POSITIVE EFFECTS OF C_{60}/OO ACTION ARE CAUSED BY FORMATION IN AN ORGANISM OF HYDRATED C_{60} FULLERENE - C_{60} HyFn

(Report of GVA on surprises that the hydrated c₆₀ fullerene presents and that it may present in the future)

To our colleagues, friends and experts at <u>LONGECITY</u> \rightarrow <u>Science & Technology</u> \rightarrow <u>Nanotechnology</u> \rightarrow <u>C60health</u> (<u>http://www.longecity.org/forum/forum/415-c60health/</u> and others (in science and life) to whom it may concern!

Upon analysing a great number of messages that have been left to date by members of the LONGECITY forum, I would like to share my conclusions with you and, at this time, draw a kind of bottom line (to dot all the it's, so to speak) in order to inform you of the main cause of the biological effects you have discovered when using C_{60} solutions in different (!!!) samples of olive oil = C_{60} /OO (and in experiments conducted on yourself, on domestic animals and on poultry).

For that purpose I want to present you my short analysis of several key points, as described in the two articles discussed here.

The first article being quoted in this forum (http://extremelongevity.net/wp-content/uploads/C60-Fullerene.pdf) is, of course, the article by Baati et al. (under research director F. Moussa, Université Paris Sud XI) "PROLONGATION OF RATS' LIFESPAN THROUGH THE REPEATED ORAL ADMINISTRATION OF [60]FULLERENE" Biomaterials, 33(26), 2012b, 6292-6294.

The authors of the second article, which was published almost at the same time as Baati's article, are Japanese researchers whose article is freely accessible so that everyone can familiarise themselves with it: Kato T.M., et al. "SUB-ACUTE ORAL TOXICITY STUDY WITH FULLERENE C60 IN RATS." J. Toxicol. Sci., 37(2), 2012, 353-61 (http://dx.doi.org/10.2131/jts.37.353).

Firstly, let me note that the works of the French and Japanese researchers were performed in accordance with requirements of international practice and standards for bioactive compound testing (surely, with due consideration of national peculiarities, e.g. the EU or JP). In other words, these works reasonably claim to be the official documents of biological testing of fullerene C60 rather than the result of some privately conducted market research. I am grateful to all of them!

So what are the similarities and what are the differences in the results obtained by the French and Japanese researchers?

- 1. All of them studied the <u>biological activity</u> of C_{60} in the form of oily solutions: in the first case of olive oil (with effects observed), in the second case of corn oil (no effects observed). And what is most important here, regardless of the type of oil used, is that no hazardous or toxicological effects of C_{60} were observed!
- 2. The French researchers tested C_{60} dissolved in olive oil (C_{60}/OO). They have actually carried out 3 independent experiments, in which they studied: (i) the distribution and bioavailability of C_{60} , (ii) its antioxidant and hepatoprotective properties and (iii) its effect in terms of any increase in a rat's life span. In the experiments mentioned, doses of C_{60} varied within a range of 1.7 4.0 mg/kg of the animal's body weight, depending upon the certain experimental design. But what is important to us is that one of the experiments revealed that, of all the total dose of C_{60} fed to rats, only its inessential part was absorbed in the gastrointestinal tract (GIT) and distributed in the other organs of the rats' organism (see below).

3. In the research carried out by the Japanese researchers, the rats were fed with fullerene C_{60} (dissolved in corn oil, CO - C_{60} /CO) in doses of up to 1000 mg/kg of the rats' weight, which is almost one hundred times as much as the doses involved in the French experiments (!!!). They revealed an important fact, proving that C_{60} is not absorbed in the gastrointestinal tract (GIT) and, therefore, cannot be detected in other organs of the rats' organism: upon absorption, all the C_{60} remained as an amorphous mass on the stomach and intestine walls (!!!) and fullerene C_{60} itself failed to show any obvious effects in terms of biological effects.

It is worth mentioning that the chemical nature of the saturated and unsaturated fatty acids forming part of the lipids (fats) of corn and olive oils is actually identical.

