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For Occupational Health

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INSTITUTE OF OCCUPATIONAL MEDICINE

A PILOT STUDY TO INVESTIGATE EXPOSURE TO ENDOTOXIN IN FARMWORKERS PERFORMING SHEEP DIPPING

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membranes). Investigation of other bath constituents which may be related to health problems is required. A health study could be performed using endotoxin concentrations in baths as a surrogate for personal exposure as this analysis is more reliable than the airborne sampling method.

TM/94/02

INSTITUTE OF OCCUPATIONAL MEDICINE

A PILOT STUDY TO INVESTIGATE EXPOSURE TO ENDOTOXIN IN FARMWORKERS PERFORMING SHEEP DIPPING

by

ER Waclawski, RT Cullen, KJM Niven, DM Brown

SUMMARY

Preliminary observations have identified endotoxin to be present in the bath used for dipping sheep. A pilot study was performed at six farms to measure airborne concentrations of endotoxin to which workers dipping sheep are currently exposed and to quantify the range of concentrations of endotoxin in dipping baths both before and after sheep dipping.

Total inhalable personal samples were collected from workers performing sheep dipping. Static samples were also obtained from two sites at each farm. Twenty-five ml samples of dip bath were collected before and after sheep dipping. In addition samples of the tapwater used to fill the dipping bath at each farm were obtained.

Pre-dipping the endotoxin concentrations in the baths ranged from 16 E units/ml to 1248 E units/ml. Post-dipping the endotoxin concentrations in the bath samples ranged from 2880 E units/ml (240 ng/ml) to 27600 E units/ml (2300 ng/ml).

The endotoxin concentrations in air from personal samples varied from 8 E units/m^3 of air to 309 E units/m^3. The range for paddlers was 8 to 67 E units/m^3; for chuckers the range was 18 to 309 E units/m^3 and for helpers the range was 14 to 150 E units/m^3. Samples collected near the draining pens ranged from 7 E units/m^3 to 242 E units/m^3.

The personal 8 hr time weighted average airborne endotoxin concentration ranged from 3 E units/m^3 (paddler farm 4) to 85 E units/m^3 (chucker/helper farm 2). The highest exposure for a paddler was 55 E units/m^3 (farm 5). The highest endotoxin concentration for a helper was 36 E units/m^3 (farm 1).

The pilot study has identified the presence of airborne endotoxin during dipping. The endotoxin concentrations from air samples was low. This may reflect the limitations of the methodology used in collecting 'total inhalable' samples which may not be able to determine the exposure from splashing and large droplets. The bath samples have confirmed the presence of endotoxin in baths post-dipping.

Further investigation is recommended into developing a suitable sampling method to quantify more clearly endotoxin exposure during sheep dipping (including splashing on to mucous

1. INTRODUCTION

Recent figures (MAFF 1993) indicate that the total sheep population in the UK was some 44 million in 1992, with half these numbers in Scotland. The figures represent an increase over the previous year of approximately 1%, continuing a general upward trend in numbers which has been maintained during the last decade.

Many infectious diseases of sheep can be controlled with programmes of disinfection, antibiotic therapy and immunisation, while dosing with antielminthics is an efficient method of treatment to control worm infestations. Ectoparasitic diseases in sheep have traditionally been treated for many years mainly by dipping but also by showering, spraying and jetting, which are generally recognised as suitable for control of blowfly only. Synthetic pyrethroid insecticides have allowed the development of pour-on formulations, which are applied in a single dose to the sheep and spread over the surface of the animal. Dipping is the only recommended method for controlling sheep scab as it is the most effective means of achieving thorough wetting of the fleece.

In the UK dipping is mainly undertaken during the spring, summer and autumn. Organophosphates comprise one of the largest groups of commercially available sheep dips. The most common used in manufactured sheep dips are diazinon, propetamphos and chlorfenvinphos. They are lipid soluble and readily absorbed through the skin. Dermal absorption of organophosphates is regarded as the predominant source of exposure in agricultural workers, while the respiratory route is generally thought to be less important, although possibly toxicologically significant, in agricultural situations where a finely divided aerosol is generated (Ballantyne, 1992).

Organophosphates cause inhibition of cholinesterase enzymes by phosphorylation and accumulation of acetylcholine at susceptible receptors. Their acute hazard is well documented (O'Brien, 1960, Ballantyne, 1992) however, the effects of chronic exposure to sub-acute levels and the risks to workers health during sheep dipping are less clear.

A non-specific influenza-like illness has been described by Murray and co-workers who suggest that this condition may be caused by exposure to sheep dips organophosphate pesticides. The relationship described between symptoms and erythrocyte cholinesterase activity four to six weeks after exposure is not convincing. Only 3 to 9 subjects with flu-like symptoms had evidence of a rise in erythrocyte cholinesterase activity of at least 25% 4 to 6 weeks after exposure (Murray *et al*, 1992).

