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Commentary

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Comments on "Diverse Radiofrequency Sensitivity and Radiofrequency Effects of Mobile or Cordless Phone near Fields Exposure in Drosophila Melanogaster"

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Abstract

The paper by Geronikolou, et al. (2014) "Diverse Radiofrequency Sensitivity and Radiofrequency Effects of Mobile or Cordless Phone near Fields Exposure in Drosophila melanogaster" [1] published in Plos One supposedly presents original work on the effects of mobile and cordless phones electromagnetic fields (EMFs) on Drosophila melanogaster reproduction. The paper reports that two of its authors "conceived and designed the experiments". This is not the case. The paper is a replication of the experimental procedures introduced by Panagopoulos, et al. (2004) "Effect of GSM 900-MHz Mobile Phone Radiation on the Reproductive Capacity of Drosophila melanogaster" [2], and applied since then in many publications (Panagopoulos, et al. 2007a; b; 2010; 2013; Panagopoulos, 2016; 2017; 2019) [3-5,15,16,20,21]. Geronikolou, et al. followed the same experimental methodology without reporting replication or even citing the original study. Then, they differentiated on secondary points - employing a different statistical method, calculating theoretically the near-field instead of measuring it, not sham-exposing the control groups, and including experiments with cordless phones based on the same procedures - which led them to serious flaws and misleading conclusions. Our present commentary is a necessary action to protect authorship and restore science in regards to experiments with mobile and cordless phones.

Keywords

Electromagnetic fields, Mobile phone radiation, Drosophila melanogaster, Reproduction

Introduction

We recently saw a paper published in Plos One titled "Diverse Radiofrequency Sensitivity and Radiofrequency Effects of Mobile or Cordless Phone near Fields Exposure in Drosophila melanogaster" by Geronikolou, et al. (2014) [1]. This paper is a replication of the main experimental methodology of Panagopoulos, et al. (2004) [2] with differentiation and certain additions on secondary points. Instead of reporting that which could be a reason for publication, Geronikolou, et al. (2014) [1] reported in the "Author Contributions" that two of them "conceived and designed the experiments".

They differentiated from Panagopoulos, et al. (2004) [2] by doing minor changes in the procedure and by rephrasing the text. Moreover they claimed they found "errors" in previous studies on the effects of microwave Electromagnetic Fields (EMFs) on Drosophila reproduction, including another study of ours [3] based on the same methods introduced by [2]. They claimed they "overcame systematic errors", by using a different statistical method to analyse the results, and by calculating theoretically the near-field intensity of the exposure device instead of measuring it with field meters. Thus, not only they replicated our experiments without reporting that, but in addition they tried to downgrade certain parts of our methodology.

Panagopoulos, et al. (2004) [2] was the first peer-reviewed study published in an international scientific journal reporting a dramatic effect of real-life Mobile Telephony (MT)

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EMFs emitted by commercially available mobile phones on animal reproduction. The experiments were performed with fruit flies and recorded an up to 60% decrease in reproductive capacity due to a single 6-min daily exposure to an active mobile phone in "talk" mode for 2-5 days. Later we found that this dramatic decrease in fecundity was due to DNA fragmentation in the reproductive cells induced by the mobile phone EMF-exposure [4-7]. Today similar effects on reproduction and DNA have been confirmed by numerous peer-reviewed published studies.

