THE PREDICTION OF MUTAGENS AND/OR CARCINOGENS IN THE AQUATIC ENVIRONMENT OF IRAQ

Muhsin A. Jabar, Medical College, University of Kerbala, Kerbala , IRAQ

ABSTRACT

The aquatic environment in Iraq is exposed to a variety of chemical mutagens and/or carcinogens. The main input sources of these agents are related to the three wars during the last three decades. Some of these pollutants may enter the human body through the food chain and drinking water creating serious health hazards. Therefore, monitoring the aquatic environment for such pollutants is very important for future plans in determination of the input sources of these agents and their subsequent prevention.

Three procedures have been developed for such purposes:
First: performing analysis on water samples in order to identify every chemical constituent within them which can then be individually tested for its genetic toxicity. This may be a formidable task because of the very large number of chemical and biological agents.

Second: collecting water samples and concentrating their chemical constituents, then exposing the resulted concentrates to a range of biological systems. This approach has two fundamental limitations, the developing a suitable concentration and perhaps more importantly, the difficulty of detecting mutagens in the presence of toxic chemicals.

Third: to reduce the masking effects of toxic chemicals, some aquatic organisms can be used as bio-indicators for detection of the potentially hazardous chemical agents.

The technical procedure for the last approach will be discussed where bacteria, yeast and tissue culture can be used. It has been approved that such approach is fast, cheap and reliable in the preliminary assessment for water quality and the determination of the most health hazard pollutants in the aquatic environment.

Introduction:

It is quite clear that the kinds and quantities of the carcinogenic and/or genotoxic materials in the Iraqi environment after the wars are numerous. A quick look to the United Nation Environmental Protection(UNEP) Desk Study (2000) on the Iraqi Environment before the last war may shows how serious is the impact of such pollutants on the human health in Iraq. Therefore, a rapid screening and identification procedures for these hazardous agent are urgently required. That is regardless of the possible argument on the strengths and weaknesses of different short-term tests when compared to the long-term carcinogenicity tests on animals.

A well conducted long-term carcinogenicity study is usually so slow and so expensive that the results will be regarded as definitive, may be because the test would never be repeated. Unfortunately, when they are repeated they do not always give the same result. However, different short-term tests may give different results, and, the more tests that are used, the more likely is that one of them will give a positive result. In this case, there will some sort of confusion which represent a more realistic picture of carcinogenic risk than the arbitrary simplicity of results from a single test in the long-term assays. Therefore, there will be a little doubt about their impact on the whole field of prediction of carcinogenicity (Green, 1980

Many research scientist are highly convinced that short-term tests must be used quantitatively rather than qualitatively for the prediction of human genetic or carcinogenic risk (Green,1980 ; Ames and Hooper,1978). This is related first to the large number of compounds for which some evidence of possible carcinogenicity exists. And secondly, short-term tests such as Ames test is sensitive and gives reasonably low frequency of both ‘ false’ positives and negatives. In addition to that the addition of the liver enzyme fraction (S9) to the *in vitro* tests will bring the gab between the *in vitro* and *in vivo* close to each other.

So that a good effect of short-term tests assays is that they encourage a critical screening of carcinogenicity data. However, there are some difficulties in classifying the short-term bioassay as negative. Nevertheless, a variety of arbitrary criteria have been proposed to validate the short-term bioassys: for instance , a two fold increase in the number of mutant colonies on the treated plates, or a statistically significant increase, a dose-related increase in the number of the mutants in Ames test.

Kinds of Short-Term Bioassays:

In this paper, the most currently used short-term bioassays for induction of DNA base-changes will be described. These are almost fast, cheap and reliable. Hence, they can be used in the rapid screening for environmental pollutants in water air and soil.

Detection of DNA Base-Changes:

This assay system is quite necessary for the preparation of a data-base to be used for assessment of the level of the indigenous pollution. It will be implemented for detecting a wide variety of chemical pollutants and predicting their possible impacts on the aquatic organisms such as fish, phytoplankton and zooplankton as will as human beings who will be exposed to them directly or indirectly through the consumption of these organisms. However the actual evaluation of any possible risk of human health may needs further research. Since the health hazard assessment of studies is usually time-consuming and of high expense, the short-term bioassays will be of considerable importance.

