REPORT

Measurement of the concentration of Ozone produced by the "Sanozone" device in a closed environment and controlled experimental conditions. Edition: 02

Report n°: MS2_2020_R94

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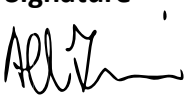
laboratory head: Alberto Ferrari, PhD alberto.ferrari@tpm.bio	**Signature** 	**Date** 03/11/2020
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Ed.	Report n°	Date	Description
01	**MS2_2020_R94**	02/11/2020	**First edition**
02	**MS2_2020_R94**	03/11/2020	**Literature research update**

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1. Internal references

Quote number: TPM_2020_1061_rev01

Technopole Mario Veronesi (TPM), Mirandola

Technopole Mario Veronesi (TPM) is a research laboratory founded by Emilia Romagna region in collaboration with Democenter, a Foundation born in the Modena area in order to develop a network innovation model, specializing in two strategic sectors: the Motor Engineering and Advanced Mechanics district and the Biomedical one. TPM provides a privileged access to all skills expressed by the Emilia-Romagna Region's High-Technology Network, which works in connection with the province and region health services, and it is a linking key point with national and international networks.
Thanks to specific skills of an excellent researchers team and the use of latest and most advanced equipment and technologies, it is a place where companies and scientific expertise meet together, to create, solve and accelerate production opportunities specifically tailored to the needs of the customer. Therefore, it represents a unique system in Emilia-Romagna, one of the few in Italy, organized on an integrated model made up of training, research, incubation and advanced services.
The unique and peculiar feature of TPM is to guarantee high level services for the applied research, industrial development and products validation, hosting training and education for technicians and researchers, consultation and assistance for participation in calls for research funding projects for large, medium and small companies in biomedical, cosmetic and agri-food sectors.

2. Object

The first purpose of the activity covered by this test report is the execution of a bibliographic survey in order to identify which is in the literature the value of the percentage of ozone dispersed in the air capable of sanitizing the surrounding environment. On the subject of ozone sanitation processes, experimental

researches are reported in the literature with particular reference to:

- sanitation of environments, -

sanitizing of food or water.

The second purpose of the activity covered by this test report is the realization of a test campaign aimed at measuring the percentage of ozone dispersed in a controlled environment (default volume) by the "Sanozone" system provided by the customer. The tests are also intended to detect the efficiency of this dispersion, considering the time required for the ozone to reach and maintain a desired concentration of ozone.

3. Materials and methods

3.1 Tools

- Literature research carried out with PubMed biomedical scientific literature search engine
- **Aeroqual 500 series probe** for measuring the ozone concentration
- "Flower 340" environmental chamber Serial Number: 011TT29 (TOP_052). Calibration performed on 14/09/2020. Certificate of validity of performances valid until September 2021 • 1.5kW electric heater
- Vaporizer

3.2 Literature research

The Ministry of Health recognized the use of ozone in the treatment of air and water, as a natural aid for the sterilization of environments contaminated by bacteria, viruses, spores, mites, with protocol n ° 24482 of 31 July 1996.

In the literature, several scientific papers have studied the sanitizing power of environments by applying ozone at different concentrations and residence times. The main research areas are:

- Inactivation of microorganisms on objects and surfaces ○ inactivation of viruses ○ inactivation of bacteria
- Sanitation of medical devices ○ Dental impressions
- Sanitation of environments ○ Industrial environments for dairy production ○ Operating theatres

In the literature, various experiments support the effectiveness of the use of ozone in the sanitation of environments ([1]-[14]). Among the advantages of the use of gaseous ozone there is evidence of the fact that its virucidal action is faster and able to effectively reach even shadows and cracks compared to techniques based on the use of ultraviolet radiation.

The inactivation of viruses has so far been less studied than that of bacteria; however, it is known that it also occurs rapidly following ozonation, even if it requires the generation of gas at higher concentrations than that necessary for bacteria.

In a very recent Italian study Cristiano et al., [4] showed how ozone is considered an efficient tool as a killer of pathogenic microorganisms. A study by the Ministry of Health [9] on the sanitation of environments for the production of food, highlighted the fact that the mechanism of action of ozone on viruses is not destructive, as in the case of bacteria, but "*would consist of an oxidation, and consequent inactivation, of the specific viral receptors used for the creation of the bond with the cell wall to be invaded*". The use of gas would therefore block the viral reproduction mechanism.

Tseng et al [12] in a recent study calculated the dose necessary for the inactivation of viruses on surfaces using ozone. In this investigation, the effects of concentration, contact time, different capsid architecture of viruses and relative humidity were evaluated. The authors observed that virus survival on surfaces decreased exponentially with increasing ozone dose. The viruses were exposed to the following doses of ozone:

- for the inactivation of 90% of viruses 20-112 min (mg / m^3) (exposure time [min] for ozone concentration [mg / m^3]) or 10 -57 min * ppm
- for the inactivation of 99% of viruses 47−223 min (mg / m^3) or 24 -113 min * ppm

The concentrations of ozone reported above are in line with those suggested by a study by the Ministry of Health [9] showing how a percentage up to 4.1ppm for an exposure time of 20 minutes is enough to inactivate some types of viruses in air, in the cheese maturing rooms.

According to the equivalence 80 min * ppm equivalent to 4.1ppm * 20min, based on the considerations reported in the study by Tseng et al., an inactivation of 99% of some types of viruses occurs, by exposing the viruses to an ozone concentration of 4.1ppm for 20min [12].

3.3 Experimental tests

The Sanozone device has been designed in order to concentrate the ozone in the environment in which it is operated in quantities of 4.1ppm, to maintain this concentration for more than 20 minutes and finally to restore the ozone concentration to tolerable levels for human health corresponding to 0.1ppm.

In order to verify the ability of the Sanozone device to produce ozone in quantities and at the times desired by the customer, 6 experimental tests were carried out in which the concentration of ozone produced in two distinct environments was monitored:

- 3 tests in ATT Flower 340 climatic chamber (useful volume 336 liters)
- 3 tests in an external TPM box (3 x 3 x 2 m).