But what is the difference between these two types of oil? And are biological effects of C_{60}/OO related with the OO lipids in the case that no such cases were reported in experiments with C_{60}/CO ,

In order to shed light on these questions, let's look at the components of OO (e.g. see the link http://www.oliveoilsource.com/page/chemical-characteristics or the Wikipedia link http://en.wikipedia.org/wiki/Olive_oil) and consider the CO components (http://en.wikipedia.org/wiki/Corn_oil).

As we can see from the above source information, as opposed to the CO, the OO contains chlorophylls (HPh = derivatives of porphyrins whose simplest analogues are phthalocyanines). Chlorophylls are natural plant pigments. For example, they determine the green colour and its gradations in the leaves of various plants, including ripe olive fruits and olive leaves. As a result, the deeper (and darker) the green colour is of OO, the more chlorophyll it contains.

Moreover, OO contains polar polyphenol compounds (Polyphenols, PPh), which, in addition to porphyrins (chlorophylls), are absent in other popular edible oil grades, including CO.

Nevertheless, different OO samples contain at least twice as much water as CO and other oils derived through extraction with organic solvents but not through the mere pressing of oil-containing raw, as is normally done when making OO.

But why is so much attention paid to chlorophyll? The thing is that, like phthalocyanines and porphyrins, HPh can form so-called donor-acceptor complexes (DA-complexes) with C₆₀, which are also scientifically known as charge-transfer complexes or CT-complexes (see Cataldo's chapter 13, page 331-332 on http://www.owndoc.com/pdf/solubility-of-fullerenes-in-oil.pdf, which were posted by Turnbuckle 09/27/2012, 12:31 PM, #67 and we can see on http://www.longecity.org/forum/topic/57849-equipment-mixing-centrifuging-and-filtering/page_st_60).

Similar DA-complexes are denoted by the general formula: $D(^{\delta+})A(^{\delta-})$, where A is the acceptor molecule which "accepts" part of the negative charge of the charge cloud of the donor molecule D as the DA forms. In our case, A is the C_{60} molecule and D is the chlorophyll molecule, while DA-complexes (HPh/ C_{60}), consisting of a C_{60} molecule and molecules analogous to HPh, have been long known and studied in detail. To date, they are considered to be important as prospective materials, for example, for transforming solar energy into electrical energy. See examples of scientific research on DA-complexes C_{60} with porphyrins/phthalocyanines and some of their properties at http://www.ncbi.nlm.nih.gov/pubmed/22938243 and http://www.ncbi.nlm.nih.gov/pubmed/23050927.

Which features of the DA-complexes HPh/C₆₀ shall be interesting for further discussion here?

1. Time and again, it has already been noted by members of this forum, and shown by several C_{60}/OO producers, that an oily solution forms when the black powder of C_{60} dissolves in greenish OO, which, in due course, tinctures with reddish, reddish-brown or similar hues. Some examples are available at the following links:

http://c60antiaging.com/c60-faq/the-color-of-the-carbon-60-in-olive-oil http://www.longecity.org/forum/topic/58922-log-andey

2. It is a scientifically known fact that such reddish, reddish-brown or similar hues are common for a wide range of solutions of DA-complexes of C_{60} with other organic nitrogen- or oxygen-containing compounds, including water. Particular examples can be found at the links below:

http://en.wikipedia.org/wiki/Buckminsterfullerene

http://www.ipacom.com/index.php/en/publications-about-c60hyfn/92

- It is necessary to note that, unlike hydrated C_{60} solutions (C_{60} HyFn, http://www.longecity.org/forum/topic/57382-hydrated-fullerene-hyfn), many DA-complexes of C_{60} may be formed with organic molecules, which can remain stable only in non-aqueous media (e.g. in the medium of low-polarity organic solvents, to which OO, CO, FO (fish oils) etc. may be referred).
- 3. Here I would like to mention one important point as a common example. The point is that the DA-complex, which is formed with two "hydrophobic" molecules (that "feel themselves convenient" in oily medium) due to the separation of electrical +/- charges therein, acquires the properties of a <u>polar</u>, hydrophilic chemical compound (composite). Such a complex is bad in terms of getting along with a hydrophobic oily medium. As a result, having formed in an oily medium, the complex will seek to leave it, to separate from it and form its own combination community (phase) of molecules with similar properties, which would be separate from oil. Scientifically this phenomenon is characterised as the transformation of homogeneous system into a heterogeneous one, which is one consisting of several separate molecular communities-phases.

