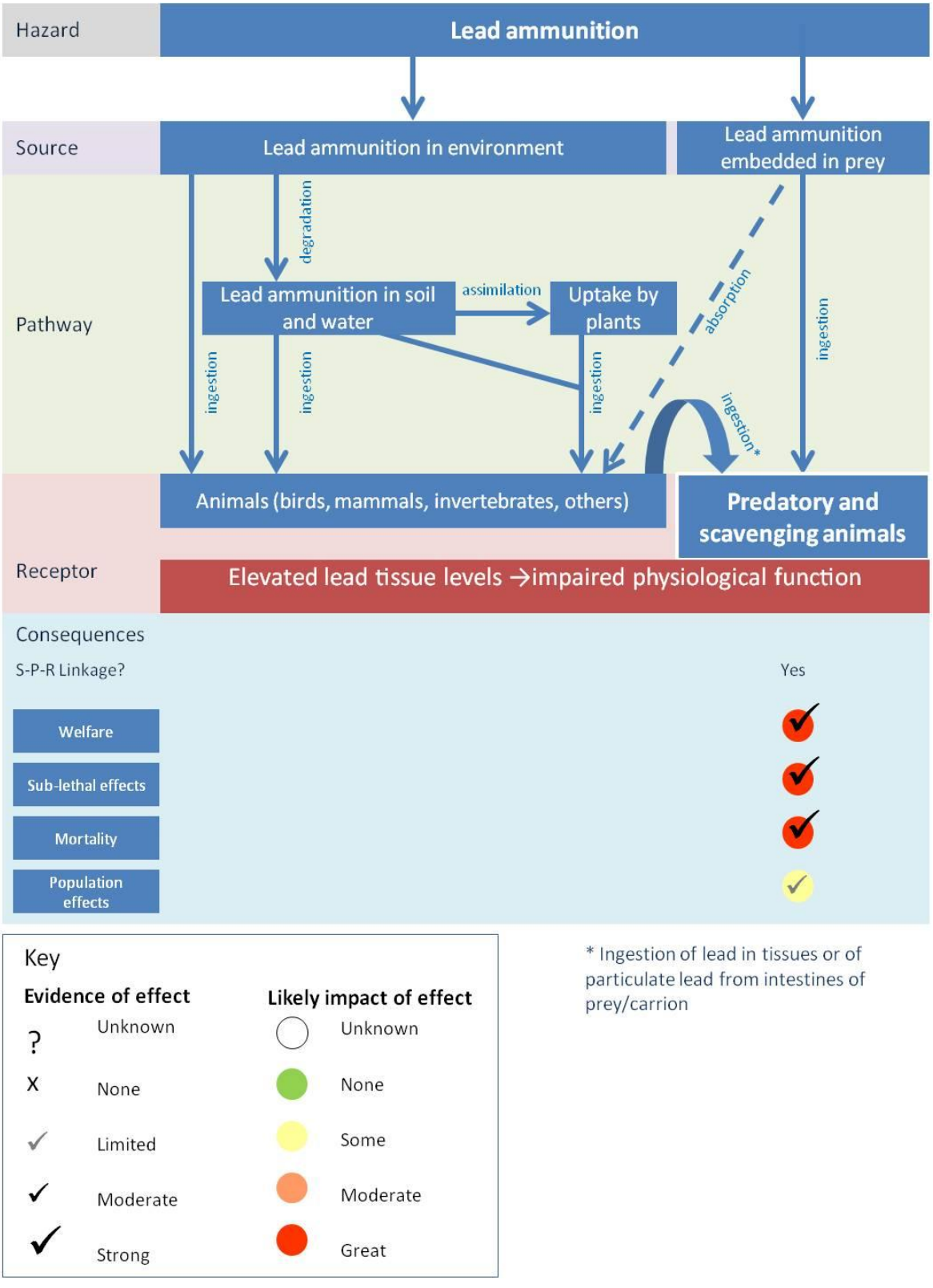


Figure B.1. Linkage Set B illustrating pathways and summary of effects on predators and scavengers from ingestion of lead within prey or carrion.

Linkage Set C: Poisoning of wildlife from ammunition-derived lead that has degraded and entered soil and water and/or become incorporated into biota

Linkage Set C examines the risk of poisoning of wildlife from ammunition-derived lead that has degraded and entered the soil and water and/or become incorporated in biota, rather than though the ingestion of lead projectiles from ammunition themselves. The source-pathway-receptor model is described in Table C.1.

Table C.1. Conceptual model of Source-Pathway-Receptor linkages which might result in lead ammunition in the environment degrading and entering soil and water, becoming incorporated in biota, and also being ingested by higher organisms and adversely affecting their health or populations.

S-P-R linkage label	Source	Pathway		Receptor
C (i)	Lead from spent ammunition in the environment being released *via* degradation and entering soil and water	Uptake by plants of lead from soil and water	Ingestion of plants by herbivorous animals	Adverse effects on the health of the animals from effects of absorbed dietary lead acting on the functioning of various organ systems and potentially leading to reduced welfare, death and impacts on population size.
C (ii)	Lead from spent ammunition in the environment being released *via* degradation and entering soil and water	Ingestion by lower animals of lead in soil, water and plants	Ingestion of animals that have taken up ammunition-derived lead from soil, water and plants by omnivorous and/or carnivorous animals	
C (iii)	Lead from spent ammunition in the environment being released *via* degradation and entering soil and water	Direct ingestion of lead in soil and water by range of animals (invertebrates and higher animals)		The receptors include both the lower animals incorporating lead from the environment and their consumers.

C.1 Source: Evaluating the probability that lead from spent gunshot and bullets degrades resulting in elevated lead concentrations in soil and water.

We first evaluate the probability that lead from spent gunshot degrades resulting in elevated lead concentrations in soil and water, then evaluate the probability that wild animals will be exposed to lead from ammunition sources *via* the identified pathways, and finally investigate the probability of wild animals being adversely affected by the hazard, and consider the risk of impacts.
Some of the information below is taken or adapted from that information presented in the 'Assessment of the risks to human health from livestock feeding in areas of lead gunshot deposition' (Green 2013). It has been adapted and repeated here to enable the risk assessments to be read in isolation.

C.1.1 Lead ammunition densities in the environment and factors influencing degradation

C.1.1.1 Ammunition densities

Shot densities in a range of habitats are presented in Section A.1.1.1 under Linkage Set A. Thirteen percent of the UK's rural land is specifically managed for shooting activities and two thirds of rural land in the UK is managed by shooting providers for a range of activities including shooting (PACEC 2006). Section A.1.1.2 illustrates that lead deposition from shooting is very widespread across the UK, that deposition rates vary with the intensity of shooting activity, and that shot accumulates over time.

Approximately 5,500-12,900 tonnes of lead gunshot (68-154 billion individual shot) enter the UK environment annually, and contamination is cumulative with soils being the ultimate environmental sink (see Section A.1.1.2 and Supporting Information 2). It is not known how many bullets enter the environment annually. Insufficient data appear available to calculating tonnage accurately although it is estimated to be small numbers of 100 of tonnes (see Section A.1.2), and a higher proportion of the bullet is likely to remain in the prey (see Section A.1.2).

C.1.1.2 Lead ammunition degradation

When lead ammunition is fired the majority of the volume of projectile (where this does not hit or passes through targets) is deposited in the environment as whole shot or whole or large fragments of bullets, although a small amount of fragmentation and loss of lead 'dust' is also likely to occur. The smaller the particles of lead the greater the immediate potential for environmental contamination as it is the compounds that result from lead weathering that create the greatest potential for lead mobility in soils. Weight for weight, lead gunshot, for example, will present a considerably greater potential for lead mobilisation that a bullet due to the increased surface area of the shot. While elemental lead is a very stable metal under neutral pH conditions, the surface of shot will be chemically transformed in the environment, and the lead compounds formed, which will vary with soil conditions, will play an important part in determining the mobility of lead. When lead comes in contact with moist air a thin lead oxide (PbO) layer generally forms at the surface of the metal and when both oxygen and water are present, metallic lead can be converted to lead hydroxide ($Pb(OH)_2$).

Lead compounds may be transformed to other lead compounds in the environment and processes including oxidation/reduction, precipitation/dissolution, adsorption/desorption, and complexation/chelation influence its mobility. In water the solubility of different compounds is related to pH, amount of calcium, salinity and the presence of humic material. While elemental lead is not generally soluble under neutral water conditions, it may occur dissolved in water as lead carbonate $PbCO_3$ or $Pb(CO_3)$. Some compounds are water soluble, lead acetate being a good example, whereas lead sulphide and phosphate tend to be very insoluble. Lead compounds are generally soluble in soft, slightly acidic water but some are also soluble under slightly alkaline conditions.

Soils and sediments act as an environmental sink for lead. Lead in soil may occur in a variety of chemical forms (e.g. as carbonates, sulphides etc.) and fractions, e.g. including exchangeable, adsorbed and organic complexes. Lead is strongly adsorbed to soil organic matter, silicate clays, peat and iron oxides. Consequently, under most conditions the majority of lead that enters soils is likely to be retained in the upper layers and not leach to any great extent into the subsoil and groundwater. However, although this is a general rule the mobility of lead in soils is nonetheless highly variable in relation to environmental conditions and is thus site specific, as illustrated in the examples below. In addition, the amount of lead in soils will also influence the potential for increased lead levels in subsoils and groundwater, and soil lead concentrations can be extremely elevated in areas of high lead ammunition deposition.

Under most environmental conditions shot degrades only slowly and in addition to the chemical processes described above, degradation may be influenced by physical erosion/abrasion, which is accelerated in course and gritty soils and/or those with considerable soil movement. Movement of lead through the soil may also be influenced by other factors, such as precipitation and snow melt.

The National Sports Shooting Foundation has produced a report on 'Lead Mobility at Shooting Ranges', a synthesis of which is given in the executive summary, available online (see http://www.nssf.org/ranges/rangeresources/library/detail.cfm?filename=facility_mngmnt/environment/executive_summary.htm&CAT=Facility%20Management). Adriano (1986) provides comprehensive information on the biogeochemistry and bioavailability of lead in the terrestrial environment.

Research into the degradation/transformation of metallic lead from shot or bullets, examples of which are outlined below, illustrates the varied impacts of soil chemistry on the rate of degeneration/transformation of metallic lead shot or bullets, the transformation products, and the rate of passage of lead and its transformation products through the soil profile.

Cao *et al*. (2003) found that at one shooting range the weathering products on the surface of the spent bullets were predominantly hydrocerussite $Pb_3(CO_3)_2(OH)_2$ and lead carbonate ($PbCO_3$), in contrast to another site where the surface lead was transformed into hydroxypyromorphite ($Pb_5(PO_4)_3(OH)$). At one site, a substantial amount of lead migrated down in the subsurface soil, possibly due to the enhanced solubilisation of organic lead complexes under the prevailing soil conditions. Where there is a high cation exchange capacity, a high proportion of lead may be retained with lower migration to subsurface levels.

McLaren *et al*. (2009) experimentally evaluated the rate of oxidation of lead shot and transfer of lead to the soil under different soil moisture and temperature regimes. They found lead to be rapidly corroded and readily released from the shot into the soil. The rate of lead shot dissolution was related to field moisture capacity. Shot corrosion, development of crust material on lead shot, and increases in lead both in solution and associated with the soil solid phase, were temperature related. Calculated free ion Pb^{2+} concentrations suggest that after six months, almost all samples contaminated with lead shot exceeded soil critical limits for lead toxicity.

Sullivan *et al*. (2012) investigated the potential of indigenous fungi to secrete low-molecular-weight organic acids and mobilise heavy metal cations at an abandoned shooting range. They found that under laboratory conditions several fungal isolates (found at high relative abundance in some of the most contaminated soils) demonstrated a marked ability to dissolve lead-carbonate at high concentrations and highlighted the need to understand more about how fungal communities may potentially impact human and ecosystem health.

Sanderson *et al*. (2012) investigated the effect of soil type on distribution and bioaccessibility of metal contaminants in Australian shooting range soils where bullets had been used. Soil lead concentrations ranged from 399 to 10,403mg/kg lead and the distribution of contamination reflected firing activity, soil properties, climate and management practices. Up to 8% of total lead occurred in soil fines (<0.075mm), due to the fragmentation of bullets on impact. Amounts of water-extractable lead varied with soil acidity, with a higher proportion extractable under acidic than alkaline conditions. Soil properties, such as cation exchange capacity, pH and dissolved organic carbon influenced both lead mobility and bioaccessibility in soils. For example, subsoils at two shooting ranges contained up to 30 and 46% of surface lead concentrations and bioaccessibility ranged from 46% -70% between different ranges. Cerussite, hydrocerussite, pyromorphite, galena and anglesite were the secondary lead minerals formed as a result of weathering in these soils, and acidic pH promotes dissolution of these and the downward movement of lead in the soil profile.

C.1.2 Lead concentrations in soils and water associated with lead ammunition

The major natural source of lead in soil is the weathering and mineralisation of parent material. In rural areas receiving little lead pollution, there is often a strong relationship between soil lead and that of parent material. The lead content in uncontaminated top soils of remote areas is generally within the range of 10 to 30mg Pb/kg (EFSA 2010). 'Normal' levels of contaminants in soils in England and Wales (see revised Environmental Protection Act Part 2A contaminated land Statutory Guidance (England and Wales) – DEFRA 2012) are considered to be those that are typical and widespread and arise from a combination of both natural and diffuse pollution contributions. In the case of lead, which has been used and widely distributed in the environment by humans for centuries, these will generally be higher than natural background concentrations. For English soils, the British Geological Survey (BGS) has identified 'Normal Background Concentrations' (NBCs) of soil lead in the principal domain (94% of the area of England) as 180mg/kg (n=34,257). NBCs are higher in the two other domains, i.e. in urban areas and areas of mineralisation of the underlying rock which generally have an anthropogenic component such as mining activity (Ander *et al*. 2013). NBCs are defined as the upper limit (the upper 95% confidence limit of the 95th percentile) of 'normal' levels of lead in soil, and soil lead concentrations are considered to be typical and widespread up to (and including, but not exceeding) 180 mg/kg in the principal domain. Mean concentrations of soil lead and 75% percentile concentrations are presented in Table C.2. These provide a comparative indication of the extent to which lead from gunshot might contaminate soils over and above other sources of contamination.

Table C.2. List of lead domains with summary statistical information for associated English topsoil (concentrations in mg/kg). Adapted from Ander *et al.* (2013).

Contaminant domain (areas of contaminant concentrations)	Sample size	Percentage area of England	Mean lead concentration	75% Percentile
Principal	34,257	94	72	66
Urban	7,529	4	276	322
Mineralisation	347	2	665	638

A range of studies in the UK and around the world have investigated lead concentrations in soil and water associated with the deposition of lead ammunition (Table C.3). In some studies a detailed history of the intensity and duration of shooting activity was known, in others shooting activity has simply been reported as 'disused target shooting site' or similar. Some have evaluated total lead concentrations, some 'plant-available' lead (using a variety of methods to evaluate availability) and some have inferred available lead from concentrations in plants and other biota. In some studies lead shot, or lead particles of above a certain sieve size have been removed prior to analysis. In the majority of cases comparable data have been provided from control sites not known to have been used for shooting. While the results of different individual studies are not always directly comparable, they nonetheless give a good general picture of the range of lead concentrations found in soil and water associated with deposited lead ammunition. We have therefore presented some detail from a broadly representative range of studies, and drawn general conclusions from these.

Table C.3. Examples of lead concentrations in soils associated with shooting activity and non-shooting control sites.

Source of lead ammunition	Shooting soil lead concentrations (mg/kg)	Non-shooting control soil lead concentrations (mg/kg)	Reference
Clay pigeon shoot (100-175m from stands). Shot. England	$\bar{x}$ = 3,038 max. = 8,172	72	Clements (1997)
Clay pigeon shoot for 20 years (80-100m from stands). Shot. *Soil sieved to remove pellets before analysis.* England	5,000 to 10,600		Mellor & McCartney (1994)
Disused shooting range (operational for 23 years). Shot. Pellets removed before analysis. Finland	8,700 (medium shot density area) 18,800 (high shot density area)	75	Rantalainen *et al*. (2006)
Game shooting wood and pheasant rearing area (200 years). Shot. *Soil sieved through 2mm mesh.* England	160 (wood) 68 (field)	60 (wood) 44 (field)	Sneddon *et al* (2009)
Shotgun clay pigeon (trap & skeet) shooting range. USA. Shot. *Pellets removed before analysis*.	75,000	74	Stansley & Roscoe (1996)
Clay pigeon fall out zone on acid peat bog. Shot. Northern Ireland	max. = 15,700 $\bar{x}$ = 306	67	White Young Green Environmental (2006)
Clay pigeon range. Shot. New Zealand	max. = 719 (used for 7 years) max = 55,958 (used for 51 years)		Rooney & McClaren (2000)
Woodland clay pigeon site used for 20 years. Shot. Finland	max = 50,000		Selonen *et al*. (2012)
Topsoil of arable area adjacent to clay pigeon shooting range used for 30 years. Shot. Czech Republic	573- 694		Chrastny *et al* (2010)
300m from edge of clay pigeon fallout range. Shot. Finland.	2,010		Hartikainen & Kerko (2009)
Shooting range, USA (top 5 cm of soil)	23,620 2m from targets 67,860 9m from targets		Vantelon *et al.* (2005)
Firing range. Bullets. Australia	399-10,959		Sanderson *et al.* (2012)

Many more data exist of soil lead concentrations but the examples above indicate the approximate range of soil lead concentrations.