The measurements were carried out in both cases using the Aeroqual 500 series probe supplied directly by the customer. The probe was connected to a computer via the USB port. At the end of the various tests, the data recorded by the Aeroqual 500 series probe were processed.

The tests in which the ozone values in the test environment reached and maintained the threshold value of 4.1 ppm for more than 20 minutes were judged as passed.

3.4 Tests in the Climatic Chamber

Both the ozone generator and the Aeroqual probe have been set in the "Flower 340" Climate Chamber, in order to carry out the experiment in a hermetically sealed environment, at controlled temperature and relative humidity (Figure 1). The Climatic Chamber allows you to set a thermo-hygrometric cycle capable of simulating the operation of the Sanozone device at specific atmospheric conditions. Once the desired set of values for the temperature and the relative humidity have been reached into the chamber, the ozone generator was started.

As soon as the value of 4.1ppm is reached, the generator switches off automatically. The total duration of the test includes both the times of achievement and those of ozone decay to the value of 0.01ppm.

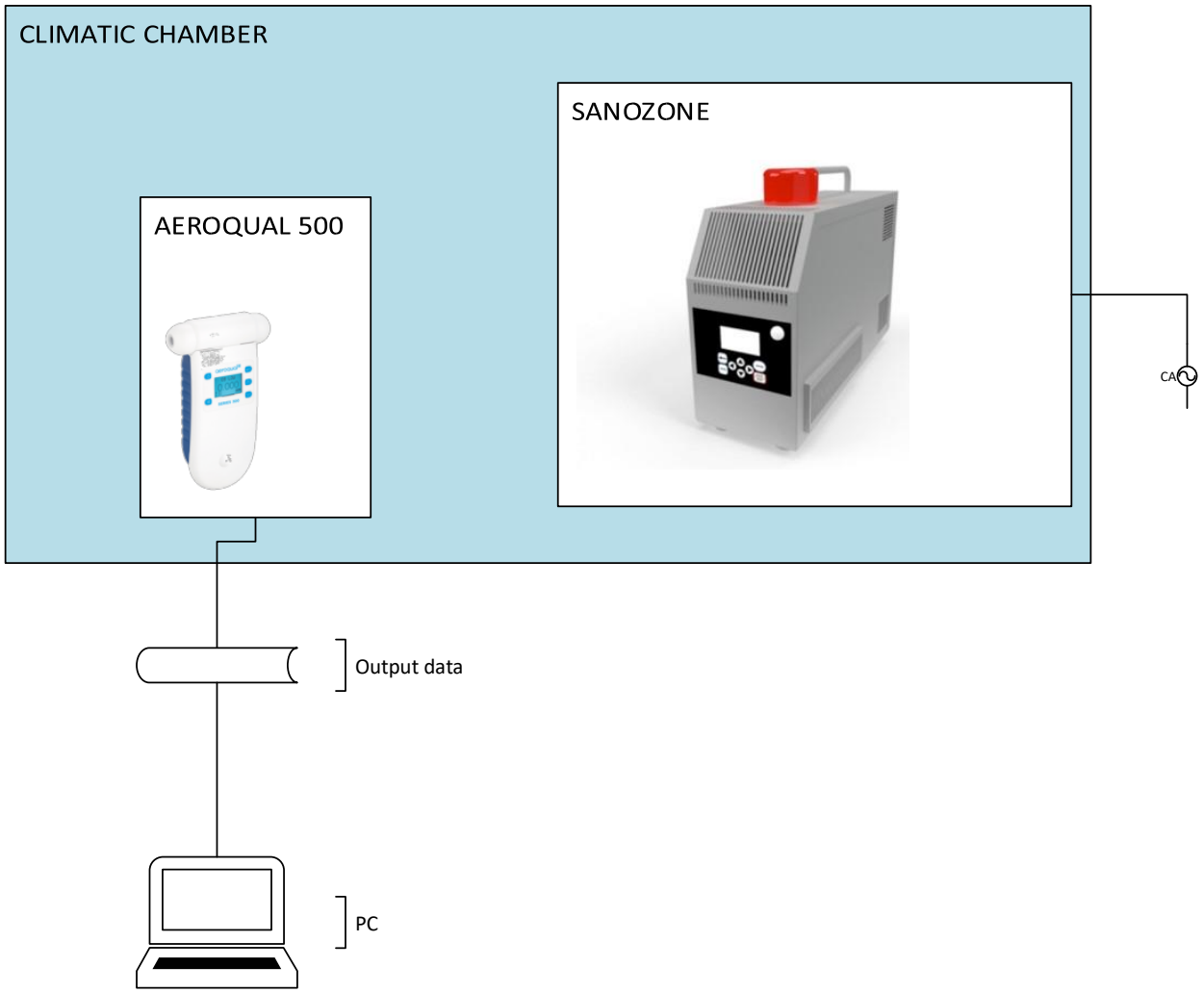

Figure 1 Diagram of the positioning of the O3 generator and the Aeroqual 500 probe in the climatic chamber

The values recorded in Table 1 are summarized as regards the temperatures and relative humidity for the climatic chamber tests

Table 1 experiments in the climate chamber

		TEMPERATURE (°C)	RELATIVE HUMIDITY (%)
CLIMATIC CHAMBER	TEST cc01	26	46,2
	TEST cc02	35	70,0
	TEST cc03	35	35,0

3.5 TESTS IN EXTERNAL BOX

The tests in the external box were performed to verify the trend of ozone diffusion in conditions closer to the volumes of a domestic or industrial chamber than those of the climatic chamber.

Figure 2 shows the scheme of the set up used. In particular, the ozone generator, the Aeroqual 500 series probe, a vaporizer and an electric heater were used.

Once the devices were positioned, the vaporizer and stove were turned on in order to reach stable values of desired temperature and humidity, then the Sanozone device was turned on.

