Analysis report ORAC Europe BV

		initials investigator
Customer name:	**Hak Agrofeed BV** ***att.* Mr. B. Hak** **Leemansstraat 2** **4251 LD Werkendam**	
Amount of samples delivered:	6 samples	
Date of sample arrival:	Tuesday, September 16, 2008	
Sample condition at arrival:	***OK*** [X] ***Damaged*** [ ] ***Other*** [ ] *(see remarks)*	
Sample storage conditions:	Upon arrival, samples were stored at 4°C, in the dark.	
Remarks:	Samples were presented as individually wrapped cucumbers, packaged in a plastic bag.	

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Customer's description of samples:

The delivered cucumbers were divided into two groups:
a control group (n=3) and an Immutines-treated group (n=3).
Control cucumbers were labeled C-1, C-2 and C-3.
Immutines-treated cucumbers were labeled I-1, I-2 and I-3.

All cucumbers were grown at the nursery of W. Limburg BV in IJsselmuiden, The Netherlands.

Further details about the cultivation procedures and additional information about immutines will be provided in Appendix A, which is attached to the end of this report.

ORAC Europe sample preparation:

Of each cucumber the total weight was determined.
From each cucumber, an equal-sized part (± 5 cm) was cut from the exact middle of the cucumber. The weight of each part was determined and also its volume was determined (*see results section*).
Each part was thoroughly grinded using a laboratory grinder.
From each of the resulting samples, 2.5 gram was weighed accurately in labeled glass test-tubes.
To extract the hydrophilic contents from each sample, 10 mL of an acetone/water/acetic acid solution (140:59:1, v/v) was added to each test-tube. This solution (abbreviated as AWA) is commonly used to extract hydrophilic constituents from food samples.
All sealed test-tubes with samples were placed in a sonication bath for 15 min. Hereafter, all samples were thoroughly vortexed for 1 min, and placed back in the sonication bath for another 15 min.
All tubes were vortexed again for another minute and finally, all tubes were centrifuged at 800 x g for 15 min.
Supernatants were carefully collected and stored in dark glass bottles at 4°C until further use in the hydrophilic ORAC assay.

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		initials investigator
Date of sample preparation:	Tuesday, September 16, 2008	
Required assay:	**Hydrophilic ORAC assay**	
Date(s) of testing:	Wednesday 17 & Thursday 18 September, 2008	
Responsible investigator:	Dr. E. van den Worm	
Report finalization date:	Tuesday, September 23, 2008	
Remarks:	*- no remarks -*	

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Short description of performed assay:

Samples provided by Hak Agrofeed BV were tested for their *Oxygen Radical Absorbance Capacity* (ORAC), using the commonly accepted and well-validated hydrophilic ORAC assay with fluorescein as fluorescent probe and with AAPH (2,2'-azobis (2-methyl-propionamidine) dihydrochloride) as a physiological relevant peroxyl radical generator. Kinetic fluorescence profiles were detected using an automated fluorescence reader (*Thermo Fluoroskan Ascent*). Fluorescence was monitored every minute for 1 hr. at 37°C, using an excitation wavelength of 485 nm and an emission wavelength of 538 nm.

In the ORAC assay, Trolox (a water-soluble derivative of vitamin E) is used as an internal standard. Therefore, the results of the ORAC assay are expressed as µmol Trolox equivalents (TE) ***per 100 g of test-sample***. This is a standard way of expressing ORAC values.

Each sample was dissolved and diluted in freshly prepared 75mM sodium phosphate buffer (pH = 7.4) shortly before the experiment.

All reagents were freshly prepared prior to the experiment. All solutions were kept in the dark at 37°C except the AAPH solution which was kept on ice (in the dark) until use.

From the obtained experimental data, final ORAC values were calculated using the 'area under the curve' (AUC).
The net AUC was obtained by subtracting the AUC of the blank from that of the sample. The relative ORAC value (expressed as Trolox equivalents) was calculated by extrapolation from the Trolox calibration curve (AUC_{Trolox} vs. [Trolox]).
ORAC values are expressed as mean values ± Standard Deviation (S.D.).

Remarks:

Before all dilution steps and final addition to test-plate, all sample dilutions were carefully vortexed.

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TEST RESULTS:

Cucumber No.	Total Weight	Weight (part)	Volume (part)
C-1 (control)	400.56 g.	64.10 g.	62 cm^3
C-2 (control)	386.52 g.	64.50 g	59 cm^3
C-3 (Control)	411.85 g.	65.17 g.	63 cm^3
I-1 (Immutines)	358.50 g.	63.80 g.	62 cm^3
I-2 (Immutines)	345.30 g.	63.85 g.	62 cm^3
I-3 (Immutines)	375.53 g.	64.82 g.	63 cm^3

	ORAC value (µmol TE / 100 g.)	
Control cucumbers	**99.13 ± 5.38 (mean ± S.D.)**	(n = 4)
Immutines-treated cucumbers	**105.15 ± 7.30 (mean ± S.D.)**	(n = 4)

** As stated previously, ORAC values are expressed as µmol TE per 100 g of test sample. If required, customer can extrapolate these ORAC values to µmol TE per cucumber or µmol TE per serving.*

	Antioxidant capacity (% of control)	Increase in antioxidant capacity (relative to control)
Control cucumbers	**100**	-
Immutines-treated cucumbers	**108.66 ± 4.82**	**8.66 %**

Responsible investigator:

Dr. E. van den Worm
(*CEO*, ORAC Europe BV)

(e-mail: E.vandenworm@uu.nl)

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APPENDIX A

Note: The following information is provided by the customer. ORAC Europe is not responsible for the contents of this appendix.

