D&F Associates

EVALUATION OF BIOAEROSOLS AT DOWN FARM, ODIHAM, HOOK, HAMPSHIRE RG29 1QX – 11 JANUARY 2013

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1. Background & Site Description:

D&F Associates Ltd have been commissioned by GK Benford & Co to evaluate bioaerosols at a composting facility situated at Down Farm, near Odiham, Hampshire RG29 1QX. The facility occupies an area of approximately 0.3 ha and processes organic and green waste using Stonemark Bunker (an in-vessel composting system) with open windrow maturation.

Down Farm is set in a rural location bounded by fields and Odiham RAF base. The nearest sensitive receptor is an RAF building situated 10m South of the composting boundary. Helicopters operate at the RAF base which could influence the dispersal of bioaerosols at Down Farm.

2. Sampling Points & Sampling Protocol:

The Environment Agency Technical Guidance Note M17 (Monitoring of particulate matter in ambient air around waste facilities, 2004) offers guidance about aerosol emissions from composting sites. The document suggests a risk-based approach based on conclusions that bioaerosol concentrations would be at or below acceptable levels by 250 metres under most atmospheric conditions (M17 4.5.2, page 28).

The Association for Organic Recycling (AfOR) has published guidance on the evaluation of bioaerosols at open composting facilities ('A standardised protocol for the monitoring of bioaerosols at open composting facilities', 2009). The Protocol aims to aid the assessment of the risk and impact that an open windrow composting system may have on the level of naturally occurring airborne micro-organisms. The Protocol cannot be used to assess the occupational exposure of workers who may be engaged in composting activities and it cannot determine if a composting facility presents a risk to the health of people who may live or work near to a site. This is because satisfactory dose-response data is not available.

M17 and the 2009 AfOR Protocol have been used for this assessment and the Filter Method given in the 2009 AfOR protocol has been used to evaluate bioaerosol levels. Deviations from the AfOR method are presented in the Summary Table attached as Appendix 2 (page 11).

2.1 Samples

Sample locations were chosen in accordance with the guidelines given in the 2009 AfOR Protocol. Sample points are dependent on weather conditions and are chosen with respect to the direction of the wind on the day of monitoring. Sample points may therefore be expected to change for each assessment.

The following sample points were used for the evaluation at Down Farm. Sample locations and relevant wind directions are illustrated on the site map attached as Appendix 1 (page 10):

Sample Point 1:	Sensitive Receptor (at South boundary of the facility, approximately 10m North of MOD office and adjacent to wood stockpile). Grid reference SU 73198 49109 (SP1).
Sample Point 2:	25m Upwind (at perimeter of cattle field, North of the facility boundary). Grid reference SU 73159 49210 (SP2).
Sample Point 3:	10m Downwind (in cattle field, East of the facility boundary). Grid reference SU 73219 49135 (SP3).

2.2 Sampling

Air sampling was carried out using Casella Tuff 4 Plus personal air sampling pumps and SKC Button Aerosol Samplers containing sterile 25mm 0.8µm polycarbonate filters. The units were calibrated to operate at 2 L/min using a float rotameter with a linear flowpath of 600-5000 ml/min (SKC 392-0650). Three samples were taken in parallel at each sampling location. The sampling heads were mounted on a tripod 1.6 metres high and spaced approximately 3-5cm apart in a horizontal hanging position. The pumps were programmed to run for 30 minutes and the sampling start and stop times were recorded. Exposed filters were aseptically removed from the sampling head using sterile tweezers and placed into 10ml aliquots of sterile buffered saline water containing Tween 80 (0.9% NaCl and 0.01% Tween 80, buffered to pH 7.5). Two unexposed filters were aseptically transferred into 10ml aliquots of sterile buffered saline solution to form control samples. An electrically controlled coolbox was used to transport the samples to the laboratory at 4ºC.

2.3 Laboratory Enumeration

The samples were shaken and allowed to stand for 10-15 minutes in order to equilibrate to room temperature. A 10-fold series of dilutions were prepared from the filter suspensions. 0.5ml aliquots were plated onto prepared agar plates containing half strength Nutrient Agar (NA for the enumeration of mesophilic bacteria) and Malt Extract Agar (MEA for the enumeration of thermophilic fungi including *Aspergillus sp.*). Gram negative

bacteria were evaluated using cooled MacConkey Agar (MAC) and 1.0ml aliquots of the filter suspensions. Plates to evaluate mesophilic and Gram negative bacteria were inverted and incubated at 37°C. Plates to evaluate thermophilic fungi and *Aspergillus fumigatus* were inverted and incubated at 40°C. All plates were counted after 48 hours. NA and MEA plates showing fewer than 100 colonies were returned to their respective incubators. Growth for *Aspergillus fumigatus* was rechecked after 3-days and growth for mesophilic bacteria was rechecked after 5-days.