The Institute of Occupational Medicine (IOM) has performed studies related to sheep dipping and observed dipping on farms using different dipping methods. These studies were primarily concerned with exposure to organophosphate insecticides (Niven *et al*, 1993).

During our observations we were impressed by the potential microbiological hazard present during sheep dipping which appears to be related to Gram-negative organisms from sheep. As a consequence, bath water is likely to become contaminated with endotoxin derived from the cell walls of faecal Gram-negative bacteria. Inhalation exposure to endotoxin from such bacteria could lead to symptoms such as fever, chest tightness, chills, cough, headache, joint and muscle pains, tiredness and throat irritation (Rylander *et al*, 1989; Haglind & Rylander, 1987) - symptoms similar to sheep flu.

Preliminary Observations

The endotoxin concentration of dip prior to and following sheep dipping was measured at one farm.
Three samples of sheep dip were obtained during a dipping session over 2 days at one farm. A sample of dip was obtained from a freshly prepared bath. The bath had been used during the previous dipping season. It had been emptied and only refilled prior to dipping. Dip was also obtained at the beginning and end of the second day's dipping after 230 and 380 animals had been dipped respectively. These samples were analysed for lipopolysaccharide (LPS) using a 'Coatest' kit (Chromogenix, Sweden), after being centrifuged and filtered to remove coarse particulate material through 0.45 μm filters.

LPS was also assayed in fresh dip and disinfectant obtained from the manufacturer and diluted in endotoxin-free water according to their instructions. The effect of these components was also investigated on the activity of the standard LPS.

Endotoxin was present in the fresh bath at a concentration of 7225 E units/ml. At the start and end of day 2 the concentration was 15258 E units/ml and 25092 E units/ml respectively. Dip components contain very little endotoxin (<35 E units/ml). Dip components reduced the activity of the standard LPS assay by approximately 20%. Thus the results for the bath samples may be an underestimate of the endotoxin levels. In addition the removal of coarser particulate material may have also reduced the amount of endotoxin in the samples prior to analysis.

We therefore recommended that a pilot study be undertaken so that further sampling be carried out during dipping to give a better estimate of endotoxin exposure and the work was commissioned.

2. AIMS

The aims of this pilot study were:

1. To measure airborne concentrations of endotoxin to which workers dipping sheep are currently exposed.

2. To establish the range of concentrations of endotoxin in baths both before and after sheep dipping.

3. To make recommendations in the light of the findings as to whether a larger scale investigation would be appropriate.

3. METHODS

3.1 Field Sampling

3.1.1 Selection of farms

Six farms were recruited in South East Scotland. The workers on these farms had participated in earlier collaborative IOM/HSE research involving occupational hygiene assessments of working practices during sheep dipping (Niven *et al*, 1993). Their selection for the present study was based on their willingness to participate and mutual agreement on available dates for conducting the dipping. The choice of proprietary brand of sheep dip was not considered to be critical and farmers therefore used their preferred dip.

3.1.2 Visual assessment

The occupational hygiene assessment was based on that used by Niven *et al* (1993).

During each dipping session an occupational hygienist recorded information on:

Names and sheep dipping occupation of team members

Details and size of the flock

Type of dip concentrate used and descriptions of dilution methods

Type of dipping bath, volume, control measures, drainage arrangements, dipping technique, speed of dipping, time sheep spent in bath

Dip bath replenishment methods

Clothing worn by each participant and protective clothing used

Ambient conditions during the dipping session

Personal hygiene arrangements and practice

Subjective visual assessments were made of:

The degree of splashing or contamination of clothing or body during the dipping session, likely to result in increased exposure to endotoxin.

The effects on exposure of specific incidents such as entering the draining pen, assisting sheep in the dipping bath and particularly excessive splashes.

3.1.3 Collection of samples

Personal inhalable samples were obtained for each member of the dipping team using battery operated pumps connected to IOM inhalable dust sampling heads (Mark & Vincent, 1986). A maximum of three individuals were involved in the tasks during dipping (ie paddler, chucker and helper). These tasks have been described previously (Niven *et al*, 1993). The sampling heads contained 25 mm diameter cellulose ester membrane filters and the flowrate was set at approximately 2 l min^{-1} using a calibrated rotameter. Flow was checked at the start of sampling, approximately midway through the dipping session and at the end. Each sampling head was located in the breathing zone of the participants (ie within 200 mm of the nose and mouth).

Area samples were also obtained at two standard locations during each dipping session. These locations were, within approximately 1 m of the paddler and within approximately 1 m of the draining pen. The same sampling ensembles and methods were used to obtain the area samples as were used to obtain the personal samples.