Hui (2002) investigated concentrations and distributions of lead at an abandoned skeet range bordering a cordgrass *Spartina* spp. marsh in Southern California, USA. Soil lead concentrations (maximum = 16,200 ppm, d.w.) were significantly correlated to the shot pellet densities.
In a PhD study by Clements (1997), lead concentrations were analysed in soil (and ryegrass *Lolium* spp., see below) on a dairy farm in southern Worcestershire where organised clay pigeon shoots had been undertaken for 10 years. Fifty soil samples were assayed for lead content using nitric acid extraction and flame atomic absorption techniques. Pellets were not removed from the soil prior to analysis. The results showed that soil lead levels were high in the zone between 100 and 175m from the shooting stands, where the maximum lead concentration detected was 8,172.42μg/g and the mean was 3,038 μg/g lead. Non-shooting areas used as controls yielded up to 72μg/g of lead.

Mellor and McCartney (1994) measured soil and crop (oilseed rape *Brassica napus*) lead levels at a clay pigeon shooting range near Bolton in Lancashire where shooting had been undertaken for 20 years. Twelve soil samples and a pooled three-plant crop sample were assayed using both nitric acid and acetic acid extraction techniques (to distinguish between total lead and "plant available" lead) with lead concentrations measured by atomic absorption spectrophotometry. Lead shot were removed from the soil by sieving through a 1.8mm sieve and counted. Control soil and pooled plant samples were taken from a distant (300m from the shooting stand) site on the same farm. The results revealed high total lead levels in the soil between 80 and 140m from the shooting stands where they ranged from 5,000 to 10,600mg/kg. "Plant available" lead ranged from 1,000 to 4,100mg/kg between 90 and 140m from the stands. This zone of high lead contamination corresponded with the results of pellet counting (up to 257 per soil core sample obtained by using a 10cm diameter corer and taking the top 15cm soil). Outside the zone of maximum pellet deposition the levels fell rapidly.

Rantalainen *et al*. (2006) reported on the contamination of a disused "shot gun shooting range". The range was divided into uncontaminated (clean), medium and highly contaminated areas based upon previous pellet counting. Organic soil (humus and fermentation layers), enchytraeid worms and microarthropods (see Section C.2.2) were assayed for lead using nitric acid extraction and atomic absorption spectrophotometry. Pellets were manually removed from the soil. Mean lead level in the control (uncontaminated) area was 75mg/kg, rising to 8,700mg/kg in the medium and 18,800mg/kg in the highly contaminated areas.

Sneddon *et al*. (2009) collected samples of soil, pore water, plants, small mammal hair and earthworms (see below) from a shooting ground in Cheshire that had been used for game shooting for 200 years and for intensive reared pheasant shooting for 20 years. Samples were taken from a woodland copse, a shot-over meadow and distant control sites and were assayed using nitric acid extraction and plasma-mass spectrometry. Soil was sieved through a 2mm mesh. The results showed elevated mean total soil lead levels in the wood (160mg/kg) compared with the control woodland (60.25mg/kg), and in the shooting field (68.3mg/kg) compared with the control non-shooting grassland (43.9mg/kg).

Rooney and McClaren (2000) describe the contamination of three clay pigeon shooting sites in Canterbury, New Zealand, which had been in used for 7, 21 and 51 years. Soil samples were assayed by both EDTA and nitric acid extraction techniques after manual removal of visible pellets and subject to flame atomic absorption spectrophotometry. Lead levels were reported for total lead (nitric acid extraction) because of unacceptable variability of the EDTA extracted results. The analyses showed that the range used for 7 years had maximum lead soil levels of 719mg/kg whilst the similar range that had been used for 51 years had levels up 55,958mg/kg. Both ranges were on the Canterbury plain in areas of similar climate and geology. The study also showed that lead was concentrated in the top 15-20cm of soil and fell to the accepted natural background level of 30 mg/kg at levels deeper than this. The soils were not disturbed by agricultural activities.

In a report to a Local Authority in Northern Ireland, White Young Green Environmental (2006) reported lead deposition from clay pigeon shooting on an acid peat bog in Northern Ireland. The control sampling area of bog, over which no shooting had occurred, returned a background lead

level of 67.4mg/kg in the top 25cm of peat with less than 12mg/kg in peat deeper than 25cm. The highest level of contamination in the fall-out zone of the shooting range was 15,700mg/kg with a mean value across the zone of 305.6mg/kg. Levels above 500mg/kg were detected down to levels deeper than 1m below ground surface.

In a similar study in Finland, Selonen *et al*. (2012) studied the decay and distribution of lead at two woodland clay pigeon shooting grounds. One site had been used for 20 years and then abandoned for 20 years; the adjacent site had been used for the 20 years leading up to the study. They found that over a 20 year period up to 4kg/m^2 accumulated on the ground, resulting in total lead levels of up to 50,000mg/kg. After one of the shooting ranges had been abandoned for 20 years lead levels in the organic topsoil had reduced, but lead levels in water leaching from the site had increased, compared with a shooting range still in use, reflecting increased bioavailability over long time periods associated with oxidization of the lead metal.

It is clear that elevated lead levels associated with clay pigeon shooting are not limited to the areas of pellet fall-out but can also include neighbouring areas. Chrastny *et al*. (2010) detected lead levels up to 694mg/kg in topsoil in an arable field adjacent to a clay shooting range and was able to show that spring barley *Hordeum vulgare* grown in the field was contaminated mainly by lead deposition on the foliage.

Hartikainen and Kerko (2009) discovered elevated lead levels in the topsoil chosen as a control sample when measuring soil contamination on a Finnish clay pigeon shooting site in use for 30 years. The control sample was taken 300m from the edge of the fall-out zone of pellets and revealed lead levels of up to 2,010mg/kg in the top 7cm of soil. The authors concluded that this contamination was the result of downwind deposition of lead dust and powder generated by impact of lead pellets with the clays in the air.

Murray *et al*. (1997) found lead concentrations in surface soils at a recreational shooting range in Michigan, USA, to be 10-100 times greater than background concentrations on adjoining properties. Distributions in subsurface soils reflected surface distributions indicating the mobilisation and movement of lead, despite the clay-rich nature of the soils. The authors postulated that this may be due to the transformation of some of the lead to soluble lead carbonate and sulphate, both of which appeared to be present in the crust material found coating many lead pellets at the site.

We have found only two studies in which lead concentrations have been analysed in intertidal sediments. Tsuji and Karagatzides (1998) found the sediments of a heavily hunted over tidal marsh (in the western James Bay region of northern Ontario, Canada) to be at background levels. The authors suggested that this may be because the alkaline soil of this region (pH range 7.4 to 7.9) rendered the lead shot relatively inert. In contrast, Jung *et al*. (2010) collected intertidal sediments from the vicinity of a small island used as a target for military air weapons shooting in South Korea for more than 50 years and found significant enrichment of the sediments with lead (concentrations ranged from 18-277mg/kg) in the vicinity of the shooting range. While lead and other trace metals resulting from the weathering of ammunition appeared to have been relatively immobilised by geochemical processes and not dispersed extensively to the marine environment, the authors suggested that metal-contaminated soils should be better contained to prevent marine contamination.

Stansley *et al*. (1992) examined lead shot densities on eight shotgun (trap and skeet) shooting ranges in the USA where the fall-out areas included wetlands and measured the lead in the surface water, streams and downstream lake. Shot densities ranged from 4.15 X 10^6 to 3.7 X 10^9 pellets/hectare. In an acid marsh environment total water lead was as high as 1,270μg/l and filterable lead was 83μg/l (for comparison, in the EU the maximum level of lead in drinking water from 1st December 2013 is 10μg/l – Council Directive 98/83/EC). They found negligible off-site transport of lead when water pH was 7 or above, but some evidence of lead mobilisation when water pH fell below 7. The shooting range with the highest pellet density in this study was the subject of a further investigation (Stansley and Roscoe 1996) in which lead take-up by small

mammals and amphibians within the shot-fall area was studied as an indicator of bioavailability (described below). Total soil lead assay after removal of pellets showed mean values of 74μg/g from the control site and 75,000μg/g from the shot fall area of the shooting range, representing a 1,000-fold increase. The authors calculate that there is likely to have been some 266,000kg of lead in the form of pellets distributed within the soil of the shot-fall area of this shooting range at the time of sampling.

Heier *et al*. (2009) showed that trout in cages receiving water from a stream in Scandinavia subject to run-off from a shooting range showed elevated liver lead levels after 11 days and Sorvari (2007) studied lead contamination at three Finnish shooting ranges and found the concentration of lead in groundwater at one of them to exceed the drinking water standard, indicating potential health risks. Stromseng *et al*. (2009) periodically monitored lead levels in a drainage stream from a Norwegian military small arms shooting range to better understand the factors affecting lead discharge and identify strategies to limit the spread of ammunition residues. Mean lead levels in the first monitoring period, ahead of snow melt, were 14μg/l, but after high flow following precipitation, increased approximately four fold compared with low discharge concentrations. Snow melt and precipitation events gave rise to a large proportion of the total lead released and the authors speculated that these sudden release events could result in increased stress and reduced survival of aquatic animals that have little time to adapt.

Conclusion C.1 Probability that lead from spent gunshot degrades resulting in elevated lead concentrations in soil and water

The available studies provide **strong evidence** that in the large majority of cases where shooting occurs regularly and/or at high intensity, both in and possibly close to the shot fallout areas, there is a **high probability** that soil lead levels (both when analysis includes and excludes discernible fragments of shot) will be elevated above those in control soils. The degree of elevation may range from a slight to thousands of times control sample concentrations, largely in relation to the extent of shot deposition. The form and chemical speciation of the transformed lead, and its mobility and distribution through the soil profile, will depend largely upon soil chemistry. The extent of soil contamination will increase as shooting with lead shot continues over time.

A limited number of studies are available either measuring lead in water from sites contaminated with lead, or lead in biota exposed to water contaminated by lead from ammunition. These provide **strong evidence** that in some areas where shooting occurs regularly and/or at high intensity, and in and possibly close to the shot fallout areas, there is a **medium to high probability** that water lead concentrations will be elevated above those at control sites. The extent to which such contamination is likely to affect sites downstream of shooting areas is unknown, but the likelihood of broader watershed contamination appears **low,** and it seems likely that the majority of the water contamination will be relatively local.

No studies have been conducted in areas where shooting is at relatively low intensity, and in such areas lack of ‘pre-shooting’ soil and water lead concentrations may render it difficult to detect relative increases in soil lead resulting from ammunition deposition.

C.2 Pathways: Evaluating the probability that wild animals will be exposed to lead from ammunition sources via the identified potential pathways

C.2.1 The uptake of lead by plants from water and soils contaminated with ammunition-derived lead

Most lead taken up from soils by plants is found in the roots, with relatively little transmitted to the foliar parts. However, transfer to foliar parts varies with plant species and environmental conditions, and foliage lead concentrations can be significantly increased with heavy exposure to lead under certain conditions (e.g. see Adriano 1986). In addition, in proximity to firing ranges, lead 'dust' can be deposited on the foliar parts of plants (see above Section C.1.2).

Clements (1997) assayed fifty (unwashed) ryegrass samples from a dairy farm where organised clay pigeon shoots had been undertaken for 10 years (in southern Worcestershire) for lead content using nitric acid extraction. Lead concentrations were below detection limits for 49 of the samples and 121.75mg/kg in one sample from within the zone of highest soil lead.

Mellor and McCartney (1994) showed that oilseed rape within the fall-out area of the clay-pigeon shooting range contained elevated lead levels, with the highest levels in the roots. Levels were 470mg/kg d.w. in the roots, 62mg/kg d.w. in the stems and 148mg/kg ppm d.w. in the seeds compared with levels of 10 mg/kg, 4mg/kg, and not detected in similar plant parts from a control site 300m distant.

Sneddon *et al*. (2009) found elevated lead levels in the above-ground portions of ryegrass *Lolium perenne* sampled from shooting field (38.4 mg/kg) compared with the non-shooting field (0.89mg/kg). Plants were washed until the washing water was shown to be trace element-free so the contamination was unlikely to have resulted from any soil residue. Ryegrass was the most abundant plant species across the sampling and control field areas.

In Switzerland, after the decommissioning of military shooting ranges, heavily contaminated soils are landfilled or otherwise processed, but other solutions are sought for less contaminated soils and many are used for grazing. Evangelou *et al*. (2012) evaluated the uptake of metals in the above ground mass of nine plant species growing on a calcareous and a weakly acidic soil. The soils differed in their lead concentration. Above ground plant parts had lead concentrations below Swiss tolerance levels for fodder plants of 40mg/kg, except for *Chenopodium album* shoots in which lead concentrations reached 62mg/kg d.w.

Labare *et al.* (2004) investigated lead concentrations in a small number of samples of soil, sediment, water, worms and tadpoles from three sampling sites and a control site at a small arms firing range in West Point Academy, New York, USA. Soil and in stream sediment lead levels were very elevated over those at control sites, reaching 11,000mg/kg and 340mg/kg w.w. respectively. Water lead levels were 1.4-4.3 times higher at the three test sites than at the control site. Plant lead levels varied considerably, with two species reported (*Phalaris* spp. and *Carex* spp.) showing 22-55 times (43.3 mg/kg w.w. for *Phalaris* spp. and 0.87mg/kg w.w. for *Carex* spp.) control levels.

Rooney *et al*. (1999) took soil samples from a heavily contaminated clay pigeon shooting range and cultivated a range of plants in the soil to measure phytoavailability. The clay target shooting ground was alternated between arable crops and pasture although no details of rotational intervals were given. Soil lead levels were measured by both EDTA extractable and nitric acid solvent techniques after sieving through a 2mm sieve and comparisons of these methods showed that the proportion of total soil lead extracted by EDTA increased with increasing lead levels in the soil. At 23mg/kg lead only 23% was EDTA extractable, whilst at 6,174mg/kg of apparent total lead measured by nitric acid dissolution, EDTA was able to extract over 8,000mg/kg. This anomaly was explained by reference to the uneven distribution of elemental lead particles (from shot) of less than 2mm in diameter. The soils used in the cultivation trials varied from an EDTA extractable lead content of 11mg/kg (equivalent to a natural background level) to 5,998mg/kg. The plants cultivated were barley *Hordeum vulgare*, lettuce *Lactuca sativa*, perennial ryegrass, radish *Raphanus sativus*

and white clover *Trifolium repens*. Plants were grown for 120 days and then harvested, washed and separated into roots and leaves.
The full results of the cultivation trial are shown in Table C.4.

Table C.4. Results of mean plant lead concentrations from a cultivation trial of various crops (from Rooney *et al*. 1999).

Soil lead mg/kg	Mean plant lead concentration in mg/kg									
	Barley		Lettuce		Ryegrass		Radish		Clover	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
11	0.2	4.6	0.2	36.1	1.2	3.3	2.7	14.8	3.1	5.1
103	4.3	16.2	4.8	43.8	5.1	3.3	10.4	48.5	6.1	353.7
813	9.1	136.0	11.4	287.0	7.5	84.7	4.0	525	10.1	343.6
1391	11.4	264.5	5.0	251.0	24.0	466.6	17.8	556	11.7	254.4
4204	46.6	1361.7	7.0	1388.1	51.4	666.7	124.2	2695	133.7	2705.1
5998	34.4	2040.2	9.6	1968.5	43.4	1347.2	49.0	3825	93.7	2167.7

All plants showed increasing tissue lead with increasing soil lead levels and, with the exception of ryegrass in low lead soil, all plants accumulated more lead in the roots than in the leaves. However, sufficient plant translocation of lead occurred for the leaves to exceed the acceptable limits for foodstuffs. The leaves of barley and ryegrass showed virtually linear relationships between soil lead and leaf tissue lead levels; both were maximal at a soil lead level of 4,204mg/kg (46.6mg/kg lead in barley leaf and 51.4mg/kg in ryegrass leaf). At increasing soil lead levels both the lead content of the leaf tissue and the productivity (growth) of the plants declined, suggesting some lead toxicity at very high soil levels, although lead in root tissue continued to increase. Assays of titanium were used to eliminate the possibility that root lead levels were ascribable to soil contamination.