Microbiological growth media was prepared in accordance with British Standard 5763 Part 0 – General Laboratory Practices. Following sterilisation and cooling to 47°C, MEA was treated with Penicillin G Sodium Salt (20 units/ml) and Streptomycin sulphate (40 units/ml), and NA was supplemented with 0.01% w/v cycloheximide (dissolved in <2.0ml acetone). Prepared plates were pre-incubated and quality checked before use.

2.4 Calculation of Results

Exposed filters placed into 10ml aliquots of sterile buffered saline water containing Tween 80 produce microbial suspensions in which the micro-organisms have been diluted by a factor of 10 (*i.e.* 1:10 or 10^{-1}). For each subsequent dilution step, the micro-organisms are further diluted by a factor of 10 *(e.g.* 1:100 or 10^{-2}, 1:1000 or 10^{-3}, 1:10000 or 10^{-4}, etc). Serial dilution allows the number of viable cells in a microbial suspension to be quantified as colony forming units per millilitre (cfu/ml).

The number of micro-organisms in 60 litres of air (pump rate of 2 L/min x 30min sampling period) was obtained by calculating the number of micro-organisms in 1.0ml of the original suspension by 10. The number of micro-organisms in one cubic metre of air (m^3) was obtained by multiplying the number of micro-organisms determined in 60 litres of air by 16.667.

The minimum level of detection for mesophilic bacteria, thermophilic fungi and *Aspergillus fumigatus* is 167cfu/m^3. Concentrations below the minimum level of detection are given as <167cfu/m^3 (*i.e.* less than 167 colony forming units per cubic metre of air).

The minimum level of detection for Gram negative bacteria is 83 cfu/m^3. Concentrations below the minimum level of detection are given as <83 cfu/m^3 (*i.e.* less than 83 colony forming units per cubic metre of air).

2.5 Other Considerations

Equipment, consumables and microbiological growth media were assessed in the laboratory for quality assurance before use or release. Results and records of QA and QC checks are maintained as part of Good Laboratory Practice procedures. Industrial Methylated Spirits (IMS) was used to sterilise equipment in the field and 'Control Samples' were collected as part of the assessment to confirm aseptic handling. Procedures are followed to prevent external contamination.

3. Meteorological/ Weather Conditions:

Wind speed and direction, air temperature and relative humidity were logged at 1-minute intervals using a Kestrel 4500 portable weather station.

4. Site Activities:

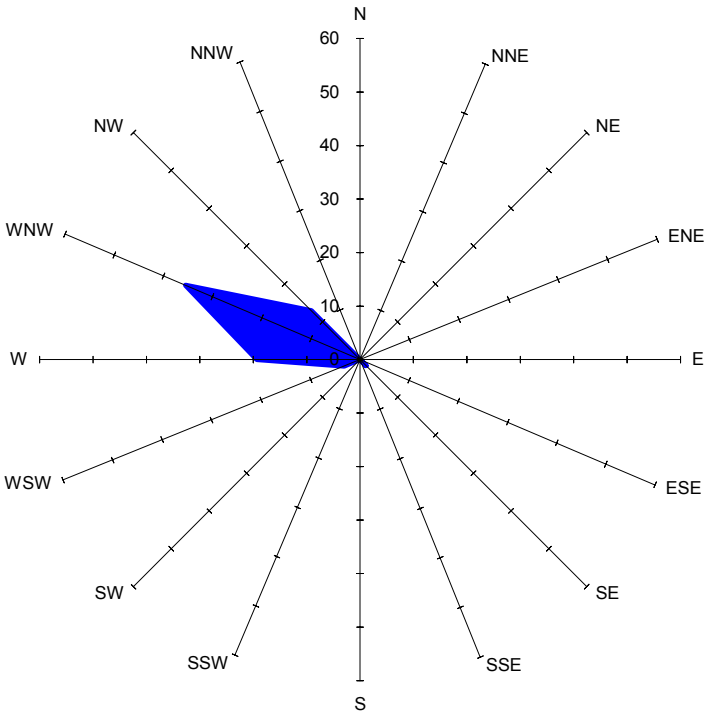
Continuous composting processes were in progress in the Stonemark Bunker System.