Approximately 25 ml samples of the liquid in each dipping bath were obtained before dipping began and at the end of the session. Samples of the water used to fill and replenish the bath were also obtained.

3.1.4 Transportation of samples to laboratory

Once samples were obtained they were immediately transferred to unused universal containers and placed in a cool box containing ice packs for storage before transportation by car to the laboratory for analysis. Samples from each day's dipping were always delivered to the laboratory within 2 hours of completion of the dipping session. All samples were cold to the touch on arrival at the laboratory.

3.2 Method for Determination of Endotoxin in Sheep-dip and Personal Samples

3.2.1 Sheep-Dip samples

All liquid samples were stored frozen on arrival at the laboratory. Before assay, post-dip bath samples were thawed, centrifuged at 2500 rpm for 10 minutes and the supernatants filtered through 0.2 μm membrane filters. For the endotoxin assay, samples from the baths, post-dipping, were diluted 1/40000 with endotoxin-free water. Other samples were diluted to 1/100 or 1/200.

3.2.2 Personal and airborne samples

Filters were stored frozen. On the day of the assay, 5 ml of endotoxin-free water was added to each filter in a universal container. Containers were rotated end-over-end at room temperature for 1 hour. The supernatants were diluted 1/10 and assayed for endotoxin.

3.2.3 Assay

The endotoxin 'Coatest' kit (Chromogenix AB, Molndal, Sweden) was used. Reagents contained in the kit were made up according to the manufacturer's instructions.

Fifty microlitres of test sample or standard was pipetted in duplicate into microtitre plate wells. The plate was incubated at 37°C for 5 minutes. Fifty microlitres of LAL (Limulus Amoebocyte Lysate) was added to each well, mixed and incubated at 37°C for exactly 7 minutes. After incubation, 100 μl substrate was added to each well and the plate incubated for a further 5 minutes at 37°C. The reaction was stopped with 100 μl of 20% acetic acid. A standard endotoxin control provided in the kit was used to prepare a standard curve ranging from 0-1.2 Endotoxin Units/ml (EU/ml)(1EU = 12 ng endotoxin).

The absorbance of the samples in each well was determined at 405 nm using an automatic plate reader (Dynatech model MR 650; Dynatech Ltd, Billingshurst, UK). The absorbance against endotoxin concentration for standard controls was plotted to give a standard curve from which the unknown endotoxin concentrations of the samples were calculated.

3.3 Statistical Analysis

The data collected on endotoxin concentrations in baths and air samples were tabulated.

Regression analysis was performed using the PC version of MINITAB, release 8.2.

4. RESULTS

4.1 Observations for each farm

The main characteristics of each dipping session are summarised in Table 4.1. Three baths were of the long swim type (F1, F3, F6), with one each of short swim (F5), circular (F4) and circular with island (F2). The volume of the baths ranged from 1350 litres to 2000 litres. Five farms used dips with diazinon as the main organophosphate with the remaining farm (F3) using a propetamphos based dip. The number of sheep dipped ranged between 120 and 1700 and the rate of dipping from 40 to 436 sheep per hour. The time each sheep spent in the bath was approximately 10 seconds (F1, F3, F6), 15 seconds (F5) or 30 seconds (F2, F4).

At farm 1 a light breeze was present which was blowing towards the paddler and away from the static samplers. A small amount of visible splashing was noted. No wind was present at farm 2. At farm 3 a light breeze blew from the draining pens towards the workers and away from the static samplers. Splashing was very noticeable at this farm.

There was no wind present on the day of dipping at farm 4. A small amount of visible splashing occurred. Dipping at farm 5 was sheltered from the wind. A moderate amount of splashing was noticed. The wind was moderate at farm 6 and was blowing away from the paddler towards the static samplers. The paddler at farm 6 wore his face visor throughout the whole of the dipping session and it appeared to obscure the sampling head. The amount of splashing noted at farm 6 was the greatest of all the six farms.

4.2 Endotoxin Measurements

4.2.1 Endotoxin concentrations in baths used for dipping sheep

Concentrations of endotoxin in the tapwater used to fill baths were, with the exception of farm 6, below 30 units/ml (Table 4.2). Endotoxin levels in the baths had increased considerably by the end of each dipping session (Table 4.2). At three of the farms, post-dipping concentrations were in excess of 16000 units/ml (Farms 3, 5 and 6).

4.2.2 Relationships between endotoxin concentrations and the number of sheep dipped

Figure 4.1 shows a scatter plot of final endotoxin concentration against the number of sheep dipped. The correlation coefficient was only 0.66. This was not statistically significant. A comparison of the number of sheep dipped per hour and endotoxin concentration gave no significant correlation.