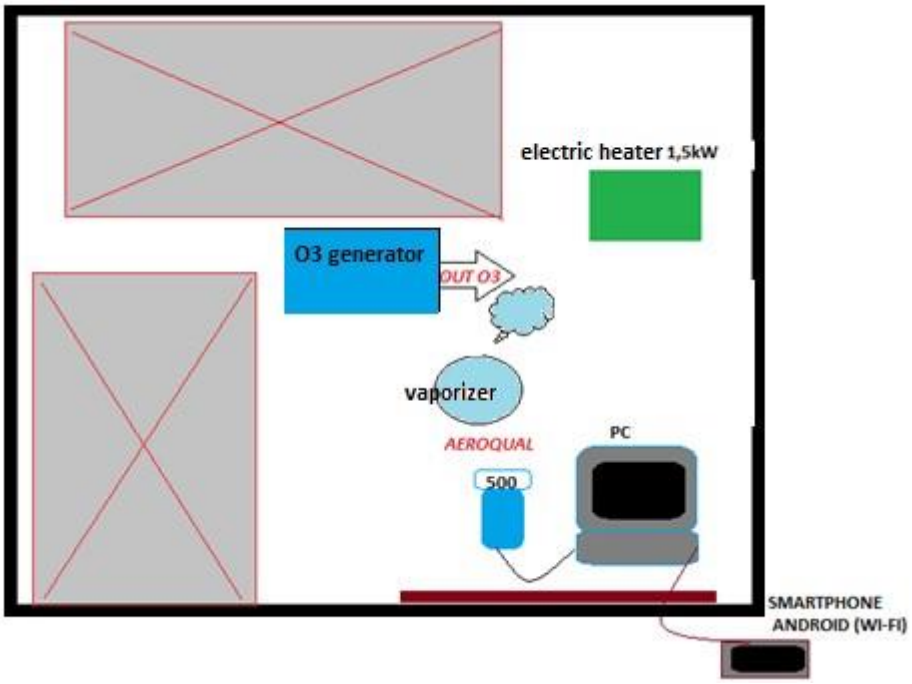

Figure 2 Set up scheme of the experiment in the external box

The tests were carried out at an increasing temperature from 15.7 to 21.8 ° C and medium-high humidity 60-66% (Table 2).

Table 2 Experiments in the climate chamber

		TEMPERATURE (°C)	RELATIVE HUMIDITY (%)
External box	Test cs01	15,7	66,0
	Test cs02	19,9	62,5
	Test cs03	21,8	59,4

4. RESULTS

4.1 Results of the tests in the climatic chamber

Figure 3, Figure 4, Figure 5 show the trends of the ozone concentration in ppm at different temperatures and relative humidity percentages.

