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Information on which to base assessments of risk from environments contaminated with anthrax spores

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SUMMARY

Although there has been a considerable amount of research conducted into *Bacillus anthracis*, the causative agent of anthrax, the data are widely disseminated in the scientific literature and are therefore not always easy to assimilate. In view of continuing concern about potential anthrax contamination in environmental materials and sites, this review brings together the currently available information relating to the health hazards from *B. anthracis*. The relevance of the available information for risk assessment purposes is assessed.

REVIEW

Bacillus anthracis *the organism*

The causative agent of anthrax is a bacterium *Bacillus anthracis*, a large, encapsulated, Gram-positive, non-motile, spore-forming rod, 1–1·5 μm by 4–10 μm. The bacteria grow vegetatively within an infected host animal and are seen as single cells, or short chains, in diagnostic blood or tissues smears. Sporulation only occurs when the vegetative form is exposed to the atmosphere and conditions are unfavourable for the continued multiplication of the vegetative form [1, 2]. As a result *B. anthracis* shed by infected animals at death is found in or on products from such animals, or in soil contamined by them, as resistant spores that may persist for years. Whether or not the bacteria have a saprophytic growth phase in soil is debatable [2].

Anthrax spores are resistant to heat and chemical disinfectants [3]. They are alleged to be destroyed by boiling for 10 min and by dry heat at 140 °C for 3 h. They may survive for 70 h in 0·1% mercuric chloride. The ability of these spores to remain viable for many years in animal products, soil and the industrial environment is an important factor in the epidemiology of anthrax [4].

Virulence determinants

The two known virulence factors of *B. anthracis* are its polypeptide capsule and its three-component toxin. Both are elaborated by the normal virulent bacteria *in vivo* during infection but require special growth conditions for *in vitro* production in the laboratory.

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The capsule is believed to act by inhibiting phagocytosis during developing infection. It has also been suggested that the toxin's main function is to overcome the host's defences so that the bacilli are more effective in colonization [5].

Genetic variation

The capsule and the toxin are encoded by genes on two separate plasmids. Loss of one or both of these plasmids, with consequent loss of ability to produce capsule and/or toxin, leads to loss of virulence. Naturally occurring mutants lacking one or both of these plasmids are proving to be not uncommon in the environment [6, 7]. Turnbull and colleagues [6] speculate that these may be modified variants of virulent counterparts, and thus indicative of virulent strains elsewhere in the system, either in the present or at some time previously.

Pathogenicity

When virulent anthrax bacilli are injected sub-cutaneously into the susceptible host, the encapsulated organisms proliferate freely and appear to resist phagocytosis by leucocytes which accumulate in the lesion. If host resistance mechanisms fail to contain the infection, the bacteria reach the lymphatics and spleen where they multiply and are ultimately released in a sudden burst. The levels of bacilli and toxin in the circulation then increase rapidly leading to fever, coma and death within the space of a few hours. The primary site of action of the toxin is still unknown. Cardiac failure, increased vascular permeability, shock, hypoxia and respiratory failure have all been implicated as the cause. Respiratory failure is regularly seen and may be of cardiopulmonary origin or due to central nervous system depression.

Forms of the disease

Anthrax is primarily a disease of herbivorous animals. Humans become infected only incidentally, when brought into contact with diseased animals, or products, such as hides, hair or bones from such animals. *B. anthracis* spores can gain access into the human body by various methods, resulting in different manifestations of the disease [2], as follows.

Cutaneous anthrax

The most common form of anthrax in humans is the cutaneous variety, accounting for 90–95% of all cases worldwide [2, 5]. A primary lesion usually develops at the site of a minor cut or abrasion on an exposed area into which anthrax spores have become accidentally inoculated. The spores germinate, and after an incubation period of 2–5 days, an inflamed papule develops, later becoming a vesicle. There is invariably a marked accompanying oedema. Eventually the vesicle breaks down and is replaced by a black eschar. In severe cases of cutaneous anthrax the regional lymph nodes become enlarged and tender and the blood stream is eventually invaded. The systemic form of the disease is frequently fatal; without treatment septicaemia and death may occur in up to 20% of patients [2, 5]. With treatment, death is rare [2] and is likely to be associated with secondary complications such as asphyxia from oedema affecting the neck and compressing the trachea, or meningitis.