4.2.3 Airborne endotoxin concentrations

The amounts of endotoxin eluted from membrane filters used to sample the air in the breathing zone of workers involved in dipping are shown in Table 4.3. Endotoxin measurements on unexposed (blank) filters were very low being less than 1.5 units for four

farms and 5.5 and 13 units for farms 2 and 5 respectively (data not shown in Table 4.3). The data are extremely variable and it is not possible to determine whether any one occupational group had greater exposure to endotoxin, particularly as at some farms chuckers exchanged roles with helpers during the dipping session.

Air was also sampled with samplers situated near to the paddlers and by the draining pens. As with the personal samples, endotoxin concentrations were very variable in these static samples. The amounts of endotoxin on the two static samples from any site tended to be of a similar magnitude. Regression analysis showed the two measurements to be highly correlated (coefficient = 0.99).

The endotoxin concentrations in air from personal samples varied from 8 E units/m^3 of air to 309 E units/m^3. The range for paddlers was 8 to 67 E units/m^3; among chuckers the range was 18 to 308 E units/m^3 and for helpers the range was 14 to 150 E units/m^3. Static samples collected from opposite the paddler ranged from 3 E units/m^3 to 417 E units/m^3. Samples collected near the draining pens ranged from 7 E units/m^3 to 242 E units/m^3.

The high static values at farm 6 may have been due to the wind direction and large amount of splashing that was observed.

Personal 8 hr time weighted average endotoxin concentrations ranged from 3 E units/m^3 (paddler farm 4) to 85 E units/m^3 (chucker/helper farm 2). The highest exposure for a paddler was 55 E units/m^3 (farm 5). The highest endotoxin concentration for a helper was 36 E units/m^3 (farm 1).

There was no correlation between bath concentrations of endotoxin and the amounts of endotoxin recovered from personal or static samplers.

5. DISCUSSION

5.1 Occupational Exposure to Endotoxin

The results of endotoxin analysis of dip from baths confirms our initial finding that dipping leads to contamination of the baths with endotoxin. The number of sheep dipped does not predict the amount of endotoxin in the bath at the end of dipping. As faecal material is the likely source of endotoxin, the concentration of endotoxin is likely to be related to the quantity left in the bath by the sheep. The bath samples in the present study were centrifuged and filtered which may lead to lower measured endotoxin concentrations than are actually present.

Gram-negative bacteria and their endotoxins are present in various work environments in agriculture including horse (Olenchock *et al*, 1992) and cattle barns (Siesel *et al*, 1991), swine confinement buildings (Haglind & Rylander, 1987) and poultry farms. Other industries with known endotoxin exposures include the wool (Love *et al*, 1986) and cotton industries (Rylander & Bergstrom, 1993).

The composition and nature of endotoxin can vary with strain and species of bacteria. Endotoxins from different sources have been shown to have different effects (Baseler *et al*, 1983).

Respiratory exposure to endotoxin-containing dusts has been associated with both an acute decline in pulmonary function (Castellan *et al*, 1987) and chronic lung disease (Kennedy *et al*, 1989) in cotton dust-exposed subjects. Endotoxins can profoundly affect both humoral and cellular immune systems in humans and experimental animals (Olenchock, 1990).

Fever and respiratory symptoms have been reported following challenge testing with endotoxin-contaminated tap-water which gave estimated doses of between 0.01 and 0.03 μg/Kg body weight, (ie 8400 to 25200 E. units for a 70 Kg man)(Muittari *et al*, 1980). Rylander *et al* (1989) suggest an endotoxin threshold for fever of 0.05 μg/Kg body weight (42000 E. units for a 70 Kg man). Contamination of a mucus membrane with two mls of liquid from the baths at Farms 5 and 6 would produce fever.

Recent inhalation challenge studies in man have shown that inhalation of 200 μg, but not 20 μg of lipopolysaccharide (LPS) caused bronchoconstriction in normal subjects (Michel *et al*, 1989; Rylander *et al*, 1989). Some asthmatic subjects developed bronchoconstriction on inhaling only 20 μg LPS (Michel *et al*, 1989). A later study found that LPS inhalation produced a fall in FEV_1 that was maximal at 60 minutes and lasted more than 5 hours. Bronchial obstruction correlated with non-specific responsiveness to histamine but not to atopy (Michel *et al*, 1992).

Despite these and many other studies showing changes in respiratory function and other symptoms such as fever and malaise following inhalation of dusts and aerosols containing endotoxin, there is no occupational exposure limit for endotoxin. Palchak *et al* (1988) have proposed a conservative exposure limit of 30 ng/m^3 as an 8-hour weighted average. This figure, which incorporates a 10-fold safety factor, was derived from an examination of a number of published studies (Castellan *et al*, 1987; Rylander *et al*, 1985; Rylander and Haglind 1984; Petsonk *et al*, 1986) which gave a median exposure associated with a 5% fall

in FEV_1 of 300 ng/m^3. These studies related to the cotton industry and humidifier fever. The maximum airborne endotoxin concentration in the present study was 7 ng/m^3. This is a low concentration relative to the proposed exposure limit.