Several enthusiasts of our forum witnessed visual proof of this process. As they noticed, during the cooling/freezing of C_{60}/OO and depending on the grade and quality of OO, a rather clear layering of C_{60}/OO into two phases (layers), can be observed: the main phase (in weight) has a hue similar to that of the initial OO and there is a separate solution layer of reddish-brown hue similar to that of the solutions of the OA-complexes of C_{60} , as I have already mentioned above.

In other words, as the temperature drops, the solubility of hydrophilic polar HPh/ C_{60} DA-complexes in hydrophobic low-polarity OO decreases, causing the layering (separation) of chemical compounds available in C_{60} /OO into two phases.

In general, on the basis of common examples of experiments held on "Large Rats" with C_{60} /OO, we may assume that the more pronounced (definite) the layering effect is, the more that electron donor D-molecules (in our case – of HPh and water) contain the initial OO.

4. In view of the above mentioned information, we come to the most essential point, which is the explanation of the true cause of the positive effects of C_{60} (whether they are only for C60?) revealed to date when applying C_{60}/OO in "Small and Large Rats" (see the example here: http://www.longecity.org/forum/topic/58783-poll-quantifying-c60oo-data/page_mode_show).

The main point is that the polar DA complexes, particularly HPh/ C_{60} , are stable in non-aqueous and rather hydrophobic media, such as vegetable oils. But as soon as such <u>DA-complexes</u> get into an aqueous medium, they start to break down there at some rate (whether this was high or low was not been studied in detail) under the influence of water molecules. In a scientific sense, this means that hydrolysis processes will take place due to the interaction of polar DA-complexes of C_{60} with polar water molecules. This will eventually result in the decomposition of these DA-complexes into the their separate hydrated components. These components are denoted here as "HPh/H₂O" and " C_{60} /H₂O".

In general and in this particular case too, this will result in the formation of strong, highly hydrophilic hydrated C_{60} fullerenes (C_{60} HyFn = C_{60} @{H₂O}n, http://www.longecity.org/forum/topic/57382-hydrated-fullerene-hyfn), and, possibly, of various hydrated forms of nanoparticles/nanoaggregates of C_{60} = (C_{60})_m@(H₂O)_n. In these formulas, the symbol "@" shall mean that the m molecules of C_{60} are enclosed in a shell consisting of n molecules of H₂O (water).

5. Based on the results of research carried out by the Japanese scientists, we can assume that, regardless of the type of oil (Oil = OO, CO, FO etc.) used to prepare various samples of C_{60} /Oil and upon feeding them to rats, almost all the oil quantity is quickly absorbed in the gastrointestinal tract. As a result, all the black C_{60} non-absorbed (digested) residues on its (GIT) walls as some amorphous mass, while no (!) portion of the C_{60} from the amount introduced is absorbed by the "small rats"!

In other words, in actually appears that the major lipid and hydrophobic components of oils (triglycerides) are a very poor and ineffective means for transporting fullerene C_{60} through the walls of the GIT and delivering it to a living organism's other organs and tissues.

However, upon feeding rats with C_{60}/OO for 7 days (daily dose of C_{60} equal to 4 mg/kg), the French researchers managed to discover fullerene C_{60} in the animals' various organs (blood, liver, spleen, brain), although the doses were very low. These doses comprised 0.003% and 0.007% of the total accumulated dose (TAD) of C_{60} administered to to the "small rats" on the first and eighth day respectively. In conversion, the average dose corresponds to one a hundred-thousandth (1:100000 or 10^{-5} , or 10 ppm) dose of C_{60} TAD.

There is also an important fact proving that many samples of OO contain HPh in quantities up to 10 ppm (http://www.oliveoilsource.com/page/chemical-characteristics).

In other words, we can observe an interesting coincidence: compared to the TAD, the portion of C_{60} that was adopted by rats is the same as the concentration of HPh in OO!

This coincidence gives rise to a rather reasonable question: what was the vehicle for distributing C_{60} within the body if the lipid components of OO are poor candidates for such a role?

There is only one answer: it is the HPh of olive oil, which, as mentioned before, is able to form polar DA-complexes with C_{60} (HPh/ C_{60}).