Pulmonary (inhalation) anthrax ('Woolsorter's Disease')

The far less common pulmonary form of anthrax results from exposure to spore-bearing dusts, usually in industrial plants where animal products were being handled. The disease is difficult to recognize and diagnose early enough for treatment to be effective. Untreated mortality is greater than 95% [5].

Studies on the pathogenesis of pulmonary anthrax in susceptible laboratory animals have revealed that some spores, inhaled in aerosols of < 5 μm particle size, penetrate to the alveoli of the lungs [4]. Here they are phagocytosed by alveolar macrophages [8], which in turn are carried via the pulmonary lymphatics to the regional tracheobronchial lymph nodes. There the spores germinate and the vegetative cells multiply rapidly causing an active bacterial infection of the nodes. Nothing is known of the detailed kinetics of spore germination following entry into the host [9]. Although many of the vegetative bacilli are destroyed by the cellular defences of the lymph nodes, some escape and are carried by efferent lymphatics to the blood stream. Subsequently they are rapidly cleared by the reticuloendothelial system (particularly the spleen) but soon overwhelm the defence system and establish a massive, fatal bacteraemia. Despite treatment, death usually follows within 24 h. There has only been one survivor among the 17 reported cases of pulmonary anthrax in the American literature since 1900 [4]. On the other hand, Christie [10] was of the opinion that milder cases occurred in at-risk occupations, manifested as undiagnosed bronchitis; and others [11, 12] have speculated on the occurrence of subclinical pulmonary infections.

Gastrointestinal anthrax

The intestinal tract, commonly the portal of entry in herbivorous animals, is a relatively rare route of infection in man. Human cases of ingestion (gastrointestinal) anthrax are more common in underdeveloped countries and are usually the result of ingesting poorly cooked or putrid meats from infected animals. In industrial nations intestinal anthrax is less common than the cutaneous or pulmonary forms of the disease; in fact there is no known record of any case of intestinal anthrax in Britain. Abdominal pain, fever, vomiting, bloody diarrhoea and shock are the principal manifestations of this form of the disease which has an incubation period of 2–7 days. As with pulmonary anthrax, mortality is relatively high because of the failure to make a diagnosis in time for treatment to be effective. Autopsy reveals haemorrhagic inflammation of the small intestine with lymphadenopathy.

Transmission

Anthrax spores are transmitted to animals through ingestion of contaminated water, hay or grazing in areas which have previously experienced anthrax. Consumption of inadequately processed feed ingredients of animal origin, such as bloodmeal or bonemeal, is another source of livestock infection [2]. Direct animal-to-animal transmission appears to be rare although, in certain endemic countries, transmission by biting flies (tabenids) is thought to be important in spreading the disease. Direct exposure to anthrax spores is generally necessary for man to

Table 1. *Summary of anthrax infections in animals*

Animal species	Route	Effect	Spore dose	Comments	Reference
Variety of mouse strains	Sub-cutaneous	LD50	5–30	Virulent strain used. Time to death varied from 3–6·5 days	25
Variety of mouse strains	Intra-peritoneal	LD50	41–151	Virulent strain	25
Variety of mouse strains	Sub-cutaneous	LD50	10^3–10^8	Toxigenic, non-encapsulated Sterne strain	25
Outbred mice	Parenteral	LD50	5	Unknown strain	25
Mice	Inhalation	LD50	14500	Virulent strain	23
Several strains of mice		Death	400	Virulent, encapsulated strain	25
Mice	Intra-peritoneal	LD50	$10{\cdot}9 \times 10^5$	Mean value from low virulence strains	31
Mice	Sub-cutaneous	LD50	$11{\cdot}7 \times 10^5$	Mean value from low virulence strains	31
Mice	Intra-peritoneal	LD50	287	Mean value from high virulence strains	31
Mice	Sub-cutaneous	LD50	22	Mean value from high virulence strains	31
Mice	Intra-peritoneal	LD50	12–24	Vollum strain	32
Mice	Intra-peritoneal	LD50	1400–15000	Pasteur No. 2 strain	32
Rats	Parenteral	LD50	10^6	Unknown strain	25
Rats	Inhalation	LD50	255000	Virulent strain	23
Guinea-pig	Parenteral	LD50	50	Unknown strain	25
Guinea-pig	Inhalation	LD50	50000	Single spores	26
Guinea-pig	Inhalation	LD50	55000	3·5 μm particles (= 18 spores)	26
Guinea-pig	Inhalation	LD50	61000	4·5 μm particles (= 36 spores)	26
Guinea-pig	Inhalation	LD50	570000	8 μm particles (= 235 spores)	26
Guinea-pig	Inhalation	LD50	860000	12 μm particles (= 680 spores)	26
Guinea-pig	Inhalation	LD50	50000	Organisms isolated from Manchester wool mill, USA	11
Guinea-pig	Inhalation	LD50	20000	—	1
Guinea-pig	Inhalation	LD50	16650	Virulent strain	23
Guinea-pig	Inhalation	LD50	40000	Virulent strain	29
Guinea-pig	Intra-muscular	LD50	< 10	—	25
Guinea-pig	Intra-peritoneal	LD50	10–50	—	25
Guinea-pig	Intra-peritoneal	LD50	10	V 1b strain, control animals	33
Guinea-pig	Intra-peritoneal	LD50	832	V 1b strain, immunized animals	33