The systems used in the measurement of induced point mutation after chemical exposure are generally based upon the quantification of the frequencies of specific genotypes detectable in the presence of various selective agents (Venitt and Parry,1984). These selective systems include resistance to the effects of toxic drugs as is widely used in mammalian cell culture systems (Cole and Arlett,1984)and growth of prototrophic cells in auxotrophic cell populations, such as those used in Salmonella / mammalian microsome system (Venitt etal.,1984).

These selective systems have provided us with methods for the *in vitro* measurement of mutant frequencies in bacteria and cultured cells. *In vivo*, the availability of point mutational assays is more limited and at present confined to methods such as the mouse spot test (Fahring,1975). More recently, assays based upon the use of transgenic animals carrying selective genetic markers particularly those of bacterial origin are in use (Gossen and Vijg, 1990).

In terms of environmental monitoring, the fundamental problem with all the above assays is that they are based upon the use of specialised laboratory methodologies that are not applicable to species in the natural environment.

Therefore, a new methodology has been developed by Prof J.M. Parry and his colleagues at the School of Biological sciences, University of Wales, Swansea, U.K; which can be, theoretically, applied to the study of DNA base changes in any gene of any species for which DNA sequence information is available. This methodology is based upon the measurement of base changes which occur in the DNA sequences which code for recognition sites for bacterial restriction enzymes (Parry, et al.,1990 ; Zijlstra et al.,1990).

The Restriction Site Mutation Assay(RSM):

As part of their work on the development of methodologies for detecting genetic damage and genetic changes in aquatic species, they were utilizing the methodology which was termed "The Restriction Site Mutation Assay (RSM)" to study the effects of geno-toxin exposure to the α globulin gene XEL HBA1 of *Xenopus laevis.* (Partngton and Baralle , 1981).

Breifly, this technique involves polymerase chain reaction (PCR) by alternating cycles of polimerisation and denaturation always the amplification of DNA sequences between two unique reaction primers (amplimers). For the XEL HBA1 gene the utilized region is between base 20 and base 203 to amplify a DNA fragment suitable for mutational analysis. Using matched base primers 5'-ATATTGTCTGAATGAATGAATG-3' and 3'-AGATGTCCTAGAAGTATCAGT 5' a region of 204 bases by 30-35 cycles of incubation with substrates and Taq polymerase in a temperature cycler can be amplified. The resulting amplified product can then be run on a polyacrylamide gel and can readily be identified as a characteristic band.

The basic principle of RSM methodology, is the DNA extracted from an organism exposed to a genotoxin which may contain a sequence changes leading to a wild type restriction site or may contain a sequences changes leading to a mutant restriction site. DNA containing wild type sequences , for example, 5'-CTAG-3' is cut by the restriction enzyme Male 1, where as DNA containing a mutant such as C to T transition at the first base of the sequence ,i.e. to 5'-TTAG-3' will be resistant to the cutting action of Mac-1. For more details see (Jones and Parry,1992.

Thus after treatment of DNA with restriction enzyme under optimal condition all wild type sequences are cut where as the 204 base region

under study containing a restriction enzyme resistant site will remain intact. The restriction enzyme resistance sequence can then be amplified

by PCR treatment to produce a sample of mutant DNA. Thus the basis of the assay is the selective amplification of mutant sequences.

Polymeras Inhibition assay (PI assay):

This technique has been developed by Jenkins et al.(2000) for a rapid screening of a large number of samples with very low concentration of genotoxic compounds; the polymeras inhibition has been shown that it is capable of detecting DNA damaging agents of biological relevance, i.e. know human carcinogens.
PI assay is based upon the inhibition of DNA polymerases (including those used in the polymerase chain reaction PCR), in countering damaged DNA bases. Hence DNA-damaging agents can be identified by corresponding reduction in PCR amplification after exposure.

