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Rapporteurs' Report

WORKSHOP

"Application of Proteomics and Transcriptomics in EMF Research" October 30 - November 1, 2005

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Helsinki, Finland
www.stuk.fi



This report has been prepared by Workshop Chairman and Rapporteur Dariusz Leszczynski, and by Workshop Rapporteur Martin L. Meltz. The report is not a comprehensive summary or review of the Workshop and the presentations of the speakers. These presentations can be found both on the WHO EMF Project and Cost 281 Web Sites, along with a list of sponsors and meeting attendees.



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This is a summary of the comments made by the Workshop participants, in response to a series of questions simultaneously discussed by three breakout groups at the end of the second day of the workshop. Each of the groups included at least one expert on genomics and one on proteomics, and an expert on metabolomics participated with one of the groups. The workshop attendees were assigned randomly to each of the groups. After the allotted time, the notes from each group were presented on screen. On the morning of third day of the workshop, all of the attendees were presented with a summary of the notes, and were given the opportunity to discuss each item together. The attendees modified the items collectively. Changes were made in a few of the initial questions, which had been prepared in advance by the Rapporteurs. After the meeting, the Rapporteurs prepared a draft summary, which was distributed to all attendees for comment. The Rapporteurs take responsibility for the final revised version, prepared after all comments were received.



1st Question: There is an uncertainty about the biological effects of EMF exposures. High throughput screening technologies (HTST) have their own technical limitations and uncertainties. What are the possible benefits of using HTST methods in the study of EMF bioeffects?

- The ultimate consensus of the assembly was that a research approach that employs HTST is useful for scientific investigation of EMF effects, but that it is not yet ready, and should not be used, for health risk assessment.
- A HTST approach is especially suitable to look for biological effects of stimuli, like EMF, where a biophysical mechanism of interaction with living matter is unknown, and therefore where the exerted biological effects might be difficult to predict.
- The advantages of employing HTST in EMF research include:
 - A high efficiency of detecting changes - A large number of changes can be detected in a single experiment.
 - A large number of changes can be followed simultaneously for defined biological, kinetic, and exposure changes.
 - New information will allow the generation of new targeted hypotheses.
 - The identification of response genes and proteins will provide a "critical mass of knowledge" that will help in defining the mechanism of any biological response observed.
 - The information will enable the discovery of "biological targets" that are otherwise unpredictable, based on the present status of the knowledge of EMF bioeffects.
 - Any HTST-discovered responder genes and proteins, after subsequent experimental confirmation that they lead to cellular or physiological changes, might become potential end-points for animal, human volunteer and epidemiological investigations.
 - The application of the HTSTs will make it clear to the public, scientific and regulatory communities that scientists in the field are applying the most sophisticated investigative tools available to detect even subtle bioeffects of EMF exposures.

2nd Question: Which of the HTSTs (transcriptomics, proteomics, metabolomics) are the most suitable for studying the possible effects of EMF exposures?

- There was a consensus among the workshop participants that:
 - The use of HTSTs in EMF research should include transcriptomics, proteomics, and metabolomics.
 - There is no single HTST that would be more applicable than the others, and the use of a particular technology will depend upon the desired endpoint to be examined.
 - The simultaneous use of several HTST methods can help to validate the findings obtained with the other technologies.

3rd Question: What problems can be expected when using HTSTs in EMF research?

- There are no specific problems of HTST that would be pertinent particularly to EMF research. All of the known limitations of HTSTs, as they exist in other biological research studies, apply and should be considered in the EMF research.
- Large-scale screening (genome-wide; proteome-wide) vs. targeted screening (e.g. aimed at certain signaling pathway) is an issue. The targeted screening can be of considerable benefit (data analysis, cost of experiments) when used to examine changes initially discovered using the genome/proteome-wide screening techniques.

4th Question: What should be the recommended number of HTST experiment replications?

- The number of replicate experiments needed to validate a change depends (at least in part) on the magnitude of the change that is being validated.
- A single HTST screening experiment, followed by one or more other screening methods, can be used for validating a change. Any change observed in HTST analysis should be further confirmed by a cellular level or physiological test.
- Acceptance of the finding should not rely only on the size of change observed in HTST analysis.
- It is not possible to give a strict recommendation concerning the number of replicates in submitted/published manuscripts. Validation of changes using non-HTST methods and physiological tests should, however, be considered as necessary.