Uptake of lead from shot by plants is indicated by the research of Rantalainen *et al*. (2006) who measured lead levels in soil from a disused shot gun shooting range in Finland, and tree growth and litter production. There were no differences in tree growth rates between uncontaminated and contaminated areas but pine needle litter production was inversely linked to levels of lead contamination. There were also subtle effects of increasing lead upon nitrogen and soil respiration rates.

Green fodder used for animal feed in the EU must have a lead concentration of below 30mg/kg with an assumed moisture content of 12% [c. 34 mg/kg. d.w.] (EFSA 2010 – Table 2). If we assume a similar ‘safe’ threshold for wildlife, this indicates that plant uptake in areas contaminated with lead from ammunition sources can exceed acceptable levels in certain plants.

Conclusion C.2.1 Probability of the uptake of lead by plants from water and soils contaminated with ammunition-derived lead

The available studies provide **strong evidence** that some of the lead from deposited ammunition is available for plant uptake, and that in areas where shooting occurs regularly and/or at high intensity, and in and possibly close to the shot fallout areas, there is a **high probability** that plant lead levels will be elevated above those in control soils. In addition to lead shot densities, the degree of elevation may vary with the part of the plant analysed, the plant species, the soil type, soil pH and other factors, and may range from a slight to many hundreds of times control sample concentrations.

C.2.2 Uptake of lead by animals from soil, water and biota contaminated by ammunition-derived lead

Rantalainen *et al*. (2006) measured lead levels in soil, enchytraeid worms and microarthropods from a disused "shot gun shooting range" in Finland. There was a greater bacterial biomass and more enchytraeid worms in the uncontaminated organic soil but biomasses of microarthropods and nematode worms were unaffected by increasing lead levels. There were no differences in tree growth rates between the areas but pine needle litter production was inversely linked to levels of lead contamination. There were also subtle effects of increasing lead upon nitrogen and soil respiration rates.

The Labare *et al.* (2004) investigation at a small arms firing range in West Point Academy, New York, USA (see above) found that worms at test sites contained between 27-90 times (15-55mg/kg), and tadpoles 8-20 times (11-27mg/kg), the lead concentration of controls.

Sneddon *et al*. (2009) collected samples of soil (see above) plants, small mammal hair and earthworms from a shooting ground in Cheshire that had been used for game shooting for 200 years and for intensive reared pheasant shooting for 20 years. No small mammal samples were obtained from the shot-over meadow. Earthworms were washed and retained until their bowel was empty before assaying; tissues of those from the shooting woodland were significantly higher in lead (111.79mg/kg) than from the control woodland (5.49mg/kg). Mixed washed and unwashed small mammal hair showed no significant variations in lead levels across the sampling sites.

In the USA, Stansley and Roscoe (1996) investigated concentrations and bioavailability of lead at a shooting range in the USA that had been used for at least 30 years. Composite soil samples, after shot removal, had very elevated lead concentrations of 75,000mg/kg d.w. Tissue concentrations were elevated, and ALAD activities depressed in small mammals and frogs, suggesting that at least a portion of the lead was bioavailable. White-footed mice *Peromyscus leucopus* tissue lead at the range was 5-64 times higher than from control animals, and green frogs *Rana clamitans* had femur and (pooled) kidney samples elevated by 1,000 and 67 fold respectively. Blood ALAD activity was also significantly reduced in mice and frogs from the range, with lesions associated with toxicity in some animals. The authors suggested that ingestion of contaminated soil may be a significant route of lead uptake in small mammals at the range.

Johnson *et al*. (2004) evaluated risks to woodchucks *Marmota monax* from lead exposure in a previously used upland skeet range and a small arms range relative to a control site. No effects were found between the sites. The authors highlighted the need for additional analyses at ranges with higher lead concentrations and using small mammals with smaller home ranges.

Heier *et al*. (2009) showed that trout in cages receiving water from in a stream in Scandinavia subject to run-off from a shooting range showed elevated lead levels in liver after 11 days.

Bianchi *et al*. (2011) investigated lead shot density and lead concentrations in sediment and red swamp crayfish *Procambarus clarkii* in the 2000ha inland marsh of Padule di Fucecchio, Tuscany,

Italy. Lead shot density was high (0-311 shot/m^2) and while slightly lower in protected areas than in hunting areas, contamination was widespread throughout the marsh, with a mean lead concentration of 115.6mg/kg d.w.. Crayfish had high lead concentrations in gut contents (43.84 +/- 47.48mg/kg d.w.) and in the hepatopancreas (3.217 +/- 4.850mg/kg d.w.).

Hui (2002) investigated concentrations and distributions of lead at an abandoned skeet range bordering a cordgrass marsh in Southern California (maximum = 16,200 mg/kg d.w soil lead). Lead concentrations in plants varied according to species' abilities to inhibit lead uptake from soil. Horn snails *Cerithidea californica* had a meat lead concentration (1,987mg/kg d.w.) over 100 times greater than the leaves of the plant species with the highest mean concentration (18mg/kg d.w.) at the same site.

Migliorini *et al*. (2004) investigated the soluble lead fraction in soils from a clay pigeon shooting range, and its bioaccumulation in the saprophagous *Armadillidium sordidum* (Isopoda) and the predator *Ocypus olen* (Coleoptera). Results showed that a significant portion of metallic lead from spent pellets is bioavailable in the soil and can be bioaccumulated by edaphic (soil) organisms, entering the soil trophic network, but without biomagnification.

Vyas *et al*. (2000) investigated lead levels in soil, earthworms and passerines at a clay pigeon (trap and skeet) range in Maryland, USA, open since 1970 and used two evenings a week since 1991. They examined both free-flying birds, and birds housed for 28 days in a purpose built outdoor aviary on site. Soils (after shot removal) had very elevated lead concentrations of 110-27,000mg/kg d.w. Earthworm samples from the site had toxicologically significant lead concentrations (840 and 660mg/kg) i.e. levels which can cause serious disease in experimental feeding studies (Beyer *et al*. 1988). The results showed that at least some ground foraging passerines were poisoned by lead from shot at the site, but the authors could not determine whether this was from one or a combination of direct shot ingestion, the ingestion of lead ammunition contaminated soil (soil is known to be a critical routes of exposure in some bird species (e.g. Beyer *et al* 1998b)) or lead contamination of other dietary components at the site.

Ma (1989) found elevated concentrations of lead in kidney, liver and bone tissue of wood mice *Apodemus sylvaticus*, bank voles *Clethrionomys glareolus*, and shrews *Sorex araneus* at a shooting range on acidic sandy soil, compared with control animals from an adjacent unpolluted area. All shrews and some voles from the range had kidney lead concentrations of >25mg/kg d.w. and were considered lead poisoned (geometric mean for shrews was 270mg/kg d.w.) with a maximum of >1,000mg/kg d.w. Under the conditions found at this site, lead from shot appeared to readily enter the food chain.

Conclusion C.2.2 Probability of uptake of lead by animals from soil, water and biota contaminated by ammunition-derived lead

The studies reviewed provide **strong evidence** that where levels of soil, water and/or biota are elevated as a result of the degradation of lead from ammunition, there is likely to be uptake of lead by certain animals. The extent to which lead uptake results from the ingestion of, or exposure to, contaminated soil and water, or to the ingestion of contaminated plants or lower animals, is not generally known. One study provided **evidence** that lead from ammunition can **bioaccumulate** but with **no evidence of biomagnification**. This is consistent with other studies indicating that plants and animals may bioconcentrate lead, but that it is not biomagnified in the aquatic or terrestrial food chain (U.S. ATSDR, 2007).

C.3 Receptor: Evaluating the probability of impacts on the health, welfare and population processes of animals consuming/incorporating ammunition-derived lead from soil and water and/or biota

It appears possible that an earthworm species *Aporrectodea rosea* may have developed tolerance to high lead exposure at a clay pigeon site. Reid and Watson (2005) found soil levels of 6,410 +/- 2,250 and 296 +/- 98mg(Pb)/kg d.w., respectively at a shooting site soil and an un-shot control site. At 6.1 +/- 1.2mg(Pb)/g d.w., shooting site body burdens of earthworms were almost 1,000 times higher than those from the control site (7.1 +/- 9.0µg(Pb)/g d.w.). However, experimental exposure of shooting site and control site earthworms to lead nitrate showed some decrease in body condition (measured semi-qualitatively) of the control site worms over the others.

Migliorini *et al*. (2004) investigated the effects of lead and other metals in soils from a clay pigeon shooting range and control sites on the arthropod community. Collembola, Protura and Diplura were positively correlated with major detected contaminants (lead and antimony), while Symphyla showed a negative correlation with these pollutants.

Sorvari (2007) studied lead contamination at three Finnish shooting ranges and found the concentration of lead in groundwater at one of them to exceed the drinking water standard. They predicted leaching using laboratory tests and a distribution model and assessed risks to biota using ecological benchmarks and exposure and accumulation models. At one site their risk analysis predicted high risks to soil biota, small mammals, terrestrial plants and aquatic organisms.

Stansley and Roscoe (1996) found depressed ALAD enzyme levels in the mice and the frogs sampled within the shot-fall area of a shooting range with high pellet density which is a recognised indicator of sub-clinical lead toxicosis in mammals (Loneragan and Gould 2002). Other haematological components appeared to be subtly but not statistically significantly affected by the lead levels to which the mice and shrews were exposed.

Stansley *et al.* (1997) exposed eggs of pickerel frogs *Rana palustris* and bullfrogs *R. catesbeiana* to 0, 25, 50, 75 and 100% lead-contaminated surface water from a trap and skeet range. Total lead concentrations in 100% range water treatments varied from 840–3,150µg/l, with the filterable form accounting for approximately 4–5% of the total. Hatching was not affected in either species but there were developmental effects and highly significant mortality (100% and 98%) in pickerel frog tadpoles after 10 days of exposure to 100 and 75% range water; mortality was not significantly increased in bullfrogs.

Bannon *et al*. (2011) experimentally dosed pigeons *Columbia livia* for 14 days with the soil fraction (< 250µm) taken from a contaminated small arms range at low (2,700µg Pb and 215µg Cu/dl) and high (5,400µg Pb and 430µg Cu/dl) doses. Results showed that ingestion of soils contaminated with lead caused increased body burdens of lead in birds, with lead absorbed in a dose-response manner in blood, tissues, and feathers. Erythrocyte protoporphyrin, a biomarker of early lead effect, was increased at blood lead levels > 50µg/dl.

Rantalainen *et al*. (2006) investigated the consequences of lead pellet-derived heavy lead contamination at a cast-off shooting range in southern Finland. They found microbes and enchytraeid worms to be negatively affected by the contamination, and results suggested that in the most contaminated areas decomposition and nutrient mineralisation processes may have been disturbed. While there was also an indication of reduced pine litter production at the contaminated areas, pine growth, soil-dwelling nematodes and microarthropods appeared unaffected, and the forest appeared healthy overall. The lead contamination therefore appeared to be affecting many components of the forest ecosystem, but the authors considered the ecosystem itself to be resilient to the lead contamination.

Lewis *et al*. (2001) describe cases of lead poisoning in birds and mammals in the vicinity of a law enforcement firing range facility in Georgia, USA, i.e. suggestive of ingestion of lead. Fatal cases, occurring over two one month time frames, included eight yellow-rumped warblers *Dendroica*

coronata, a solitary vireo *Vireo solitaries* and grey squirrel *Sciurus carolinensis*. Further investigations to evaluate exposure to lead were undertaken over a short period of time with some 72 wild animals (37 mammals and 35 birds) from 22 different species being collected and euthanized from a 24 ha area surrounding the shooting range. Five mammals and five birds were collected from areas 1.5–3 km outside the shooting range area as a small control group. Some 12 individuals collected nearby the facility (17%) (both birds and mammals) had lead tissue levels of >2.00ppm which can be classified as sub-clinical lead poisoning, including a grey squirrel with levels indicative of severe lead poisoning. Carcasses of one brown-thrasher and two white-tailed deer contained lead fragments. None of the 10 control animals had levels above 2.00 ppm (although one bird had elevated kidney lead concentration yet below the suggested 2.00 ppm cut off point for sub-clinical poisoning). It is assumed by the authors that the wild animals consumed the bullet-derived lead from the environment. However, it was not known whether this wildlife had been exposed to lead that had already been abraded or otherwise released from the rifle bullets and was in the soil component.

Vyas *et al*. (2000), described above, found that at least some ground foraging passerines in the vicinity of a clay pigeon shoot in Maryland, USA, were poisoned by lead from shot at the site (with some mortality), but as with the previous study described, the authors could not determine whether this was from one or a combination of direct shot ingestion, the ingestion of lead ammunition contaminated soil (soil is known to be a critical routes of exposure in some bird species (Beyer *et al.* 1998b)) or other lead-contaminated dietary components.

Conclusion C.3 Probability of impacts of ammunition-derived lead in soil and water and/or biota on health, welfare and populations of wildlife

The literature reviewed provides **strong evidence** that where invertebrate and vertebrate animals are exposed to elevated levels of lead of ammunition origin in soil, water or biota, lead will exert sub-lethal negative effects on animal physiology (i.e. both welfare and individual survival) in many species, and in some animals may cause mortality. The literature also suggests the possibility that some invertebrates may develop a high level of tolerance to lead, and one study suggested that while lead may affect the functioning of many components of a forest ecosystem, the ecosystem itself was resilient.

The studies also provide **strong evidence** that **local populations** of certain invertebrates can be negatively affected by exposure to elevated levels of lead from ammunition. There is potential for contaminated wildlife (e.g. from earthworms to small mammals and passerines) to be consumed by scavengers and predators, including raptors, and for this to present a **local risk** to these groups that is additional to those described in Section B.

Figure C.1 summarises the pathway and effects of ammunition-derived lead that has degraded and entered soil and water and/or become incorporated into biota. Table 1 provides a synthesis of explanatory text.

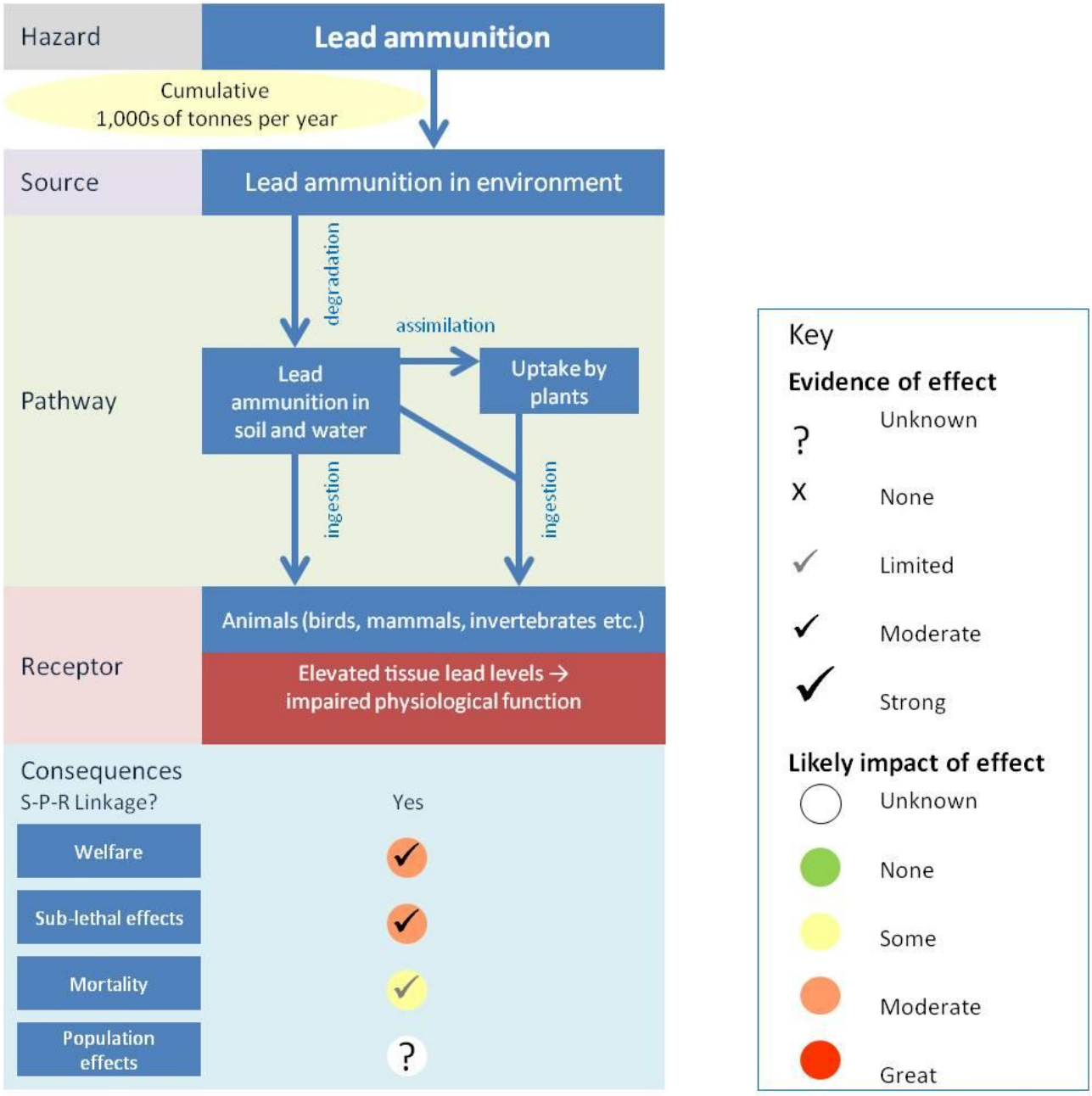


Figure C.1. Linkage Set C illustrating pathways and summary of effects on wildlife of ammunition-derived lead that has degraded and entered soil and water and/or become incorporated into biota.

