H14-170

NUMERICAL MODELLING OF MICROORGANISMS DISPERSION IN URBAN AREA: APPLICATION TO LEGIONELLA.

Frédéric Tognet[1], Cyrille Turmeau[2], Ti L. Ha[3], Eric Tarnaud[1], Laurence Rouïl[1], Bertrand Bessagnet[1], Enric Robine[3], Yannick Morel[2]

[1]Institut National de l'Environnement Industriel et des Risques (INERIS), 60550 Verneuil en Halatte, France.
[2]Direction Générale de l'Armement, DGA maîtrise NRBC, 91710 Vert-le-Petit, France.
[3] Centre Scientifique et Technique du Bâtiment (CSTB), 77447 Marne La Vallée, France.

Abstract: Dispersion modelling is often used to estimate potentially contaminated areas in case of accidental release of microorganisms in the atmosphere. In the specific case of *Legionella*, accidental spread in the atmosphere due to contaminated cooling towers system may occur over distance larger than 10km. In addition, most cooling towers are located in urban areas where dispersion due to obstacles is complex. In this case, dispersion models have to take into account complex flows and microphysical processes that occur within the plume and may have an impact on the survival of the microorganisms.
To estimate the concentration of microorganisms in these areas, a specific module has been developed within the lagrangian dispersion model Micro Swift Spray (MSS, Aria technologies). This module takes into account microorganisms outside or inside water liquid droplets and microphysical interaction inside the plume. A simple biological module governing the survival of airborne microorganisms has also been implemented in the dispersion model.
In order to evaluate this model, a field campaign of biological aerosols dispersion was performed by CSTB (Champs-sur-Marne, France) on June 23rd, 2009. Spores of *bacillus atrophaeus* (usually referred to as BG) initially contained in a water tank were disseminated in a suburban area from a source at 3 meters above ground level. Air was sampled by DGA MNRBC (Vert-Le-Petit, France) at 4 various locations from 50 to 300 meters from the source to monitor NG concentration. Direct impaction onto Petri dishes was performed with slit-samplers and six-stage Andersen impactors. Wetted-wall cyclones and SKC Biosamplers were also implemented in order to sample air and generate liquid samples.
Dispersion modelling for this campaign has been carried out by INERIS using the microorganism module developed in MSS. The results show that predicted concentrations and in situ measurements are in agreement. MSS Model was implemented to simulate legionella airborne dispersion from a virtual cooling tower at the same location. The biological model has been activated. Results show that the impact of biological model on airborne concentration is significant.

Key words: *Legionella, microorganism, biological aerosols, dispersion, numerical modelling, biological model, biosamplers.*

INTRODUCTION

Legionella is an opportunistic bacterium responsible for Legionnaires' disease, a respiratory infection for humans. The occurrence of Legionella community has sometimes been linked with transmission of an infectious agent from cooling towers (CT). During the episode that occured in Pas-de-Calais (France) in 2003, a transport of *Legionella* up to a distance of 12 km has been observed (Nguyen, 2006). In such an incident, the use of a numerical dispersion model would be helpful to test the influence of several potential sources to guide the search of the CT involved as well as to estimate the extent of the potentially contaminated area.

Although the description of the CT plumes is conventionally based on the use of numerical models; their effectiveness to assess or anticipate the impact of dispersion on the local population's exposure remains to be validated. Moreover, just a few models allow the simulation of the dispersion of microorganisms. For instance, Lighthart and Kim (1989), introduced a bioaerosol droplet dispersion model with evaporative processes and biological decay in the aerosol droplets, to obtain a reasonable fit to observed data from a field spray of a General Ecosystem Model within the first 30 meters from the source (Ganio and al. 1995). In the specific case of *Legionella*, accidental release in the atmosphere due to contaminated cooling towers may occur over large distance. Moreover, most CTs are located in urban or sub-urban areas where accounting for the dispersion due to obstacles is challenging. In this case, a dispersion model has to take into account complex flows and microphysical processes that act within the plume. That is why a specific module was developed inside the lagrangian model MSS (Tinarelli and al. 2007). The objective of this study supported by the AFSSET(Agence Française de Sécurité Sanitaire de l'Environnment et du Travail) consisted in estimating the ability of this tool to predict the potential spread of microorganisms by an anthropogenic source resembling a CT, by comparing data from the simulation with experimental measurements.