Panagopoulos, et al. (2004) [2] introduced original methods in handling and exposing the fruit flies, and in assessing their reproductive capacity/fecundity by the number of F_1 pupae (chrysalides) which under specific conditions coincides with the number of laid fertilized eggs (oviposition). In this way errors were minimized and procedures of fecundity assessment became considerably simpler. Before, fecundity was assessed by counting the number of laid eggs under a stereo-microscope [8-13]. This was subjected to large errors in the counting - since one cannot mark which eggs are already counted from tens or hundreds of eggs usually laid one upon another and within the mass of the food - and moreover one could not tell whether a laid egg was fertilized or not (non-fertilized eggs do not develop). By keeping the males and females of each group in separate vials for the first 48 h of adult lives, and placing them together for the next 72 h while both males and females are sexually mature and still young, we ensured: a) That all laid eggs were fertilized, and b) Zero mortality of eggs and larvae, meaning that all embryos (laid fertilized eggs) developed into pupae, in contrast to embryos from older insects which display significant mortality. In this way, the number of F_1 pupae coincides with the number of laid eggs, and thus, instead of counting eggs under the microscope, we counted pupae (six days after the completion of the 72 h mating period) which can be seen with bare eyes immobilized on the walls of the glass vials (and checked with a marker during counting). Thus the assessment of reproductive capacity/oviposition became much easier, faster, and with no error at all. By introducing these simple innovations - after detailed study of the insect's development - we improved significantly the procedures for fruit fly fecundity assessment. This study also introduced the use of a commercially available mobile phone handset as the exposure device, which is now widely accepted as the only realistic exposure methodology to assess the biological effects of real-life EMFs emitted by mobile phones and other telecommunication devices [14-17]. For the above reasons, Panagopoulos, et al. (2004) [2] is a widely recognized study, never challenged, and cited up to today by more than 120 other published studies. We had worked for years to conceive and design these methods, and we had already presented results [18,19]. Following this, we published numerous studies that utilized and extended the above experimental procedures to investigate the effects of different types of EMFs on Drosophila melanogaster reproduction and ovarian cells [3-7,15,16,20,21].

Unreported Replication of the Main Experimental Methodology

In page 2, right column, 1st paragraph ("Experimental Procedure") Geronikolou, et al. (2014) [1] write: "Each experiment included a collection of newly emerged flies from the stock. The newly emerged insects were anaesthetised with ether and separated under stereoscopic microscopy (Carl Zeiss 4773117) into sex groups. Male and female insects were placed in different glass vials with food at 25 °C. Each vial was then exposed continuously for 20 min every day for two days, until insects were sexually mature. The mature insects were anaesthetised again and placed in new glass vials with food; each vial contained 8 male and 8 female insects. These new cultures were exposed for the same time period for three more days. Six days after the last day of radiation we measured the number of chrysalides on the vial wall"… and in the 3rd paragraph they write "We kept the field characteristics same as in typical use during speaking and we have placed the phone device in contact with the glass vial during the experimenting time".

The corresponding parts of the procedure in Panagopoulos, et al. (2004) [2] read: "In each experiment, we collected newly emerged adult flies from the stock; we anesthetized them very lightly with diethyl ether and separated males from females". "In each group we kept the 10 males and the 10 females for the first 48 hr of the experiment in separate glass tubes". "Keeping males separately from females for the first 48 h of the experiment ensures that the flies are in complete sexual maturity and ready for immediate mating and laying of fertilized eggs. After the first 48 h of each experiment, the flies were anesthetized very lightly again and males and females of each group were put together (10 pairs) in another glass tube with fresh food and allowed to mate and lay eggs for 72 hr". "After 5 days from the beginning of each experiment in all three sets of experiments, the flies were removed from the glass vials and the vials were maintained in the culture room for 6 additional days, without further exposure. After the last 6 days, most F_1 embryos (deriving from the laid eggs) are in the stage of pupation, where they can be clearly seen with bare eyes and easily counted on the walls of the glass tubes". "We exposed the flies within the glass vials by placing the antenna of the mobile phone outside of the vials, in contact with the glass wall and parallel to the vial's axis". "The experimenter could speak on the mobile phone during connection (this we called ''modulated'' or ''speaking'' emission)"… "to simulate the actual conditions to which a user is subjected while speaking".

As it is evident (in spite of rephrasing the text), Geronikolou, et al. (2014) [1] followed exactly the same experimental procedures as Panagopoulos, et al. (2004) [2], except that they had 8 males plus 8 females in each group (instead of 10 plus 10), and they exposed them to the EMF for 20 min daily (instead of 6 min). These are secondary changes that do not provide originality. Thus, Geronikolou, et al. (2014) replicated Panagopoulos, et al. (2004) experimental methodology and their declaration that they "conceived and designed the experiments" is not true.

In page 2, right column, end of 1st paragraph, Geronikolou, et al. (2014) [1] made confusing statements: "Three days later we counted the newly emerged insects as introduced by Panagopoulos, et al. (2004) [18]", and gave an irrelevant ref-

erence regarding stroke epidemiology (!), while Panagopoulos, et al. (2004) [2] was not included in the reference list. Plos One issued a "Correction" and included the reference after we sent a letter, but responded that they do not publish comments [22; personal communication with Plos One]. But according to their previous statements and their results they did not count the newly emerged insects (flies), but the pupae (as in [2]). In their next sentence they write: "The newly emerged flies (pupae) encounter low mortality during their transformation risk in larvae [23]". It is thus evident that they confuse newly emerged flies with pupae which are totally different developmental stages, and gave another irrelevant reference ([23] in their paper) regarding the development of the nucleolus of the ovarian nurse cell (!). Even with the addition of the reference issued by the Plos One "Correction" [22], they cite Panagopoulos, et al. (2004) [2] only for the pupae counting, whereas the whole experimental procedure is replicated without any citation.