Guinea-pig and rabbits	Ingestion	None	10^8	—	26
Rabbit	Parenteral	LD50	5000	—	23
Rhesus monkey	Parenteral	LD50	3000	—	23
Rhesus monkey	Inhalation	LD50	80000	Virulent strain	23
Cynomolgus monkey	Inhalation	LD50	4130	Compilation of several experiments, 1–10 min exposure to particles $< 5\ \mu m$	8
Cynomolgus monkey	Inhalation	LD50	6000	Organisms isolated from Manchester wool mill, USA	11
Rhesus monkey	Inhalation	LD50	53000	Single spores. Air breathed 1200 ml/min	26
Rhesus monkey	Inhalation	LD50	760000	12 μm particles (= 680 spores)	26
Cynomolgus monkey	Inhalation	43·8% mortality	Daily mean: 530, peak: 5685, total dose: 16962	*B. anthracis* containing-particles $< 5\ \mu m$. Exposed for 32 of 47 days	16
Cynomolgus monkey	Inhalation	22·6% mortality	Daily mean: 198, total dose: 4959	*B. anthracis* containing-particles $< 5\ \mu m$. Exposed for 25 of 41 days	16
Cynomolgus monkey	Inhalation	7·1% mortality	Daily mean: 312, total dose: 947	*B. anthracis* containing-particles $< 5\ \mu m$. Exposed for 55 h	16
Cynomolgus monkey	Inhalation	0% mortality	Daily mean: 1041, total dose: 1347	Exposed for 31 h but inadequate for post-exposure observation period	16
Chimpanzee	Inhalation	LD50	50000	Virulent strain	30
Dog	Parenteral	LD50	5×10^{10}	—	23
Dog	Inhalation	LD50	18×10^6	Virulent strain	23
Sheep	Inhalation	Produce disease	200000	—	23
Pig	Parenteral	LD50	10^9	—	23
Pig	Inhalation	LD50	27×10^6	Virulent strain	23
Man	Inhalation	Some mortality	600–2150	Calculated dose, 150–700 spores were $< 5\ \mu m$	11
Man	Inhalation	None	600–1300	25–50% of spores were $< 5\ \mu m$	20

contract any form of the disease. Person-to-person transmission is apparently extremely rare.

During World War II, Gruinard Island, which lies off the West Coast of Scotland, was the site for the well known scientific trial of *B. anthracis* as a potential biological warfare agent [13]. Despite enormously high concentration levels in the soils of certain 'hot spots' for 40 years afterwards there is no reason to believe that transmission to the mainland with resulting infection ever occurred. The island was finally decontaminated in 1986 [13].

Treatment

Most strains of *B. anthracis* are susceptible to penicillin, tetracycline, erythromycin and chloramphenicol. These drugs are usually effective in cutaneous anthrax (30 mg/kg of penicillin V in four equal doses for 5–6 days). As indicated earlier, in pulmonary anthrax, chemotherapy (5 million units of aqueous penicillin G intravenously every 6 h, plus 500 mg streptomycin intramuscularly every 12 h) will only be effective if the disease is recognized before bacteraemia has developed.