Although the basis for our assay for LPS, enzyme activation in the lysate from limulus (horseshoe crab) amoebocytes, is common to the majority of endotoxin assays, there are a number of ways of performing the measurements and there is no internationally agreed protocol (Renolds & Milton, 1993). It is therefore difficult to compare endotoxin results between different laboratories. Another confounding factor is that endotoxins from different species and strains of bacteria may give differing results in the assays. There is a need to standardise both airborne sampling and measurement of endotoxins worldwide.

The air sampling performed in this study indicates that endotoxin does become airborne during dipping. The results may underestimate the quantity of airborne endotoxin to which the workers are exposed, because of limitations in the sampling method. In the absence of validated sampling methods for the determination of airborne endotoxin, the method used was that recommended by HSE in their guidance document MDHS 14 (General methods for the gravimetric determination of respirable and total inhalable dust) (HSE, 1993).

MDHS14 defines total inhalable dust as the fraction of airborne material which enters the nose and mouth during breathing. The definition is expressed numerically as a fraction of particle aerodynamic diameter. At 100 μm the inhalable fraction is defined to be 50%. There is no definition for particles with an aerodynamic diameter greater than 100 μm. Specific recommendations are not made in regard to whether the aerosol is solid or liquid.

Recommendations in the use of specific samplers are made. The IOM personal inhalable sampler used in this study is one of the recommended samplers for the sampling of inhalable aerosol. There is no guidance for measurement of particles greater than 100 μm.

The size distribution of the aerosol generated during sheep dipping is unknown. However, it is very likely that much of the aerosol will be greater than 100 μm, and may extend up to several mm. The extent to which these 'massive' droplets are or should be considered inhalable is unknown as is the efficiency with which they will be measured with the current instruments. In addition the possibility of sampled liquid 'running out' from the samplers has not been excluded.

Assessment of personal exposure from results of the airborne sampling for endotoxin is therefore difficult. The absence of a correlation between airborne and bath concentration may well be related to the limitations of the airborne sampling method. Similar problems would be encountered in measuring other substances in the sheep dip, including organophosphate, by this method. Further work to attempt to quantify the size distribution of the aerosols produced and characterise a suitable sampling method is clearly indicated.

This pilot study has demonstrated a 10-fold difference in endotoxin concentrations in baths after sheep dipping. The limitations of the airborne endotoxin monitoring suggest that this method is not currently suitable for use in any studies which would relate airborne exposure to health effects. The analysis of endotoxin in bath samples is more reliable and could be used for such studies.

5.2 Illness Associated with Sheep Dipping

The ill-health which is said to be associated with sheep dipping requires to be investigated further. Case reports and case series lack the necessary information on the nature of the exposure that is associated with ill-health. A formal epidemiological study including objective exposure assessment is necessary.

Sheep dipping is associated with potential exposure to organophosphate pesticides, solvents, biocides and other contents of dip formulations including impurities. The work also produces potential close exposure to bacteria, endotoxin and other biological contaminants on the sheep. The work is performed outdoors and exposure to ultraviolet radiation, sunlight, ozone and other pollutants may occur.

There are potential interactions between these 'exposures' which could modify the toxicity of the organophosphate pesticide. For example, malathion and parathion are both known to be converted to -oxon derivatives by photochemical transformation (Brown *et al*, 1993). These have greater toxicity than the original compound (Wolfe & Seiber, 1993). In California, these can lead to problems during the re-entry to orchards treated with these agents (Glotfelty *et al*, 1990). A 'chain of chance' (Lem, 1967) may be necessary for an adverse outcome associated with sheep dipping to be noted in farmers. We have found that pure dip components modify the measured activity of the endotoxin assay. This may suggest that interactions in the sheep dipping bath can take place. Whether these can then increase OP toxicity in a subject requires further investigation. The previous HSE sponsored study (Niven *et al*, 1993) identified metabolites in the urine which were 'unexpected' given the use of diazinon. Whether these metabolites are derived from impurities in the original formulation or are produced following exposure to a modified diazinon-compound also requires further investigation.

The current No-Observed Adverse Effect Level (NOAEL) for diazinon is based on a healthy volunteer ingestion study of a relatively short-term nature (FAO/WHO, 1967). The effect on individuals with clinical or sub-clinical physical or psychiatric disease is not known. A differing susceptibility in the general population cannot be ruled out. In addition, the end point used to determine the NOAEL (cholinesterase inhibition) may not be the only observation to use. The possibility that adverse effects may occur without peripheral cholinesterase inhibition requires to be considered. Such effects may be due to impurities or chemical modifications of the original OP.