This work refers only to the transport of bioaerosols in the air. The numerical model and the modelling methodology is first presented, then the experiments, which consisted of *in situ* dispersions by a biological tracer (spores of *Bacillus atrophaeus*) on a suburban site. Results and comparison are presented. An approach of the dispersion modelling of *legionella* airborn from a virtual CT is made. The work was broken down as follow: the DGA has provided measurements of concentration and bioaerosol particle size which were then compared with the results of simulations performed by INERIS. CSTB provided the technology for assembling the source, some measurements concentration as well as the set up of the biological model.

NUMERICAL MODEL

In this study, the lagrangian model MSS (Aria technologies) was chosen. The wind field is reconstructed from average measured variables (temperature, relative humidity, wind direction and intensity) with the meteorological preprocessor (Micro Swift). The final wind field (which is mass consistent) is diagnosed using analytical corrections induced by the presence of obstacles, considering the topography and buildings. Thus, the recent module "obstacle" of MSS improves the accuracy of the results for near field and urban areas.

The source code must also be accessible in order to implement the modules described above which are specific to bioaerosol (cultivability of bacteria) and their sources (evaporation-condensation in the plume of a CT). Thus, a specific module was developed inside the lagrangian model MSS. These developments were conducted in partnership with ARIA Technologies. The underlying principle is that the virtual particles created by the lagrangian model include droplet spectrum of liquid water, vapor and microorganisms. Initially, the microorganisms are contained in water droplets. Evaporation rate is computed for each droplet and the evolution of the temperature of the droplet is driven by the generic Lagrangian equation (RS Miller and al, 1998). Water vapour diffusion into the air is solved on a eulerian grid. Finally, the evolution of the density and temperature within the plume is evaluated using the Glandening (1984) equations. When water droplets are totally evaporated, microorganisms are released and transported as free airborne tracers.

With respect to microorganisms, a consideration of sustainability must also be included in the code. An experimental approach specific to *L. pneumophila* has been applied by CSTB. This approach is used to set a module of sustainability that is superimposed on the transport calculations.This biological model based on an exponential survival of microorganisms is applied at the specific moment when microorganisms are transported as free airborne and are directly exposed to radiation, air temperature and humidity.

EXPERIMENTAL DEVICE

Dispersion system

The dispersion system has been designed by CSTB to mimic a small CT. The emission is performed at 3 m height above the ground, using 8 high pressure nozzles located on the section of a vertical pipe mounted on a mobile scaffolding (Figure 27). The nozzles produce droplets that have a median volume diameter of 30 microns, similar to that of ART available. The device is operated for up to 30 minutes above a tank containing the spore in suspension.

Biological agent

Spores of *B. atrophaeus* (known as *B. globigii* or BG strain reference: ATCC 9372, CIP7718, DSM 675, FRBD Batr001) are similar in size to *Legionella*: their average dimensions is 0.7 x 1.4 microns (Figure 27. Dispersion system.

Figure 28. Spores of *B. atrophaeus* (MEB)). It is an environmental (telluric) microorganism devoid of pathogenicity or toxicity, used as environmental tracer. Another advantage of such a tracer is our ability to detect these harmless particles at very low concentrations. In confined spaces, the NMAD of one spore is 1.4 microns (measured by Andersen). Assuming the natural tendency of these spores not to aggregate in diluted suspensions, a concentration below $3{,}5\times10^{7}$ spores / ml lead to an average of 1 spore for 2 drops of 30 microns diameter. This induces a low probability for aggregates formation.

Figure 27. Dispersion system.