Linkage Set D: Primary poisoning from direct absorption of lead from embedded lead ammunition

The conceptual model source-pathway-receptor that suggests a hazard to animals from embedded lead ammunition i.e. shot and bullets that have entered the body, not *via* ingestion, but as a result of wounding but surviving, is given in this section (Table D.1).

Table D.1. Conceptual model of Source-Pathway-Receptor linkages which might result in lead being absorbed from lead ammunition embedded in tissues of living animals which have been shot at but survived, adversely affecting their health or populations.

S-P-R linkage label	Source	Pathway	Receptor
D (i)	Embedded lead shot and/or bullets, or fragments thereof, within tissues of animals which have been shot but survived	Lead ammunition (shot and/or bullets) which has been shot into an animal but the animal has survived with ammunition derived lead embedded in tissues; a fraction of the lead then dissolves over time and becomes bioavailable to the host and enters the bloodstream.	Adverse effects on the health of the animals from effects of absorbed lead from tissues acting on the functioning of various organ systems and potentially leading to reduced welfare, death and impacts on population size.

D.1 Source: Evaluating the probability that non-lethal embedding of lead ammunition occurs

Section B.1.2 reviews the presence of ammunition shot into the tissues of wounded but surviving animals. Numerous papers report on radiographic studies to investigate proportions of live birds, predominantly wildfowl but also other gamebirds, carrying embedded shot pellets. A substantial proportion of many species of wildfowl carry embedded shot, the studies presented in Table B.2 suggests this to be an overall average of approximately a quarter (see Table B.2). Relatively few studies have been undertaken on the prevalence of embedded ammunition in mammals, however, as mentioned in Section B.1.2, Elmeros *et al*. (2012) found recent levels of 8.5% and 6.9% in foxes and otters, respectively, in Denmark.

D.2 Pathway: Evaluating the probability that non-lethal embedding of lead ammunition causes elevated lead tissue levels

Studying the effects of embedded lead ammunition in terms of lead toxicity in wildlife is complex and has rarely been conducted. Sanderson *et al*. (1998) found no apparent effects of lead shot in game farm mallards after embedding five shot of three types (lead, iron, and a bismuth/tin alloy) into the breast muscle. Body weight, hematocrits and survival did not differ between the groups and both the lead and bismuth alloy shot were encapsulated by connective tissue. However, this single study does not alone provide conclusive evidence of the potential impacts of embedded shot. De Francisco *et al*. (2003) suggest toxicity does not occur when pellets are located subcutaneously or intramuscularly as “the pH conditions do not permit the dissolution of the lead”. Given the mechanisms of toxicity of lead, it is reasonable to assume that these are similar across vertebrate hosts and thus research on lead toxicity from retained lead ammunition in humans (which *have* been studied) may provide an appropriate model. There are numerous case reports of this occurrence (e.g. Dillman *et al*. 1979, Linden *et al*. 1982, Magos 1994, McQuirter *et al*. 2004)

with effects ranging from increased blood lead levels and associated effects through to lead-related fatality. Factors affecting probability of lead toxicity risk to the host include location of ammunition in the body and, for some cases, length of time during which body tissues are exposed to absorbable lead. The relatively shorter lifespans of many species of wildfowl e.g. ducks, and some other wild animals, may reduce the likelihood of substantial lead mobilisation, but there is an absence of evidence regarding this pathway in wildlife.

D.3 Receptor: Evaluating the probability of impacts of lead from embedded ammunition on the health, welfare and population processes of wildlife

Tavecchia *et al*. (2001) reported on effects of lead exposure on survival of mallard in the Camargue, France. They analysed recovery data for 2,740 mallard captured and x-rayed during 1960-71 to determine the prevalence of lead pellets both ingested and embedded after being shot, using innovative modelling and statistical procedures. They reported a 19% relative decrease in survival of adult birds from the tissue-embedded pellets (in addition to the same relative decrease in survival due to ingested lead shot (two or more pellets) with the separate effects being additive).

Madsen and Noer (1996) reported reduced survival in pink-footed geese with embedded gunshot (as measured by re-sighting previously-radiographed birds with embedded pellets). Beyond this reduced survival, they were not able to quantify other impacts or identify its direct causes. Merkel *et al*. (2006) investigated the effect of embedded lead shot on body condition of eider ducks *Somateria mollissima* shot in Greenland. No long-term effects were found for immature or adult birds but juvenile birds were in poorer body condition than un-wounded birds. The authors considered this likely to add to other causes of mortality.

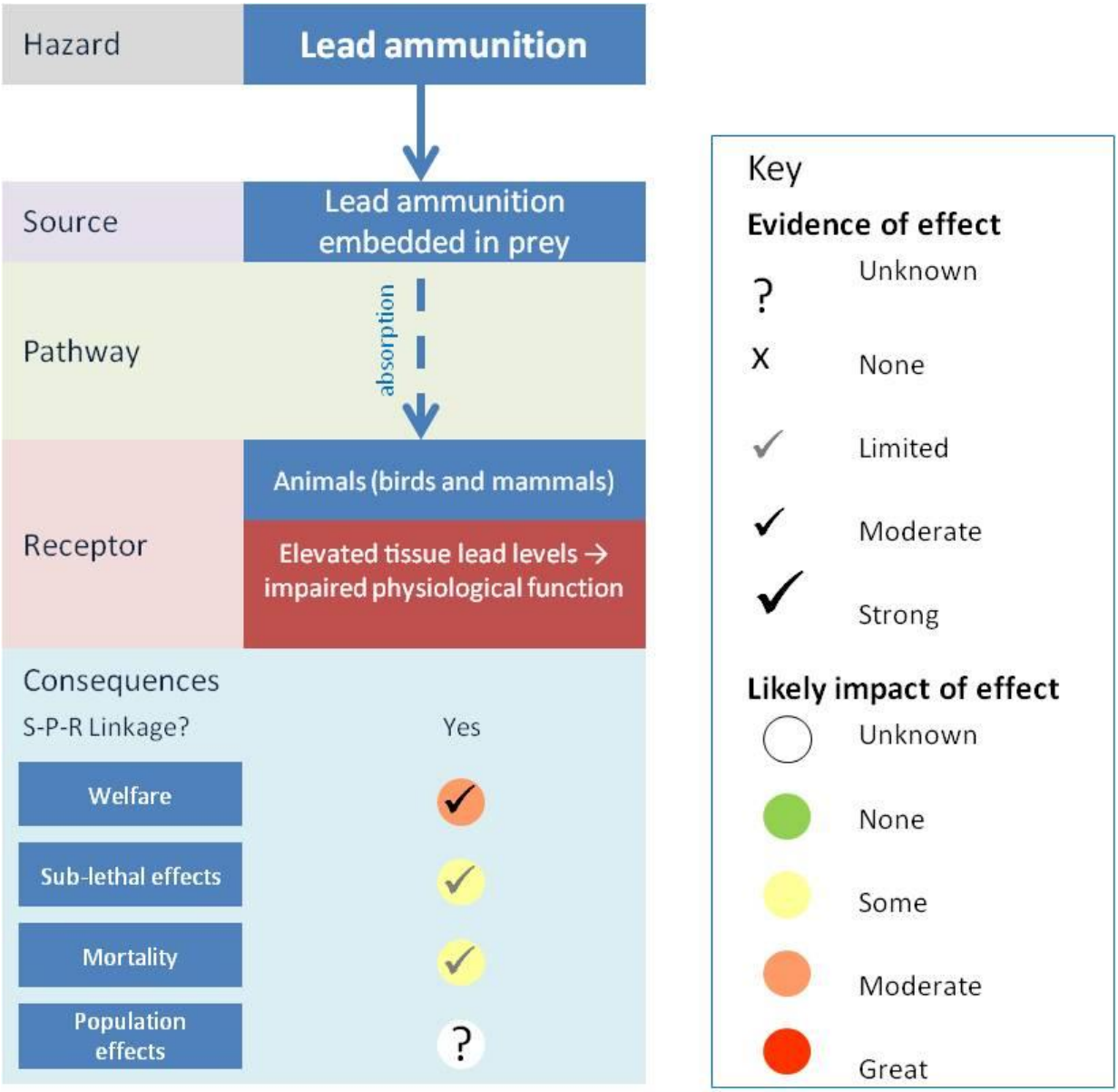
The study of Guillemain *et al*. (2007) of teal trapped in the Camargue from 1957 to 1978 found no effect on survival from embedded pellets.

In all of the above cases reduced survival of birds with embedded lead shot, where this occurred, may have resulted from the injuries themselves and their associated sequelae such as reduced competitiveness, increased likelihood of predation or secondary infection. Any additional component from the dissolution of embedded shot, if this occurred, is unknown.

Conclusion D.3 Probability of impacts of embedded lead ammunition on the health, welfare and population processes of wildlife

There is **strong evidence** that embedding of lead ammunition occurs, with **uncertainty** about whether this causes increase in tissue lead levels (studies from humans provide **limited evidence** that this may happen but their transferability to many species of wildlife is uncertain). There is **evidence** and a **probability** that embedded lead ammunition affects survival, however, this evidence does not allow us to conclude whether or not embedded lead played a part in the reduced survival, where this occurred.

Figure D.1 summarises the pathway and effects of lead ammunition which is embedded in wildlife. Table 1 provides a synthesis of explanatory text.



Note: most studies unable to differentiate effects of lead vs trauma and its sequelae (or a combination of both)

Figure D.1. Linkage Set D illustrating pathways and summary of effects on animals with embedded lead ammunition.

Overall conclusions

See Table 1 (page 270) for a summary of overall risk assessment conclusions.

References

Some of the information for Linkage Set C has been taken from the 'Assessment of the risks to human health from livestock feeding in areas of lead gunshot deposition' for which references have already been comprehensively evaluated. In these cases abstracts only have been scrutinized to summarize the main conclusions.

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Supporting information

1 Review of the exposure of wildlife to lead from a variety of sources

1.1 Lead in the environment and exposure of wildlife

Lead in the environment can be attributed to two sources: (1) natural sources, i.e. releases of naturally-occurring lead in rocks and soil by natural processes, e.g. volcanic activity and weathering; and (2) anthropogenic sources, i.e. releases of naturally-occurring lead by human processes, and releases of lead used intentionally in human products and processes (UNEP/DTIE 2008).

Lead is naturally found in soil and rock in low concentrations. When lead is found in rock, concentrations generally range from 2 to 150 mg/kg depending on the type of rock (Wedepohl 1978, Adriano 1986). The average global lead concentration in soil has been reported to be 22 mg/kg and ranges from 0.2 to 115 mg/kg (Richardson *et al*. 2001). These natural lead sources can be transported and mobilised by a number of natural processes, including volcanoes, sea spray, forest fires and weathering (UNEP/DTIE 2008). In 1983, volcanoes emitted between 540 and 6000 tonnes of lead into the atmosphere (Nriagu, 1989). The weathering of rocks plays a significant role in the global lead cycle, releasing lead into soils and aquatic systems, but rarely results in overall elevated concentrations (UNEP/DTIE 2008).

The accumulation of lead in the environment in situations where it can potentially pose a threat to wildlife health has largely occurred as a result of human activity. Elevated lead levels have been found in soil, air and water, where there has been contamination from industrial lead sites as well as from a range of human products, including leaded paint, leaded petrol and lead ammunition. The distribution of lead in the environment varies from location to location. Lead paint on buildings remains a source of exposure to humans and occasionally to wild animals that live in close proximity to such buildings. For example, at the Midway Island National Wildlife Refuge, established on the site of a decommissioned military base, Laysan albatross *Phoebastria immutabilis* chicks nesting close to buildings had elevated blood lead and lead poisoning as a direct result of the ingestion of paint chips (Finkelstein *et al*. 2003).

Lead is now so widespread it can be found in most animals (US ATSDR 2007). For example, the levels found in human bodies in the 1990s were estimated to be three orders of magnitude higher than those of prehistoric humans (Flegal and Smith 1995, Patterson *et al*. 1991). Historically, lead emissions peaked during the 1970s, with annual global emissions estimated at 400,000 tonnes per year (Nriagu 1996). There has been a significant decrease in emissions in the developed world in recent decades (UNEP/DTIE 2008), due to the removal of lead from various industrial and domestic products (e.g. petrol and paint) and processes, and other widespread efforts to reduce human exposure. Consequently, exposure of both humans and wildlife to lead contamination from industry and significant widespread sources such as petrol has decreased dramatically.

Countries that have removed lead from petrol have seen substantial reductions in air lead concentrations, e.g. a 91% decrease in the national average air lead concentration in the USA from 1981-2012 (http://www.epa.gov/airtrends/lead.html). However, the many uses of lead spanning centuries, and the environmental distribution of lead waste or by-products especially through widespread diffuse routes such as the atmosphere, have resulted in soil lead concentrations that, almost universally, exceed those that would have occurred in the absence of human intervention.

In Great Britain, the Environmental Protection Act (1990) makes provision for the control of environmental pollution and statutory guidance has recently been produced (for England and Wales) on Part 2A of the Act, dealing with contaminated land (DEFRA 2012). Within this, 'Normal' levels of lead in soils have been defined. 'Normal' for these purposes is considered to reflect those that are typical and widespread, and arise from a combination of both natural and diffuse pollution contributions. For English soils, the British Geological Survey (BGS) has identified 'Normal

Background Concentrations' (NBCs) of soil lead in the principal domain (i.e. in the majority of soils, covering 94% of the area of England) as 180 mg/kg (n=34,257). NBCs are higher in the two other identified domains, i.e. in urban areas (4% of the area of England) and areas of mineralisation of the underlying rock (2% of the area of England) (Ander *et al*. 2013). Concentrations of lead in topsoils from UK lead mineralisation domains are in almost all cases strongly influenced by mining and mineral processing activities. This lead accumulation in the topsoils is mainly a result of the dispersion of (i) mineral processing waste products, and (ii) lead in fumes from smelting in the major historical mining areas of Derbyshire, N. Pennines and Somerset (Appleton *et al*. 2013).

NBCs for lead and other contaminants are not average concentrations but are defined as the upper limit (the upper 95% confidence limit of the 95th percentile) of 'normal' levels of lead in soil. Soil lead concentrations in England are considered to be typical and widespread up to (and including) the NBC of 180 mg/kg. Mean concentrations of soil lead and 75% percentile concentrations for all three domains are presented in Table C.2. These provide a useful comparative baseline for studies of environmental lead contamination and risk analyses, and the BGS's maps of contaminant distribution in soil are available online (http://mapapps2.bgs.ac.uk/bccs/home.html).

Sediment lead concentrations can be high in rivers and estuaries as they receive effluents from domestic, industrial and agricultural activities. This is particularly the case when estuaries receive waste from (non-ferrous) metal mining areas (see below).

Animals are primarily exposed to lead via inhalation or ingestion, and, following the removal of lead from petrol, the main lead exposure pathway is via ingestion, whether directly from ingesting soils (e.g. Paustenbach, 2000), ingesting lead objects (e.g. angler's lead weights or lead from ammunition), or ingesting lead that has become biologically incorporated in dietary items or in water. The examples below illustrate some of the main sources of exposure to wildlife and how these have changed over time.

1.2 Lead exposure to wildlife in the urban and industrial areas

Birds, especially pigeons, have been suggested as biological indicators of exposure to lead, showing comparatively high lead body burdens in urban and industrial areas (Ohi *et al*., 1981, Schilderman *et al*. 1997, Nam and Lee 2006). Pigeons are exposed both through inhalation of atmospheric lead and the ingestion of lead deposited on surfaces where they feed and on soil. Pigeon body lead burdens have been correlated with traffic density; high blood lead concentrations in areas of high traffic density showed lead isotope ratios consistent with those of leaded gasoline in the Netherlands (Schilderman *et al*. 1997). In Tokyo, lead in pigeons dropped following a ban on the addition of tetraalkyl lead to petrol and the subsequent reduction in atmospheric lead concentrations (Ohi *et al*., 1981). Similarly, lead concentrations in the feathers of Tawny Owls *Strix aluco* declined by >90% in Central Norway (broadly in the area surrounding Trondheim) between 1986 and 2005, consistent with the termination of the use of lead as a petrol additive (Bustnes *et al*. 2013). While legislation banning certain uses of lead has undoubtedly reduced exposure in wildlife, urban areas continue to have elevated lead levels relative to rural areas. For example, Roux and Marra (2007) found that despite U.S. federal ban on lead-based paint and gasoline in 1978 and 1986, respectively, passerines captured in urban sites continued to have significantly higher blood lead concentrations than their rural counterparts.

