Ministero dell'Ambiente
e della Tutela del Territorio
e del Mare

DIREZIONE GENERALE PER LA PROTEZIONE DELLA NATURA E DEL MARE

DIVISIONE VI – TUTELA DELL'AMBIENTE MARINO E COSTIERO

MINISTERO DELL'AMBIENTE E DELLA TUTEL
DEL TERRITORIO E DEL MARE

REGISTRO UFFICIALE - USCITA
Prot. **0029911 - 16/04/2013** - PNM-VI

Spett.le Directa Plus S.p.A.
c/o Comonext Science Park
Via Cavour,2
22074 Lomazzo (CO)

OGGETTO: Prodotti composti da materiali inerti di origine naturale o sintetica, ad azione assorbente, per la bonifica dalla contaminazione da idrocarburi petroliferi, ai sensi del DD 31 marzo 2009 e ss.mm.ii.

Con riferimento all'istanza avanzata da codesta Società relativa al riconoscimento per l'impiegabilità in mare di prodotti composti da materiali di origine naturale o sintetica, ad azione assorbente, per la bonifica dalla contaminazione da idrocarburi petroliferi ai sensi del decreto citato in oggetto, si comunica che sulla base delle verifiche svolte, il prodotto "GRAPHENE PLUS" distribuito da codesta Società, rispetta le condizioni poste dal Decreto 31 Marzo 2009 e ss.ii.mm..

Pertanto tale prodotto verrà inserito nell'elenco dei prodotti composti da materiali ad azione assorbente di origine vegetale o animale o minerale o sintetica e inerti dal punto di vista chimico e biologico, impiegabili in mare per la bonifica dalla contaminazione da idrocarburi, pubblicato sul sito istituzionale del Ministero dell'Ambiente e della Tutela del Territorio e del Mare.

Il Direttore
Dr. Oliviero Montanaro

Via Cristoforo Colombo, 44 – 00147 Roma Tel. 06-57225340- Fax 06-57225373
e-mail: digirolamo.irene@minambiente.it

m_amte.PNM.REGISTRO UFFICIALE.USCITA.Prot.0021157.29-10-2015

Ministero dell'Ambiente
e della Tutela del Territorio
e del Mare

DIREZIONE GENERALE PER LA PROTEZIONE DELLA NATURA E DEL MARE

DIVISIONE III - DIFESA DEL MARE

MINISTERO DELL'AMBIENTE E DELLA TUTELA
DEL TERRITORIO E DEL MARE
Direzione Generale per la Protezione della Natura e del Mare

REGISTRO UFFICIALE - USCITA
Prot. 0021157/PNM del 29/10/2015
DIV III

Spett.le Directa Plus S.p.A.
c/o Comonext Science Park
Via Cavour,2
22074 Lomazzo (CO)
PEC: directa.plus@arubapec.it

OGGETTO: Richiesta di inserimento del nome commerciale Grafysorber™ al prodotto oleoassorbente Graphene Plus, approvato dal Ministero dell'Ambiente e della tutela del territorio e del mare il 16.4.2013 con protocollo n. 0029911 ai sensi del DD 31 marzo 2009 e s.m.i..

Con riferimento alla nota di pari oggetto inviata da Codesta Società in data 28.10.2015, nella quale viene dichiarato che il prodotto Graphene Plus non ha subito alcuna variazione nelle componenti chimico-fisiche e considerata la documentazione tecnica allegata, si comunica che nulla osta alla sostituzione del prodotto Graphene Plus con il prodotto Grafysorber™ nell'elenco ufficiale dei prodotti impiegabili pubblicato sul sito WEB di questo Ministero ai sensi del Decreto 31 marzo 2009 e s.m.i..

Si provvederà pertanto ad aggiornare l'elenco ufficiale dei prodotti impiegabili sopra citato come richiesto da codesta Società.