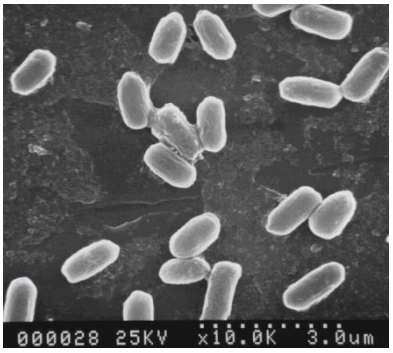

Figure 28. Spores of *B. atrophaeus* (MEB)

Biocollectors

Instrumentation consisted in Agar impactor (yielding particle/L_{air}results; one such particule can contain one or more bacterial cells) or in a liquid medium (cfu/L_{air}; cfu : colonny-forming unit ie. viable isolated bacterial cell), arranged on a support of 80 cm. Reproducibility of these instruments in controlled conditions is usually good (relative standard deviation of the air concentration of 5% for agar impactors and 10% in a liquid medium).

- ✓ impactor slot STA 203, New Brunswick (NBSS; Fig. 3) that operates at a aspiration flow rate of 50 L / min, with a $D_{ae}50$ smaller than 0.6 microns.
- ✓ Andersen 6-stage impactor TE-10-800 Tisch Environmental (AND, Fig. 4) that operates at an aspiration flow rate of 28.3 L / min with an omni-directional sampling head. There are six Petri dishes containing suitable growth medium, kept under sieves of different pore size. Each sieve has 400 pores. The $D_{ae}50$ falls from 7 to 0.65 microns on the different stages.
- ✓ Soprano wet wall cyclone Bertin Technologies (CYC; Fig. 5) set at a rate of 550 L / min (± 5%). The air inlet of the sampling head is multidirectional.
- ✓ Impinger Biosampler of SKC (SKC, Fig. 6): aspiration from 12.5 L / min for an initial 15 mL of liquid.


Figure 29. Impacter STA203

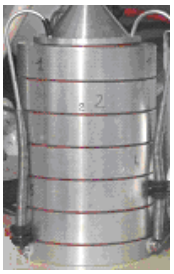
Figure 30. Andersen


Figure 31. Cyclone Soprano


Figure 32. Biosampler

Meteorology

Conventional sensors were setup in ventilated shelters measuring pressure, temperature or relative humidity. Wind velocity and wind direction were recorded using 3D sonic anemometers at a frequency of 32 Hz in three locations (Young, model 81000V). Values are spatially averaged over 12 cm (distance transmitter-receiver).

Campaign set up

A first campaign was carried out to set up the dispersion system and to check the sensitivity of the biocollectors. For this test, the source was installed on a roof. All instruments downwind and within 300 m (on roofs and on the ground) detected bioaerosols. The second campaign ran a few months later on the same day time to take benefit of similar weather conditions for the three tests (dispersions C, D and E). For the final test (dispersion E), the concentration of the spore suspension was multiplied by a factor 7. The instruments were located 20 to 300 m away from the source according to the distribution of Table 1. Points 2 and 4 were located on the roofs of the buildings.

Table 4: instruments location

Collection points and distance from source		20m pt1	100m pt2	100m pt3	100m pt4	200m pt5	200m pt6	200m pt7	300m pt8
Biosensors	NBss		X	X	X	X	X	X	
	AND	X		X			X		
	SKC		X	X	X		X		
	CYC			X			X		X
Meteorology (3 points)		a) 30m above ground and 30m away from the source – b) 2m above ground at source location– c) 2m above ground at point 3							

The final disposition was adjusted at the beginning of the testing phase (Figure 7), according to local meteorological measurements (the generator, central site and 30 m above ground).