- **Test cc01 T=26°C; HR=46,2%**

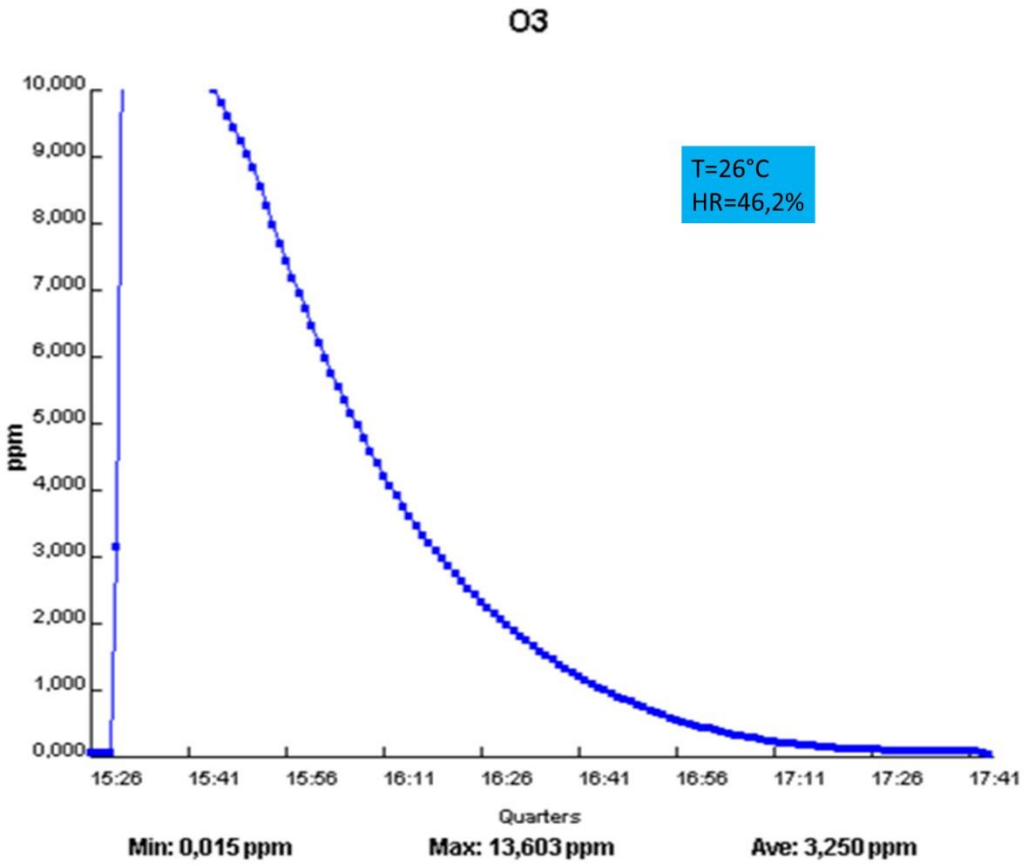

Figure 3 Tests in the climate chamber Test cc01 T=26°C; HR=46,2%

- **Test cc02 T35°C; HR70%**

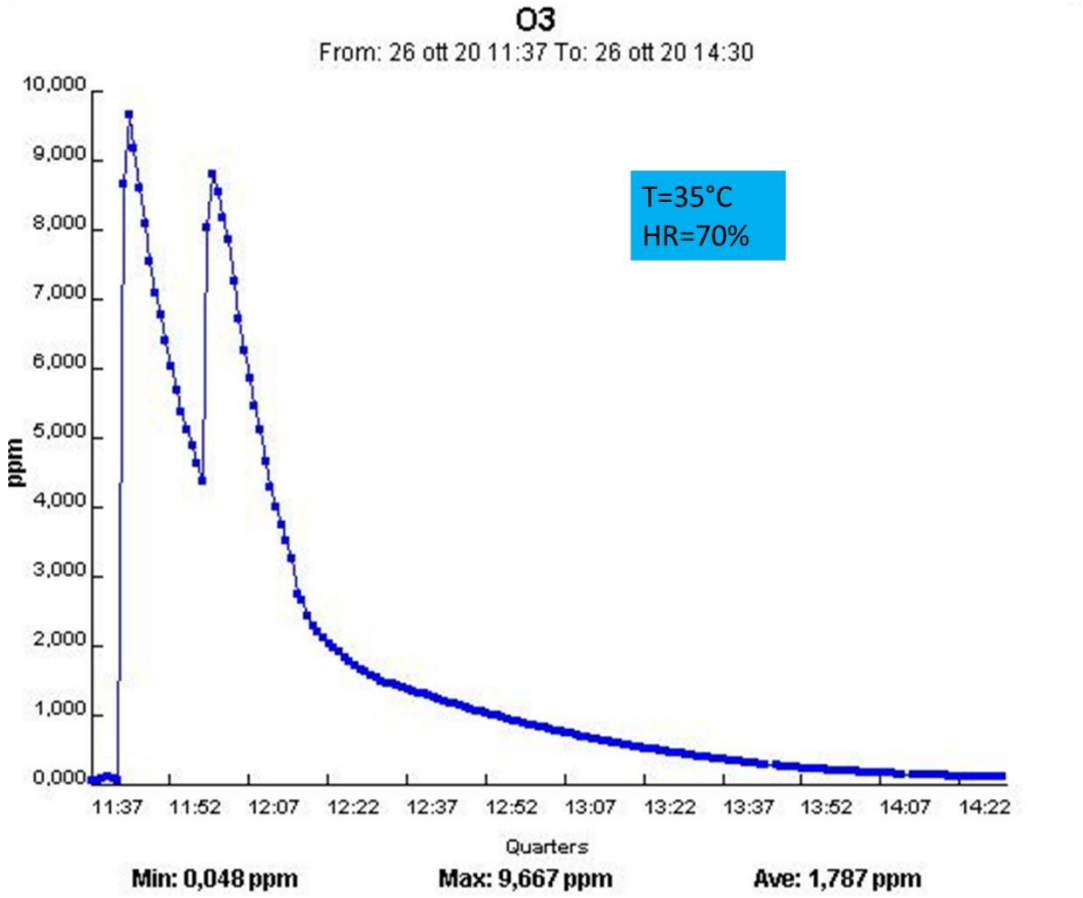

Figure 4 Tests in the climate chamber Test cc02 T35°C; HR70%

- **Test cc 03 T=35°C; HR=35%**

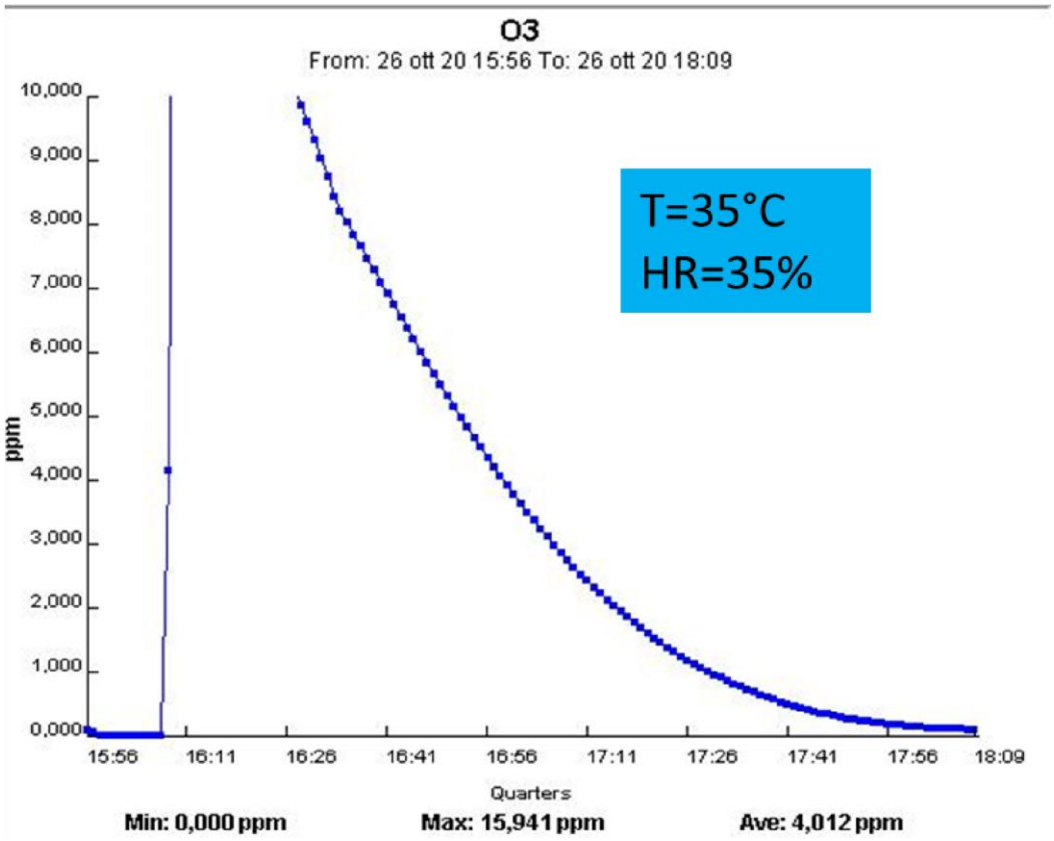

Figure 5 Tests in the climate chamber Test cc 03 T=35°C; HR=35%

The comparison between the recordings of the three tests performed in the climatic chamber is illustrated in Figure 6.

Due to the small acquisition volume (336L) the tests have seen an ozone production above the desired threshold already in the first minutes of operation. However, the comparisons between the trends detected at different temperatures and humidity remain indicative. It can be noted that at the same temperature of 35°C, the increase in relative humidity from 35% to 70% induces a reduction in the ozone generation capacity (even remaining much above the minimum desired threshold of 4, 1 ppm).