In England, Pain (1987) monitored blood lead concentrations in Canada geese from Hyde Park, central London, in June 1985 and 1986, before and after the permissible level of lead in petrol was reduced (from around 0.34 g/l to 0.143 g/l in January 1986). Canada geese were exposed through both inhalation of atmospheric lead directly and lead deposited from the atmosphere onto the grass that they grazed in the park. There was both a significant reduction in the bird's blood lead level over this period, and a significant increase in the activity of the blood enzyme ALAD (delta aminolevulinic acid dehydratase), the activity of which is reduced by exposure to even low levels of lead (Pain 1987, Figure 7.30a).

These and many other studies, including those on blood lead in humans, illustrate the efficacy of removing lead from petrol at reducing one of the most widespread sources of environmental lead contamination (see http://www.epa.gov/airtrends/lead.html).

Via a different route, there was one case of avian mortalities in England reportedly associated with the use of organic lead as a petrol additive. In the Mersey Estuary, local bird mortalities between 1979 and 1982 were almost certainly due to trialkyl lead compounds originating from a factory manufacturing tetraethyl lead additives for petrol (Bull *et al*. 1983; Wilson *et al*. 1986).

Despite the reduction in atmospheric lead, exposure levels to lead remain elevated in urban and industrial areas as illustrated by soil lead levels (see above and Table C.2). Sediment lead concentrations can also be elevated, especially, for example, in estuaries that receive waste from old lead mines. Such sediments, along with the benthic invertebrates associated with them, can present a source of contamination to birds, including some species of wader that feed in estuarine and coastal areas (Bryan and Langston 1992).

1.3 Lead exposure to wildlife in areas of mineralisation

In areas of mineralisation of metalliferous ores, especially around mining and mineral processing areas, elevated lead concentrations can occur in soils and toxic lead concentrations can be found in local wildlife (e.g. Beyer *et al*. 2004, 2013). Areas of mineralisation (2% of the area of England) are classified as a different domain for the purposes of defining NBCs of soil lead (see above). An example of the transfer of such lead to wildlife is the UK is given by a study on red grouse *Lagopus lagopus* (Thomas *et al*. 2009). These authors found that lead isotope ratios in red grouse with elevated bone lead from two estates in Scotland indicated gunshot as the sources of contamination, whereas on one Yorkshire estate, in a lead mining region, lead isotope ratios suggested that lead ammunition combined with lead from galena (PbS) mining were the sources of exposure. Isotopic studies showed that lead from gasoline was not a likely source of lead in bones of any of the grouse.

1.4 Current widespread sources of lead exposure to wildlife

Diffuse pollution of soil and water across the globe has resulted from the deposition of lead distributed in the atmosphere which resulted from industrial emissions and, especially, from the use of leaded petrol. However, while lead from natural and anthropogenic sources is still distributed and deposited from aerosols, this has reduced dramatically following the removal of lead as a petrol additive (in most developed countries at least). For example, air quality pollutants inventories for England estimated emissions of lead at 41 tonnes in 2011. This represented a decline of 98% since 1990, dominated by a 1,799t reduction in transport sources due to the phase-out of leaded petrol (Thistlethwaite *et al.* 2013).

The examples above relating atmospheric deposition of lead, largely from petrol sources, to wildlife exposure in urban and industrial areas applies also to rural areas, although exposure levels will have been lower. For example, bone lead levels in Eurasian otters *Lutra lutra* found dead in southwest England between 1992 and 2004 were studied by Chadwick *et al*. (2011). These authors found that bone lead levels were spatially positively correlated with modelled lead emissions and stream sediment lead, and over time lead levels in otters fell by 73% following UK legislative control of lead emissions implemented since the mid 1980s.

Following an earlier population increase, mute swan numbers in Great Britain decreased sharply in the 1960s and then changed little between 1970/71 and 1984/85, with lead poisoning from the ingestion of lead fishing weights shown to be the largest single cause of death in a number of areas. In 1982, voluntary measures were introduced to address this problem, and the use of most lead weights was banned in 1987. Subsequently, the incidence of lead poisoning reduced and

mute swan numbers increased dramatically, with the timing of increases corresponding closely with the introduction of legislation (Kirby *et al*. 1994).

This background gives context to an assessment of the risks that wildlife faces from lead in the environment. Today in the UK, legislative controls and monitoring of industrial, municipal and agricultural lead emissions are such that cases of lead contamination in wildlife are likely to be rare occurrences. Nevertheless, levels of exposure are likely to be higher in urban areas and near some centres of current and historical industrial activity, especially lead mining (which represents approximately 2% of the area of England). However, beyond these, the literature does not currently suggest any geographically widespread and common sources of environmental lead contamination to which wildlife is likely to be exposed, beyond lead angler's weights, which appear particularly to be a problem for swan populations, and lead ammunition.

1.5 Conclusions

As knowledge of the impacts of lead emissions on people increased in the latter half of the last century, many sources of lead emissions have been regulated including the removal of lead from most paints and from petrol. In addition, regulations have been introduced and strengthened to control industrial, municipal and agricultural emissions. Significant sources of lead exposure in England are likely to occur primarily in areas of soil mineralisation (especially areas of current or historical mining activity or areas receiving their products) and in urban and industrial areas. These areas cover a small proportion of the land surface of England. Incidental cases of wildlife poisoning from lead may also arise occasionally as a result of accidents. More broadly, widespread environmental contamination with lead in a form that can cause significant harm to wildlife appears most likely where lead from ammunition or lead angler's weights are used and becomes available to feeding animals. The contribution of lead from ammunition and the level of risk that this presents to wildlife are dealt with in this risk assessment.

2 Calculation of estimation of numbers of bird shot in UK annually to assist in calculation of tonnage of lead ammunition used.

PACEC (2006) gives estimates of the number of wild gamebirds and waterfowl shot in the UK in 2004 as just under 19 million, of which about 79% were pheasants. This total excludes woodpigeons, which PACEC (2006) treats as pests, rather than game. Results from game bag records collected by the Game and Wildlife Conservation Trust and presented by Aebischer (2013), show that numbers of pheasant, red-legged partridge, grey partridge and mallard shot in 2011 were 12 – 23% higher than they were in 2004, with the scale of increase varying among the four species. Because of the preponderance of pheasants in the national bag of gamebirds and waterfowl, we took the value for the increase in bag of this species (12%) to represent the recent increase in bag for all gamebirds and waterfowl combined. Multiplying by the 2004 total of 19 million gives an estimated UK total for 2011 of 21.3 million gamebirds and waterfowl shot, excluding woodpigeons. PACEC (2006) reports that 3.6 million pigeons were shot, not as part of a job, in 2004 and that 53% of the total number of pigeons shot were killed not as part of a job. Hence, the estimated total number of pigeons shot is 6.8 million. Adding these to the total of other birds shot and assuming that the 2004 pigeon total also applies to 2011, gives a total of 28.1 million birds shot in 2011. This is a conservative estimate because we used the lowest of the four species estimates of the 2004 – 2011 increase in bag.

Appendix 5.
Consensus conclusions from two risk assessments carried out by members of the Primary Evidence and Risk Assessment Subgroup (PERASG) of the Lead Ammunition Group (LAG), 21 February 2014

This is an appendix to the report, "Lead Ammunition, Wildlife and Human Health"
by the Lead Ammunition Group, 2 June 2015.

PERASG Terms of Reference

The terms of reference of PERASG are as follows.

Purposes

a. To gather and list sources of evidence for assessing the risks of lead in ammunition under the categories outlined below.

b. To advise on the quality, applicability and therefore inclusion of such evidence for risk assessment.

c. To propose a risk assessment method.

d. To use the proposed evidence sources to prepare an initial risk assessment under the categories outlined below.

1. Risks to wildlife from ingested lead from ammunition. This will include welfare considerations, individual and population level risks.

2. Risks to human health from the ingestion of lead from ammunition. This will include both risks associated with the ingestion of lead gunshot/bullets or fragments thereof in game animals, and the ingestion of animals that have themselves ingested and assimilated lead from ammunition. (It may also include any other perceived risks arising from lead ammunition).

3. Risks to human health through livestock feeding in areas of lead shot deposition. This will include risks from lead deposited through inland shooting, including clay-pigeon and other target shooting.

The process

From the many papers on lead risks and impacts, we will aim to list those that provide comprehensive coverage of the key issues and the most current, relevant, science-based information and reviews available. Where authoritative reviews are used we will provide an opinion on the soundness of the authors' interpretations. Additional references may be added if deemed appropriate at any time.

The primary evidence gathered will cover the risks from lead in ammunition and not comparative risks of other materials used for non-lead ammunition types.
We will cover the following types of information:

1. Published in independently peer-reviewed[103] established journals.

2. Published in independently peer-reviewed other literature (e.g. proceedings of conferences) or published reports written by 'accredited' expert specialist groups (which may have an ISBN number or be freely available online).

3. Other reports, e.g. commissioned by government, academic institutions and NGOs - not published, or published but not peer-reviewed.

4. Other literature considered to be of sufficiently high quality in total or with some information useful to the Sub-Group.

We may consider it necessary to send literature for independent or further independent peer-review.

References will also be tagged as having the following applicability: UK, EU (Europe), INT (International). This is to assist judgment about their relevance to the UK and reflects the fact that, while not necessarily carried out in the UK, they may contain information of relevance to the LAG's purposes.

PERASG Chair and membership

N.B. The ToR for LAG members is both for risk assessment (RA) AND for risk management(RM) and that is why the ToR for the RM is important as it restricts their ability and scope to RM to England in the case of Defra and the UK in the case of the FSA. The LAG is thus responding to Defra and the FSA in its remit and scope.

PERASG is only concerned with RA and not with issues of RM. This is why the PERASG ToR are different and why they use studies from anywhere worldwide as long as they judge that the findings, in part or whole, are reasonably expected to be applicable to the England/UK situation.

Chair: Prof Len Levy

Members: Peter Green, Prof Rhys Green, Dr John Harradine, Dr Alastair Leake, Dr Debbie Pain

Purpose of statement of consensus conclusions

Two assessments of risks to wildlife from ingested lead from ammunition were produced: "Lead Ammunition and Wildlife in England (UK)" by John Harradine and Alastair Leake (WRA1) and "An evaluation of the risks to wildlife in the UK from lead derived from ammunition", by Debbie Pain and Rhys Green (WRA2). PERASG was unable to agree a single risk assessment and therefore WRA1 and WRA2 have been submitted to the LAG as minority reports. However, the PERASG authors of the two minority risk assessments have agreed the following statement of consensus.

[103] *Peer review* is a process used for checking that research methodology and conclusions are sound, before being published, typically undertaken by other specialists in the field of study.

Is wildlife in the UK exposed to lead from ammunition?

It is estimated that at least 6,000 tonnes of lead are dispersed annually from ammunition in the UK. This comes from gunshot and/or bullets from game shooting, clay shooting, pest control and deer stalking. Potentially this is available to wildlife. Exposure potentially occurs wherever shooting takes place and spent ammunition remains on/in soil or water or in the bodies of shot animals.

Are there pathways from spent lead ammunition to wildlife?

Five potential source-pathway-receptor models are identified.

1. Direct ingestion of spent lead ammunition from the environment.

2. Indirect ingestion of spent lead ammunition by predators/scavengers in the bodies of their prey.

3. Movement of spent ammunition lead via plants into their consumers.

4. Movement of spent ammunition lead via soil ingestion or soil organisms/invertebrates into their consumers.

5. Movement of spent ammunition lead from embedded shot/bullets into body tissues/organs.

Evidence of pathway 1 is available for many species of wildfowl, some other waterbirds and gamebirds, in the UK and overseas. Evidence of pathway 2 exists for some raptor species in the UK and overseas. Evidence exists for pathway 3 and 4, but there are few studies from the UK. A possible pathway 4 is identified for woodcock. There are few studies of pathway 5.

A range of other species of wildlife may be exposed, although few relevant studies have been done in the UK. These include corvids and other scavengers (pathway 2). Ground-foraging passerines and pigeons, as well as other forms of wildlife, including small mammals/frogs may be exposed through pathway 3 and 4 in areas of high shot-fall (such as clay target grounds).

The lack of studies in the UK of any or all of the pathways in wildlife species does not necessarily mean they do not exist.

Are there significant risks to the welfare of wildlife?

Sub-clinical and clinical, behavioural, developmental and reproductive impacts of above-background tissue lead levels from ingested lead ammunition in wildlife are expected to cause welfare impacts for many birds which ingest ammunition, as well as all those animals which die from lead poisoning.

From the known pathology and physiology of the poisoning plus its effect on coordination and mobility, there is a high probability of serious welfare impacts in the majority of the individual birds that ingest lead.

Are there significant risks to wildlife in terms of effects on individuals and population processes?

Deaths and impaired reproduction of individual animals, caused by direct and indirect ammunition lead poisoning, will affect death rates and birth rates and therefore population processes. Adverse effects from ingested ammunition lead, including death, occur or are likely to occur in individual birds (and some other animals) where source-pathway-receptor linkages occur or are likely to occur. To date they are recorded in the UK for wildfowl and some other waterbirds, some gamebirds, red kite, and possibly other raptors (buzzard/peregrine falcon).

Are there significant risks to wildlife in terms of effects on population size?

The extent of lead exposure in some species suggests the potential for effects on population size, although the detailed studies necessary to establish this have not been undertaken in species found in the UK. The extent to which lead poisoning mortality may be compensated for by other factors affecting survival is unknown, and therefore population size may or may not necessarily be impacted.

Significant knowledge gaps

Woodcock exposure through contaminated earthworms; corvid/raptor/mammal exposure through scavenging lead-shot bird/animal carcases/discarded intestines; woodpigeon exposure; invertebrate/passerine/small mammal etc exposure in high density shot fallout areas; extent of impacts derived from localised high density shot fallout areas on wildlife populations at regional/other level; impacts of ammunition lead on UK wildlife population size and processes using measurements of effects of lead on demographic rates and simulation models of population processes.

Differences between risk assessments

Differences remain in the following areas:

a. WRA1 focuses on England (UK) whereas WRA2 cites more studies from elsewhere because evidence about pathway steps from non-UK studies is used where applicable to the situation in the UK.

b. Detail of source-pathway-receptor linkages assessed is greater detail in WRA2 than WRA1.

c. Assessments of reliability/applicability of evidence sources are given for whole studies addressing multiple topics in WRA1 whereas WRA2 uses only those parts of studies sufficiently sound and relevant to a particular pathway step and mentions caveats.

Appendix 6.
Register of risks and mitigation

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health”
by the Lead Ammunition Group, 2 June 2015.

This appendix is provided as a separate Excel file (‘LAG Report June 2015 Appendix 6’) which includes a cover page (worksheet 1) and three tables on separate worksheets:

1. Human health risks and mitigation measures (worksheet 2).

2. Wildlife health risks and mitigation measures (worksheet 3).

3. **Combined summary of risk reduction efficacy: a colour-coded table combining risk reduction measures for both human and wildlife health and their efficacy** (worksheet 4).

Appendix 7.
Numbers of terrestrial game birds, wildfowl, raptors and scavengers dying annually after ingesting lead ammunition

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health” by the Lead Ammunition Group, 2 June 2015.

Dr Pain and Professor Green’s estimates in expert opinion provided in September 2014 to the Lead Ammunition Group’s PERA Subgroup follow. These have been reviewed and slightly updated (March 2015).

These estimates give a broad-brush indication of numbers of birds likely to be affected (e.g. hundreds, thousands, tens of thousands, hundreds of thousands etc.). The estimates are based on a number of assumptions as stated. More formal analyses could not be conducted in the short time available to provide this opinion, but have subsequently been conducted for wildfowl (Pain *et al.* in prep).

Terrestrial gamebirds

An estimated hundreds of thousands of terrestrial gamebirds could potentially die each winter as a result of ingesting spent lead shot. While the data do not exist to estimate likely mortality with precision, sufficient data are available to give a very broad estimate of numbers of birds that may potentially die from lead poisoning each winter.