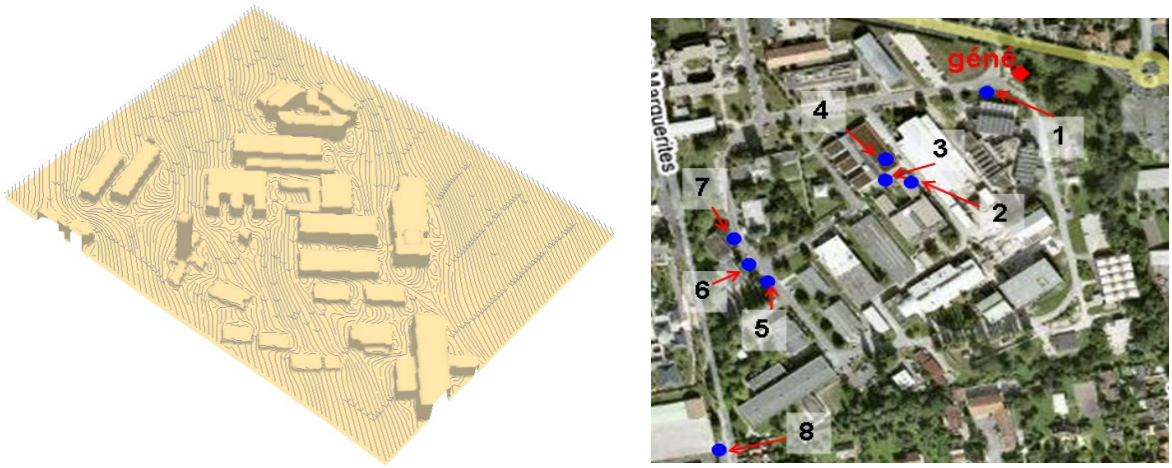

Figure 33. Modelling of the site, location of the generator and locations of collection points.

RESULTS

Table 5 summarizes the operating conditions for the three tests on Tuesday, June 23rd, 2009, a fair day with dry air, and unstable atmosphere (Pasquill class: A).

Table 5: summarize of the three dispersion experiment.

	Duration disp.	Concentration of suspension	dispersed Volume	T°	RH	Pressure	Wind direction	Wind intensity
Disp C	22,5 min	4.6 x10^6 cfu/mL	6,4 L	21 °C	35 %	1019 hPa	59 deg.	2,5 m/s
Disp D	25 min	4.3 x10^6 cfu/mL	7 L	23 °C	31 %	1018 hPa	76 deg.	3,4 m/s
Disp E	25 min	3.1 x10^7 cfu/mL	7,6 L	23 °C	31 %	1017 hPa	54 deg.	2,8 m/s

For the two concentrations of the tracer suspension and the three distances to the source, cultivable particles diameter are between 1.1 and 2.1 microns. The stability of NMAD with the distance shows that the aerosol is rapidly "dry" 20 m away from the source. Furthermore, the value of NMAD does not change when the concentration of the source is multiplied by a factor 7 (still below the theoretical limit concentration to avoid Bg aggregates). This implies an absence of aggregates. Therefore, the high value of NMAD (slightly above one spore size) could be explained by an agglomeration of inert particles on the spores in the dispersion (an effect of scavenging when the droplets are not totally dry). Otherwise, we have considered only particles containing a single cultivable spore, offering thus a good consistency between the biocollectors units (ie. 1 part./Lair = 1 cfu/Lair).

To check the model ability to predict the aerosol dilution according to the distance to the source, we compared the evolution of average concentrations from the NBSS and MSS simulations. Normalization to 10 cfu/m^3 of the point 6 results (exposed to lower turbulence) overcomes a possible systematic bias between biocollectors and simulations. For each experiment, results show a decrease in average concentrations of cultivable spores away from the source (examples in Figure 34), with a good agreement between model results and observations (discrepancies are discussed below). However, a significant difference appears at point 8 (300 meters), for which a relatively high concentration measured is not captured by the model. An accumulation due to a recirculation zone behind a building may have broken away from the obstacle and then impacted point 8. However, such a phenomenon cannot be represented by our dispersion model performed on a 1 minute stationary situations basis.

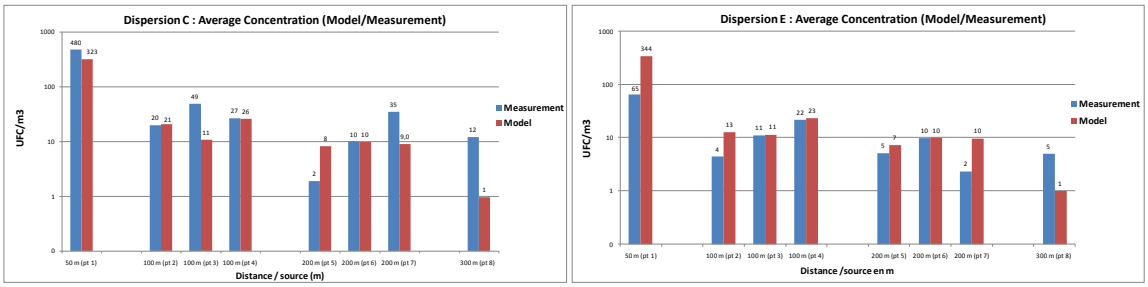

Figure 34. Evolution of normalized concentrations depending on the distance to the source (disp C and E; NBSS and model)