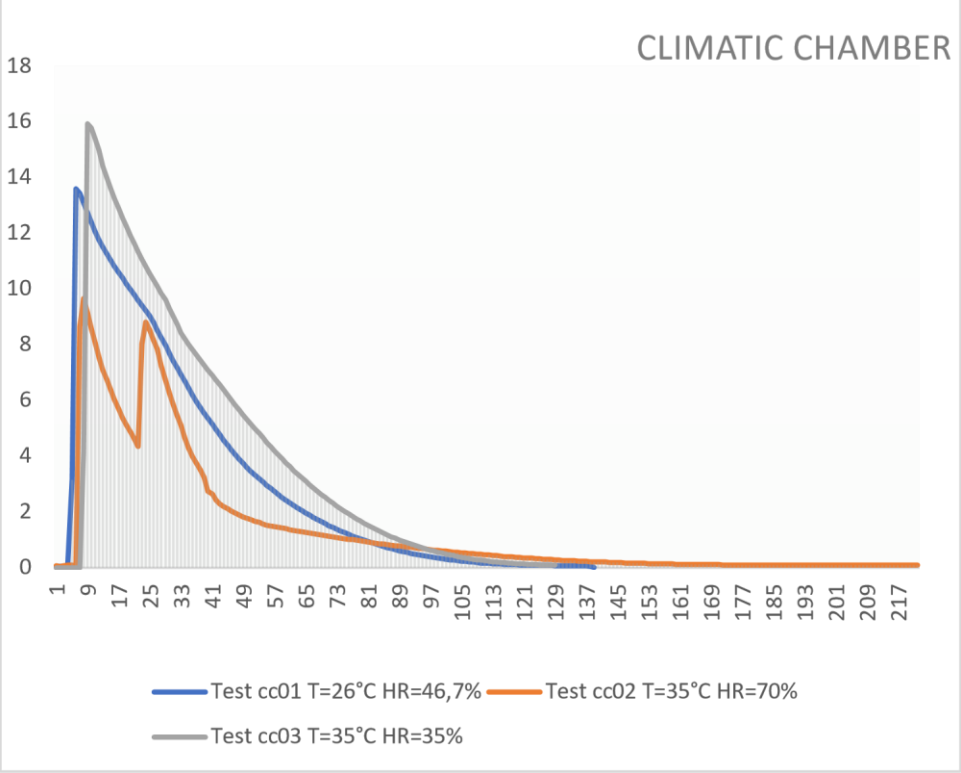

Figure 6 Comparison between the recordings of the three tests performed in the climatic chamber

4.2 Results of the tests in the external box

Figure 7, Figure 8, Figure 9 show the trends of the ozone concentration in ppm at different temperatures and relative humidity percentages.

