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MICROBIALLY DERIVED TOXIC GASES AS A POSSIBLE CAUSE OF AN EPISODE OF ILL HEALTH IN FORESTRY

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Abstract: Workers who were emptying a tank which had been used to dip sapling trees suffered nausea, requiring hospitalisation. Environmental investigations led us to conclude that their ill health probably was caused by exposure to hydrogen sulphide generated by sulphate reducing bacteria in sludge in the tank.

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INTRODUCTION

Exposure to microorganisms in agriculture is known to cause adverse health effects, including respiratory sensitisation such as farmer's lung disease, infection such as zoonoses, or toxicosis such as organic dust toxic syndrome caused by exposure to endotoxins [1, 2]. Toxicosis can also result from exposure to gases released from highly contaminated slurries, such as manure, sometimes with fatal consequences where toxic gases have built up in confined spaces [3, 6, 7]. This report presents an example of where exposure to microbially derived gases probably was the cause of health effects suffered by two forestry workers.

CASE REPORT

Young sapling trees are susceptible to attack by bark eating beetles, such as the pine weevil *Hylobius abietis*. To protect against attack, the trees are dipped prior to planting in a 0.8% solution of the insecticide permethrin in water. At a site where this operation is done, forestry workers place the trees in a long carrier, which encloses the roots, but the trunk and foliage remain exposed. The exposed parts are then lowered into a large dipping tank containing the insecticide solution. After dipping, excess solution is drained from the trees, then they are dried in a moving air current in a tunnel prior to planting.

The dipping tank is usually drained and cleaned at the end of the dipping season in spring each year. However, on the occasion we report, the dipping tank was not emptied until July, several weeks after it was last used. Two operators (both male, one aged 57 yrs and one aged 32 yrs, both non-smokers and in good general health) undertook the task of emptying the tank. They wore protective coveralls, nitrile gloves and disposable dust masks, and began to empty the tank using a probe lance and pump. Within half an hour they both felt ill with headache and nausea. They began to vomit and were then taken to hospital where they were treated with an anti-emetic preparation and allowed to go home. The symptoms reported were inconsistent with the toxic effects of permethrin, which typically include itching, tingling or burning sensations of the skin [9]. Their experience prompted us to undertake an examination of the circumstances which caused their symptoms.

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Environmental investigation. The most apparent feature, on visual examination of the dipping shed, was the presence of moulds and scum on the surface of the dipping solution, which prompted site examiners to initiate a microbiological investigation. Five samples were taken:

A: From the solution in a drain tank;

B: From the solution in a second drain tank where scum was evident;

C: Sludge from the dipping tank;

D: Unused dipping tank solution;

E: A brown scum from the surface of the solution in the dipping tank.

These samples were analysed for their microbiological content by diluting them in mineral salts solution then preparing spread plates on agar media chosen to reveal a range of microbial groups. Agar plates were incubated until colonies emerged. These were counted to calculate total numbers. Representative colonies were isolated for identification purposes. The results of this examination are summarised in Table 1. Total numbers were calculated as colony forming units (cfu), which is taken to relate to the number of viable cells. Identification was by gross and microscopic morphology supplemented where necessary by biochemical tests. Predominant bacteria isolated included Gram-negative rods and Gram-positive sporing and non-sporing rods. Fungi included *Acremonium, Botrytis, Cladosporium, Penicillium, Rhizopus, Trichoderma* and *Verticillium* spp.

Health hazard. Microbiologically-related health hazards to consider are infection, allergy or toxicosis, placed into context by the scale and likely route of exposure for the worker and the degree of protection afforded by the work conditions. The speed of onset of symptoms, within 1/2 hour of starting work, ruled out infection and the symptoms described, of sickness and nausea, ruled out respiratory allergy. Another possibility was endotoxin-related toxicosis; organic dust toxic syndrome (ODTS) or inhalation fever [10]. There were over 10^4 colony forming units of Gram-negative/coliform bacteria per ml present in the sludge, which might suggest a high level of endotoxin, but the evidence for this being the cause of the ill-health was not particularly strong because of the speed of onset of the symptoms. Another consideration was an indirect microbiological problem. When the samples were collected, a “rotten egg” smell was noted, which can be considered as an indication of the presence of hydrogen sulphide (H_2S). There was no evidence of any significant levels of chemicals present in the dip formulation, or other chemical additives at the site, which would have led to the generation of toxic gases, so this led us to consider whether H_2S may have been generated by the metabolism of anaerobic sulphate-reducing bacteria (SRBs) in the sludge in the dip tanks. Further microbiological tests were performed by inoculating suspensions of the five samples into SRB test kits (SigTest; ECHA, Cardiff, UK). Following incubation at 30°C for up to 6 days, the presence of SRBs was demonstrated in this test by blackening of the agar media, the speed and extent of which can be used to estimate the approximate concentration. The results of these tests are summarised in Table 2.

Table 1. Microbiological content of samples taken from tree dipping tanks (numbers = colony forming units/ml or /g original sample, mean of duplicate plates inoculated from a series of dilutions).