Differentiations and Flaws

Geronikolou, et al. (2014) [1] differentiated from Panagopoulos, et al. (2004) [2] methodology in the following points which led them to serious flaws:

1. They criticized the use of the Analysis of Variance statistical test by Panagopoulos, et al. and others, as a "systematic error" (page 2, left column, "In addition, all of them arbitrarily presumed that the statistical distributions of the egg laying were normal."). Analysis of Variance is one of the most common statistical methods especially in Drosophila reproduction studies [8-12,23]. This method assumes that the experimental counts - in this case the average number of F_1 pupae per maternal fly - follow the Normal (Gaussian) distribution around the mean. This is a most reasonable assumption under well-controlled conditions for the specific animal. Oviposition counts are evenly/"normally" distributed around a mean value which can be controlled by laboratory conditions (temperature, humidity, light, food, etc.) [9,13,24,25]. Moreover the method is still robust with moderate deviations from the Normal distribution [26,27]. Thus this is by no means a "systematic error".

2. They criticised the mobile phone near-field intensity measurements and instead they suggested theoretical calculation of the near-field (pages 2-4). Intensity measurements in the near-field especially in microwave telecommunication including MT antennas may indeed include significant error due to increased variability and even possible capacitive coupling between the antenna and the sensor of the field meter. The error can be effectively minimized by increasing the number of measurements and reporting average intensity and standard deviation (SD), and even by excluding certain unrealistically high measurements which could be possibly attributed to capacitive coupling [28]. This provides a representative estimation of the field. "Accurate" estimation of the intensity of MT EMFs, especially in the near-field, has no meaning as they are highly varying any moment, due to the varying information they transmit and other reasons [14-16,28]. Similarly, "calculating" accurately the near-field of modern telecommunication devices theoretically is actually impossible and introduces an even larger error since the parameters of the fields in the applied formulas are equally variable as in the measurements and in addition the formulas themselves are simplified. For example, in order to calculate the field Geronikolou, et al. (2014) [1] assumed "cylindrical flow of the power output" within an angle θ corresponding to the antenna radiation lobe $50° \leq \theta \leq 80°$ (thus they assumed that the emitted power is evenly distributed each moment upon a cylindrical surface within the angle θ), and that the antenna emits constantly with maximum power (2 W or 0.25 W for the mobile or the cordless phone respectively) within this lobe (page 3, left column). Both assumptions oversimplify reality. Such simplifications in combination with the high variability of the signal introduce much larger error than when the field is measured and averaged by a scientist/engineer experienced in such measurements. Important reason why such formulas are oversimplified especially in the case of MT antennas is that they do not take into account variations due to signal reception, number of subscribers sharing the frequency band each moment, air conductivity, location in relation to base antennas, presence of objects and metallic surfaces, "speaking" versus "non-speaking" mode, etc. Such variations may exceed ± 100% of the average signal intensity [28]. Finally, calculating the emission theoretically is impractical. For all the above and more reasons it is established to measure EMF-emissions from MT antennas by field meters/spectrum analysers even with approximation.

Although they presented simplified formulas for the calculation of the near-field intensity, they did not provide the results of their "calculations" but instead they provided "three dimensional illustrations" of the power density without units and without explanations. Interestingly, they admit that near-field measurements are necessary (page 4, left column, first paragraph). What is then the meaning of suggesting theoretical calculation instead of measurements, and presenting this as a "correction" in Panagopoulos, et al. dosimetry?