Immutines:

Immutines are immune-stimulating elements. D&H discovered this group of elements in seawater. In order to be classified as such, the elements should be present in the forms of cofactors, prosthetic groups, transitional elements or as monomeric elements. Some are direct or indirect immune-stimulants, some function as growth factors and some elements catalyse the production of antioxidants.
Drying, mechanically or chemically processing of sea water destroys the immutines. Years of research, solved this problem. Lack of necessary plant immutines in the soil causes plant deficiencies and diseases. Lack of immutines in the soil may not harm the plants, but without them, animals feeding on those plants develop deficiencies, diseases, and lower levels of antioxidants. In the production of plants, roughly 16 elements are given in the form of fertilizer. Still plant, animals and humans requires some 55 more elements.

Practically all elements are required, or essential for the processes of metabolism, growth and immune responses. In the absence of these elements, the plant, animal or human is unable to complete its life cycle (growth and reproduction). The particular structural, physiological, and biochemical roles of each element cannot be replaced by any other constituent. Each element is unique and directly or indirectly involved in metabolism (as part of an enzyme or organic cellular constituent).
Plants, animals and humans, depend on these elements in the form of immutines.

Seawater contains large amounts of elements bound to protein components or other compounds at various valences. They are manifested as cofactors, prosthetic groups, transitional elements and monomeric elements.

The ocean provides the necessary balanced condition for survival of the immutines. The extraction method developed by D&H guarantees that the immutines do not polymerize or regress into a solid elementary form.

An element analysis of the used *Immutines* formulation is presented at page 4.

Cultivation details of cucumbers:

The delivered cucumbers are traditionally grown at the nursery of W. Limburg BV in IJsselmuiden, The Netherlands. The cucumbers are grown in greenhouses and the plants are rooted in rockwool.
During cultivation, the cucumbers are divided in a control group (non-treated) and an *Immutines*-treated group.
Cultivation conditions are similar for both groups, only the composition of the watering solution is different for each group.

The control group is daily watered with a solution containing the following ingredients. (the following ingredients are present in 20.000 liters of water):

Nitrocalcite	175 kg.
Nitric acid	105 kg.
Iron chelate	6 kg.
Red iron	3 L.
Phosphoric acid	40 kg.
Nitre	150 kg.
Magnesium sulphate	75 kg.
Manganous sulphate	300 g.
Zinc sulphate	200 g.
Sodium borate	600 g.
Copper sulphate	38 g.
Sodium	24 g.
Acid Container	30 kg.

Control Group (CG):

The above-mentioned solution is administered 20 times during the growing period of the cucumbers. The average administration is 5 L. per m^2 per day. The average wash-out is approximately 5-10% (this wash-out is partly dependent on the use of pesticides).

***Immutines*-treated Group (IG):**

To the above-mentioned solution (20.000 L.), 7 liters of *Immutines* are added during the first 10 times that the plants are watered. After about 20 days, the mixture containing 7 liters of *Immutines* is replaced by a mixture to which 9 liters of *Immutines* per 20.000 L are added.
The average administration is 5 L. per m^2 per day. The average wash-out is approximately 5-10% (this wash-out is partly dependent on the use of pesticides).

Photo 1) Cucumbers of the control group (CG)

Photo 2) Cucumbers of the Immutines-treated group (IG)

Element analysis of Immutines formulation:

	PPM
Aluminum, Al	.265
Antimony, Sb	.056
Argon, Ar	.315
Arsenic, As	1.437
Barium, Ba	.032
Beryllium, Be	.016
Bismuth, Bi	-
Boron, B	247.892
Bromide, Br	755.203
Cadmium, Cd	-
Calcium, Ca	358.167
Cerium, Ce	.003
Cesium, Cs	.002
Chloride, Cl	134,376.0
Chromium, Cr	.159
Cobalt, Co	.028
Copper, Cu	.163
Dysprosium, Dy	.001
Erbium, Er	-
European, Eu	-
Fluoride, F	.91
Gadolinium, Gd	-
Gallium, Ga	.00053
Germanium, Ge	.00041
Gold, Au	-
Hafnium	.00017
Helium, He	-
Holmium, Ho	-
Hydrogen, H	91,6300.0
Indium, In	.385
Iodine, I	60.0
Iridium, Ir	.00006
Iron, Fe	6.127
Krypton, Kr	-
Lanthanum, La	.001
Lead	-
Magnesium, Mg	40,593.66
Manganese, Mn	.182
Mercury, Hg	-

	PPM
Molybdenum, Mo	.157
Neodymium, Nd	.034
Neon, Ne	.0001
Nickel, Ni	.062
Niobium, Nb	.00023
Nitrogen, N	.5
Oxygen, O	728,450.0
Phosphorus, P	4.118
Polonium, Po	-
Potassium, K	602.447
Praseodymium, Pr	.00019
Protactinium, Pa	-
Radium, Ra	-
Radon, Rn	-
Rhenium, Re	.0037
Rubidium, Rb	.433
Ruthenium, Ru	-
Samarium, Sm	-
Scandium, Sc	.048
Selenium, Se	4.194
Silicon, Si	10.377
Silver, Ag	.006
Sodium, Na	6,968.9
Strontium, Sr	4.652
Sulfur, S	4,886.0
Tantalum, Ta	-
Tellurium, Te	.000147
Terbium, Tb	-
Thallium, Tl	.026
Thorium, Th	.0001
Thulium, Tm	-
Tin, Sn	.064
Titanium, Ti	.002
Tungsten, W	.056
Uranium, U	.019
Vanadium, V	1.184
Xenon, Xe	-
Ytterbium, Yb	-
Yttrium, Y	.02
Zinc, Zn	.58
Zirconium, Zr	.034