Data on the proportion of terrestrial birds with ingested shot are available for several species in the UK, i.e. shot red-legged partridge (1.4%, Butler 2005[104]), shot pheasant (3%, Butler *et al.* 2005) and grey partridge (4.5% average for found dead adults and juveniles - Potts 2005), so data exist to allow for order of magnitude estimates of mortality. To do this we took breeding population estimates (from Musgrove *et al.* 2013) of these species and the other most numerous gamebird, Red Grouse, potentially susceptible to shot ingestion and added the numbers of pheasant and partridge released for shooting each year (taken from PACEC 2006). We doubled the numbers of territories to give numbers of individuals for Red-legged and Grey Partridges. For pheasant, we assumed that the ratio of males to females was 1:4.6 (Cramp & Simmons, 1980). We ignored the many young wild-bred birds hatched in the previous breeding season that are present during the shooting season because good estimates of the immature population were not readily available. However, the number of wild-bred young gamebirds hatched in the previous breeding season, averaged over the shooting season is typically about twice the number of older birds. Our estimate of numbers of terrestrial gamebirds birds that may potentially ingest shot is therefore an underestimate. We also omitted other potentially susceptible game species. We assumed that hunter-shot Grey and Red-legged partridges would have similar levels of shot ingestion (1.4%) because Grey Partridges found dead would be expected to have higher levels of shot ingestion if some had died of lead poisoning, and we assumed a similarly low 1.4% level of shot ingestion in Red Grouse and used the 3% reported for pheasant. We then assumed that shooters are twice as likely to kill birds that have ingested lead shot (due to their weakened state) than to kill birds that

[104] Earlier data for red-legged partridges (1933-1992) were excluded as Butler (2005) considered it possible that cases of lead ingestion were missed by the pathologists and considered it unlikely that a detailed search was part of all post-mortem examinations, particularly when no clinical signs of lead poisoning were evident.

had not ingested shot, and corrected for this (e.g. see Bellrose 1959). We then calculated the number of birds in the population likely to have ingested shot at any one time (c. 615,000). Given that we only have estimates for the proportion of gamebirds with ingested shot at the time they were killed and shot has a residence time in the alimentary tract that rarely exceeds 30 days (on average about 20 in wildfowl – Bellrose 1959), the number of birds likely to ingest shot at some time during the winter shooting season will be many times higher than this. All birds that ingest lead shot may suffer some welfare effect, and a proportion of them, probably in the order of hundreds of thousands, are likely to die from lead poisoning.

Wildfowl

An estimated 30,000-60,000 wildfowl, or a few percent of the population, may die each winter as a direct result of lead poisoning in Britain and sub-lethal effects of lead may have additional consequences. Additional wildfowl may die outside the winter. Some additional non-wildfowl waterbird species may also be affected but insufficient data have been collected to estimate numbers.

Wintering wildfowl populations in Britain have been taken from Musgrove *et al.* (2011). We have not included those species that feed predominantly in areas that are not shot over or where shot is unlikely to be available. For a few species that feed both in areas that are shot over and other areas, we have (in a rough but approximately proportionate way) reduced the size of the population that could potentially be affected by lead shot ingestion. We have used figures on the proportions of wildfowl with ingested shot in the UK as summarized in Mateo (2008) and from Mudge (1983). Proportions of birds with ingested shot are not available for all goose species. We have therefore combined information for all goose species for which there are data and averaged proportions of shot ingestion across them all and used that value for all species. We have assumed that a similar proportion of Mute Swans and geese ingest shot, and assumed a 10% ingestion level in Whooper Swans which could be an underestimate as a very high proportion have elevated blood lead (Newth *et al.* 2012). We have then broadly estimated mortality by using the estimate for mallard cited in Mudge (1983) based on an unpublished report from 1981. Mudge used the methods described by Bellrose (1959) and conservatively estimated that 2.3% of the wintering population of Mallard in Britain die from lead poisoning. Bellrose's method takes into account both the number of shot ingested and the overall proportion of birds with shot ingestion. Details of number of shot ingested (i.e. 1,2,3,4,5 shot etc.) are not available for many species and, as Mallard have intermediate levels of shot ingestion, we have simply used the ratio from Mudge's 1983 paper, i.e. 2.3% winter mortality and a 4.2% shot ingestion level (2.3/4.2 = 0.547) to estimate mortality from shot ingestion levels. This gives an estimate of 42,100 wildfowl potentially dying of lead poisoning each winter.

Given uncertainties about current ingestion levels in UK wildfowl, but accepting that these are not likely to have changed significantly over time (e.g. see Newth *et al.* 2012), we could simply extrapolate the figures from Mudge's paper (1983) as he sampled wildfowl from a wide range of inland and coastal areas. Mudge found that that 2.72% of 2,278 hunter shot wildfowl of 15 species susceptible to shot ingestion (i.e. excluding seaduck species) contained ingested shot. If shot ingestion rates are averaged across these 15 species (rather than pooling the numbers of individuals sampled and numbers having ingested shot), this gives an average shot ingestion level of 3.8% across all species. Applying a similar average ingestion level to the approximately 2.6 million wildfowl that winter in the UK and are potentially susceptible to shot ingestion would give an estimated 71,000-99,000 birds with ingested shot at any one time. If the relationship between levels of shot ingestion and mortality is broadly similar to that estimated by Mudge (1983) for mallard, this would result in an estimated annual winter mortality of 39,000-54,000 birds. There are various assumptions and uncertainties in this simple calculation, but we suggest that the true value is likely to be tens of thousands and lie broadly within the range of 30,000-60,000 individuals. Had there been widespread compliance with regulations banning the use of lead shot for shooting wildfowl, this estimate, based upon data from before the ban, might overestimate the numbers affected currently. However, compliance with English regulations banning the use of lead gunshot

for shooting wildfowl has been shown to be very low (Cromie *et al.* 2010) and most ducks sampled have been found to have been shot with lead ammunition throughout the period since the ban was introduced. Hence, deposition of lead gunshot in wetland wildfowl habitats is likely to have continued at similar levels to those at the time of Mudge's estimate and it has certainly continued in terrestrial habitats used for foraging by several wildfowl species. For this reason the estimate of number affected is likely to be approximately correct. The estimate is for winter and excludes birds that die outside of the winter, excludes the sub-lethal effects of lead, which can also influence mortality, and excludes non-wildfowl wetland bird species that are also known to be susceptible to lead poisoning (e.g. waders).

Predators and scavengers

A proportion of deaths of avian predators and scavengers is likely to result from lead poisoning in the UK. Data are available for a few UK species suggesting that this may be 2-9% in these species, but are inadequate to estimate the proportion with accuracy. However, the source and exposure pathways exist and this lack of information should not be treated as evidence that there are negligible effects of ammunition-derived lead on scavenging and predatory birds in the UK. Measurements of lead concentrations in tissue samples from carcasses of dead predatory and scavenging birds have been used, together with post mortem examinations, to assign the cause of death to lead poisoning and other causes. Such studies in the USA, Canada and Europe report proportions of deaths caused by lead in species likely to be at risk of ingesting ammunition-derived lead ranging from 3% of deaths to 35% of deaths. In Europe, the bird species with the most consistently high proportions of deaths attributed to lead poisoning is the White-tailed Sea Eagle (14 – 28% of deaths attributed to effects of lead). In the UK, there is evidence of death being caused by lead poisoning in 9% of a sample of Red Kites carcasses collected in England. Two percent of carcasses of Buzzards and 4% of carcasses of Peregrine Falcons collected in Britain had tissue concentrations of lead consistent with death being caused by lead toxicosis. However, the necessary measurements of tissue lead concentration have not been reported from sufficient numbers of carcasses of several species potentially at risk to draw any reliable conclusions about the proportion of predatory and scavenging birds dying from lead poisoning in the UK. In particular, sufficient observations are lacking for White-tailed Sea Eagle, Golden Eagle and Western Marsh Harrier. For some of these and other species, especially Buzzard, the geographical distribution of carcass collection for analysis probably does not sufficiently cover areas, such as parts of Scotland, where culling of deer and disposal of discarded viscera containing remnant of lead ammunition are prevalent. Hence, whilst there is strong evidence that a sometimes-substantial proportion of predatory and scavenging birds die from lead poisoning from studies in North America and Europe, equivalent studies with sufficient statistical power to measure the proportion of birds affected have not yet been conducted in the UK. This lack of information should not be treated as evidence that there are negligible effects of ammunition-derived lead on scavenging and predatory birds in the UK. For a full review of the global evidence concerning lead ammunition risks to scavenging species see Johnson & Rideout (2014).

Appendix 8.
The numbers of people potentially at risk from health and neurodevelopmental effects

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health”
by the Lead Ammunition Group, 2 June 2015.

Professor Green and Dr Pain’s estimates in expert evidence provided in September 2014 to the Lead Ammunition Group’s PERA Subgroup.

Kidney disease

An estimated 27,000 - 62,000 adults from the shooting community consume at least one game meal per week averaged over the year (Ellis 2014). Calculations in the Lead Ammunition Group PERA Subgroup Human Health Risk Assessment indicated that 71,000 adults eat at least one game meal per week. Both of these estimates deal only with consumers of game meat in the shooting community and not the general public. The Lead Ammunition Group PERA Subgroup human health RA calculated that the consumption rate of gamebird meals required to give the EFSA BMR (10% increase in risk of CKD) was 1.2-1.9 gamebird meals/week for one statistical model used by EFSA, and 4.0-6.5 gamebird meals/week for another of their models. The upper and lower bounds for each model refer to two alternative bioavailability values that were used. Using information on the distribution of numbers of game meals per week within the set of high-level consumers derived from the Food Standard Agency Scotland (2012) survey of high-level consumers in Scotland, the number of high-level consumers eating various numbers of game meals per week can be calculated. If there are 27,000 adult high-level consumers (the lower bound in Ellis 2014) the number of adults eating at least 1.2 game meals is 23,127; 1.9 meals - 13,417; 4.0 meals - 2,467; and 6.5 meals - 129. The equivalent figures if there are 71,000 adult high-level consumers (the Lead Ammunition Group PERA Subgroup RA value) are 1.2 game meals - 60,816; 1.9 meals - 35,289; 4.0 meals - 6,486; and 6.5 meals - 338. Hence, the estimated size of the ‘at risk’ population is strongly dependent on the statistical model and the bioavailability value used to calculate the number of meals needed to give the BMR. The ‘at-risk’ population within the shooting community can range from hundreds of adults to tens of thousands, depending on these assumptions.

A way to perform this calculation for the whole adult population of the UK, using completely independent dataset, is as follows. Taylor *et al.* (2013) found from NDNS** data that 40 of 1,031 adults (3.88%) aged over 18 ate gamebird meat during a 4-day sampling period, with average consumption rates of 19.6 g/d (the equivalent of 137.5 g/week) The range of daily consumption rates for the 40 respondents who ate gamebird meat was 1.8-79.0 g/d. The UK population over 18 years of age in June 2012 (Office of National Statistics 2012) was 49,667,327. Assuming that the proportion of these that ate gamebird meat was the same as for the NDNS sample (3.88%), this gives an estimate of 1,927,092 adults in the UK that eat some gamebird meat. The Lead Ammunition Group PERA Subgroup human health RA calculated that the consumption rate of gamebird meat required to give the EFSA BMR (10% increase in risk of CKD) was 240-380 g/week for one statistical model used by EFSA, and 800-1,300 g/week for another of their models. The upper and lower bounds for each model refer to two alternative bioavailability values that were used. The estimated number of adults eating at least 240 g/week is 288,097; 380 g/week – 116,915; 800 g/week – 16,848; 1300 g/week – 3,419. As with the previous method, the estimated size of the ‘at risk’ population is strongly dependent on the statistical model and the bioavailability value used to calculate the quantity of gamebird meat needed to give the BMR. The ‘at risk’

population in the general population as a whole can range from thousands of adults to hundreds of thousands, depending on these assumptions. There is also a problem with extrapolating from 4-day reported diet data to long-term average consumption rates. Long-term average mean daily consumption for those that eat gamebird meat at all is likely to be lower than the mean from a 4-day sampling period. However, the proportion adults that eat any gamebird meat at all over a long period (such as a year) will be higher than that from a 4-day sampling period.

Heart disease

An estimated 27,000 - 62,000 adults from the shooting community consume at least one game meal per week averaged over the year (Ellis 2014). Calculations in the Lead Ammunition Group PERA Subgroup Human Health Risk Assessment indicated that 71,000 adults from the shooting community eat at least one game meal per week. Both of these estimates deal only with consumers of game meat in the shooting community and not the general public. The Lead Ammunition Group PERA Subgroup human health RA calculated that the consumption rate of gamebird meals required to give the EFSA BMR for the effects of hypertension on the risk of heart disease and stroke (a 1% increase in systolic blood pressure) was 3.2-5.2 gamebird meals/week. The upper and lower bounds refer to two alternative bioavailability values that were used. Using information on the distribution of numbers of game meals per week within the set of high-level consumers derived from the FSAS (2012) survey of high-level consumers in Scotland, the number of high-level consumers eating various numbers of game meals per week can be calculated. If there are 27,000 adult high-level consumers (the lower bound in Ellis 2014) the number of adults eating at least 3.2 game meals is 2,322; 5.2 meals – 403. The equivalent figures if there are 71,000 adult high-level consumers (Lead Ammunition Group PERA Subgroup RA value) are 3.2 game meals – 6,107; 5.2 meals – 1,060. Hence, the ‘at risk’ population within the shooting community is numbered hundreds or thousands.

A way to perform this calculation for the whole adult population of the UK, using completely independent dataset, is as follows. Taylor *et al.* (2013) found from NDNS** data that 40 of 1,031 adults (3.88%) aged over 18 ate gamebird meat during a 4-day sampling period, with average consumption rates of 19.6 g/d (the equivalent of 137.5 g/week). The range of daily consumption rates for the 40 respondents who ate gamebird meat was 1.8-79.0 g/d. The UK population over 18 years of age in June 2012 (Office of National Statistics 2012) was 49,667,327. Assuming that the proportion of these that ate gamebird meat was the same as for the NDNS sample (3.88%), this gives an estimate of 1,927,092 adults in the UK that eat some gamebird meat. The Lead Ammunition Group PERA Subgroup human health RA calculated that the consumption rate of gamebird meat required to give the EFSA BMR for the effects of hypertension on the risk of heart disease and stroke (a 1% increase in systolic blood pressure) was 640-1,040 g/week. The upper and lower bounds refer to two alternative bioavailability values that were used. The estimated number of adults eating at least 640 g/week is 32,090; 1040 g/week – 7,356. The at-risk population within the general population is numbered in the thousands to tens of thousands. There is a problem with extrapolating from 4-day reported diet data to long-term average consumption rates. Long-term average mean daily consumption for those that eat gamebird meat at all is likely to be lower than the mean from a 4-day sampling period. However, the proportion adults that eat any gamebird meat at all over a long period (such as a year) will be higher than that from a 4-day sampling period.

Neurodevelopmental impairment especially in children

It was estimated by an unpublished CA/BASC telephone survey that 9,000 (midpoint of 5,500-12,500) under 8-year olds from the shooting community consume at least one game meal per week averaged over the year (Ellis 2014).

Calculations in the Lead Ammunition Group PERA Subgroup Human Health Risk Assessment indicate that 11,000 children of all ages from the shooting community eat at least one game meal

per week. Both of these estimates exclude high-level consumers of game meat outside the shooting community.

Both of these surveys refer to all types of game, but it is likely that the vast majority of it was wild game killed using lead ammunition. Much of the game would be expected to be from gamebirds, for which the best information on contamination with ammunition-derived lead levels is available, but the precise proportions of gamebird meals are not known.

These estimates are for the live-quarry shooting community only and for children eating one or more game meals a week, which generally exceeds the amount of game required to give the Bench-mark Dose for neurodevelopmental effects. Hence, it seems probable that the population of children at potential neurodevelopmental risk from ammunition-derived lead in game meat in the live-quarry shooting community in the UK may be more than 10,000.

In the general population of the UK, 5,516,357 children were age 6 or under in 2012 (June 2012 Office of National Statistics). Taylor *et al.* (2013) using data from the National Diet and Nutrition Survey **(2008-2010), reported that an estimated 0.877% of children under 6 years old ate gamebird meat, with an average consumption of 6.8g/d (equivalent of 48 g per week). Hence, the number of children <6 likely to absorbing some ammunition-derived dietary lead from gamebirds in the course of a year is estimated as at least 48,378. This number is a minimum because it is based on a diet survey of 4 days duration (see below).

The NDNS survey results provide the best available data for the general population as a whole (i.e. including the majority who are not connected with live-quarry shooting) and is considered as representative of the general population. The NDNS results showed that gamebird consumption patterns did not vary seasonally. This estimate of the number of young children exposed to at least some ammunition-derived lead may be conservative for several reasons. The survey dealt with gamebirds only and did not cover other types of game that may be shot with lead. The results are based upon reported diet within a 4-day period. A higher proportion of children than this is likely to eat gamebirds over a longer period, such as a year.

The authors also note that NDNS data were collected from 2008-2010 and game has become more popular and widely available in supermarkets since then, and along with promotion of the health benefits of eating game consumption levels may have increased. The number of young children in the general population eating >1 gamebird meal per week (rather than some gamebird meals) cannot be established precisely from the NDNS data because of the small sample size for consumers.