Human infection data

One hundred and twenty cases of anthrax were treated at the London Hospital between 1884 and 1954; only one case resulted from inhalation of anthrax spores, the remainder were cutaneous forms of the disease. Of the 120 cases, 102 were occupational and 18 non-occupational (16 from an unknown source and two from contaminated shaving brushes) [14]. In Britain 56 cases with four deaths were reported in the period 1961–5, 20 with five deaths from 1971–5, and only seven cases with no deaths in 1981–6 [15]. In the USA only four cases were reported between 1980 and 1988. The disease in man is still prevalent in southern Europe and various countries in Asia, Africa, the Middle East and former USSR.

Only two epidemics of inhalation anthrax are on record. The first occurred in 1957 in a goat hair processing mill in Manchester, New Hampshire, USA [4]. A total of 9 cases of anthrax were involved; 5 of these cases were pulmonary anthrax occurring during a 10-week period and resulting in 4 fatalities [11]. The remaining 4 cases were cutaneous infections. The employees who contracted pulmonary anthrax were involved with carding, combing and weaving goat hair imported from Pakistan. In order to investigate the levels of airborne contamination that occurred in the mill, 91 cynomolgus monkeys were exposed to the air in a similar working mill [16] (see Table 1 for details). The monkeys had a 10% mortality rate caused by pulmonary anthrax from a calculated inhaled dose of 1000–5500 *B. anthracis* organisms over 3–5 days. Autopsy revealed findings similar to that for humans who developed pulmonary anthrax after industrial exposure to similar *B. anthracis*-containing aerosols. A worker in the card room of the Manchester mill was calculated to have inhaled approximately 600–2150 anthrax-bearing particles per 8 h shift, of which 150–700 were less than 5 μm in diameter [11].

The second epidemic, involving at least 42 cases of inhalation anthrax, occurred in 1979 in Sverdlovsk (now Ekaterinburg) Russia, and was the result of an accidental explosion release of anthrax spores from a military facility [17]. For years the events remained enshrouded in secrecy and at this time information on exposure levels and case rates remain unpublished.

While the majority of cases of pulmonary anthrax have occurred in individuals heavily exposed to industrial aerosols, several cases have involved minimal exposure [4]. For example, one case was diagnosed in an individual who walked by the open door of a tannery in which contaminated hides were being handled [12]. Environmental sampling confirmed the presence of *B. anthracis* and it has been hypothesized that as he walked by the tannery, he inhaled an aerosol containing *B. anthracis* that was generated in the receiving area. During the ensuing investigations, a previously proven case of fatal inhalation anthrax in a housewife was uncovered. The woman may have been exposed by a similar route although the cases occurred 8 years apart [12]. A third case occurred in another housewife who lived 200 yards from a plant processing wool and hair, although no causal link was proved here [12]. Another unusual case of inhalation anthrax occurred in a man handling contaminated goat hair at his home. While handling the yarn he is presumed to have inhaled an infecting dose of *B. anthracis* [4]. In London, 1954, a grinding machine operator developed inhalation anthrax and died within 2 days after briefly handling contaminated hessian sacks [18].

Paradoxically, the infectivity of *B. anthracis* for man is normally regarded as low. This statement is supported by the consideration of the amount of potentially infected raw materials imported into developed countries in the past and the rarity of human infection, even by the cutaneous route [18, 19]. Dahlgren and colleagues [20] found that in the dustiest parts of a plant processing goat hair in America, the workers were inhaling between 600 and 1300 anthrax spores during the working day and that between 25% and 50% of these spores were associated with particles less than 5 μm in diameter. Apparently these workers suffered no ill effect, although pulmonary anthrax occurred in the Manchester mill which had a similar level of air contamination. There is speculation that some degree of immunity is possible as a result of sub-clinical infections in those routinely exposed [11, 12].

The low infectivity of anthrax spores for man is further borne out by the observation that *B. anthracis* was recovered from the nose and pharynx of 14 out of 101 healthy, unvaccinated workers at two goat hair mills [21]. Also, Pienaar [22] noted that large teams of workmen, employed for tracking down and burning anthrax contaminated animals in a wildlife reserve, were definitely exposed but none contracted the disease. This is also reported by other authors [19].