The PI assay exploits the well-reported fact that DNA damage blocks DNA synthesis both in vitro and in vivo (Moore and Strauss, 1979; Villani, etal, 1978). This DNA damage can take the form of DNA adducts or a basic sites and/or strand breaks.

In practice, templates containing DNA damage are known to be poor substrate for the polymerase chain reaction (PCR) (Govan et al. 1990, Jennerwein and Eastman, 1991) whereas non adducted template can be readily amplified. Hence the presence of DNA damage in target DNA sequences can be deduced from the accompanying reduction in PCR amplification. The use of PCR allows us to harness the exponential amplification of this technique to distinguish between undamaged and damaged DNA templates (Mc Carthy et al., 1996).

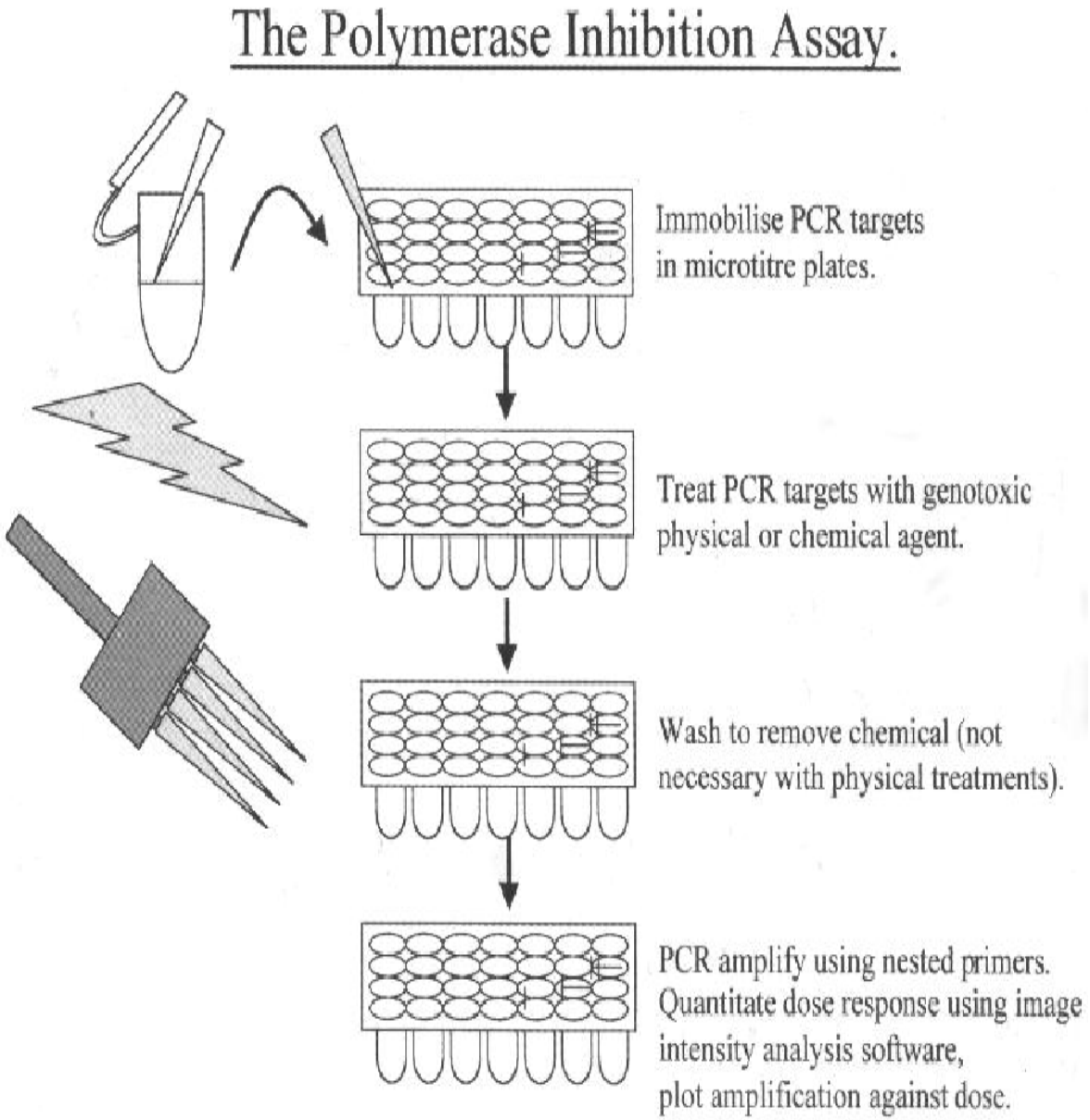
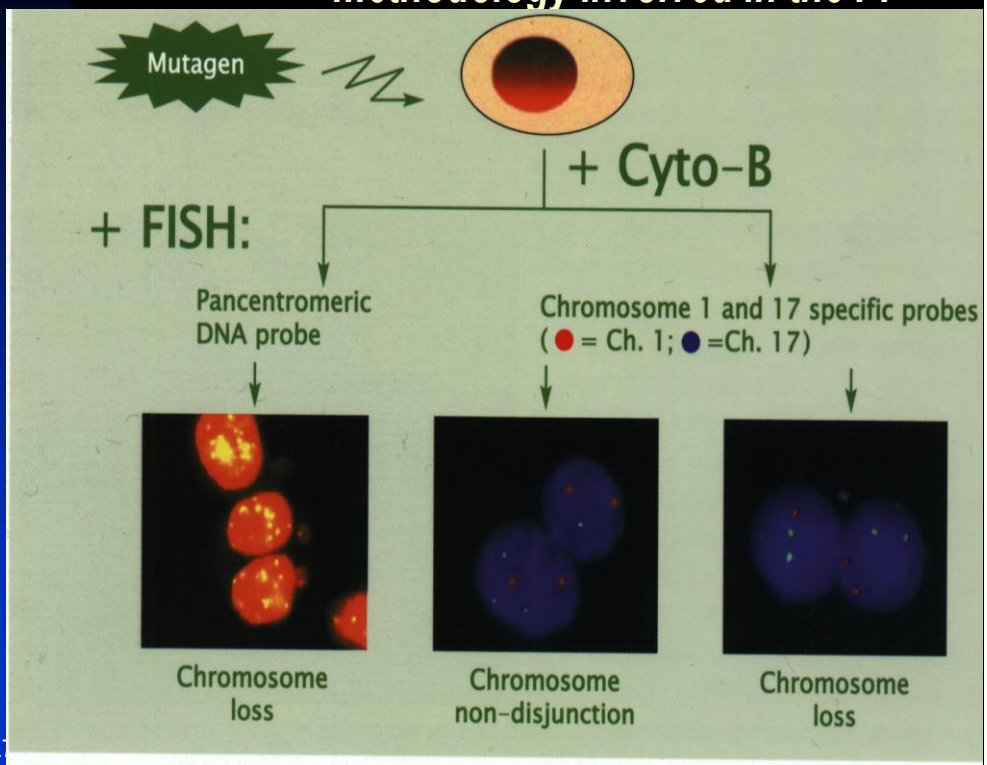


Figure 1. Schematic representation of the methodology involved in the PI assay.

Schematic representation of the methodology involved in the PI



In vito test system to determine induction of chromosome loss

CONCLUSSION:

These are examples of the most widely used techniques for the prediction of environmental carcinogens. However, the classic bioassays may also be implemented as they are also valid for such purpose. These include the detection of prototrophic mutants in auxotrophic cell population. For example, Salmonella/manmalin microsomal (Ames et al.,1973) yeast (Parry and Al-Mossawi,1979) or Neurospora (Bridges,1972).

These procedures deal with such huge amount of chemical pollutants, many of which may be carcinogenic, it is essential to chose a quick, cheap and reliable procedure. By these techniques we can assess the water supply resources especially drinking water resources for the presence of the mutagenic/ carcinogenic pollutants. Medicines, food, air quality and even the industrial environment can also be assessed. However, these assays will play an important role in chemical identification of the most known carcinogens. In this case, they chemical analysis and the biological assay will go side by side and the later will be used as a guide in determination of the most active carcinogene.

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