6. RECOMMENDATIONS

1. Further investigation is necessary into a suitable sampling method (eg gauze patches, half-facepiece mask, swabbing, nasal/buccal swabs) to be tried out in a range of dipping situations to measure exposure by splashing as well as inhalation.

2. Sheep dipping aerosol should be characterised in terms of size.

3. Sampling methods should be developed for droplets and aerosol of large size.

4. The presence of other constituents of the bath liquid which may be related to illness should be investigated.

5. A health study could be undertaken of farmworkers performing sheep dipping using endotoxin concentrations as a surrogate for exposure.

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REFERENCES

Ballantyne B, Mars TC, eds. (1992). Clinical and experimental toxicology or organophosphates and carbamates. Oxford: Butterworth Heinemann.

Baseler MW, Fogelnark B, Burnell R. (1983). Differential toxicity of inhaled Gram-negative bacteria. Infection and Immunity; 40: 133-138.

Brown MA, Petreas MX, Okamoto HS, Miscuke TM, Stephens RD. (1993). Monitoring of malathion and its impurities and environmental transformation products on surfaces and in air following an aerial application. Environmental Science and Technology; 27: 388-397.

Castellan RM, Olenchock SA, Hankinson JL, Millner PD, Cocke JB, Bragg CK, Perkins HH, Jacobs RR. (1984). Acute bronchoconstriction induced by cotton dust; dose-related responses to endotoxin and other dust factors. Annals of Internal Medicine; 101: 157-163.

Castellan RM, Olenchock SA, Kinsley KB, Hankinson JL. (1987). Inhaled endotoxin and decreased spirometric values. New England Journal of Medicine; 317: 605-610.

Food and Agricultural Organisation of the United Nations, World Health Organization. (1967). Evaluation of some pesticide residues in food. Geneva: WHO/Food Series. (Add/67.32).

Gotfelty DE, Schomburg CJ, McChesney MM, Sagebiel JC, Seiber JC. (1990). Studies of the distribution, drift and volatilization of diazinon resulting from spray application to a dormant peach orchard. Chemosphere; 21: 1303-1314.

Haglind P, Rylander R. (1987). Occupational exposure and lung function measurements among workers in swine confinement buildings. Journal of Occupational Medicine; 29: 904-907.

Health and Safety Executive. (1993). General methods for the gravimetric determination of respirable and total inhalable dust. London: HM Stationary Office. (MDHS 14).

Kennedy SM, Christiani DC, Eisen EA, Wegman DH, Greaves IA, Olenchock SA, Ting-Ting Y, Pei-Lan L. (1987). Cotton dust and endotoxin exposure-response relationships in cotton textile workers. American Review of Respiratory Disease; 135: 194-200.

Lem S (1978). The chain of chance. London: Mandarin. (Originally published in Polish as Katar in 1975).

Love RG, Smith TA, Jones CO, Gurr D, Soutar CA, Seaton A. (1986). Respiratory symptoms in wool textile workers. An epidemiological study of respiratory health in West Yorkshire wool textile mills. Edinburgh: Institute of Occupational Medicine. (IOM Report TM/86/04).

Maizlish N, Schenker M, Weisskopf CP, Seiber JN, Samuels S. (1987). A behavioral evaluation of pest control workers with short-term, low level exposure to the organophosphate diazinon. American Journal of Industrial Medicine; 12: 153-172.

Mark D, Vincent JH. (1986). A new personal sampler for airborne total dust in workplace. Annals of Occupational Hygiene; 30: 89-102.

Michel O, Duchateau J, Sergysels R. (1989). Effects of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. Journal of Applied Physiology; 66: 1059-1064.

Michel O, Ginanni R, Sergysels R. (1992). Relation between the bronchial obstructive response to inhaled lipopolysaccharide and bronchial responsiveness to histamine. Thorax; 47: 288-291.

Ministry of Agriculture, Fisheries and Food. (1993). Agriculture in the United Kingdom 1992. London: HM Stationery Office.

Muittari A, Rylander R, Salkinoja-Salomon M. (1980). Endotoxin and bath water fever. Lancet 1980; ii: 89.

Murray VSG, Wiseman HM, Dawling S, Morgan I, House IM. (1992). Surveillance of sheep dip exposures 23 September to 3 November 1991. Final Report Oct. 1992. London: National Poisons Unit.

Niven KJM, Scott AJ, Hagen S, Waclawski ER, Lovett M, Cherrie B, Bodsworth PL, Robertson A, Elder A, Cocker J, Nutley B, Roff M. (1993). Occupational hygiene assessment of sheep dipping practices and processes. Edinburgh: Institute of Occupational Medicine. (IOM Report TM/93/03).