Despite these uncertainties, the NDNS data indicate the extremes in exposure to ammunition-derived dietary lead from gamebirds. At one extreme, if the 0.9% of children recorded to eat gamebirds in an average 4-day period also ate gamebird meals in all other 4-day periods in a year and no other children did so, the proportion of children in the general population eating gamebirds in a year would be 0.9% and they would be exposed annually to a quantity of ammunition-derived lead about five times that from the rest of their diet combined. However, if, taking the other extreme, a totally different 0.9% of children consume gamebirds in each 4-day period of the year, this would result in 82% of all children eating gamebird in the course of the year with an increase in annual dietary exposure of about 5% relative to exposure to dietary lead from the baseline diet. So at the extremes 48,000 children (0.9% of the population of 6 or younger) could experience a 500% increase in their annual exposure to dietary lead, or 4.5 million children (82% of the population of children of 6 or younger) could experience a 5% increase in annual dietary lead. Reality is likely to lie in between these extremes, but is more likely to be nearer to the first (tens to hundreds of thousands) rather than the second because humans generally have quite consistent dietary habits.

We note that as the sample sizes presented for children eating game in Taylor *et al.* (2013) were small, additional data could increase or decrease these estimates considerably thus the estimates are imprecise. However, they would still be of the order indicated so the ranges are likely to be

reasonably accurate. Additional years of data have been and are being collected by the NDNS and this will increase confidence in estimates.

Spontaneous abortion

Taylor *et al.* (2013), using NDNS** data, found that 2.87% of women of childbearing age reported eating gamebird meat in a 4 day period. Based on this, the equivalent weekly consumption of gamebird meat in those women that consumed it was 14-650 g per week. From these values it can be estimated that about 0.22% of women are likely to have consumed more than 560 g of gamebird meat per week and 0.09% to have consumed 920g (the amounts required to increase abortion risk by 1%). The number of live births in the UK in 2012 was 812,970. If the proportion of pregnant women eating gamebird meat was similar to that for all women of child-bearing age and their frequency of consumption of gamebird meat was consistent over time, then the number of women potentially exposed to sufficient to increase the risk of abortion by 1% would be in the range 745 – 1,700 women per year.

Appendix 9.
Environmental and biodiversity impact

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health”
by the Lead Ammunition Group, 2 June 2015.

Judgement of environmental and biodiversity impact follow as closely as possible a number of parameters and considerations set out in Defra guidance for identifying and assessing the environmental impact of policy options[105]. The guidance lists seven environmental policy areas of priority, but the most relevant to this report are “potential changes that the policy (in this case the continued use of lead ammunition) may have for biodiversity”.

Judgment of biodiversity impacts focuses on the likelihood of gains or losses for certain biodiversity features, namely:

1. The variety of species;

2. Variety and abundance within species;

3. The amount of space for ecosystems and habitats;

4. Physical connectedness between ecosystems and habitats; and

5. Environmental changes within ecosystems and habitats.

The question is therefore whether or not the evidence adduced in the risk assessments shows that the continued use of lead ammunition affects (compromises) any of these biodiversity features. The guidance points out that consideration should also be given to locations that are recognised as biodiversity rich e.g. locations subject to designation for wildlife, as well as impacts in areas that are already managed such as farmland; and should take account of the policy having possible impacts outside UK.

The guidance recommends three steps for measuring biodiversity impacts:

1. Judging trends relative to baseline data leading to loss or change in biodiversity (various sources of baseline data are available e.g. bird counts);

2. Assessing the magnitude of the impact by quantifying and then monetising impacts in terms of costs and benefits wherever possible (not attempted within this report); and

3. Evaluating the significance of all the non-quantifiable impacts qualitatively.

These steps are difficult to apply to the continued use of lead ammunition because it is not possible to know what biodiversity would look like if lead ammunition were not so widely used as it has been for the past hundred years. The current state of biodiversity is the product of environments long subjected to the widespread deposition and availability of lead from ammunition in one form or another. Building a motorway through a prime habitat will cause a reasonably predictable impact;

[105] Defra. Assessing Environmental Impact: guidance. 9 April 2013. https://www.gov.uk/assessing-environmental-impact-guidance

but impacts arising from the continued use of lead ammunition have to be estimated by the piecing the evidential sources together from a wide range of scientific fields and experience. The mute swan population in the Thames valley following the ban of fishing weights provides the only English wildlife example of what happens if a major source lead exposure is effectively removed from the animals' environment; but even that does not tell us whether other wildlife in the Thames valley was similarly benefitted. It remains a matter of conjecture.

The significance of this fundamental problem (that baseline data are already shaped by widespread use of lead ammunition) means that all assessments of impacts in this report are made on the balance of probabilities or the potential for such and such impact. This will not satisfy those looking for absolute proof, but it is the best that can be done in the circumstances.

For the purposes of this report and with that cautionary note in mind, the guidance is applied as follows:

For wildlife impacts, in line with Defra guidance, might be graded:

High: having major environmental impact; "major" being a chief or key impact on the biodiversity feature stated; with strong evidence of loss or change in biodiversity relative to established baseline data; impacts on a majority of individuals in wildlife populations providing high value ecosystem services (even though not monetised).

Medium: significant environmental impact; i.e. falling short of major but being noteworthy and important for the biodiversity feature stated; with evidence suggesting potential for losses or changes in biodiversity relative to baseline data; impacts on a significant number of individuals in the stated wildlife feature (whatever their notional ecosystem service value); with significant qualitative, non-quantifiable impacts.

Low: minor environmental impact; "minor" being low in rank or severity; with evidence of a credible possibility of changes in biodiversity but not meeting tests for higher impact; impacts on some individuals in some wildlife populations; the possibility of some qualitative, non-quantifiable impacts.

Very low: negligible environmental impact is likely.

Appendix 10.
Summary extracts: conclusions from EFSA 2010 and 2012 relevant to game meat products

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health”
by the Lead Ammunition Group, 2 June 2015.

EFSA 2010

Control measures taken to regulate lead in paint, petrol, food cans and pipes in Europe since the 1970s have led to a substantial decrease in exposure.

Following a call for data, 14 Member States and Norway submitted approximately 140,000 results of lead concentrations in various food commodities and tap water.

A total of 94,126 results covered the period from 2003 to 2009 and were suitable for calculating lead concentrations in the various food categories. The lead level in approximately two thirds of the samples was below the limit of detection or limit of quantification.

Mean lead dietary exposure estimates for adults across European countries ranged from 0.36 to 1.24 µg/kg b.w. per day and from 0.73 to 2.43 µg/kg b.w. per day for high consumers, based on lower bound and upper bound assumptions for the level of reporting, respectively.

Overall, cereals, vegetables and tap water were the most important contributors to lead exposure in the general European population. More specifically, the following food groups were identified as the major contributors to lead exposure: cereal products, followed by potatoes, cereal grains (except rice), cereal-based mixed dishes and leafy vegetables and tap water. Considerable variation between and within countries in the contribution of different food categories/groups exists.

Lead in blood is considered to be the biomarker of choice for the concentration of lead in soft tissues, and hence recent exposure, although in part it also reflects long term exposure. Bone lead in vivo reflects the long-term uptake and body burden.

Absorption of lead appears to be highly variable and tends to be higher in children than in adults. It is lower in the presence of food. Absorbed lead is transferred to soft tissues, including liver and kidneys, and to bone tissue, where it accumulates with age.

Half-lives for lead in blood and bone are approximately 30 days and 10 to 30 years, respectively, and excretion primarily is in urine and faeces.

The CONTAM Panel identified the following potential adverse effects of lead, the developmental neurotoxicity in young children, cardiovascular effects and nephrotoxicity in adults as the basis for the risk assessment.

A decrease in Full Scale IQ score was considered to reflect a change in cognitive function in children at ages 4 and higher as it is the most consistently used end-point of cognitive ability assessed in such studies and was used as the critical endpoint for neurodevelopmental effects. An increase in SBP and an increase in the prevalence of CKD as assessed by a decrease in glomerular filtration rate were used as endpoints for adults.

The computed BMDL were as follows:

1. Developmental neurotoxicity: $BMDL_{01}$ = **12 µg/L** (B-Pb)
2. Effects on SBP in adults: $BMDL_{01}$ = **36 µg/L** (B-Pb); 8.1 µg/g (TB-Pb)
3. Effects on kidney in adults: $BMDL_{10}$ = **15 µg/L** (B-Pb)

Using the equation of Carlisle and Wade (1992), dietary lead intake values in adults, in whom there is negligible exposure from air and from soil and dust (<1 µg B-Pb/L), corresponding to the respective BMDL dietary intake values were as follows.

1. Effects on SBP– B-Pb 36 µg/L ~ 90.0 µg/60kg **= 1.50 µg/kg per day**
2. Effects on kidney – B-Pb 15 µg/L ~ 37.5 µg/60kg **= 0.63 µg/kg b.w. per day**.

Using the IEUBK model, a B-Pb level of 12 µg/L, the $BMDL_{01}$ dietary intake value for developmental neurotoxicity in 6 year old children, corresponds to a dietary lead intake value of **0.50 µg/kg b.w. per day**.

The CONTAM Panel concluded that the present PTWI of 25 µg/kg b.w. is no longer appropriate and noted that there was no evidence for a threshold for a number of critical endpoints including developmental neurotoxicity and renal effects in adults. Therefore, a margin of exposure approach was applied to risk characterisation.

Dietary exposures to lead based on LB and UB assumptions for average adult consumers in Europe are lower than the BMDL intake value for effects on SBP (1.50 µg/kg b.w. per day), but vary from above to below the BMDL intake value for effects on the prevalence of CKD, (0.63 µg/kg b.w. per day). The respective MOEs range from 1.2 to 4.2 and from 0.51 to 1.8, respectively. **Hence, if exposure were closer to the upper bound estimates, the possibility of effects in some consumers cannot be excluded.**

Consumer groups with higher lead exposure levels include high consumers of game meat (1.98 to 2.44 µg/kg b.w. per day) and high consumers of game offal (0.81 to 1.27 µg/kg b.w. per day). The estimated dietary exposures of these groups are also within, or at the higher end of the range of the respective BMDL intake values.

Estimated exposure in children up to age seven exceeds the $BMDL_{01}$ intake level of 0.50 µg/kg b.w. per day for neurodevelopmental effects. The MOE in average 1 to 3 year old child consumers ranged from 0.16 to 0.45, and was only slightly higher in 4 to 7 year old children. **Therefore, the possibility of effects in some children cannot be excluded. It was not possible to estimate the potential numbers of children who might be affected, as even in average consumers the MOE was <1.**

EFSA did not examine the effects of high level game consumption on children as the possibility of effects in some children on average diets could not be excluded even in the absence of this additional source of dietary lead exposure.

Women of 20 to 40 years of age were used as a surrogate for pregnant women to calculate the risk of lead exposure in utero on neurodevelopment in the offspring. Estimates of exposure were at or above the BMDL for neurodevelopmental effects, and the **CONTAM Panel concluded that it was not possible to exclude a risk to the developing fetus through exposure of some pregnant female consumers**.

CONTAM Panel concluded

1. Further efforts should be made to increase the understanding of the lead dose-response relationship.

2. At the same time, work should continue to reduce exposure to lead, from both dietary and non-dietary sources.

EFSA 2012

Due to its long half-life in the body, chronic toxicity of lead is of most concern when considering the potential risk to human health. The central nervous system is the main target organ for lead toxicity. In adults, lead-associated neurotoxicity was found to affect central information processing and short-term verbal memory, to cause psychiatric symptoms and to impair manual dexterity. There is considerable evidence demonstrating that the developing brain is more vulnerable to the neurotoxicity of lead than the mature brain. A number of studies in adults have identified an association between blood lead concentration, elevated systolic blood pressure and chronic kidney disease, at relatively low blood lead levels. The International Agency for Research on Cancer (IARC) classified inorganic lead as probably carcinogenic to humans (Group 2A) in 2006.

Using an alternative measure, the 2010 EFSA opinion identified a 95th percentile lower confidence limit of the benchmark dose of 1 % extra risk ($BMDL_{01}$) of 0.50 µg/kg b.w. per day for developmental neurotoxicity in young children. It also lists cardiovascular effects and nephrotoxicity in adults as potential critical adverse health effects of lead with respective $BMDL_{01}$ and $BMDL_{10}$ of 1.50 and 0.63 µg/kg b.w. per day.

In light of the particular concern for lead exposure in children, it is important to better identify major dietary sources of lead. The current report provides updated information on the levels of lead found in a range of foods on the European market and estimates exposure using detailed individual data from the Comprehensive European Food Consumption Database covering seven age groups from infants to the very elderly.

The 144,206 analytical results for lead in food retained in the current study were submitted by 20 countries, expanding the coverage of Europe compared to the 94,126 results from 15 countries retained for the calculations in the EFSA opinion published in 2010 (EFSA, 2010). This constituted an increase of 53 % in the number of reliable results available to EFSA for the period 2003 to 2011, of which 23,876 covered the period after 2008. However, a handful of countries still dominate the coverage of lead results providing a potential bias.

The 144,206 lead occurrence results retained in the current study were sorted into the four different levels of the FoodEx 1 classification system. More than half of the foods tested had levels of lead at less than detection or quantification limits. The mean lead levels varied between 0.3 µg/kg for infant follow-on formulae to 4,300 µg/kg for dietetic products with an **overall median across all categories of 21.4 µg/kg**. Eighty-two food categories out of 734 at FoodEx level 3 with quantified discrete results had mean lead levels exceeding 100 µg/kg. **The highest individual sample maximum of 232,000 µg/kg was found in game meat**, followed by 155,000 µg/kg in seaweed, **117,000 µg/kg in edible offal from game animals** and 59,900 µg/kg in dietary supplements.

Adult exposure was estimated at 0.50 µg/kg b.w. per day in the current study or 31 % lower than the exposure calculations presented in the EFSA opinion of 2010, mainly due to modelling differences and more accurate inputs in the present study.

The lower dietary exposure assessment results of the current study compared to the estimates presented in the EFSA opinion of 2010 can be attributed in part to the more accurate calculation methods used and the better matching of occurrence and food consumption results, but also to some extent to the decreasing lead levels in food.

The overall results, although lower than what was reported in the previous EFSA opinion, might thus still be overly conservative. The selected way of handling the left-censored data in this study was specific to the unique circumstances and would not be recommended as a future standard method.

Including the new results lowered the overall sample mean by 10 %. The distribution of the lead levels reported differed between the two sampling occasions, as can be seen in Figure 14. The

latest data collection included fewer very high values compared to the initial data collection, which can explain part of the decrease in the overall mean when including the new results.

Nevertheless, excluding the sampling year 2011 with few results, an overall decrease in lead levels of 23 % was estimated for the 8-year period across all sample results. This is consistent with findings of decreasing dietary lead exposure in other surveys across the world (WHO, 2011). It is also consistent with decreases in European blood lead levels. For instance, the mean level of lead in children's blood in Germany has fallen by more than 50% over a longer 14-year period (ENHIS, 2009).

A comparison between the new exposure assessment and the results from the 2010 EFSA opinion for the adult population indicated a 31 % reduction. In the previous calculations, the adult survey median for MB mean lead dietary exposure was 0.75 µg/kg b.w. per day (not shown in the opinion), while it was estimated at 0.50 µg/kg b.w. per day in the current study. About half of the reduction in dietary exposure to lead might be attributable to the combined effect of the new sample data received covering a further three-year period of decreasing lead levels and the modified handling of reported left- censored results. The use of detailed individual consumption patterns and exclusion of surveys covering less than two days as well as a more accurate matching of occurrence and consumption data is believed to account for most of the remaining reduction. A similar reduction would be expected for the other age groups, although a direct comparison with the previous opinion is not possible because of a lack of detail in the latter. Often it is not the food with the highest lead levels, but foods that are consumed in larger quantities that have the greatest impact on lead dietary exposure.

Dietary lead exposure for all age groups is considerably lower than the previously established, but now invalidated, health-based guidance values. With an alternative approach, the 2010 EFSA opinion identified a 95^{th} percentile lower confidence limit of the benchmark dose of 1 % extra risk ($BMDL_{01}$) of 0.50 µg/kg b.w. per day for developmental neurotoxicity in young children, which is lower than the estimated mean exposure. The 2010 EFSA opinion also lists cardiovascular effects and nephrotoxicity in adults as potential critical adverse health effects of lead with respective $BMDL_{01}$ and $BMDL_{10}$ of 1.50 and 0.63 µg/kg b.w. per day, which both are higher than the estimated mean exposure for adults.

In conclusion, it seems that lead dietary exposure in Europe is less than previously anticipated and that lead levels in food are decreasing. On the other hand, there is no threshold for the potential critical health effects of lead. It is considered important to confirm the seemingly decreasing lead levels in food by future testing.

Dietary lead exposure was found to be about a third lower than previously estimated with mean levels for the four older age groups not exceeding the $BMDL_{01}$ and $BMDL_{10}$ of 1.50 and 0.63 µg/kg b.w. per day established for cardiovascular and nephrotoxicity effects in adults. However, mean exposure for the three children age groups exceeded the $BMDL_{01}$ of 0.50 µg/kg b.w. per day for developmental neurotoxicity in young children.