However, when such HPh/C60 appears in the body and then quite certain biological effects appear, what was the real factor causing these effects?

This factor is simple, as you can see.

Regardless of where HPh/C_{60} happened to appear (whether they deposited on the surface of the GIT or entered its cells and intracellular space), all of them primarily interact with water, and there is always enough of it!

We shall also not forget that the rats, taking C_{60} /OO periodically, drank water regularly and that their GIT and any residues on its walls left upon after absorption of OO, were constantly wetted (hydrated) with water.

In general and as mentioned before, HPh/ C_{60} , when in contact with water – the dominating formation of hydrated fullerenes (C_{60} HyFn or, generally, C_{60} /H₂O) – is accompanied by displaying of a wide range of positive biological effects that have been revealed and confirmed in numerous tests of aqueous solutions of C_{60} HyFn (C_{60} FWS)

(see the examples provided at the following links:

http://www.longecity.org/forum/topic/57382-hydrated-fullerene-hyfn/

http://www.longecity.org/forum/topic/59259-baati-rats-autopsied-no-cancer/

http://www.ipacom.com/index.php/en/publications-about-c60hyfn/70

http://www.ipacom.com/index.php/en/fullerenes-and-water-left/74

http://www.ipacom.com/index.php/en/production-left/68

http://www.ipacom.com/images/Articles/annotation_en.pdf).

These links are not provided for advertising purposes and their $C_{60}FWS$. They are provided in order to enable users of this forum to see the amazing similarity of the positive biological effects that have been scientifically determined in the case of $C_{60}HyFn$ and which, in the case of C_{60}/OO , have been determined and summarized by enthusiastic volunteers, or "Large Rats" in the "C60health" forum (see, particularly:

http://www.longecity.org/forum/topic/58783-poll-quantifying-c60oo-data/page mode show and

http://c60antiaging.com/health-benefits-of-c60/buckyballs-the-secret-behind-centuries-old-health-spa/ (the Section "Health benefits of C60 in humans")

Since, in this post, I have discussed the question about hydrated C_{60} fullerene, it follows from the description presented on the IPAC LLC website, the physics-chemical and biological properties of C_{60} HyFn, as well as a wide range of its positive biological activity, are determined not by the C_{60} molecule itself, but relate to the unique properties of special extended water structures that C_{60} HyFn creates around and supports for an indefinite period of time.

In other words, the C_{60} molecule is only structure-organising principle (source). It is biologically inert and it does not get immediately involved in any biochemical reactions, including reactions with free radicals. This being said, however, C_{60} HyFn exceeds any known antioxidants in terms of its antioxidant effect by hundreds or even thousands of times. In general, C_{60} HyFn is a catalyst of the recombination ("self-neutralisation") of free radicals in aqueous media. This is what differentiates it from the behaviour of the C_{60} molecule in the medium of non-polar organic solvents, in which C_{60} , as is supposed, operates as a kind of "sponge" for trapping free radicals. In the aqueous medium, it's completely different!

See the following links to obtain information on the relevant mechanisms:

http://dx.doi.org/10.1016/j.freeradbiomed.2009.06.016

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http://api.ning.com/files/SX8ermG9424lhdvFkeQcWoojpoYy-

<u>3icjwBcTJcYVN88FF4uhrWq7fO9GK7WHeymsW7tRjTfFmFwnbsPS9Xj5Ibp1v6K4sym/FRBMfulltext 1.pdf</u>, as well as in the scientific publications and reports by IPAC LLC.

By the way, the above-mentioned article on the mechanisms of the antioxidant and radio-protective effect of C_{60} HyFn, which is also cited in the Baati at al article, may explain the strange fact that the French and Japanese researchers were unable to detect any products of C_{60} 's chemical interaction with free radicals, as is usually common for typical antioxidants. They also found no so-called C_{60} metabolites with other biological molecules, which usually come into chemical interaction with allogenic (foreign) molecules in order to dispose (deallocate) of them.

In their earlier studies, F. Moussa et al. detected metabolites of C_{60} with endogenous vitamin A (then, actually, they used micronised C_{60} in an aqueous mixture of detergent and cellulose derivative – http://dx.doi.org/10.1021/nl051866b). However, no metabolites were found in their experiments with C_{60}/OO , as are being discussed here. Why is this the case?