5. Results for Airborne Micro-organisms:

5.1 Meteorological Information:

Meteorological data recorded at each sample point is summarised in the Meteorological Conditions Table (page 6). The mean wind direction for the assessment was calculated from 285° (West Northwest) with influences recorded from the Southeast, West Southwest, West, West Northwest and Northwest.

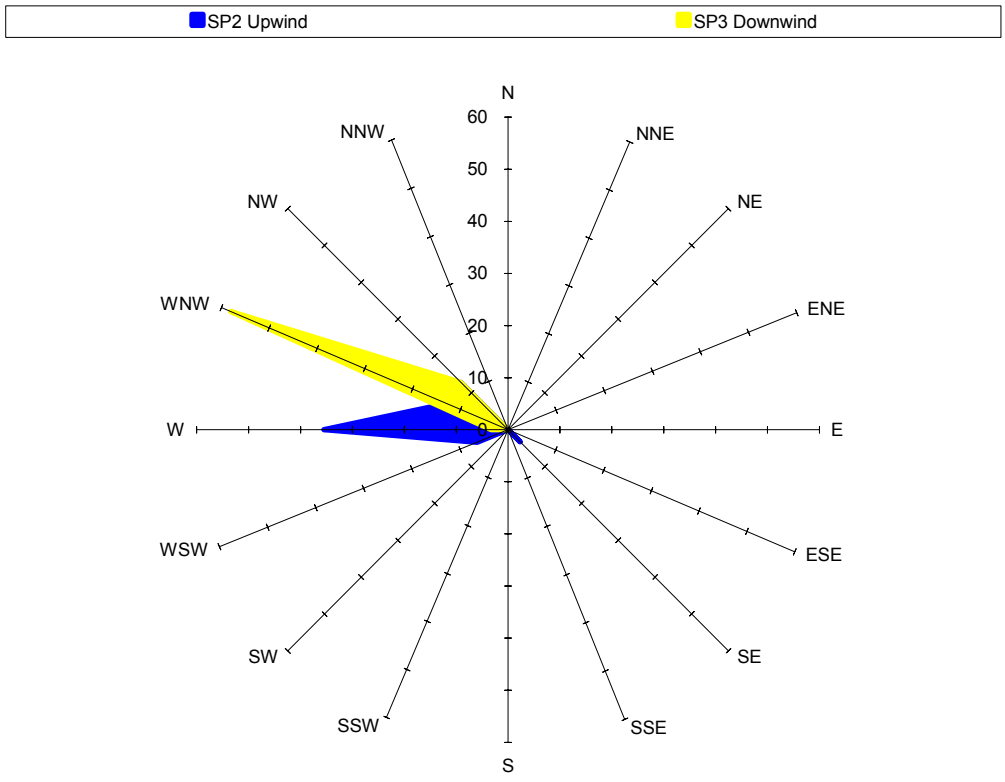
Figure 1: Wind Rose Illustrating Primary Wind Components from the West Northwest.



Note: Units in Percentage Contribution. Wind speed ranged from 0.0-1.2 m/s.

Figure 2 illustrates the wind directions at each sample location. Mean wind directions have been calculated using the AfOR method as described on page 30 of the Protocol. The wind directions are illustrated on the site map attached as Appendix 1 (page 10).

Figure 2: Wind Rose Illustrating Wind Direction at Sample Locations SP2 & SP3.



No/ negligible air movement was measured during sampling at SP1 (Sensitive Receptor).

The mean wind direction at SP2 (25m Upwind) was calculated from 280° (West). Wind influences were recorded from the Southeast, West Southwest, West, West Northwest and Northwest. Wind speeds were measured within the range 0.0-1.2 m/s with an average wind speed of 0.5 m/s calculated.

The mean wind direction at SP3 (10m Downwind) was calculated from 290° (West Northwest). Wind influences were recorded from the West, West Northwest and Northwest. Wind speeds were measured within the range 0.0-1.1 m/s with an average wind speed of 0.5 m/s calculated.