3. They did not sham-expose the control animals as in Panagopoulos, et al. but kept them "away from any electromagnetic source under the same room and temperature conditions" (page 2 right column, end of 3rd paragraph). They do not clarify whether this took place during the exposures or at a different time. a) Having the controls within the same room during the exposures even at a few meters distance they also get exposed in some degree which the authors of Geronikolou, et al. (2014) [1] did not measure (neither calculated). b) Even if they did that at a different time or at a different room, all rooms in any laboratory are exposed to stray 50 Hz Extremely Low Frequency (ELF) EMFs from devices and electric power lines within the walls which the authors of [1] also did not measure (or calculated). If these stray fields were stronger at this location than at the location of exposure, this would induce a decrease in reproduction in the control groups which would result in a smaller (and statistically weaker) difference from the exposed groups [20]. Thus, they did not ensure that the control animals were in identical conditions with the exposed ones apart from the mobile/cordless phone exposure. The only way they could do this was to sham-expose the control animals with a turned-off handset at exactly the same location of the lab and for the same

duration of time as the exposures, in order to take also into account any possible stress on the animals due to the view of the exposure devices outside the vials or even the voice of the experimenter while doing the exposures in "talk" mode (as in Panagopoulos, et al. studies).

This additional flaw in Geronikolou, et al. (2014) [1] can explain the weaker effects they found than in Panagopoulos, et al. studies in spite of their significantly longer daily exposure duration (20 min instead of 6), and the same (interestingly identical) average count in their control groups as in the sham-exposed of Panagopoulos, et al. (2004) [2], although they enriched the food (of identical other composition as in Panagopoulos, et al.) by adding diluted yeast on its surface which can increase oviposition by up to 300% [9] while Panagopoulos, et al. did not.

4. They performed experiments with GSM 900 MHz (Global System for Mobile telecommunications) mobile phone exposure (as in our studies), and with DECT (Digital Enhanced Cordless Telecommunications) cordless phone exposure which emits a different EMF (1880 MHz, with different intensity, ELF pulsing, and modulation). [Experiments with cordless phone EMFs using the specific fruit fly experimental protocols were also already published by a coauthor of ours [29] but even if they were not, Geronikolou, et al. (2014) [1] should report that they applied the experimental protocols introduced by Panagopoulos, et al. (2004) [2] to test the bioactivity of DECT phones]. In both cases they found a weaker effect than in Panagopoulos, et al. studies with mobile phones, but with the cordless phone they found a weaker and statistically less significant effect ($P = 0.0445$) than with the mobile phone ($P = 0.0090$). Then they interpreted the weaker effect with the cordless phone as questioning our attribution of the effect with mobile phone to its EMF in two other studies of ours [3,30] (page 5, left column, last paragraph, "Unlike other research studies in literature [6,16]"). But since the effect they reported with either mobile or cordless phone is in both cases statistically significant ($P < 0.05$) by such a statement they question their own findings. From the two studies of ours that they cite in regard to this, the one [30] describes a widely acknowledged and cited biophysical mechanism for the action of EMFs on cells, and the other [3] applies the same experimental protocols as in Panagopoulos, et al. (2004) [2] to compare the effect on fruit fly reproduction between GSM 900 MHz and GSM 1800 MHz mobile phone exposure. Thus, the reference to these Panagopoulos, et al. studies is irrelevant to cordless (DECT) phone exposure.

An obvious reason why they did not find a statistically stronger effect with cordless phone exposure (apart from the fact that they did not sham-expose the controls) is the different and weaker EMF emitted by the cordless phone than that of the mobile phone. Indeed the cordless phone EMF had a significantly smaller max power (0.25 W) than the GSM 900 mobile phone (2 W), a higher carrier frequency (1880 MHz instead of 900 MHz), a different pulsing frequency (100 Hz instead of 217 Hz and other ELF frequencies), etc. The difference in power/intensity alone is enough to explain a weaker effect, while the difference in pulsing frequency is also very important [16].

5. Even though Geronikolou, et al. (2014) [1] did not think of the above obvious explanation for the weaker effect of the cordless phone, they provided an arbitrary explanation of "diverse radiofrequency sensitivity i.e. due to genetic factors" (page 5, left column, last paragraph) which they claimed it was "validated" by their results (!) "that bring to light that lower frequency (900 MHz herein) is a more intense stress factor than 1880 MHz". But the observation that lower frequency EMFs are more bioactive, was shown by us experimentally between GSM 900 MHz and GSM 1800 MHz under equal intensities and other factors e.g. ELF pulsing, modulation etc. [3,4], and it is also predicted theoretically by our above mentioned biophysical mechanism [30] which finds that bioactivity is inversely proportional to frequency. Again the authors of [1] not only they did not cite our studies and presented this as their own explanation, but they arbitrarily attributed the weaker effect to the higher carrier frequency (1880 MHz) of the DECT EMF without considering the differences in power/intensity, pulsing, etc.