- **Test cs 01 T=15,7°C; HR=66%**

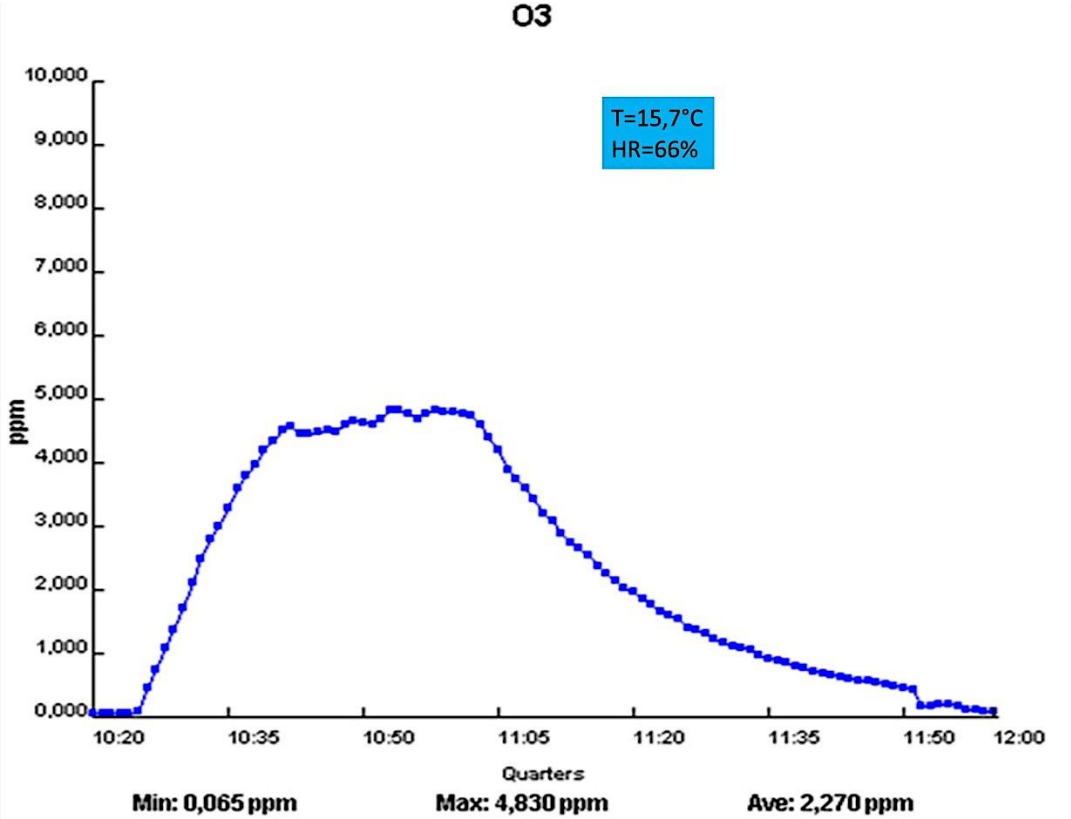

Figure 7 Test cs 01 T=15,7°C; HR=66%

- **Test cs 02 T=19,9°C; HR=62,5%**

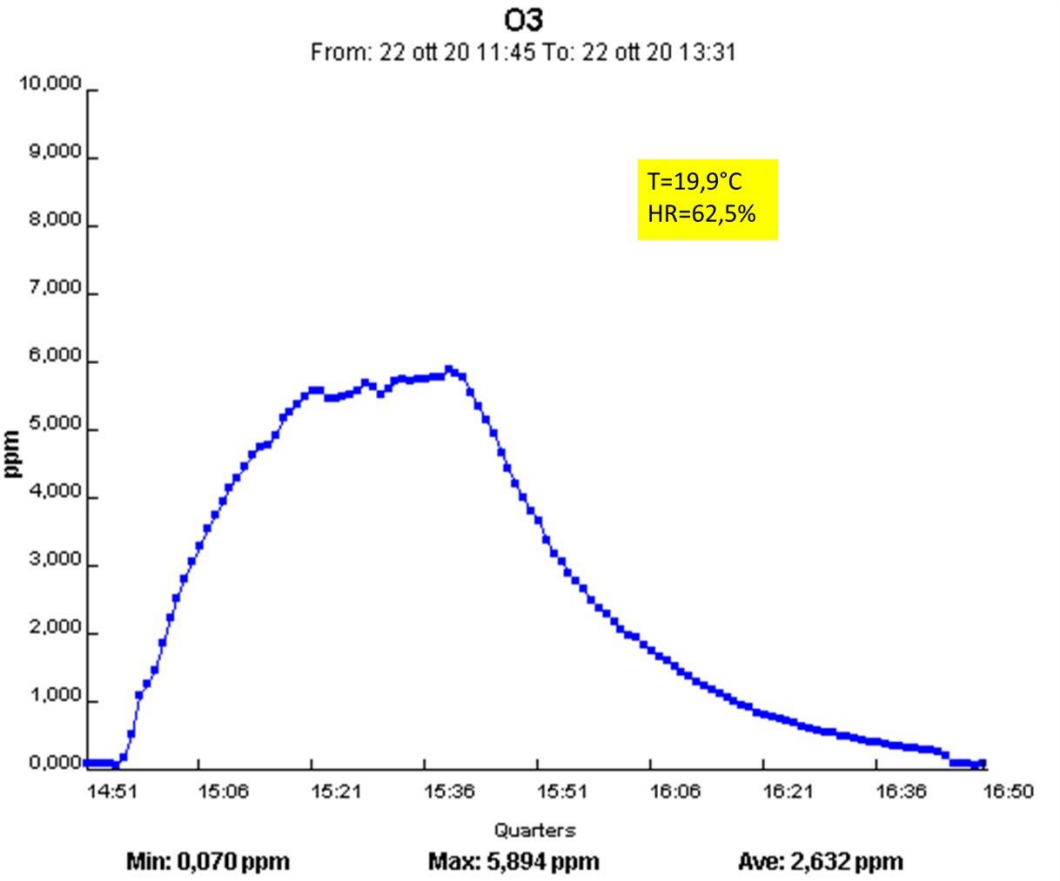

Figure 8 Test cs 02 T=19,9°C; HR=62,5%

- **Test cs 03 T=21,8°C; HR=59,4%**

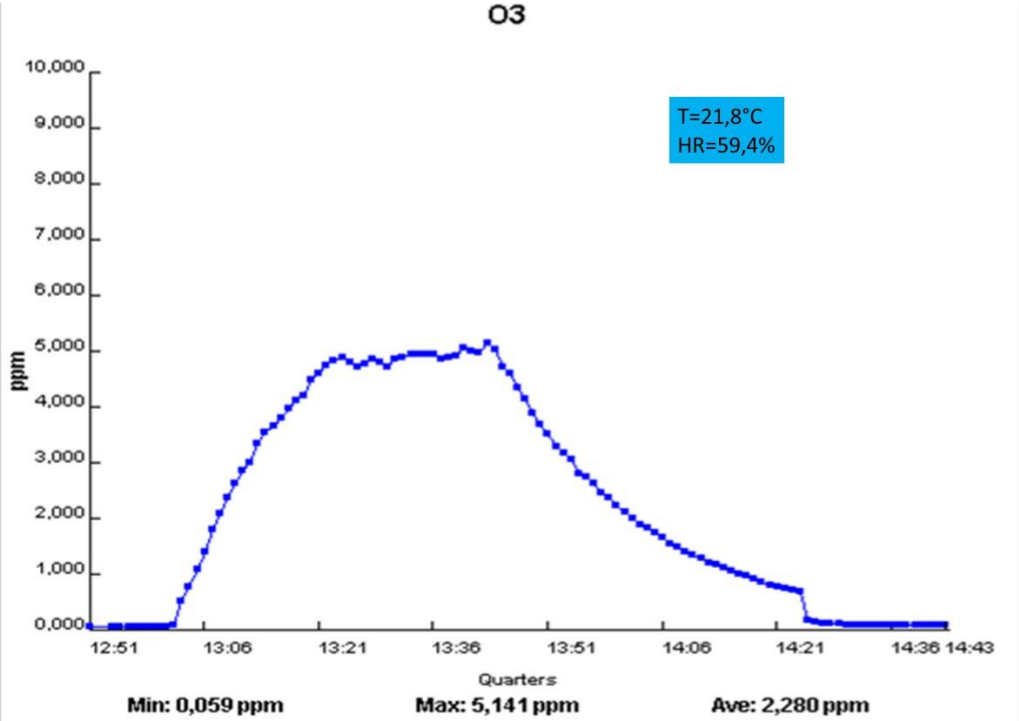

Figure 9 Test cs 03 T=21,8°C; HR=59,4%

The comparison between the data recorded in the external box is shown in Figure 10. It can be noted that in conditions close to the operating setting such as the ones of offices the diffusion behavior of ozone does not undergo significant variations despite deviations in temperature and relative humidity.