Il Dirigente
Dott. Giuseppe Italiano

Via Cristoforo Colombo, 44 – 00147 Roma Tel. 06-57228004/8510 - Fax 06-57228390
e-mail: PNM-3@minambiente.it

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

March 31, 2022

OFFICE OF
LAND AND EMERGENCY
MANAGEMENT

Mr. Giulio Cesareo
Founder and CEO
Directa Plus Plc
3rd Floor 11 -12 St. James Square
London SW1Y4LB
United Kingdom

Dear Mr. Cesareo:

We have received and reviewed the information you submitted on your company's sorbent "GRAFYSORBER® Pillows, Booms and Pads." Our review indicates that these products meet the definition of a "sorbent" as specified in Title 40 of the Code of Federal Regulations (CFR), sections 300.5 and 300.915(g) of the National Contingency Plan (NCP). Based on this review, "GRAFYSORBER® Pillows, Booms and Pads" is not required to be listed on the NCP Product Schedule. Please note the product clarifications listed below.

So that you may be prepared to provide On-Scene Coordinators with a certification as referenced in section 300.915(g)(4) of the NCP, the following statement should be reproduced, dated, and signed on your corporate letterhead:

> [SORBENT NAME] is a sorbent material and consists solely of the materials listed in section 300.915(g)(1) of the NCP.

It is recommended that your product is not left in situ after a spill. It should be completely collected and properly disposed of after use. It is also recommended that any loose product is not used on open water. Enclosed for your review is a copy of section 300.915(g) from the NCP. If you any have questions, please contact me at the Office of Emergency Management at (202) 564-1974 or DeHaven.Leigh@epa.gov.

Sincerely,

Leigh DeHaven

Leigh DeHaven
NCP Product Schedule Manager
U.S. Environmental Protection Agency
Office of Emergency Management (OEM)
Regulations Implementation Division
1200 Pennsylvania Ave., NW (5104A)
Room: 517E WJC North
Washington, DC 20460

COMPLIFE
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ANALYSIS OF THE ECOTOXIC POTENTIAL OF A PRODUCT

DIRECTA PLUS

GRAFYSORBER® G+

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

Sede legale: Via Guido Rossa, 1 20024 Garbagnate M.se (MI) Italy, I P.I./C.F. 11093320155
Capitale Sociale € 161.834 i.v., C.C.I.A.A. MI 1437929 - Registro Società N. 342215
Centro di Saggio operante in BPL - Sistema di gestione qualità certificato ISO 9001
Iscritto al Registro Regione Lombardia ai fini dell'autocontrollo alimentare (N 030015309008)
Laboratorio di Prova Accreditato ACCREDIA LAB N 1318L (UNI CEI EN ISO/IEC 17025:2018)
L'elenco delle prove accreditate è consultabile su www.accredia.it

INDEX

KEY PERSONNEL

Customer

DIRECTA PLUS SPA
Via Cavour, 2
22074 Lomazzo (CO)

Experimenter

Dr Andrea Poggi
Biologist
Complife Italia S.r.l

Quality Control

Dr Silvana GIARDINA
Biologist
Complife Italia S.r.l

Complife Italia S.r.l

Sede di I Location
Via Angelini, 21
27028 San Martino Siccomario (PV)
Italy
tel. +39-0382 25504 - fax +39-0382 536006
Mail: info@complifegroup.com

REPORT CHANGE RECORD

The table here below reports the change log of all approved changes made to the document that make up the course after initial approval.

Rev. no	Date	Description
00	24/11/2020	Draft Report release

1. STUDY DESIGN

1.1 Title
Evaluation of the ecotoxic potential of a product

1.2 Study aim
The aim of the test is to determine the ecotoxic effects of products in biological systems. In particular, a growth inhibition test on the alga *Pseudokirchneriella subcapitata* was conducted according to OECD 201 and the ISO8692 guidelines.

1.3 Tested sample

DIRECTA PLUS

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Graphite

1.4 Evalued parameters and experimental models
The experimental study evaluated the alga *Pseudokirchneriella subcapitata* growth inhibition (OECD 201).

2. EXPERIMENTAL PROTOCOL

2.1 Sample preparation
The sample was prepared according to the indications of the EN 14735: 2005 standard. In particular, the product was extracted in aqueous medium (100 g/L, dry solid/liquid ratio 1:10) and stirred for 24 hours at 15-25°C temperature. The solution was centrifuged for 7 minutes at 5000 rpm. The eluate was filtered on 0.45 µm filter. The tests were performed on the extracted filtered diluted solution at 0.063, 0.125,0.25, 0.5, 1 g/L.