To represent the dispersion dynamics, we have normalized the maximum concentrations of NBSS and the MSS model and compared their time evolutions. Only items 4 and 6 (supposed to be better captured by the model) were studied. The general dynamical model is close to experimental results (examples in Figure 35). The duration of the bacterial cloud and amplitude fluctuations are thus highly correlated. In some situations, hot concentrations may be missing or added according to field measurements. The use of stationary weather conditions averaged over one minute can be responsible for this feature.

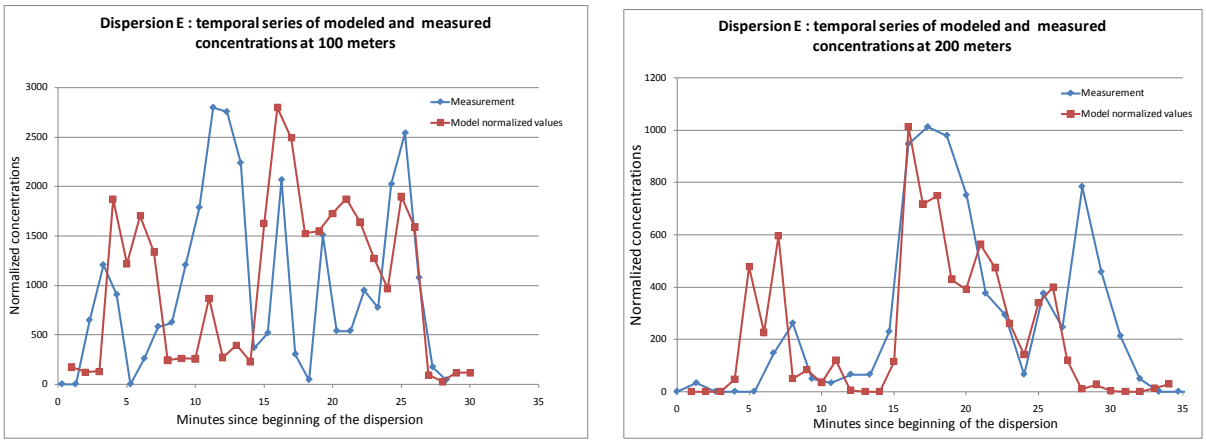

Figure 35. Time evolution of normalized concentrations (disp E- -pt4 pt6; NBSS and model)

For the non-normalized values, significant differences were found between the concentrations measured by some biocollectors and simulations. The average ratios of concentrations (model / biocollector) are respectively 9 - 1.9 - 1.6 and 5 for the NBSS, the AND, the SKC and CYC. If the model can be questioned, an experimental bias must also be considered because of the existence of a ratio of 1 to 6 between each type of biocollector. In addition, obtaining ratios below a factor of 1.3 between the NBSS and the AND when used in a controlled environment indicates that the problem is specific to an external metrology. If the relationship between our four types of biocollectors and with the MSS model are consistent on average, the dispersion of ratios remained significantly higher than in a controlled way of testing (laboratory facility). Thus, the relationship model {/} biocollectors on these tests span an order of magnitude and differences in relative efficiencies of biocollectors reach a factor of 5 (less than 1.5 in laboratory conditions). The origins of this variability would be distributed between the model and therefore the fact of using instrumentation outdoors.