5.2 Results for Airborne Micro-organisms:

5.2.1 Current Limits

The Environment Agency Position Statement 031 refers to sensitive receptors as people who are likely to be within 250 metres of a composting operation for prolonged or frequent periods. Dwellings (including associated gardens) and workplaces where workers would frequently be present are sensitive receptors. Operators of composting facilities and their staff are not considered to be sensitive receptors as their health is covered by Health and Safety legislation.

The Agency considers acceptable bioaerosol levels as:

i. Those before the start of the composting process; or
ii. Bioaerosol levels that are no greater than 1,000 cfu/m^3 for total bacteria, 500 cfu/m^3 for the thermophilic fungus *Aspergillus fumigatus* and 300 cfu/m^3 for Gram negative bacteria.

5.2.2 Microbiological Results

The microbiological results obtained for this assessment are given in the Estimated Concentrations of Airborne Micro-organisms Tables (pages 7-9). The following results discuss the colony forming units per cubic metre of air (cfu/m^3) as calculated average means.

Sensitive Receptor (SP1)

Sampling was undertaken 10m North of an RAF office building on the pad boundary and directly adjacent to a wood stockpile. Mesophilic bacteria were estimated at less than 389 cfu/m^3, thermophilic fungi (including *Aspergillus fumigatus*) at less than 167 cfu/m^3 and Gram negative bacteria at less than 139 cfu/m^3.

25m Upwind (SP2)

Sampling was undertaken at the perimeter of a cattle field North of the site boundary. Mesophilic bacteria were estimated at less than 167 cfu/m^3, thermophilic fungi (including *Aspergillus fumigatus*) at less than 167 cfu/m^3 and Gram negative bacteria at less than 111 cfu/m^3.

10m Downwind (SP3)

Sampling was undertaken in a cattle field to the East of the site boundary. SP3 was downwind of active processes in the composting bunkers. Mesophilic bacteria were estimated at less than 167 cfu/m^3, thermophilic fungi (including *Aspergillus fumigatus*) at less than 167 cfu/m^3 and Gram negative bacteria at 306 cfu/m^3.

6. Conclusions:

1. Mesophilic bacteria and *Aspergillus fumigatus* were estimated below EA guidance levels upwind and downwind of the composting area and active processes in the Stonemark Bunkers, *i.e.* SP2 and SP3 and at the Sensitive Receptor, *i.e.* SP1.

7. Summary:

The evaluation undertaken on 11 January 2013 completed an operational appraisal of airborne micro-organisms at Down Farm Composting Facility. Mesophilic bacteria and *Aspergillus fumigatus* were estimated below EA guidance levels upwind and downwind of the composting area and active Stonemark Bunkers.

METEOROLOGICAL CONDITIONS

Site:	Down Farm Composting Facility	**Site Operator:**	G.K. Benford & Co
Sampling Date:	11 January 2013	**Commissioning Laboratory:**	D&F Associates Ltd
Estimated Mass of Materials:	<500 Tonnes	**Type of Material Processed:**	Organic/ Green Waste

Location	Sample Reference Number	Bearing of samplers from boundary of operational area (° from true north) Grid Reference	Mean wind direction (° from true north)	Difference in bearing between location of samplers from boundary/ source and mean direction of wind (°)	Mean wind speed during sampling (m/s)	Arithmetic mean of air temperature (°C)	Arithmetic mean of relative humidity (%)	Prevailing weather conditions
Sensitive Receptor	SP1	310-5 SU 73198 49109	N/A	N/A	N/A	4.8	94.1	Cloud cover = 10/10 Dry and overcast.
25m Upwind	SP2	130-160 SU 73159 49210	280	120-150	0.5 (Range 0.0-1.2)	4.7	98.4	Cloud cover = 10/10 Dry and overcast.
10m Downwind	SP3	230-325 SU 73219 49135	290	0	0.5 (Range 0.0-1.1)	5.1	99.5	Cloud cover = 10/10 Dry and overcast.