2.2 ALGA TEST

2.2.1 Assay system characterization and maintenance

Test organism:	Green alga
Species:	*Pseudokirchneriella subcapitata* (ex *Selenastrum Capricornutum*)
Family:	Chlorophyceae
Order:	Chlorococcalles

According to ISO8692 (section 5.1), a suitable commercially available product of Pseudokirchneriella subcapitata microalgae, immobilized in an inert matrix, was used. 10^4 cells/ml of algae were inoculated in algal culturing medium by using disposable spectrophotometric cells of 10 cm path-length, called "long cells".

Culture medium is prepared mixing the 4 stock solutions specified in the following table:

SOLUTION N.	NUTRIENT	QUANTITY IN 500 ML OF DEIONIZED WATER
1	NH_4Cl	0,75 g
	$CaCl_2$ $2H_2O$	0,6 g
	$MgCl_2$ $6H_2O$	0,9 g
	$MgSO_4$ 7 H_2O	0,75 g
	KH_2PO_4	0,08 g
2	$FeCl_3$ $6H_2O$	0,04 g
	Na_2EDTA $2H_2O$	0,05 g
3	H_3BO_3	0,0925 g
	$MnCl_2$ $4H_2O$	0,207 g
	$ZnCl_2$	1 ml of the following solution: 150 mg in 100 ml of water
	$CoCl_2$ $6H_2O$	1 ml of the following solution: 75 mg in 100 ml of water
	$CuCl_2$ $2H_2O$	1 ml of the following solution: 25 mg in 500 ml of water then diluted 1 ml in 10 ml
	Na_2MoO_4 $2H_2O$	1 ml of the following solution: 35 mg in 10 ml of water
4	$NaHCO_3$	25 g

Solutions 1,2,3 are sterilised by autoclaving (120°C, 15 min).
Solutions 4 is sterilized by membrane filtration (0.22 µm).
10 ml of solution 1 and 1 ml of solutions 2,3,4 are made up to 1000 with deionized water.

2.2.2 Experimental design

GROUP	. REPLICA N.	CELL DENSITY (cell/ml)	TEST ITEM (EXTRACT) (g/L)	OBSERVATION TIMES (h)
Treated	1 2 3	10^4 10^4 10^4	1 0.5 0.25 0.125 0.063	24-48-72
Control	1 2 3	10^4 10^4 10^4	Culture medium	24-48-72

2.2.3 Assay conditions

Starting cell density: 10^4
Replica number: 3 treated (for each concentration) + 3 (control)
Concentration number: 5 (0.063, 0.125,0.25, 0.5, 1 g/L)
Bottom lightning: Continuous 3000-4000 lux
Temperature: 23 ± 2° C
Assay period: 72 hours

2.2.4 Treatment

Algal suspension coming from stock culture was placed in the treated and control “long cells”. Cells were placed in a transparent holding tray at a temperature of 23°C ± 2°C with continuous lightning for a total period of 72 hours.

2.2.5 Observations

After 24, 48 and 72 hours from the beginning of the test cellular concentration was determined, by means of spectrophotometer OD density measurement, of treated and control samples. pH was measured at the beginning and at the end of the assay.

2.2.6 Results interpretation

The average growth rate for a specific period μ_{i-j} is calculated as the logarithmic increase in the number of cells from the following equation for each control and treated vessel:
Calculate the average specific growth rate over the entire period using the nominally optical density of the inoculated cells as the starting value (X_i).

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i}$$

Where:
μ_{i-j} = average specific growth rate from time i to time j
X_i = OD at time i
X_j = OD at time j

The percentage inhibition of growth rate was calculated as follows:

$$\%I_r = \frac{\mu c - \mu t}{\mu c} \times 100$$

Where:
% I_r = mean percentage inhibition of specific growth rate
μ_c = mean growth rate in control group
μ_t = mean growth rate in treated group

2.2.7 Quality criteria

The assay is valid if the following criteria are satisfied:

- the cell concentration in the control cultures should have increased by a factor of at least 16 within three days, that corresponds to a specific growth rate of 0.92/day;
- the mean coefficient of variation for section by section (CV_{ss}) specific growth rates in the control cultures must not exceed 35%;
- the coefficient of variation of average specific growth rates during the whole period (CV_{wp}) in replicate control cultures must not exceed 7%;
- pH must not vary for more than a 1.5 units during the assay.