The above data raise the question of whether a large dose of anthrax spores of < 5 μm particle size is sufficient to provoke infection, or whether an additional precipitating factor is necessary. The fact that in the pre-vaccination era, cutaneous anthrax occurred only quite rarely and pulmonary anthrax even more rarely under circumstances in which contaminated materials were handled every day, argues for a precipitating cause of the disease. However, observers of the nineteenth century epidemics could find no common thread to link their cases except work with contaminated material and exposure to the dust [14]. It appears that the hair and wool workers exposed to *B. anthracis* aerosols inhaled hundreds of spores into their alveoli every day without contracting the disease. It is probable however, at least for pulmonary anthrax, that a minimum lethal dose exists, although the magnitude of this dose depends very heavily on the strain of *B. anthracis* and the state of health of the host.

Animal infection data

Available data on anthrax infection in animals are summarized in Table 1.

Considerable variation in innate susceptibility to anthrax exists among animal species (see Table 2). Resistant animals appear to fall into two groups; (i) those resistant to infection by the bacteria but which, once infection is established are sensitive to the toxin; and (ii) those susceptible to establishment of disease but resistant to the toxin [1]. Herbivorous species are most susceptible to the disease (e.g. cattle, sheep, mice and guinea-pigs) whereas carnivores and omnivores are more resistant (e.g. rats, dogs and pigs).

The LD50 values (lethal dose required to kill 50% of an exposed population) by parenteral inoculation have been reported as 3000 spores for the rhesus monkey, 5 for the mouse, 50 for the guinea-pig, 5000 for the rabbit, $0{\cdot}7–1{\cdot}5 \times 10^6$ for the rat, 10^9 for the pig and 5×10^{10} for the dog [23]. Turnbull and colleagues [24] recorded that the LD50 for guinea-pigs by intramuscular injection is usually < 10 spores, and for mice by intraperitoneal inoculation 10–50 spores. Welkos and colleagues [25] found LD50 values for various strains of mice to range from five spores (sub-cutaneous, virulent strain) to 10^8 spores (sub-cutaneous, non-virulent strain) (Table 1).

To produce a fatal pulmonary (inhalation) infection it is necessary to introduce a relatively large number of spores into the respiratory tract. Moreover, particle size is critical with only particles $< 5\ \mu$m in diameter able to penetrate to the alveoli and thus be available for phagocytosis [26]. Spore median size is also important [1, 27]. High inhalation LD50s, even in susceptible animals, are a result of these factors since it is probable that, once in the appropriate site in the deep lung, the number of spores actually required to establish the disease in a susceptible host is very small, generally < 10 [23, 28, 29].

For rhesus monkeys the LD50 dose of virulent anthrax spores inhaled as single spore particles was found to be about 50000 spores [26], and this indicates the small chance of a single spore causing lethal infection. The LD50 value increased to approximately 760000 when the animals were exposed to particles of $12\ \mu$m diameter. Similarly, LD50s in guinea-pigs exposed for 1 min to clouds of anthrax spore clusters ranging from single spores to particles of $12\ \mu$m diameter (each particle containing approximately 680 spores), increased with increasing particle size from 50000 (single spores $< 5\ \mu$m) to 860000 ($12\ \mu$m particles) (Table 1).

In studies of virulence by the respiratory route of the strain of *B. anthracis* recovered from infected workers in the Manchester woollen mill, New Hampshire [11], the respiratory LD50s were approximately 6000 and 50000 inhaled spores respectively in cynomolgus monkeys and guinea-pigs. Interestingly, tenfold enhancement of the respiratory virulence of this strain in guinea-pigs was produced by adding the detergent used for scouring the wool [11].

Brachman and colleagues [16] exposed cynomolgus monkeys to naturally contaminated air from a wool mill in the US. Data from this study (Table 1) showed that exposure to approximately 1000 *B. anthracis*-containing particles of $< 5\ \mu$m in diameter over 3–5 day periods resulted in a 10% mortality rate in the monkeys. Exposure to 3500 to 5500 particles over 5 days resulted in 20–25% mortality.