N/A = Not Applicable

ESTIMATED CONCENTRATIONS OF AIRBORNE MICRO-ORGANISMS

Site:	Down Farm Composting Facility	**Site Operator:**	G.K. Benford & Co
Sampling Date:	11 January 2013	**Commissioning Laboratory:**	D&F Associates Ltd
Estimated Mass of Materials:	<500 Tonnes	**Type of Material Processed:**	Organic/ Green Waste

Location	Sample Reference Number	Distance From Boundary of Operation (Metres)	Sample Volume (Litres)	Microbial Type	Site Activity	Materials Processed	Calculated Concentration of Airborne Micro-organisms (cfu/60 L)	Calculated Concentration of Airborne Micro-organisms (cfu/m^3)	Calculated Mean of Samples (cfu/m^3)	Comments
Sensitive Receptor **SP1** (07:42:41-08:12:45 hrs)	1A	0	60	MB	Continuous Composting Processes in Stonemark Bunkers	Organic/ Green Waste	<10	<167	**<389**	**Mesophilic bacteria and *Aspergillus fumigatus* were estimated below EA guidance levels.** SP1 was at the South boundary of the site, 10m North of an RAF building and directly adjacent to a wood stockpile. No/ negligible air movement was measured during the sampling period.
	1B						10	167		
	1C						50	833		
	1D			MEA			10	167	**<167**	
	1E						<10	<167		
	1F						<10	<167		
	1D			AF			10	167	**<167**	
	1E						<10	<167		
	1F						<10	<167		
	1G			MAC			10	167	**<139**	
	1H						<5	<83		
	1I						10	167		

MB - Mesophilic Bacteria MEA - Thermophilic Fungi MAC - Gram Negative Bacteria AF - *Aspergillus fumigatus*

N/A - Not Applicable TNTC - Too Numerous To Count **Controls = <10 cfu/ filter**

ESTIMATED CONCENTRATIONS OF AIRBORNE MICRO-ORGANISMS

Site:			Down Farm Composting Facility				**Site Operator:**			G.K. Benford & Co
Sampling Date:			11 January 2013				**Commissioning Laboratory:**			D&F Associates Ltd
Estimated Mass of Materials:			<500 Tonnes				**Type of Material Processed:**			Organic/ Green Waste
Location	**Sample Reference Number**	**Distance From Boundary of Operation (Metres)**	**Sample Volume (Litres)**	**Microbial Type**	**Site Activity**	**Materials Processed**	**Calculated Concentration of Airborne Micro-organisms (cfu/60 L)**	**Calculated Concentration of Airborne Micro-organisms (cfu/m^3)**	**Calculated Mean of Samples (cfu/m^3)**	**Comments**
Upwind **SP2** (08:21:11-08:51:16 hrs)	2A 2B 2C	25	60	MB	Continuous Composting Processes in Stonemark Bunkers	Organic/ Green Waste	10 <10 <10	167 <167 <167	**<167**	**Mesophilic bacteria and *Aspergillus fumigatus* were estimated below EA guidance levels.** SP2 was at the perimeter of a cattle field to the North of the site. SP2 was upwind of the composting facility. The mean wind direction was calculated from 280° (West).
	2D 2E 2F			MEA			10 <10 <10	167 <167 <167	**<167**	
	2D 2E 2F			AF			10 <10 <10	167 <167 <167	**<167**	
	2G 2H 2I			MAC			10 <5 <5	167 <83 <83	**<111**	

MB - Mesophilic Bacteria MEA - Thermophilic Fungi MAC - Gram Negative Bacteria AF – *Aspergillus fumigatus*