2.5 Bibliography

OECD Guidelines for the Testing of Chemicals / Section 2: Effects on Biotic Systems Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. 2011.

ISO 8692:2012. Water quality — Fresh water algal growth inhibition test with unicellular green algae

3. RESULTS

Assay quality criteria were satisfied.
The % I for all the tested concentrations at 72 hours are reported in the following table.

	0,063 g/L	0,125 g/L	0,25 g/L	0,5 g/L	1 g/L
% I	-6,16	0,77	-5,57	1,75	-4,21

In compliance with OECD 201 validity criteria the product extract inducing the 50% alga growth inhibition is > 100 mg/l (EC_{50} % > 100mg/L).

4. CONCLUSIONS

The sample extract

DIRECTA PLUS

GRAFYSORBER® G+

in the applied experimental model showed to have

NO ECOTOXIC POTENTIAL

Experimenter	Study Director
Dott. Andrea Poggi	**Dott.ssa Silvana GIARDINA**

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info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

Sede legale: Via Guido Rossa, 1 20024 Garbagnate M.se (MI) Italy, I P.I./C.F. 11093320155
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ANALYSIS OF THE ECOTOXIC POTENTIAL OF A PRODUCT

DIRECTA PLUS S.P.A.

GRAFYSORBER® G+

COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
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KEY PERSONNEL

Customer

DIRECTA PLUS SPA
Via Cavour, 2
22074 Lomazzo (CO)
Italia

Experimenter

Dr Andrea Poggi
Biologist
Complife Italia S.r.l

Quality Control

Dr Silvana GIARDINA
Biologist
Complife Italia S.r.l

Complife Italia S.r.l

Sede di I Location
Via Angelini, 21
27028 San Martino Siccomario (PV)
Italy
tel. +39-0382 25504 - fax +39-0382 536006
Mail: info@complifegroup.com

REPORT CHANGE RECORD

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Rev. no	Date	Description
00	06/04/2021	Report release

1. STUDY DESIGN

1.1 Title
Evaluation of the ecotoxic potential of a product

1.2 Study aim
The aim of the test is to determine the ecotoxic effects of products in biological systems. In particular, a growth inhibition test on the alga *Phaeodactylum tricornutum* was conducted according to ISO10253 guidelines.

1.3 Tested sample

DIRECTA PLUS S.P.A.

GRAFYSORBER® G+

Graphite

1.4 Evalued parameters and experimental models
The experimental study evaluated the alga *Phaeodactylum tricornutum* growth inhibition (ISO 10253:2016).

2. EXPERIMENTAL PROTOCOL

2.1 Sample preparation
The sample was prepared according to the indications of the EN 14735: 2005 standard. In particular, the product was extracted in aqueous medium (100 g/L, dry solid/liquid ratio 1:10) and stirred for 24 hours at 15-25°C temperature. The solution was centrifuged for 7 minutes at 5000 rpm. The eluate was filtered on 0.45 µm filter. The tests were performed on the extracted filtered solution at LIMIT TEST dilution of 0.100 g/L as requested by Sponsor.

2.2 ALGA TEST

2.2.1 Assay system characterization and maintenance

Test organism:	Green alga
Species:	*Phaeodactylum tricornutum*
Family:	Phaeodactylaceae
Order:	Bacillariales

According to ISO 10253:2016, a suitable commercially available product of *Phaeodactylum tricornutum* microalgae inoculum was used. 10^4 cells/ml of algae were inoculated in algal culturing medium by using disposable spectrophotometric cells of 10 cm path-length, called "long cells".

Culture medium is prepared mixing the content of 7 vials and 3 stock solutions specified in the following table:

SOLUTION N.	NUTRIENT	QUANTITY IN 2000 ML OF DEIONIZED WATER
1	NaCl	26.4 g/l
2	KCl	840 mg/l
3	$CaCl_2$	1670 mg/l
4	$MgCl_2$	4600 mg/l
5	$MgSO_4$	5580 mg/l
6	$NaHCO_3$	170 mg/l
7	H_3BO_3	30 mg/l
A	Stock solution A	15 ml
B	Stock solution B	1 ml
C	Stock solution C	2 ml