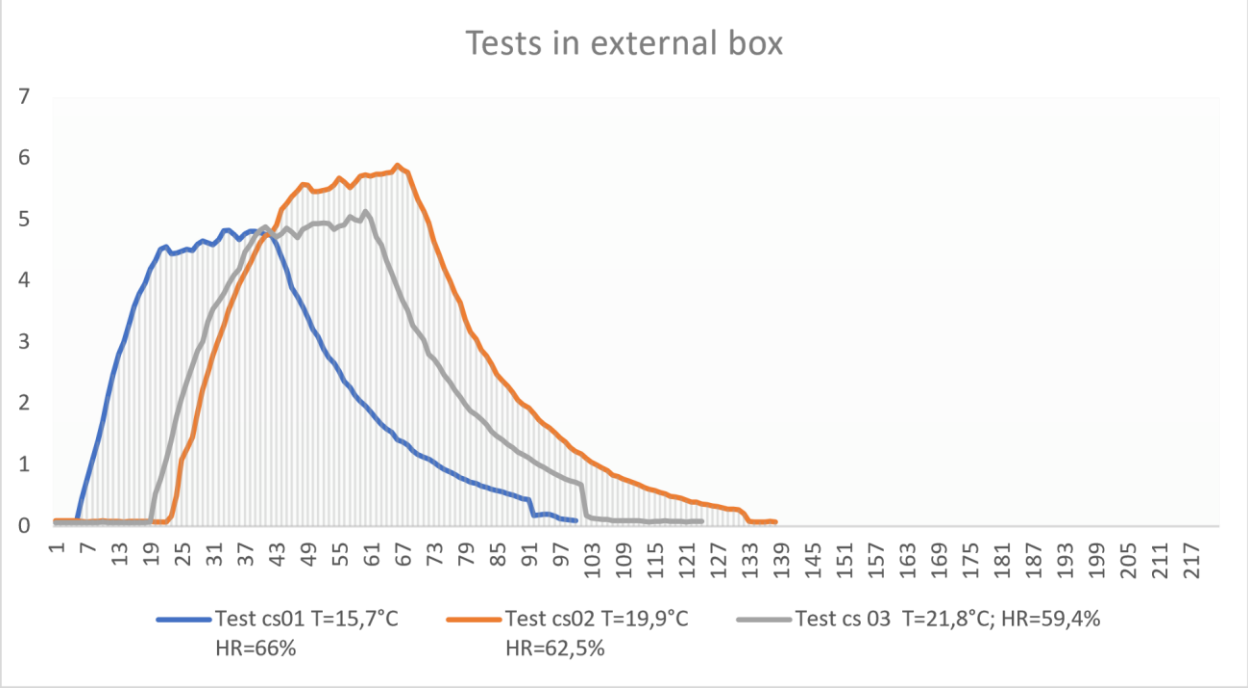

Figure 10 Comparison between the recordings of the three tests performed in the external box

5. Conclusions

The results of the tests carried out in the climatic chamber and in the external box confirm that the generator is able to produce ozone for a concentration higher than 4.1ppm for at least 20 minutes. The tests are therefore considered passed on the basis of the established criteria.

6. Appendix

The appendix shows the data recorded for the tests carried out in the climatic chamber (Table 3) and in the external box (Table 4)

Recordings of ozone percentage concentrations in the climate chamber

Data reported inTable 3 are related to the following tests:

- test Cc01 T = 26 ° C HR = 46.7%

- test Cc02 T = 35 ° C HR = 70%
- test Cc03 T = 35 ° C HR = 35%.

Table 3 Results of the tests carried out in the climate chamber

Time (min)	test cc01 T=26°C HR=46,7%	test cc02 T=35°C HR=70%	test cc03 T=35°C HR=35%
1	0,062	0,064	0,0000
2	0,062	0,048	0,0000
3	0,062	0,086	0,0000
4	0,062	0,102	0,0000
5	3,151	0,094	0,0000
6	13,603	0,058	0,0000
7	13,446	8,651	0,0000
8	13,088	9,667	4,1440
9	12,74	9,171	15,9410
10	12,396	8,596	15,7820
11	12,067	8,08	15,3920
12	11,763	7,547	14,99
13	11,517	7,088	14,405
14	11,28	6,766	14,014
15	11,046	6,404	13,618
16	10,819	6,023	13,254
17	10,599	5,687	12,891
18	10,398	5,374	12,552
19	10,186	5,12	12,235
20	9,987	4,89	11,927
21	9,808	4,634	11,636
22	9,6	4,36	11,338
23	9,422	8,029	11,082
24	9,229	8,809	10,809
25	9,039	8,54	10,566
26	8,818	8,176	10,328
27	8,529	7,844	10,096
28	8,264	7,244	9,861
29	7,982	6,725	9,613
30	7,692	6,255	9,31
31	7,425	5,856	9,035
32	7,183	5,465	8,754
33	6,931	5,104	8,43
34	6,704	4,654	8,212
35	6,448	4,282	8,018
36	6,198	3,988	7,826
37	5,972	3,753	7,645
38	5,751	3,523	7,459

39	5,545	3,246	7,279
40	5,356	2,741	7,093
41	5,151	2,658	6,906
42	4,958	2,418	6,735
43	4,759	2,273	6,552
44	4,571	2,188	6,379
45	4,404	2,118	6,197
46	4,213	2,031	6,009
47	4,047	1,969	5,832
48	3,903	1,903	5,665
49	3,753	1,834	5,491
50	3,589	1,774	5,326
51	3,456	1,722	5,153
52	3,326	1,664	4,983
53	3,207	1,636	4,83
54	3,083	1,569	4,666
55	2,962	1,533	4,502
56	2,853	1,494	4,355
57	2,736	1,471	4,202
58	2,621	1,451	4,058
59	2,521	1,422	3,91
60	2,419	1,401	3,765
61	2,322	1,375	3,631
62	2,231	1,351	3,49
63	2,141	1,328	3,366
64	2,056	1,305	3,233
65	1,969	1,278	3,104
66	1,886	1,254	2,979
67	1,81	1,233	2,864
68	1,731	1,205	2,747
69	1,655	1,184	2,639
70	1,585	1,164	2,527
71	1,513	1,139	2,419
72	1,451	1,12	2,321
73	1,384	1,096	2,218
74	1,321	1,071	2,119
75	1,264	1,053	2,031
76	1,201	1,029	1,935
77	1,145	1,011	1,852
78	1,093	0,992	1,765
79	1,04	0,968	1,682
80	0,991	0,948	1,604