N/A - Not Applicable TNTC - Too Numerous To Count **Controls = <10 cfu/ filter**

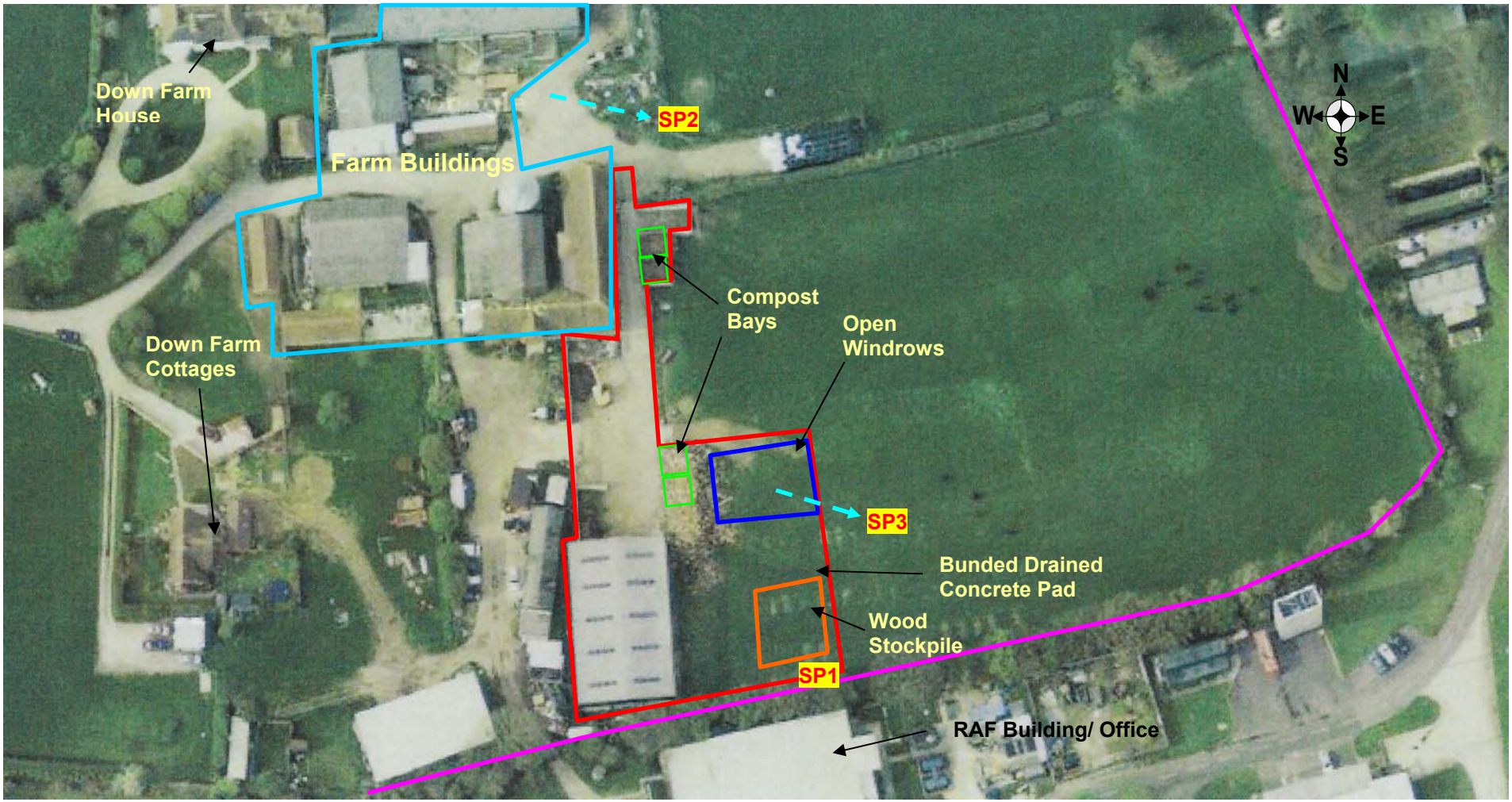
ESTIMATED CONCENTRATIONS OF AIRBORNE MICRO-ORGANISMS

Site:			Down Farm Composting Facility				**Site Operator:**			G.K. Benford & Co
Sampling Date:			11 January 2013				**Commissioning Laboratory:**			D&F Associates Ltd
Estimated Mass of Materials:			<500 Tonnes				**Type of Material Processed:**			Organic/ Green Waste
Location	**Sample Reference Number**	**Distance From Boundary of Operation (Metres)**	**Sample Volume (Litres)**	**Microbial Type**	**Site Activity**	**Materials Processed**	**Calculated Concentra-tion of Airborne Micro-organisms (cfu/60 L)**	**Calculated Concentra-tion of Airborne Micro-organism (cfu/m^3)**	**Calculated Mean of Samples (cfu/m^3)**	**Comments**
Downwind **SP3** (09:02:13-09:32:17 hrs)	3A	10	60	MB	Continuous Composting Processes in Stonemark Bunkers	Organic/ Green Waste	10	167	**<167**	**Mesophilic bacteria and *Aspergillus fumigatus* were estimated below EA guidance levels.**
	3B						10	167		
	3C						<10	<167		
	3D			MEA			<10	<167	**<167**	SP3 was in a cattle field to the East of the site.
	3E						<10	<167		
	3F						10	167		
	3D			AF			<10	<167	**<167**	SP3 was downwind of the composting area and Stonemark Bunkers.
	3E						<10	<167		
	3F						10	167		
	3G			MAC			10	167	**306**	The mean wind direction was calculated from 290° (West Northwest).
	3H						15	250		
	3I						30	500		