6. Additional parts of the text of Geronikolou, et al. (2014) [1] rephrased from Panagopoulos, et al. (2004) [2], easily found by simple comparison between the two papers, are in the description of previous studies (in the Introductions of the two papers), the description of the vials used in the experiments, the food description and the food amount in each vial ("Food and culture"). Additional flaws are the many irrelevant statements throughout their paper such as downgrading the epidemiological and environmental studies altogether ("most of the epidemiological studies were retrospective and were subjected to biased scientific criteria"-page 1, right column), or confusing statements like "the food had been prepared and thickened in room temperature conditions ", or "the glass vials with food were kept at 40 °C" (page 2, "Food and culture"). [How was it prepared in room temperature when it was boiled? And shouldn't perhaps be kept at 4 °C instead of 40 °C? (This was also corrected in the Plos One "Correction" after our letter to the journal)]. They also have terminology mistakes, such as "DNA linkage" (instead of "DNA damage") (page 1), or "Specific Absorbance rate" (instead of "Specific Absorption Rate") (page 4, Discussion) and throughout the paper they provide irrelevant references with irrelevant content.

Discussion and Conclusion

It follows that the Geronikolou, et al. paper titled "Diverse Radiofrequency Sensitivity and Radiofrequency Effects of Mobile or Cordless Phone near Fields Exposure in Drosophila melanogaster" [1] is a replication of Panagopoulos, et al. "Effect of GSM 900-MHz Mobile Phone Radiation on the Reproductive Capacity of Drosophila melanogaster" [2], with certain differentiations on secondary points. This is obvious by simple comparison of the corresponding parts of the text between the two papers, especially the parts referring to the main experimental procedure. They also included experiments with a DECT phone, apart from the GSM 900 phone, based on the same methodology which was also not novel as it was already published [29]. Again Geronikolou, et al. did not report that their experiments with DECT phone were

based on the methodology introduced by [2].

We showed that the points in which Geronikolou, et al. (2014) [1] differentiated from Panagopoulos, et al. (2004) [2] led them to serious flaws and misleading "conclusions" such as 1) Characterizing "systematic error" the use of Analysis of Variance statistical test, 2) Suggesting theoretical calculation of the antenna near-field instead of measuring it with field meters, 3) Not sham-exposing their control groups in the experiments, and 4) Arbitrarily attributing the weaker effect of cordless phone to its higher frequency alone, without considering the lower power/intensity and differences in pulsing, etc. from the mobile phone EMF.

In spite of all the above serious issues, Geronikolou, et al. (2014) [1] claimed that they "overcame systematic errors" in previous published fruit fly studies…. "The aim of our study was to investigate the effect of the 900 MHz and 1880 MHz near fields electromagnetic emission on Drosophila melanogaster oviposition in a way to overcome the above mentioned systematic errors" (!) (end of Introduction, page 2). Actually, as explained above, every single point on which they differentiated from Panagopoulos, et al. led them to serious/fatal flaws.

Thus, Geronikolou, et al. (2014) [1] paper not only did not add anything new to the EMF-bioeffects literature to justify publication as original paper, but in addition it is full of serious flaws and misleading "conclusions". If Geronikolou, et al. had not differentiated, their paper could be of importance as a replication study of Panagopoulos, et al. (2004) [2] with the additional application of the DECT exposure. Unfortunately, instead of doing this, they claimed that two of them "conceived and designed the experiments", and tried to downgrade certain parts of our methodology by discovering "errors".

A basic principle in scientific (and any) publications is acknowledgement of previous findings, and proper citation of the corresponding studies. This is why all science journals proclaim that they check for plagiarism. Replicating previous studies is important and justifies publication when this is clearly reported. Unfortunately that was not the case with the Geronikolou, et al. (2014) [1] paper.

The editor of the Plos One journal who handled the Geronikolou, et al. (2014) [1] paper bears great responsibility and should insist that its authors report replication and provide proper citations, especially when the reviewers had noted the issue (personal communication). Moreover should have recognized the many flaws of the paper and ask for extensive revisions. Finally, the Plos One journal should publish Comments on its published papers alike every other journal, providing the scientific community with the opportunity of challenging a peer-reviewed published paper by a peer-reviewed commentary.

Declarations of Interest

The authors report no conflicts of interest.

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