COOLING TOWER ("CT") DISPERSION MODELLING

A characterization of the source term "CT" was conducted by CSTB. It focuses on the size of the droplets and transfer of microorganisms from the water to aerosols during operation. A functional type CT was made available by the company Balticare and installed on the site of the CSTB. Its operating characteristics were controlled by prior device instrumentation. This gave information on emission parameters required for simulations. In addition, based on the work of the CSTB for the survival of *L. pneumophila* in aerosol according to the ambient humidity (TL Ha, 2005), two coefficients α were defined: one describing the loss of cultivability due to stress at the time of the emission (α = 0.006), the other describing the loss of cultivability during transport (α = 0.002). From the physical parameters of the CT, and the meteorological data measured during the dispersion E, we simulated a dispersion from the CT placing it at the source studied previously and using the biological module which describes the conditions of survival and cultivability of *Legionella*. Figure 36 shows a simulated contamination of 10^5 CFU / L within a small CT (French regulated maximum concentration value in the liquid water of a CT before its activity is stopped) where atmospheric concentrations are highest in the first 400 meters downwind of the tower. Results show that applying the biological model leads to a cultivability of 40% at 400 meters from the source. The resulting concentration values are very low and show that contamination by isolated bacteria seems unlikely given the characteristics of CT and investigated the initial concentration used.

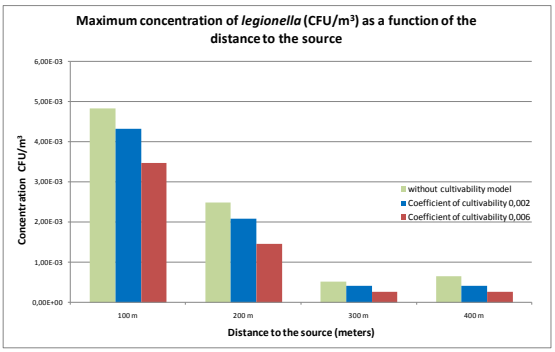

Figure 36. Maximum concentration of *legionella* depending from the distance to the source

CONCLUSION

Qualitatively, experimental measurements and numerical results from software simulations are similar as to spatial and temporal variability of air concentrations. However, significant differences remain over short time scales. Using an Eulerian model for the diagnosis of an unsteady wind field would have limited this behavior. This remark also applies to the quantitative analysis of the results since the model predicts average concentrations about twice as high than the experimental results. Nevertheless, additional tests and sampling farther away from the source would be helpful to validate its operational use.

The simulation of the health risk caused by *L. pneumophila* aerosolization from a CT is possible but would require a finer characterization of the environment, the air flow conditions and source term, as well as additional knowledge on the ecology of Legionella before and after aerosolization (isolated bacteria in biofilm or with other interaction), the latter may be critical to the sustainability module. The type of model (Gaussian, Lagrangian, Eulerian, with specific modules or not) will depend on the expected accuracy and duration of the simulations requested by the authorities for the decision making process. Experimentally, the dispersion of relative efficiencies of biocollectors is three times higher for these tests compared to controlled environment. While this does not preclude the ability of biocollectors to detect low concentrations of aerosolized microorganisms, the development of metrology is an important goal for improvement.

REFERENCES

Ganio L. M., Mohr A. J., Lightart B. 1995: A comparison between computer modeled bioaerosol dispersion and a bioaerosol field spray event. *Aerobiologia*, **11, 3**, 183-188

Ha T.L., Etude de l'aérosol de *Legionella pneumophila*. Phd rapport, Paris XII University.

Lighthart B., Kim J., 1989: Simulation of airborne microbial droplet transport, *Applied and Environmental Microbiology*, **55**, 2349-2355.

Nguyen T. M. N, Ilef D., Jarraud S., Rouil L., Campese C., Che D., Haeghebaert S., Ganiavre F., Marcel F., Etienne J., Desenclos J.C., 2006 : A community-wide outbreak of legionnaires disease linked to industrial cooling towers--how far can contaminated aerosols spread? *The Journal of Infectious Diseases*, **193**, 1, 102-111.

Glendening J.W., Businger J.A., Farber R.J., 1984: Improving plume rise prediction accuracy for stable atmospheres with complex vertical structure. *Journal of air pollution*, **34**, 1128-1133.

Miller R.S., Harstad K., Bellan J. 1998: Evaluation of equilibrium and non-equilibrium evaporation models for many-droplet gas-liquid flow simulations, *International Journal of Multiphase Flow*, **24**, **6**, 1025-1055.

Tinarelli G., Brusasca G., Oldrini O., Anfossi D., Trini Castelli S., Moussafir J. 2007: Micro-swift-spray (mss): a new modelling system for the simulation of dispersion at microscale. General description and validation. Air pollution modelling and its application XVII, 5, 449-458.