Table 2. *Relation between dose to establish anthrax infection/number of organisms per ml of blood at death, and susceptibility to toxin challenge (from Davis 1980 [1])*

Species	Relative resistance to parenteral spore challenge	Parenteral spore dose to establish anthrax	IV Toxin dose causing death (units/kg)	Quantification at death	
				Bacilli/ml	Toxin units/ml
Mouse	V. susceptible	5	1000	$10^{5\cdot9}$	—
Guinea-pig	Susceptible	50	1125	$10^{8\cdot3}$	50
Rhesus monkey	Susceptible	3000	2500	$10^{6\cdot8}$	35
Chimpanzee	Susceptible	—	4000	$10^{8\cdot9}$	110
Rat	Resistant	10^6	15	10^4–10^6	15
Dog	V. resistant	50×10^6	60	—	—

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Reporting previously unpublished data for cynomolgus monkeys, Glassman [8] records an LD50 value, based on a total of 1236 exposed animals, of 4130 spores. Statistical analysis of the results showed that large changes in the dose of inhaled particles will result in comparatively small changes in per cent mortality. For example, a one hundred-fold range of dose (tenfold above and tenfold below the calculated LD50) will only vary the predicted mortality from 25–75%.

Other reports give LD50 doses of virulent anthrax spores by aerosol inhalation as around 80000 for rhesus monkeys [23], 50000 for chimpanzees [30], 16650 [23] and 40000 [29] for guinea-pigs, 14500 for mice, 255000 for rats, $18–27 \times 10^6$ for dogs and pigs [23] and 200000 for sheep [23]. The above data indicate that the ability of the anthrax spore to produce disease via the respiratory route is not high, even in a species regarded as very susceptible such as the guinea-pig or sheep.

RELEVANCE OF AVAILABLE DATA TO HUMAN OCCUPATIONAL RISKS

The data available on human exposure to *B. anthracis* spores do not allow us to establish the minimum critical dose required to establish any of the forms of the disease. From the information available, it can be said that man appears to be moderately resistant to anthrax. It is crucial to note that any critical dose will depend very heavily on the strain of *B. anthracis*, particularly the presence of the virulence factors, and on the health of the individual human host.

Because of the lack of extensive human exposure data, assessment of risk inevitably depends on information from animal tests. Again, such information must be interpreted in the light of strain-to-strain differences in virulence of *B. anthracis* and host species or strain differences in susceptibility to infection.

No direct information on the establishment of cutaneous anthrax in animals was found. Studies involving subcutaneous and intramuscular inoculation must be considered conservative models of natural cutaneous infection because the spores have been inoculated beneath the skin. LD50 values for fully virulent strains of *B. anthracis* in mice [25] and guinea-pigs [24] are tens of spores only. Use of these data as a model for human infection adds further conservatism since mice and guinea-pigs are considered more susceptible to anthrax infection than man. Thus, a conservative cutaneous critical dose for clinical infection in man may be considered to be approximately 10 spores.

The inhalation critical dose leading to lethal infection in man is also difficult to estimate. Data available on the exposure of monkeys of anthrax spores via the respiratory route would appear to be the most relevant. The extrapolation of monkey data to man will, however, be conservative as the majority of information in the literature would suggest that this species is the more susceptible to anthrax infection. Furthermore, it is possible that infectious dose may be related to some extent to body weight. The lowest LD50 value found in monkeys was 4130 spores [8] although this figure was estimated from a variety of experiments presumably using a range of anthrax strains. Brachman and colleagues [11] estimated an aerosol LD50 for monkeys of 6000 spores of a strain of virulent anthrax which had already caused deaths in man. The size distribution of the inhaled particles was not reported and thus the number of particles which reached the alveoli of the lung cannot be estimated. However, as the lowest single strain LD50 value reported for

monkeys, 6000 spores could be considered as 'worst case' inhalation critical dose to man.

Estimating the critical dose of *B. anthracis* spores in humans is only the first step in assessing the risk to the public, or workers, from individual contamination sources. To fully assess the risks to human health the level of contamination would need to be quantified, the virulence of the anthrax strain determined and all possible exposure scenarios listed. Only then could the situations in which individuals may be exposed to the critical dose be identified. For example, walking through an area containing contaminated dust may not pose a threat to health, whereas sweeping the same area may result in air-borne spore concentrations high enough to cause pulmonary anthrax if respiratory protection is not worn.

Consideration of the results of a risk assessment exercise would allow recommendations to be made regarding the need for decontamination of the site, the use of protective clothing and apparatus or a vaccination programme for workers.

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