5th Question: What issues must be considered in relating genomic, transcriptomic, proteomic and/or metabolomic changes in EMF research?

- There are no specific problems of HTST that would be pertinent particularly to EMF research. All of the known, from other biological studies, limitations of HTST apply and should be considered in the EMF research.
- While metabolomics can be of limited guidance for determining when to collect samples for genomic and proteomic assays post-exposure, it can be informative of its own accord.

6th Question: How can the data obtained with the use of HTSTs help in discovering the biophysical mechanism if a molecular or biological effect of EMF is substantiated?

- The results of EMF studies performed using HTST should be collected by establishing an EMF HTST database.
- Participating laboratories with access to the database can use the information to design new experiments that are hypothesis driven, and which employ a systematic design.
- HTST data can lead to the investigation of specific pathways. This information in turn can lead to a determination of the molecules and/or structures responsible for the initiation of any response.
- Localization and function of correlated responding genes and/or proteins might give clues about the potential biophysical mechanism. The more responding genes and proteins that are

experimentally determined, the higher will be the probability of detecting a "pattern" (if there is any "pattern" to be seen).

7th Question: EMF technologies are continuously being developed, and new EMF frequencies and modulations continue to be introduced. Are HTSTs suitable for evaluation of their effects (if a molecular or biological effect of EMF is substantiated)?

Evaluations could be done with e.g.:

- DNA chip/array with selected genes,
 - Protein array/chip with selected proteins,
 - Protein array/chip for screening of the activity of proteins,
 - Metabolomic approaches,
 - Combinations of the above?
- Yes, it is possible to develop standardized testing. However, the experimental conditions (exposure, cell type, mRNA/protein sample processing, data analysis, etc.) should be very precisely standardized and agreed upon between participating laboratories.
 - Whole-genome and whole-proteome HTST analysis studies are not currently sufficiently mature and cost efficient for common use in screening. However, changes in genes and/or proteins discovered initially in HTST experiments, followed by less costly targeted hypothesis-driven studies based on the initial HTST observations, might be considered as more efficient for current use.

8th Question: Is it possible to develop a standardized test for screening of the future EMF signals in order to compare their effects with the substantiated effects of EMF signals which are being already in use?

- Yes, it is possible. But such an approach would require agreement on the use of a limited number of HTST methods, and a limited number of biological models.
- The current benefit of HTSTs is in their use as research tools. However, the use of HTSTs as standardized screening tools should continue to be considered, and an effort should be made to practically develop these tools for screening.

9th Question: Should there be a coordinated effort to provide funding for well designed, meaningful, studies? If yes, then, who could be the coordinating entity?

- Yes, a coordinated effort would be appropriate.
- Any such effort should be truly international.
- Even under the present circumstances, where there is not sufficient substantiation of EMF-induced biological effects, it might be beneficial to have a few laboratories in different parts of the world funded to maintain the capability of examining newly emerging wireless technologies.
- There should be a substantial effort directed at the development of some selected HTST methodologies for the special purpose of use in the risk assessment.



- A multi-center world-wide HTST study, coordinated for example by EU, could be initiated to gather information for a to-be-established EMF HTST database.
- A coordination approach similar to that of the REFLEX program could be considered.

Final Remarks:

Based on the above, the following general approach to perform HTST studies to detect EMF effects should be considered:

- First - HTST screening should be performed after purposefully "over-exposing" the biological material to an EMF signal, providing the greatest probability of detecting all responding genes and/or proteins.
- This could be followed by targeted hypothesis driven experiments, to examine the wide range of variables with identified endpoint(s). These follow-up experiments could be less expensive and less technically demanding. They include both targeted HTST methods focused on certain pathways, as well as non-HTST methods.
- Subsequently perform experiments at lower EMF exposure levels, and shorter times, as a means to determine:
 - a) if there is an exposure threshold for a response,
 - b) if there is a definable dose-response,
 - c) if the response can only be observed at lower exposure levels, because the higher level exposure "shuts down" a lower exposure level response.
- Validation and confirmation of effect through collaborative effort - analysis of the same sample using the same method but in one or more different laboratories should be attempted whenever possible.

WHO interests stated at the conclusion of the workshop

- Establishment of a database to receive, and allow qualitative evaluation of new data
- WHO is interested in the inclusion of HTSTs in its research agenda*
- WHO is interested in basic research, particularly with the potential for future risk assessment

* EMF research using HTST has been incorporated in 2006 WHO Research Agenda