2.2.2 Experimental design

GROUP	. REPLICA N.	CELL DENSITY (cell/ml)	TEST ITEM (EXTRACT) (g/L)	OBSERVATION TIMES (h)
Treated	1 2 3	10^4 10^4 10^4	0.100	24-48-72
Control	1 2 3	10^4 10^4 10^4	Culture medium	24-48-72

2.2.3 Assay conditions

Starting cell density: 10^4
Replica number: 3 treated + 3 control
Concentration number: 1 (0.100 g/L LIMIT TEST)
Bottom lightning: Continuous 3000-4000 lux
Temperature: 23 ± 2° C
Assay period: 72 hours

2.2.4 Treatment

Algal suspension coming from stock culture was placed in the treated and control "long cells". Cells were placed in a transparent holding tray at a temperature of 23°C ± 2°C with continuous lightning for a total period of 72 hours.

2.2.5 Observations

After 24, 48 and 72 hours from the beginning of the test cellular concentration was determined, by means of spectrophotometer OD density measurement, of treated and control samples. pH was measured at the beginning and at the end of the assay.

2.2.6 Results interpretation

The average growth rate for a specific period μ_{i-j} is calculated as the logarithmic increase in the number of cells from the following equation for each control and treated vessel:

Calculate the average specific growth rate over the entire period using the nominally optical density of the inoculated cells as the starting value (X_i).

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i}$$

Where:

μ_{i-j} = average specific growth rate from time i to time j;

X_i = OD at time I;

X_j = OD at time j;

i = time of test start;

j = time of the last measurement.

The percentage inhibition of growth rate was calculated as follows:

$$\%I_r = \frac{\mu c - \mu t}{\mu c} \times 100$$

Where:

$\%\ I_r$ = mean percentage inhibition of specific growth rate

μ_c = mean growth rate in control group

μ_t = mean growth rate in treated group

2.2.7 Quality criteria

The assay is valid if the following criteria are satisfied:

- the cell concentration in the control cultures should have increased by a factor of at least 16 within three days, that corresponds to a specific growth rate of 0.92/day;
- pH must not vary for more than a 1.0 units during the assay.

2.3 Bibliographic Reference

ISO 10253:2016. Water Quality - Marine Algal Growth Inhibition Tests with Skeletonema costatum and Phaeodactylum tricornutum

3. RESULTS

Assay quality criteria were satisfied.
The percentage of inhibition (% I) for the tested item at the concentration of 100 mg/L at 72 hours is here reported:

Sample	% INHIBITION
DIRECTA PLUS S.P.A. **GRAFYSORBER® G+**	**LIMIT TEST** **I% = -9.03** **IC50 > 100 mg/ml**

In compliance with ISO 10253:2016 validity criteria the product extract inducing the 50% alga growth inhibition is > 100 mg/l (EC_{50} % > 100mg/L).

4. CONCLUSIONS

The sample extracted

DIRECTA PLUS

GRAFYSORBER® G+

in the applied experimental model showed to have

NO ECOTOXIC POTENTIAL

Experimenter	Study Director
Dott. Andrea Poggi	Dott.ssa Silvana GIARDINA

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COMPLIFE Italia S.r.l.
info@complifegroup.com
complifeitalia@legalmail.it
complifegroup.com
GARBAGNATE MILANESE (MI), Via Guido Rossa 1, 20024 I +39.02.990.25138
SAN MARTINO SICCOMARIO (PV), Via Monsignor Angelini 21, 27028 I +39.0382.25504

Sede legale: Via Guido Rossa, 1 20024 Garbagnate M.se (MI) Italy, I P.I./C.F. 11093320155
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C.E.A.R.
LABORATORI RIUNITI s.r.l.

SEDE: 22046 MERONE CO - Via Nuova Valassina, 5/b - Tel. 031.640372 (3 linee r.a.) - Fax 031.645700
www.cear.it - E-mail: info@cear.it - P.IVA/C.F. e R.I. n. 01615720131 - R.E.A. Como 203428

Messer
DIRECTA PLUS SPA Cod. Cli. 6211
C/O COMONEXT SCIENCE PARK
VIA CAVOUR, 2
22074 LOMAZZO CO

SUBJECT: Ecotoxicology test with Daphnia magna after 24h in water sample.

