

DETOXIFICATION EFFECT OF TACHYONS

ALLIUM TEST was conducted in plant biogenetic laboratory by biology professor Peter Firbas. The Allium test provides a rapid screening procedure for chemicals, pollutants, contaminants, etc. which may represent environmental hazards. Root growth inhibition and adverse effects upon chromosomes provide an indication of likely toxicity. Allium cepa, has shown that this plant is particularly sensitive to the harmful effects of such environmental contaminants. Gross effects can be quantified by measurement of inhibition of growth of the newly developing root system, whereas examination of the chromosomes of the individual cells of the root tip can indicate likely mutagenic effects.

Normal mutation of cell's chromosomes is circa 4%. That mutation allows living beings to evolve. This is shown best in completely purified water. Everything what is above this percentage is a risk. Tested bottled water showed percentages from 5-20%. Waters above 20% percent of mutation are prohibited to drink. We took tap water which has showed 9,92% of mutations. That is better result than many bottled water on the market. When we exposed the same tap water to 12 hours tachyon process, it has shown significant decrease of mutation. It has dropped from 9,92% to almost what is normal in nature- 5,55%.

Conclusion is that tachionized tap water has lesser level of genotoxicity than ordinary tap water. From that point of view tachionized tap water is better quality than ordinary tap water. This experiment excludes placebo effect, because it was performed on unconscious plant.

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RATIONALE

The root tip is often the first part of any plant which is likely to come into contact with chemicals and pollutants to be found in soil and water supplies. Observation of the root tip system of the onion, Allium cepa, has shown that this plant is particularly sensitive to the harmful effects of such environmental contaminants. Gross effects can be quantified by measurement of inhibition of growth of the newly developing root system, whereas examination of the chromosomes of the individual cells of the root tip can indicate likely mutagenic effects.

BASIC PROCEDURE

Twelve onions (Allium cepa) are prepared by removal of the outer scales and brownish bottom plate and placed onto test tubes filled with test liquids for 4 days, the liquid being changed every day. A further series of twelve onions are similarly prepared and maintained on pure water to provide a control population. The ten onions which appear to be developing the best in each series are selected for examination. On day 2 one root tip from each of 5 onions is prepared for

microscopic examination. One hundred mitoses are scored from each of the 5 slides, as is the mitotic index (MI) for 400 cells. On day 4 the root length of each bulb is measured and the series photographed. (A recovery experiment can be performed by changing the medium for 5 of the 10 onions of each test series to control water after measurement on day 4, replenishing the liquid on day 5 and, finally, measuring the root length and photographing the series on day 6). Toxicity is measured by both macroscopic parameters (e.g. growth inhibition), where the degree of damage is used to assess the toxic status of the chemical tested, and microscopic parameters, where the rate of chromosome breakage and damage may be used to predict mutagenesis.