MB - Mesophilic Bacteria MEA - Thermophilic Fungi MAC - Gram Negative Bacteria AF - *Aspergillus fumigatus*

N/A - Not Applicable TNTC - Too Numerous To Count **Controls = <10 cfu/ filter**

Appendix 1: Sample Points Evaluated on 11 January 2013



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Appendix 2: Summary of Deviations from the 2009 AFOR Protocol - Filter Method

Deviation/Amendment	Reason for the Deviation/Amendment
5.5. Quality assurance.	All equipment, consumables and microbiological growth media was checked for sterility in the laboratory before use or release. Results and records of QC checks are maintained. Equipment may be swabbed in the field using IMS and additional field controls are collected to assess aseptic handling. Procedures are followed to prevent external contamination. **Impact on the results:** None.
5.6. Summary of equipment.	SKC Button Aerosol sampling heads were used to house the filters instead of IOM sampling heads. The sampler is a patented reusable filter holder with a porous curved surface sampling inlet. SKC claim that the sampler improves the collection of bioaerosols for viable or non-viable analysis whilst minimising wind sensitivity, and provides equal distribution of particle loading and low intersample variation. The sampler follows the ACGIH/ISO sampling criteria. **Impact on the results:** The porous surface minimises the collection of particles >100µm which may be projected into the IOM sampler. Bioaerosols attached to particulate matter >100µm will not be assessed. Particles >100µm are thought to be less inhalable and bioaerosol levels may therefore be lower than those obtained using IOM samplers. The sample is less likely to be affected by wind direction variations at the sample location during the sampling period.
6.2. Filters stored in separate resealable bags.	Individual filter membranes were wrapped and sterilised in tinfoil pouches. QC and sterility checks were carried out on the sterilised batch of filters before use (5.5) **Impact on the results:** None.
6.2. Transportation of loaded filters to the laboratory.	Exposed filters were aseptically removed from the filter holder using sterile tweezers on site. The filters were placed into 10ml aliquots of sterile buffered saline water containing Tween 80 to prevent osmosis. Two unexposed filters were also aseptically placed in 10ml aliquots of sterile buffered saline water on site to i) demonstrate aseptic handling, and ii) to create blank controls. **Impact on the results:** Micro-organism quality and recovery is safeguarded during the storage and transportation of exposed filters as long as the filter is not exposed to other contamination sources. Good aseptic handling is critical.
6.2. Initial suspension of the impacted filter into 5ml of sterilised physiological saline solution.	The sterilised physiological saline solution is chemically the same as the buffered saline water used by D&F but the D&F solution is buffered to pH 7.5. The impacted filters were placed into 10mls of solution instead of 5mls. The resulting suspension was plated out directly and this allowed the minimum limit of detection to be reduced from 417 cfu/m^3 (AFOR) to 167 cfu/m^3 (in 60L of air). **Impact on the results:** Improved detection level and reduced calculation errors due to the multiplication factor that is applied.
6.2. Filters shaken at 35ºC to 40ºC for 15-minutes.	The filter suspensions were allowed to stand at room temperature for 10-15 minutes to allow them to equilibrate. This was to reduce the effects of thermal shock on the microbes following transportation at 4ºC. The samples were shaken but they were not heat-treated at 35-40ºC prior to plating out. The total number of viable bacteria in the mesophilic temperature zone (20-45ºC) including the recovery of stressed micro-organisms were therefore assessed following standard incubation at 37ºC. **Impact on the results:** Heating the sample above 37ºC will adversely affect the recovery of micro-organisms that are grown at 37ºC.
6.2. 0.1ml of each respective step is plated on to culture medium with a pipette and spread out by circular movements.	For the enumeration of mesophilic bacteria and *Aspergillus fumigatus*, 0.5ml of each dilution (including the original suspension) was plated onto culture media and the bacterial suspension was gently spread across the surface of the medium using a sterile L-shaped spreader. This is a traditional microbiological technique used for the cultivation of micro-organisms. The inoculated plates were left for a few minutes to dry prior to inversion and incubation. **Impact on the results:** None.
11.1. Data calculation for filters.	The results were calculated as described in section 2.5 of this report.
Additional: Enumeration of thermophilic fungi and Gram negative bacteria	In addition to mesophilic bacteria and *Aspergillus fumigatus*, total thermophilic fungi and Gram negative bacteria were assessed.