Sample information

Received	2013/03/14
By	DIRECTA PLUS SPA C/O COMONEXT SCIENCE PARK VIA CAVOUR, 2 - 22074 LOMAZZO (CO)
Samplied by	Client
Sample type	Filtered water using one gram of G+
Start test date	2013/03/14
End test date	2013/03/15

Analitical result

Acute toxicology test with Daphnia magna **% 0 ***

(APAT CNR IRSA 8020b Man. 29 2003, without annex A)

*the result is expressed as a percentage of dead/immobile organisms.

English certificate traslated by C.E.A.R. For any dispute, the original Italian one will be considered.

Best regards.

Il Responsabile Garanzia Qualità
Dr. Alessandro Taiana

ALBO DEI CHIMICI DELLA LOMBARDIA - N° 2721 - Dr. Alessandro Taiana

L'Analista Biologo
Dr. Luca Caslini

ORDINE NAZIONALE DEI BIOLOGI ALBO PROFESSIONALE ★ DOTTORE LUCA CASLINI Nr. 46502

Merone, 2013/04/30

The result of the present relationship refer exclusively to the champion subordinate to the executed tests. The partial reproduction of this test report is only admitted after written authorization of the laboratory.

Laboratorio accettato dal gruppo di coordinamento del Ministero della Sanità per l'analisi dell'amianto

Laboratorio che effettua analisi nell'ambito dell'autocontrollo alimentare inserito nel registro della Regione Lombardia DECRETO n. 12927 del 29/12/2011 (n. di registrazione 030013303001)

C.E.A.R.
LABORATORI RIUNITI s.r.l.

SEDE: 22046 MERONE CO - Via Nuova Valassina, 5/b - Tel. 031.640372 (3 linee r.a.) - Fax 031.645700
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Client
DIRECTA PLUS SpA
Via Cavour 2,
22074 Lomazzo (CO)
Italy

Cod. Cl. 6211

SUBJECT: Determination of GRAPHENE PLUS absence of toxicity for aquatic fauna

Graphene Plus - Sample Information
Received: 08/10/2013
By: Directa Plus SpA, Via Cavour 2, 22074 Lomazzo (CO), Italy
Sample type: Graphene Plus_Basic G+

Artemia franciscana - Sample information
Received: 04/10/2013
By: Micro Bio Test inc. Belgium
Sample type: ARTOXKIT, n° AF106
Sample expiry date: 28/02/2014
Sample age: 30 h

Methods

Italian Environmental Ministry, Decree of 25th February, 2011 (SO n. 87, GU 31th March 2011 n. 74); APAT-IRSA-CNR 8060 (2003) "96 hours toxicity test with *Artemia franciscana*".

Results

Tab. 1 – Toxicity of Graphene Plus on Artemia sp. (96 h exposure). Sample: 20 g/l eluted Graphene Plus in synthetic sea water (48 h; 25°C). Blank: synthetic sea water (in accordance with table n°1, Annex 4, Decree of the Italian Environmental Ministry, 25/02/2011). SO = n° of Static Organisms after 96 h of exposure; NS = Not Statistically Significant; NSD = Not Statistically Different if compared with the blank sample. Test Validity: the percentage of static organism in Blank sample must be ≤ 10%.

	Exposed Organism in each Replica	Total Exposed Organism	Rep.1 SO	Rep.2 SO	Rep.3 SO	Rep.4 SO	Rep.5 SO	Rep.6 SO	SO	VC%	Test x^2	Fisher's exact test
Blank	10	60	1	0	1	1	0	1	4	77,4596669	NS	-
Samp. 1	10	30	1	0	0	-	-	-	1	173,205081	NS	NSD
Samp. 2	10	30	0	1	1	-	-	-	2	86,6025404	NS	NSD
Samp. 3	10	30	0	2	1	-	-	-	3	100	NS	NSD

The number of static organisms in the 3 samples is not significantly different from that of the blank.

The Biologist
Dr. Luca Caslini

ORDINE NAZIONALE DEI BIOLOGI ALBO PROFESSIONALE
DOTTORE
LUCA
CASLINI
Nr. 46502

The Laboratory Responsible
Ind. Per. Giovanni Tentori

COLLEGIO PERITI INDUSTRIALI - LECCO -
GIOVANNI
TENTORI
N° 83
CHIMICO

Merone (CO), 25th October, 2013

The results reported in this document refers to the only sample which is the object of the tests.
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