That is the reason that the properties of C_{60} HyFn itself were seen as the cause of all the biological effects they detected here, but not the properties of the very C_{60} molecule dissolved in OO!

First and foremost, this can be explained due to the numerous common physicochemical properties of C_{60} HyFn (http://www.ipacom.com/index.php/ru/published-works/71), which prove the impossibility of the C_{60} molecule, as part of C_{60} HyFn, entering into chemical reactions with other molecules and free radicals under normal conditions. The reason for that is the strong hydration (water) shell of C_{60} , which no molecules or free radicals are able to "break through" in order to form new chemical derivatives of C_{60} .

The only conclusion that can be derived from the above information is that the causes of the positive biological effects, as revealed in the work by Baati at al and by our "Large Rats", are determined (all of them) either due to the properties of C_{60} HyFn or the processes of its formation in the body through the hydrolysis of HPh/ C_{60} complexes.

In such a case, we can assume that, upon the "Large and Small Rats" taking the C_{60} /OO, the anticipated and detected biological effects, as well as their intensity, will depend significantly upon the content of HPh in OO (by the way, the more HPh there is, the thicker the green colour of the oil).

But then it is hard to escape from making a simple conclusion: in order to discuss and make some interim and objective conclusions as to the biological effect of C₆₀ dissolved in some OO, it would be ideal if everyone used the same standardised sample (or grade) of OO (see the example on http://olivecenter.ucdavis.edu/, "Imported Extra Virgin olive oil often fails international and USDA standards"

events/news/files/olive%20oil%20final%20071410%20.pdf).

But that's not the whole point since questions about the standardisation of processes for obtaining C_{60}/OO remain, and there are many of them.

To put it bluntly, problems exist now and they will undoubtedly emerge in the future as well!

To conclude this long monologue, let's return to the question of extending life span.

In particular, the question is as follows: should we seek to extend the life span in any way (for example, to affect the telomeres or take heavy doses of certain antioxidants in attempts to saturate our mitochondria with them) and then, having extended it, turn into a "plant"? Or, if you seek to extend your life span, is it not correct to start it early in life, and to maintain and improve the quality of our everyday life? Let's hope that this fullerene C_{60} did not appear in our life accidentally and that, being a unique symbiosis of carbon and water, C_{60} HyFn can really help to improve our lives!

GVA 12/26/2012

P.S.

- 1. By no means getting into scientific detail, let's note that, in accordance with their chemical nature, the DA-complexes of C_{60} with polyphenols (compounds of "C-O" types) are less stable than the complexes of C_{60} with porphyrins (compounds of "C-N" types). Hence, the intensity of the biological effects of C_{60} /OO shall be determined according to the availability of porphyrin compounds in the oil.
- 2. Let's indicate that porphyrin chlorophylls are compounds produced by plants but not by fish. Thus, as noted in the forum (http://www.longecity.org/forum/topic/57619-c60-in-fish-oil/, e.g. "zorba990" posted on August 01, 2012 about "C60 in Fish Oil", http://www.ncbi.nlm.nih.gov/pubmed/20621447), the experiments involving mixtures of fish oil (FO) with C_{60} are unreasonable and cannot reveal any of fullerene C_{60} 's additional positive effects upon the organism (!) when ingesting it as a mixture with various FO. Similar mixtures can be reasonably studied in terms of protecting the lipid components of fish oil against oxygenation and deterioration (racidification) due to the antioxidant properties of C_{60} (it serves a kind of "sponge" for free radicals, but not in non-aqueous media)).
- 3. Upon analysing the data received in the process of working with Baati et al. and considering the invaluable experience being reported with respect to the "Large Rats" in the <u>LONGECITY</u> \rightarrow <u>C60health</u> forum, I can note that, in order to achieve positive biological effects that are similar in their nature and intensity (including that pertaining to antioxidants), as revealed in the application of C_{60} HyFn (as C_{60} FWS, see the example at: http://www.ipacom.com/images/Articles/annotation_en.pdf), it is necessary to apply doses of C_{60} (in form of C_{60} /OO) that are approximately one million (10^6) times higher than the doses of C_{60} HyFn!