Sample	Malt agar 25°C fungi	Nutrient agar 25°C bacteria	MacConkey agar 37°C coliforms	RS agar 55°C Actinomycetes/ thermophilic bacteria
A	7×10^2	2×10^3	2×10^2	ND
B	7×10^5	4×10^4	ND	ND
C	1×10^3	1×10^5	2×10^4	3×10^3 (bacteria)
D	7×10^1	1×10^3	ND	ND
E	1×10^4	3×10^3	2×10^3	ND

ND - not detected.

In addition, the samples were examined for the presence of volatile chemicals by headspace GCMS on a Fisons Instruments MD800 with an HS800 sampler. Samples of the air above the sealed samples (incubated at 60°C) were injected into the GCMS operating in the electron impact (70eV) mode. Chromatograms were examined and peaks searched against the NIST (National Institute of Standards and Technology, USA) library.

Apart from background levels of toluene and other aromatic chemicals, the tests failed to detect the presence of H_2S or any other significant concentrations of volatile chemicals. However, as these analyses were done some days after the initial sample collection and the samples had been opened to the atmosphere in the interim, the opportunity to collect any volatiles liberated from the samples probably had been lost.

However, circumstantial evidence from the tests, which demonstrated that SRBs were present in the sludge, suggested a strong possibility that H_2S had been present in the workplace atmosphere during the tank cleaning operation. Calculations have been performed by an expert

Table 2. Summary of SRB tests performed on tree dip samples.

Sample	Extent of blackening of medium in test tube after:			Interpretation: number of SRBs/ml
	48 hours	5 days	6 days	
A	None	~50%	75–100%	between 1 and 10^2
B	25–50%	75–100%	75–100%	10^2 to 10^3
C	To depth ~3 cm	100%	100%	$>10^3$
D	None	None	None	no detectable SRBs
E	None	None	None	no detectable SRBs

in work relating to the presence of SRBs in contaminated tanks on ships. These suggest that at equilibrium in a closed tank at 25°C, dilute solutions of 0.5, 1.0 and 2.0 ppm H_2S will result respectively in higher concentrations of 140, 280 and 560 ppm H_2S in the air space above the solution in the tank (E.C. Hill, pers. comm.). To relate these values to human health effects, H_2S is detectable by smell at 25 ppb and is offensive at 3–5 ppm, but the olfactory receptors rapidly become fatigued so that the smell is no longer detectable. Eye irritation occurs at 150 ppm and central nervous system effects at 200 ppm [4].

DISCUSSION

This report has highlighted a case where exposure to hazardous substances in the workplace led to hospitalisation of two workers. In investigating the incident, we have demonstrated that risks in the workplace are not always easy to predict, but that a multidisciplinary approach to tackling the problem led us to what we consider to be the most credible explanation for the workers' ill health. As with any case study where the cause is not immediately apparent, retrospective analysis may give limited information. Similarities may be drawn between this case study and one in a factory setting where several workers became ill and there was one fatality [8]. On that occasion, symptoms were consistent with hydrogen sulphide toxicity and it was concluded that this was caused by exposure to manure gases, although analysis of the factory air two days after the accident showed no elevated levels of H_2S because it had been dispersed by ventilation of the problem area.

In the case presented in this report, the most obvious signs of contamination, mould and scum present on the surface of the tree dipping solution, proved unlikely to be the major health hazard. Their presence however initially diverted attention from other possible causes. Conventional microbiological analysis failed to provide adequate information, but recognition of the environmental and microbiological circumstances pointed to a likely cause, brought about by the following sequence of events:

- During its use, the dipping bath became contaminated with soil and vegetation washed from the saplings.
- Build up of sludge resulted in a highly nutritive solution conducive to the growth of microorganisms introduced with the soil. Anaerobic conditions at the bottom of the tank could have been exacerbated by the presence in relatively large numbers of aerobic fungi and bacteria in the solution, which would also deplete oxygen. The presence of microbial contamination also showed that the permethrin had no antimicrobial effect under these circumstances.
- Conditions in the tank favoured the growth of SRBs – concentrations greater than 10^3/ml were found to be present in the samples even some days after they had been collected.
- The situation was further exacerbated by the failure on this occasion to empty the tank until several weeks after it was last used, allowing the development of SRBs during a period of stagnation.

This probably led to the workers being exposed to a high concentration of H_2S during tank cleaning. The symptoms reported were consistent with those of H_2S toxicosis at concentrations around 200 ppm (central nervous system involvement, including dizziness and headache, also nausea and vomiting) [4], but not of the toxic effects of permethrin.

Such a situation, of H_2S being liberated by the metabolic activity of SRBs, is recognised as a problem in a wide range of workplaces in chemical factories, or especially in confined spaces such as in sewers, or in contaminated fuel tanks of ships and on oil rigs [4]. In agriculture, death by asphyxiation is a well recognised hazard where H_2S is liberated from liquid manure into enclosed spaces. Groves and Ellwood [5] provided a detailed review of the hazard, together with typical levels of H_2S associated with manure systems on farms. A recent report of accidents associated with liquid manure systems and manure pits on farms included two anecdotal reports of intoxication attributed to H_2S inhalation [6]. However, we are not aware of any other reported cases of intoxication in agriculture during forestry work.

Our recommendations for the future are:

- to clean out tank sludge as soon as possible after final use, taking precautions to ensure adequate ventilation when doing so;
- not to allow tanks to stagnate;
- to be aware of the possible problems that could arise from the build up of anaerobic bacteria in sludges in tanks in any work situation.

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