Appendix 11.
Summary Extracts: Opinion of the Panel On Contaminants of the Norwegian Scientific Committee for Food Safety[106]: Risk Assessment Of Lead Exposure From Cervid Meat In Norwegian Consumers and in Hunting Dogs

This is an appendix to the report, "Lead Ammunition, Wildlife and Human Health"
by the Lead Ammunition Group, 2 June 2015.

Lead is accumulating in the body and is known to be harmful to humans and animals. In 2010 and 2011, respectively, both the European Food Safety Authority (EFSA) and JECFA (WHO) have concluded that there is no evidence for a threshold for critical endpoints of lead exposure i.e. under which there is no increased risk of adverse health effects. Public authorities work to reduce the lead exposure in the population.

Lead can be used in rifle ammunition for cervid hunting, but the use of lead shots for smaller animals including wild birds has been prohibited in Norway since 2005. Norwegian researchers have reported findings of high lead levels in minced meat from moose hunted by use of expanding lead-based ammunition. Maximum levels of lead (0.1 mg/kg) apply for meat from livestock animals, but not for game meat.

The Norwegian Food Safety Authority requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the risk of lead exposure to the Norwegian population by consumption of cervid meat, including any subpopulations with an increased risk. Further, VKM was asked to describe the distribution of lead from ammunition in the carcass and to estimate the tissue area associated with the wound channel that has to be removed in order to reduce the risk. VKM was also asked to present, if any, other appropriate measures in addition to removing tissue in order to limit the content of lead residues from ammunition in cervid meat. Finally, VKM was asked to assess the significance of lead exposure to the health of dogs if they were fed with trimmings from the wound channel.

The risk assessment is restricted to lead exposure from cervid meat consumption. Cervid animals include moose, red deer, fallow deer, roe deer, and wild reindeer.

Expanding lead-containing bullets produce a cloud of lead particles in the meat around the wound channel. Lead fragments from disruptively expanding, unbonded and some bonded expanding lead-containing bullets were found by radiography of various species (roe deer, red deer, wild board, sheep, chamois) in an average radius of 15 cm around the wound channel. The maximal penetration length of visible fragments was in average 29 cm. In a study on sheep, fragments from more stable types of expanding lead–containing bonded bullets were found at distances less than 5 cm. This is comparable to fragments from non-lead disruptively expanding bullets and non-lead expanding-nose bullets measured in the same study. Corresponding studies on moose have not been found. An available study indicate that lead concentrations above 0.1 mg/kg can be found at 25 cm distance from the wound channel in red deer and wild boar shot with various unknown ammunition. The majority of a limited number of hunting teams participating in a Norwegian study reported removal of meat in a radius of 10-20 cm from the wound channel. Some hunting teams reported removal of less than 10 cm.

[106] 18.06.13 11-505-final ISBN 978-82-8259-096-9

Provided that farmed deer in Norway are harvested by headshot, it is not expected that lead is found in edible meat. However, if farmed deer are shot with lead-containing ammunition in the chest, it can be expected that the meat contain lead to similar extent as in wild cervids harvested in similar manner.

Whereas most cervid meat contains low levels of lead, high concentrations have been found in some samples, including Norwegian samples of minced moose meat, where lead concentrations from below 0.03 mg/kg to maximum 110 mg/kg have been reported. Ammunition commonly used for cervid hunting contains metallic lead. Metallic lead is less absorbed in the gastro-intestinal tract than ionic lead compounds. The absorption rate of ionic lead compounds has been found to be higher in children than adults. Metallic lead can be transformed to ionic lead in the stomach, as well as by acidic cooking conditions. Absorption of metallic lead increases with decreasing size of the lead particles. However, the present risk assessment is based on lead concentrations in blood, thus uncertainty related to bioavailability of metallic lead is not applicable.

Lead exposure from cervid meat can be seen as an addition to the exposure from other food sources.

According to the most recent (2012) representative national dietary survey in Norway, mean game (including cervid) meat consumption was low, approximately 5-7 meals per year. However, in other Norwegian population studies including hunters, a large proportion (70%) of the participants consumed cervid meat at least once a month or more often. No information on cervid meat consumption among Norwegian children has been found. However, it can be expected that children eat cervid meat equally often as the rest of the family.

Associations between game meat consumption and blood lead concentration have been studied in four population studies in Norway. In the three studies performed in the years 2003-2005, a significant association between game meat consumption and higher blood lead concentration was only seen in the subgroup of male participants in one of the studies (the Norwegian Fish and Game study).

In the fourth study, the Norwegian Game and Lead study conducted in 2012, the median blood lead concentration was in the lower range of medians measured in most European and Norwegian studies over the past 10 years. This study also showed association between cervid meat consumption and concentrations of lead in blood. **Those with frequent (monthly or more often) cervid meat consumption had about 30% higher average levels of lead in blood than those with less frequent consumption. However, there was a wide range, and many participants with high or long-lasting game meat intake had low blood lead concentrations. The increase in blood lead concentrations seemed to be associated with consumption of minced cervid meat, particularly purchased minced meat. Blood lead concentration was significantly higher in participants who reported self-assembling of lead-containing bullets.**

The blood lead concentrations measured in participants in the Norwegian population studies are in the range of, and partly exceeding, the reference values for increased risk of high blood pressure and increased prevalence of chronic kidney disease in adults, and for neurodevelopmental effects in children. The additional lead exposure from cervid meat in frequent (monthly or more often) consumers of such meat is therefore of concern.

At the individual level, the risk for adverse effect is likely to be small. At present lead levels, adults with for example normal blood pressure will most likely not experience any clinical symptoms by a small increase, although it may add to the burden of those individuals who are at risk of experiencing cardiovascular disease. A small reduction in the intelligence of children will not be notable at the individual level, but at the population level it can for instance increase the proportion not able to graduate from school. Lead exposure was declining in the population on which the reference value for increased prevalence of chronic kidney disease was based. EFSA noted that this reference value (15 µg/L) is likely to be numerically lower than necessary. The implications of having a concurrent blood lead concentration above the reference value cannot fully be

interpreted, since it is not known when and at which level of lead exposure the kidney disease was initiated. However, an eventual increased risk of chronic kidney disease would be higher among those who consume cervid meat regularly or often than those who rarely consume such meat.

For these reasons, continued effort is needed in order to reduce lead exposure in the population.

Removal of meat around the wound channel reduces the lead exposure from cervid meat consumption. Lead fragmenting and distribution is dependent on several variables, and there are no available studies in moose. The available studies do not allow a firm conclusion on the amount of meat needed to be trimmed around the wound channel in order to remove lead originating from the ammunition. Other possible measures to reduce lead exposure from cervid meat would be to use lead based ammunition with low fragmentation or ammunition without lead.

VKM's Panel on contaminants has identified some data gaps during the course of this risk assessment. More data on lead concentration in Norwegian game meat, and in particular commercially available minced meat, are needed. There is a lack of data on fragmentation pattern of bullets in moose. Furthermore, more data are needed to assess bioavailability of metallic lead in food. Finally, no blood lead data or consumption data for children were available. This is needed for a refined risk assessment of lead exposure from game meat consumption in children.

The Norwegian risk assessment noted that farmed deer are likely to be headshot, which reduces meat contamination risks. For comparison purposes Norwegian game bag statistics record 34,230 red deer shot annually. There are 70 red deer game farms with on average 100 head representing a stock of some 7,000 animals. Most red deer consumed are harvested by hunters.

There is uncertainty related to how practice of meat removal around the wound channel affects lead exposure.

There is uncertainty associated with the amount of cervid meat consumption, since both over- and under reporting is expected to occur. The reporting of frequency of game meat consumption is dependent on the participants' memory of consumption and their ability to transfer consumption into frequencies. The conversion from frequency to g/day is based on standard portion sizes, which in itself is associated with high uncertainty.

There is uncertainty associated with the release of lead from different bullet types. Furthermore, types of bullets used could not be associated with the participants in the Game and Lead study, and could not be taken into account in regression analyses.

The VKM concludes that the uncertainties mentioned above most likely attenuate the associations between cervid meat consumption and lead concentrations in blood, and would thus underestimate the impact of cervid meat consumption on lead concentrations in blood.

This assessment is based on lead concentrations in blood, thus uncertainty related to bioavailability of metallic lead is not applicable.

Appendix 12.
Summary extracts: other risk assessments on lead exposure from game meat consumption

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health” by the Lead Ammunition Group, 2 June 2015.

The Federal Institute for Risk Assessment in Germany

The Federal Institute for Risk Assessment in Germany (BfR) has assessed the additional health risk of lead exposure through the consumption of game meat in comparison to the mean lead exposure from the general diet, which previously had been evaluated by EFSA (2010). Lead-containing ammunition is widely used in game hunting of wild boar and deer in Germany, and bullet fragments may penetrate deeply into the surrounding muscle tissue, resulting in lead intake through game meat consumption.

The BfR has performed several scenario computations modelling the additional exposure to lead by game meat in different consumer groups based on a national survey on environmental contaminants in foods (LExUKon-Projekt, 2010). It was found that in view of the relatively high lead concentration in other types of food on the German market, such as grain products, fruit, and vegetables, the additional lead exposure by game meat represents a negligible contribution to the overall health risk for adult normal consumers (two portions per year) or high consumers (10 portions per year). However, families of hunters (50 to 90 portions game meat per year) have an increased risk. Children up to the age of seven and foetuses are subpopulations given special attention due to their enhanced sensitivity to lead. Therefore, the BfR-report concludes that women of childbearing age, pregnant women and children are especially vulnerable consumer groups and recommended to abstain completely from wild game meat consumption. Hunters are encouraged to avoid lead-containing bullets and rather use alternative ammunition.

British Deer Society, UK

A report (Green P, 2009) written for the British Deer Society reviewed the reported associations between use of lead ammunition and adverse health effects for humans, animals and the environment. This report concluded that lead-contaminated animal carcasses represent a significant risk for raptors and carrion eaters, and that for humans, only meat from regions more than 30 cm way from the radius of the wound channel would be safe to eat. Moderate consumption of minced meat from contaminated areas will lead to elevated lead concentrations in blood; however, the exposure from venison meat would be minor in comparison with the lead exposure caused by occupational, recreational, and environmental pollution.

Swedish National Food Agency

A Swedish risk management report[107] on lead in cervid meat was based on a Swedish pilot study where samples of minced meat from moose were mostly collected from private freezers and a few samples from game handling establishments ("slaughterhouses for game") were analysed for lead.

[107] In the process of being updated.

The results were used to estimate human lead exposure from consumption of minced moose meat (Swedish National Food Agency, 2011). The National Food Agency issued the following advice for consumption of meat from cervid shot with lead-based ammunition: pregnant women, women planning pregnancy (three months before pregnancy) and children under the age of seven are advised to avoid consumption of cervid meat coming from parts of the animal which is close to the wound channel such as minced meat and pot pieces. Hunters, hunting families and other groups of the population are advised to limit their consumption of cervid meat coming from parts of the animal which is close to the wound channel such as minced meat and pot pieces to once a week. Other parts of the cervid (e.g. legs, neck, thighs, and fillets) are not expected to have elevated levels of lead from the ammunition and can therefore be consumed.

Food Standards Agency, UK

In the UK, the Food Standards Agency (FSA) in 2012 conducted a risk assessment on lead exposure from game meat consumption, based on a consumption survey of high-level consumers of lead-shot wild-game meat in Scotland and pre-existing data on lead levels in these types of food in the UK. The risk assessment concluded that regular consumption of game meat could increase exposure to lead, and that this increased exposure would be a concern in the case of toddlers, young children and pregnant women, because of the neurotoxicity of lead to the developing brain (FSA, 2012). The report highlighted that lead levels were higher in smaller game (birds) than larger game (venison). Following the risk assessment, the UK Food Standard Agency issued the following advice: “To minimise the risk of lead intake, people who frequently eat lead-shot game, particularly small game, should cut down their consumption. Pregnant women or women trying for a baby are particularly advised to minimise their exposure to lead” (FSA, 2012).

Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN)

In 2012 AESAN published an assessment of the risk associated with the presence of lead in wild game meat in Spain. Although the information available in Spain regarding the Pb content in wild game meat and its consumption is incomplete, following the analysis of data available in Spain, it has been shown that the average Pb content in pieces of large and small game exceeds the European Union general limits for meat and offal (there are no specific limits for this food) and these contents are similar to those found throughout Europe and other countries.

It has been proven that wild game meat is consumed in Spain, although it is more common in hunters and their families. It is not restricted to the hunting season, and its consumption or products that come from it, such as cured sausage or pâté, by the general public in restaurants is not negligible.

The risk assessment associated with consuming wild game meat in Spain shows a situation almost identical to the one described by the EFSA for the entire population of Europe. No negative effects can be discarded in the adult population that has diet that includes a lot of wild game meat. Regarding managing measures that could be taken for game meat, fixing specific limits would not be an adequate solution. Firstly this is due to the big differences in Pb content (even within the same animal), and secondly because official controls for this food would not be effective enough, as most of this meat is consumed directly by the hunters and their families, without going through the usual distribution channels for food that is subject to regulations.

In the AESAN Committee’s opinion, considering the situation in Spain, the most appropriate measure regarding the consumption of wild game meat that is contaminated with Pb as a result of using Pb ammunition would be to reduce the possible exposure to Pb from this source. This would be done following specific recommendations for consuming and preparing food for groups of the

population that consume this type of meat, and encouraging banning Pb ammunition and/or replacing it with existing alternatives.

Appendix 13.
Knowledge gaps

This is an appendix to the report, “Lead Ammunition, Wildlife and Human Health”
by the Lead Ammunition Group, 2 June 2015.

Human health

The following information gaps were identified during the preparation of this RA.

1. Data on consumption levels of wild shot game in the UK. Accurate data on the size, type and frequency of wild game meals consumed in England and the other UK countries would improve the accuracy and precision of the estimate that at least tens of thousands of people are high-level consumers of game meat. However, we consider that this estimate is likely to be of the correct order of magnitude.

2. While few records exist of appendicitis associated with lead retention in the appendix, an epidemiological assessment comparing high-level consumers of wild game meat in the UK with the general population would be required to accurately evaluate the level of potential risk.

3. The extent to which lead is retained in the appendix by high-level game consumers, with subsequent health consequences, has not been evaluated by studies conducted in the UK. The level of risk from this source could not therefore be accurately evaluated.

4. We could not readily find published monitoring data on levels of exposure to and absorption of lead aerosol and dust from firing ranges in the UK and could not evaluate the level of risk from this source.

Human health via livestock

The primary evidence used in this risk assessment would be strengthened by further reliable data concerning:

1. The levels of gunshot-derived lead on agricultural pastures in the UK where rough shooting and occasional shooting occurs.

2. The levels of gunshot-derived lead in the vicinity of repeatedly used shooting stands and the extent to which the fall-out includes livestock pasture.

3. The effect of soil type and management on the mobilisation of ammunition derived lead.

    a. The plant lead levels on pasture where occasional shooting occurs compared with appropriate controls.

    b. The investigation of the physical and chemical dynamics and bioaccessibility of ammunition derived lead in pastures.

4. The patho-physiological distribution of lead into skeletal muscle [meat] and eggs in livestock and poultry consuming varying levels of lead.

5. The transfer of lead into milk and meat in chronically subclinically poisoned cattle.

6. The transfer of lead from pellets ingested by game birds and chickens to meat and eggs in these birds.

7. The uptake and patho-physiology of lead in deer.

Wildlife

(Harradine and Leake)

No single study, or combination of studies, adequately demonstrates a pathway between spent lead ammunition and adverse effects on any wildlife receptor at the population level in this country. Similarly, no studies indicate contraction of range in any wildlife species exposed to spent lead ammunition. This is not to say that they do not necessarily exist rather, perhaps, that research has not been focused in this area to determine whether or not they exist, and their extent.

It is widely assumed that different birds and animals respond to lead (above their normal background levels) in similar ways. It is not known if this is correct or what the main variables are. Similarly it is not clear whether the same background *versus* toxic levels of lead relationships, even in the same tissue/organ, are appropriate across all species.

It is also assumed that elevated tissue/organ lead levels are associated with adverse welfare impacts for individual birds or animals but the extent to which this is true and what is actually incurred by the individual is not known.

The potential for secondary poisoning from consumption of lead-shot birds or animals is not confined to birds of prey yet they are the primary predators and scavengers that have been studied to date. Corvids and gulls also utilise carcasses of wildlife and potentially too are exposed to lead from lead-shot game. No information is available on (small) mammalian predators' or scavengers' risk of secondary lead poisoning through this pathway.

There is a possible link between woodcock and spent lead shot mediated through their consumption of lead-accumulating earthworms in heavily-shot areas, that warrants investigation.

Contemporary environmental risk assessment typically involves quantitative approaches and modelling to help assess different scenarios of exposure to, and consequences of, an environmental contaminant. None was available for the current dWRA. Provided appropriate and adequate data become available then such an approach would have a place in future studies of wildlife risk arising from spent lead ammunition in this country.