O'Brien RD. (1960). Toxic phosphorus esters. New York: Academic Press.

Olenchock SA. (1990). Endotoxins in various work environments in agriculture. In: Pierce GE, ed. Developments in industrial microbiology. Vol 31. Arlington, VA: Society for Industrial Microbiology: 193-197.

Olenchock SA. (1990). Endotoxins. In: Morey PR, Feeley JC Sn, Otten JA, eds. Biological contaminants in indoor environments. Philadelphia, PA: ASTM: 191-200.

Olenchock SA, Murphy SA, Mull JC, Lewis DM. (1992). Endotoxin and complement activation in an analysis of environmental dusts from a horse barn. Scandinavian Journal of Work Environment and Health; 18 (Suppl 2): 58-59.

Palchak RB, Cohen R, Ainslie M, Hoerner CL. (1988). Airborne endotoxin associated with industrial-scale production of protein products in Gram-negative bacteria. American Industrial Hygiene Association Journal; 49: 420-421.

Petsonk EL, Olenchock SA, Castellan RM, Banks DE, Mull JC, Hankinson JL, Bragg KC, Perkins HH, Cocke JB. (1986). Human ventilatory response to washed and unwashed cottons from different growing areas. British Journal of Industrial Medicine; 43: 182-187.

Reynolds SJ, Milton DK. (1993). Comparison of methods for analysis of airborne endotoxin. Applied Occupational and Environmental Hygiene; 8: 761-767.

Rylander R, Haglind P. (1984). Airborne endotoxins and humidifier disease. Clinical Allergy; 14: 109-112.

Rylander R, Haglind P, Lundholm M. (1985). Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. American Review of Respiratory Disease; 131: 202-213.

Rylander R, Bake R, Fischer JJ, Helander IM. (1989). Pulmonary function and symptoms after inhalation of endotoxin. American Review of Respiratory Disease; 140: 981-86.

Rylander R, Bergström R. (1993). Bronchial reactivity among cotton workers in relation to dust and endotoxin exposure. Annals of Occupational Hygiene; 37: 57-63.

Siegel PD, Olenchock SA, Sorenson WG, Lewis DM, Bledsoe TA, May JJ, Pratt DS. (1991). Histamine and endotoxin contamination of hay and respirable hay dust. Scandinavian Journal of Work Environment and Health; 17: 276-80.

Swift DL. (1983). Aerosol sampling requirements to assess the health risk of special particulates. In: Marple VA, Liu YH, eds. Aerosols in the mining and industrial work environments. Ann Arbour: Ann Arbour Science Publishers: 219-231.

Wolfe MF, Seiber JN. (1993). Environmental activation of pesticides. In: Shusterman DJ, Paterson JE, eds. De novo toxicants. Hanley & Belfus, Philadelphia: 561-573. (Occupational Medicine: State of the Art Reviews 8(3)).

Table 4.1 Details of dipping bath, proprietary brand of dip used and sheep dipped for each of the farms studied

Farm	Type of Bath (Volume/litres)	Type of Dip (OP)	Approximate number of sheep dipped (% lambs)	Approximate Rate dipped/hr	Approximate time in bath (secs)
1	Long (1800)	Ciba-Geigy Topclip Gold Shield (Diazinon)	120 (50%)	40	10
2	Circular + island (1800)	Coopers Powerpack Summer Dip (Diazinon)	836 (50%)	304	30
3	Long (1800)	Youngs Flyte 1250 (Propetamphos)	1200 (33%)	436	10
4	Circular (1350)	Bimeda Golden Fleece (Diazinon)	300 (50%)	100	30
5	Short (2000)	Summer Dip (Diazinon)	1700 (33%)	227	15
6	Long (2000)	Ciba-Geigy Topclip Gold Shield (Diazinon)	600 (33%)	120	10

Table 4.2 Endotoxin (LPS) concentrations in tap water and in sheep dip baths before and after dipping sessions at 6 farms

Farm	Volume of bath (litres)	Sample	Endotoxin Units/ml[a]
1	1800	tap water	26
		pre-dip	18
		post-dip	6720
2	1800	tap water	2
		pre-dip	384
		post-dip	3360
3	1800	tap water	1
		pre-dip	96
		post-dip	16200
4	1350	tap water	4
		pre-dip	1248
		post-dip	2880
5	2000	tap water	2
		pre-dip	624
		post-dip	27600
6	2000	tap water	120
		pre-dip	16
		post-dip	24720

a. Note that 12 endotoxin units is approximately equivalent to 1 ng of LPS.