CRITICAL ASSESSMENT

Plants are easy to store and handle, and are plentiful and inexpensive. In general the chromosome condition of plant cells is good, thus providing a high standard in control conditions. The *Allium* test is a relatively rapid, easy to perform test as well as being both highly sensitive and reproducible. It also provides comparable results to a number of other test systems. Both macroscopic and microscopic effects may be observed and there appears to be a good correlation between the two. The macroscopic effect (inhibition of root growth) appears to be the most sensitive parameter. This is to be expected as any deleterious effect, direct or indirect, is likely to result in inhibition of growth. Microscopic examination allows assessment of chromosome damage and cell division disturbances, thus providing additional information as to the severity or mechanism of the toxic effect, or potential mutagenicity. The root cells possess certain enzymes, the mixed function oxidases, which are instrumental in the activation of many promutagens to mutagens. This activating system will improve the detection of those chemicals which exert their toxic effect via a reactive metabolite. The system has a wide range of applications, e.g. for testing pure chemicals, drinking water, natural water, industrial waste, etc., and is useful for evaluating and ranking environmental chemicals with reference to toxicity. The test can also be used to measure the relative toxicities of non-water soluble compounds, provided they can be dissolved in a suitable solvent and then diluted in water so that the final concentration does not exceed certain limits. In cases such as these solvent controls must also be incorporated into the test regime. The system operates over a wide pH range (3.5-11.0) without any obvious effects upon the growth of the root systems. Thus moderately acidic/alkali water samples, chemical solutions, etc. can readily be tested without pH correction being necessary. N.B. Although the pH itself may not affect the growth of the roots, it should be taken into consideration when assessing the toxicity of compounds as, in many cases, the pH can dramatically alter the toxic potential of these, e.g. by changing the state of ionisation. Disadvantages of the system concern problems associated with the state of compounds being tested. The influence of pH upon compounds present in solution and the resultant change in toxic profiles has already been mentioned. Another problem concerns the presence of insoluble compounds in water-ways, industrial effluent, etc.. It is very difficult to look at the biological effect of such complex mixtures in the *Allium* system as particulate matter may exert indirect harmful effects such as the prevention of uptake of nutrients etc.. It is therefore recommended that samples such as these are also chemically analysed. The *Allium* test is highly sensitive and, as such, positive toxic effects may result for a number of compounds which would not necessarily be deemed harmful when tested in other systems (especially higher organisms such as fish). Although this may occasionally result in false positives it also ensures that contaminations will not be overlooked; this is especially important when complex mixtures are to be tested. A positive result in this test system should, therefore, be taken to indicate a potential biological hazard. False negatives, on the other hand, have been shown to rarely occur in either the *Allium* test or other similar plant tests (Ennever et al., 1988), therefore, any compound tested giving a negative result can be reliably considered nonmutagenic. An extrapolation of results from one test system to another (and eventually to humans) should, however, be based on the results of a battery of tests and with due consideration to the metabolic pathways of the compound tested. Development of the test The *Allium* test system was first used in 1938 to examine the effect of colchicine (Levan, 1938; Östergren, 1944) and has received much attention since that time (for review, see Grant, 1982). Certain modifications of the basic test system have been introduced to

enable environmental monitoring of complex mixtures such as those present in river water, industrial waste, etc. (Fiskesjö, 1985a). The major modifications include the use of a series of bulbs (i.e. 10) for each condition tested (thus permitting an EC50 determination), and the immediate exposure of bulbs to test solutions (the old test allowed an initial growth period in pure water until roots reached an appropriate length, 1-2 cm, after which they were exposed to test compounds). Comparison to other short-term alternative toxicity test systems This test has shown good agreement with results from other test systems, using many different organisms, eukaryotic as well as prokaryotic. Results of such comparisons are summarised below (taken from Fiskesjö, 1985a). Chinese hamster cell line V79 In the absence of a metabolic activating system V79 cells appear less sensitive than the Allium test in response to organic mercury compounds. The relative sensitivity is reversed when the cells are incubated in the presence of mixed function oxidases. Despite changes in sensitivities the overall results between the two systems are comparable. Human lymphocytes The Allium test seems slightly more sensitive to the effects of organic mercury compounds than do human lymphocytes, although the overall ranking of chemicals by toxicity is similar. It should also be noted that when studied microscopically both cell types respond in a similar way (c-mitosis). Autotrophic algae, heterotrophic microorganisms and activated sludge A number of chemicals have been tested in the Allium test and the results compared to those found using 16 different plankton algae (green algae and silicious algae), yeast (*Saccharomyces cerevisiae*), protozoa (*Tetrahymena pyriformis*) and activated sludge (composition of bacteria, yeast and protozoa). The tests were all comparable when the rank ordering of chemicals according to toxicity was examined, although differences in sensitivities were apparent. The majority of the algae were more sensitive than the Allium test whereas the yeast, protozoa, and activated sludge were less sensitive. Aquatic animals (*Daphnia magna*, *Brachydario rerio* - egg or spawn & Microtox bacteria test) and plants (Lens & Unicellular algae) A number of aquatic plants and animals appear to be less sensitive to certain classes of compound compared to the Allium test e.g. fish (*Gasterosteus aculeatus*). The Allium test, in this case, is probably a better test for environmental screening due to its higher sensitivity. Other animals (e.g. the crustacean *Nitocra spinipes*) and plants (e.g. Lens and unicellular algae) give comparable results to the Allium test.

CHEMICALS TESTED

Wide range of metals, industrial pollutants, compounds, etc. have been tested. Water from a variety of sources has also been examined. For examples see references below.

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