81	0,947	0,928	1,524
82	0,897	0,912	1,45
83	0,855	0,891	1,381
84	0,815	0,867	1,31
85	0,767	0,851	1,243
86	0,73	0,833	1,182
87	0,688	0,817	1,12
88	0,649	0,798	1,062
89	0,615	0,781	1,007
90	0,585	0,766	0,95
91	0,553	0,746	0,902
92	0,522	0,732	0,849
93	0,493	0,712	0,802
94	0,467	0,697	0,758
95	0,44	0,687	0,714
96	0,415	0,667	0,673
97	0,391	0,651	0,633
98	0,37	0,637	0,595
99	0,348	0,627	0,56
100	0,328	0,607	0,526
101	0,308	0,597	0,493
102	0,289	0,58	0,463
103	0,272	0,568	0,434
104	0,254	0,55	0,407
105	0,242	0,541	0,38
106	0,226	0,527	0,355
107	0,212	0,513	0,331
108	0,202	0,505	0,31
109	0,189	0,485	0,289
110	0,178	0,479	0,271
111	0,169	0,466	0,253
112	0,159	0,456	0,236
113	0,151	0,445	0,221
114	0,142	0,432	0,206
115	0,135	0,422	0,191
116	0,128	0,408	0,18
117	0,122	0,395	0,168
118	0,116	0,388	0,159
119	0,112	0,376	0,149
120	0,108	0,367	0,141
121	0,105	0,358	0,133
122	0,1	0,35	0,125

Recordings of ozone percentage concentrations in the external box

Table 4 reports the recorded data related to the tests carried out in the external box. Data are related to the following tests

- Test cs01 T=15,7°C HR=66%
- Test cs02 T=19,9°C HR=62,5%
- Test cs03 T=21,8°C; HR=59,4%

Table 4 Recordings of the ozone concentrations for the tests in the external box

Time (min)	Test cs01 T=15,7°CHR=66%	TestHR=62,5% cs02 T=19,9°C	Test cs 03 T=21,8°C; HR=59,4%
1	0,066	0,087	0,065
2	0,068	0,095	0,063
3	0,068	0,092	0,065
4	0,068	0,089	0,063
5	0,086	0,089	0,063
6	0,447	0,083	0,063
7	0,756	0,076	0,062
8	1,084	0,083	0,063
9	1,377	0,078	0,063
10	1,72	0,09	0,068
11	2,122	0,085	0,061
12	2,477	0,08	0,06
13	2,81	0,08	0,065
14	3,014	0,076	0,061
15	3,291	0,085	0,059
16	3,59	0,077	0,062
17	3,795	0,077	0,062
18	3,971	0,082	0,06
19	4,195	0,082	0,074
20	4,332	0,074	0,527
21	4,522	0,073	0,762
22	4,567	0,07	1,083
23	4,451	0,165	1,41
24	4,453	0,504	1,79
25	4,485	1,083	2,095
26	4,525	1,256	2,358
27	4,495	1,457	2,622
28	4,606	1,859	2,868
29	4,655	2,236	3,01

30	4,624	2,5	3,342
31	4,597	2,793	3,543
32	4,682	3,053	3,67
33	4,828	3,274	3,799
34	4,83	3,546	3,968
35	4,763	3,741	4,101
36	4,68	3,953	4,199
37	4,775	4,133	4,483
38	4,819	4,289	4,599
39	4,811	4,469	4,74
40	4,79	4,636	4,84
41	4,765	4,742	4,889
42	4,756	4,781	4,791
43	4,606	4,913	4,718
44	4,398	5,168	4,776
45	4,189	5,26	4,866
46	3,897	5,383	4,806
47	3,747	5,474	4,707
48	3,589	5,575	4,85
49	3,415	5,567	4,882
50	3,214	5,458	4,94
51	3,095	5,463	4,941
52	2,891	5,482	4,955
53	2,747	5,51	4,938
54	2,665	5,567	4,85
55	2,534	5,681	4,893
56	2,367	5,619	4,918
57	2,27	5,522	5,056
58	2,136	5,606	5,004
59	2,037	5,719	4,981
60	1,968	5,736	5,141
61	1,868	5,719	5,028
62	1,762	5,748	4,718
63	1,661	5,748	4,594
64	1,587	5,767	4,333
65	1,534	5,781	4,136
66	1,412	5,894	3,89
67	1,379	5,824	3,688
68	1,324	5,78	3,523
69	1,231	5,542	3,277
70	1,175	5,33	3,174
71	1,128	5,152	3,048

72	1,095	4,946	2,805
73	1,046	4,643	2,732
74	0,979	4,438	2,62
75	0,928	4,205	2,464
76	0,888	4,011	2,358
77	0,848	3,8	2,216
78	0,796	3,649	2,114
79	0,766	3,374	1,987
80	0,723	3,173	1,885
81	0,697	3,058	1,82
82	0,662	2,879	1,749
83	0,636	2,783	1,659
84	0,606	2,65	1,548
85	0,582	2,48	1,478
86	0,559	2,37	1,414
87	0,529	2,292	1,334
88	0,506	2,183	1,285
89	0,479	2,061	1,212
90	0,445	1,984	1,166
91	0,435	1,931	1,113
92	0,18	1,838	1,057
93	0,183	1,741	1,002
94	0,196	1,667	0,961
95	0,201	1,61	0,904
96	0,164	1,527	0,859
97	0,12	1,442	0,812
98	0,114	1,379	0,773
99	0,099	1,29	0,739
100	0,092	1,22	0,717
101		1,178	0,675
102		1,106	0,178
103		1,05	0,139
104		1,002	0,126
105		0,953	0,112
106		0,913	0,108
107		0,838	0,093
108		0,813	0,087
109		0,774	0,095
110		0,745	0,092
111		0,707	0,089

112		0,677	0,089
113		0,636	0,083
114		0,608	0,076
115		0,581	0,083
116		0,551	0,078
117		0,53	0,09
118		0,488	0,085
119		0,476	0,08
120		0,455	0,08
121		0,427	0,076
122		0,401	0,085

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