Table 4.3 Endotoxon concentrations in air in the vicinity of sheep dipping using personal and static samplers

Farm	Filter position	Endotoxin[a] from filter (E units)	Air volume sampled (litres)	Endotoxin[b]		
				Sample duration (mins)	Units/m^3 air sampled	8 hr TWA
1	chucker	74	240	120	309	77
	paddler	13	236	118	53	13
	helper	35	232	116	150	36
	static (paddler)	4	302	153	12	4
	static (drain pen)	2	277	158	7	2
2	chucker	80	322	165	248	85
	paddler	sampler	fell in bath	-	-	-
	helper/chucker	39	294	159	133	44
	static (paddler)	42	302	151	141	44
	static (drain pen)	32	310	159	103	34
3	chucker	64	274	155	232	75
	paddler	6	293	160	21	7
	helper	17	270	153	63	20
	static (paddler)	1	305	152	4	1
	static (drain pen)	8	269	155	21	10
4	chucker/helper	7	327	197	21	9
	paddler	3	348	196	8	3
	helper/chucker	5	322	193	14	6
	static (paddler)	1	332	195	3	1
	static (drain pen)	2	355	190	5	2
5	chucker	10	549	392	94	77
	paddler	11	790	395	67	55
	helper	none	at this farm			
	static (paddler)	11	764	385	70	56
	static (drain pen)	11	770	382	69	55
6	chucker	2	450	229	18	9
	paddler	2	466	225	24	11
	helper	2	247	200	50	21
	static (paddler)	41	439	227	462	218
	static (drain pen)	17	355	192	242	97

a. Total amount of endotoxin, in endotoxin units, eluted from membrane filters
b. Note that 12 endotoxin units is approximately equivalent to 1 ng of LPS.

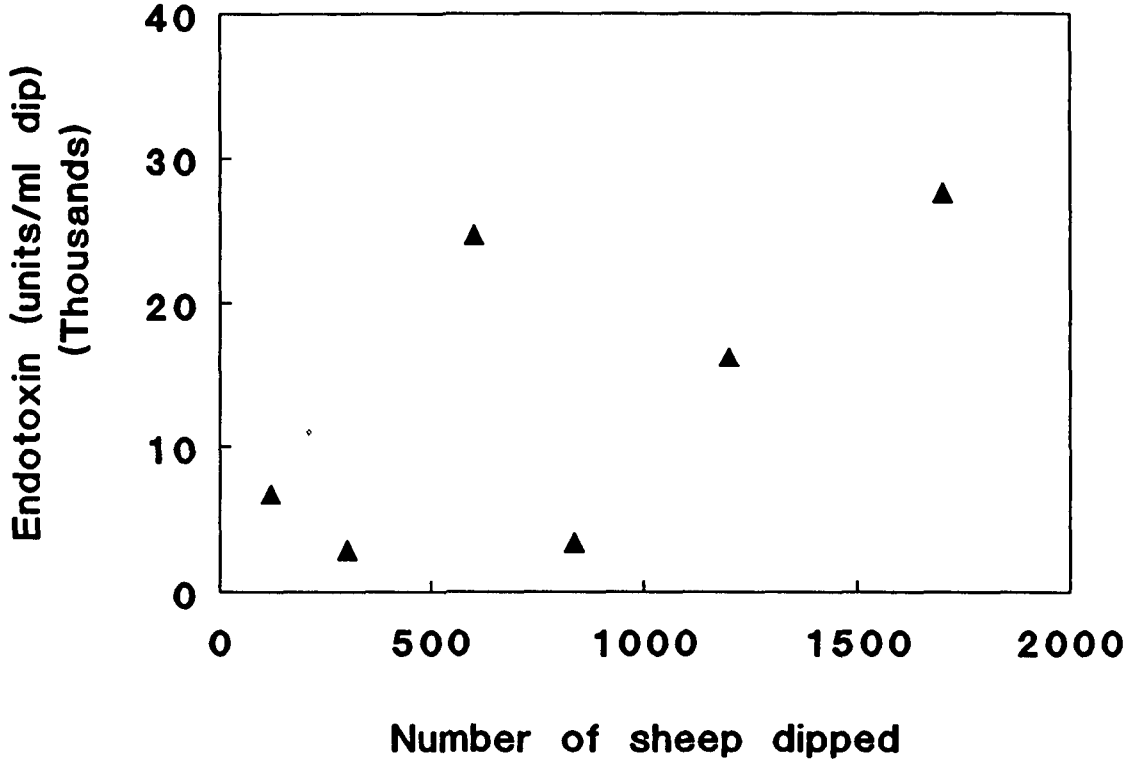

FIGURE 4.1 : Scatter plot of endotoxin concentration in baths at the end of a dipping session against the number of sheep passing through the baths.

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