ANNEX I - Basic Concepts in Clinical Epidemiology

The present Annex has the aim of providing a brief and basic explanation about the methods commonly used by epidemiological studies, in order to allow the non-specialized reader to better understanding how they are carried out, particularly in the field of research of electromagnetic radiation and its effects on human health (see Chapter II).

There are four main kinds of analytical epidemiological studies: case-control, cohort, case-cohort and cross-sectional. They can be **prospective** or **retrospective**, i.e. they can either analyze and compare subjects that were already exposed to the environmental agent (also called the *historical* approach), or collect longitudinal data as the study progresses (also called the *current* approach).

Cohort studies start with the exposure variable, and collect data from a selected, initially healthy, group within the population (the *cohort*) over a given period of time, who are known to be exposed to an agent. It aims to compare incidences of endpoints or outcomes, in subjects who were exposed (*index* subjects). with outcomes in subjects who were not exposed,. The measure of disease in cohort studies is the **incidence rate**, which is the proportion of subjects who develop the disease under study within a specified time period (the number of diseased subjects divided by the number of person-years of observation). Separate incidence rates are calculated for the exposed and non-exposed subjects and compared statistically. The measure of association between exposure and disease in cohort studies is the relative risk (RR). The **relative risk** is the ratio of the incidence rate of exposed to unexposed. . A RR of 1.0 means that the incidence rate is the same among exposed and non-exposed subjects and indicates a lack of association between exposure and disease. If it is less than 1, it means that the incidence rate of disease among the exposed is lower than non-exposed, whereas a RR above 1.0 indicates that exposed people are at higher risk of disease than non-exposed persons. The magnitude of the RR shows the strength of association between exposure and disease And the confidence interval shows a precision of the estimate. In summary, the fact that study participants are exposed continuously or not to the environmental agent is what defines to what he/she will be included.

Case-control studies have subjects with the target disease and compare two controls sampled from a population from which case arose., The purpose of the control group is to provide an estimate of the frequency and amount of exposure in subjects in the population without the disease being studied. So a case-control study is concerned with the frequency and amount of exposure in subjects with a specific disease (*cases)* and people without the disease (*controls*). No measure of disease incidence rate or risk ratios can be estimated, so measures of association, such as the **odds ratio**, are used instead. The odds ratio (OR) is generally a good estimate of the relative risk for rare diseases, and is obtained by the probability (*odds*) of exposure in disease subjects, divided by the probability of exposure in non-diseased subjects. *Matching* between the groups is done according to

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several criteria, such as gender, age,

Both approaches have their own methodological problems in terms of confounding variables, sources of bias, quantification of exposure, identification of effects, etc., which we will briefly discuss at the end of this section, so as to qualify the scientific relevance and power of evidence of such epidemiological studies.

Since these are essentially observational research methods, they are potentially subject to the effect of extraneous factors which may distort the findings. **Confounding variable** or factor refers then to an extraneous element that simultaneously is a risk factor for the disease being studied, and is associated with the exposure being studied but is not one of its consequences (Meirik. 2007). There are several ways of controlling, or adjusting for, confounding factors, **stratification** (i.e., the subdivision of groups according to presence/absence of these factors), **randomization** (which is hoped to distribute uniformly unkown confounding variables among groups) and **multivariate analysis** (which takes into account these variables in the statistical model). **Matching** strategies (in order to make subjects of both groups be the most similar possible for all known variables, except the study variables) is usually not recommended except for basic variables such as age and gender.

Bias, on the other hand, is any systematic error in the design, conduction, or analysis of a study that results in a mistaken estimate of an exposure's effect on the risk of disease. There are several sources of bias in epidemiological studies, such as selection bias, recall bias, reporting bias, proxy bias, etc.

Biases and confounding variables must be identified and understood as soon as possible in the observational design, and compensated for or post-adjusted, in order to avoid the distortion of statistical inferences that will inevitably arise and that can possibly invalidate, partially or totally, the findings of the study.

The decision whether to use cohort or case-control studies depend on many factors and is a complex one (Meirik, 2007). For putative RF-exposure induced or promoted disease, which is the main focus of epidemiological studies, cohort studies are to be preferred over case-control studies (Leitgeb, 2006), but they present many problems, such as the need for a large number of cases in rare diseases, unsuitability when there is a very long latency between exposure and disease manifestation, when there is change of exposure patterns along the collection of data, and the high rate of loss of follow-up. All this also make long term cohort studies very expensive.

Case-control studies are easier, faster and cheaper, can study rare diseases with long latencies, but also have their score of drawbacks for RF exposure studies: they have a high recall and proxy biases, validation of past exposure is many times difficult or impossible, and selection bias is common.

Finally, the induction of causal relationships from epidemiological statistical studies implies a number of requirements (Hill, 1969). They are nine:

1. strength of association;
2. intra- and inter-studies consistency;
3. specificity of the association;

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4. temporality (precession of cause in relation to effect);
5. existence of a dose-response relationship;
6. biological, physical and chemical plausibility;
7. coherence with biological knowledge;
8. consistent support from experiments;
9. and analogy to other similar, discovered cause-effect relationships.

Hill noted that "*none of my nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required as a sine qua non*".

ANNEX II: Basic Concepts in the Design of Experimental Studies

Differently from epidemiological studies, which do not manipulate independent variables in a controlled manner, experimental studies in vitro and in vivo approaches, including experiments with humans (so-called provocation studies), have the goal of testing cause-effect relationships. Their methods are more straightforward and more robust in relation to biases and other issues.

The most frequent experimental designs used in such studies are:

Self-control designs: in these experiments, a baseline of the dependent variable(s) is recorded for some time under normal conditions, with all subjects in the same standard situation. Exposure to RF is then applied, also for some time, and the dependent variables are collected again during and/or after the exposure, and compared with the baseline. Thus, subjects are their own controls, facilitating the statistical, pair-wise comparison or pre- and post-irradiation comparisons. This kind of design provides low-strength evidence, because other confounding or intervening variables may be acting simultaneously with irradiation, experimenter and subject biases, or a pos-hoc influence may be operating and are hard to detect and to avoid.

Controlled designs: in these studies, a better strength of evidence is achieved by adding a control group, as similar as possible to the experimental group, with the exception that it is subjected to a sham, or fictitious RF irradiation. The statistical power and strength of evidence of such studies are much better than self-control, but problems may arise if involuntary differences between real and sham groups exist (for example, a clicking or whirring noise when real irradiation starts).

Crossover designs: in order to avoid the effect of different confounding variables present in the experimental and control groups, and to maintain the convenience and statistical power of pair-wise comparison, the crossover designs switches alternatively the subjects between the groups, allowing sufficient time to the effect to wear out, if any. This design may present a problem if the effects have a long duration or if this parameter is of interest of the study;

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Randomized and blinded experiments: the final improvement to experimental tudies is to avoid experimenter and selection biases, by randomization and single or double blinding (avoiding totally that the experimenters, the subjects or both detect to what group they were assigned to).

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