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Mutagenesis Testing Program

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Until recently, mutagenicity testing was done on preselected compounds in a manner in which the testing laboratories knew the identity of the substances under test and the "expected" results, i.e., positive for compounds selected because of their carcinogenicity and negative for food additives. There is no completed study in which substances were tested blind using a standardized protocol. Also, little attention has been placed on reproducibility and variability within and between laboratories. These aspects are currently under investigation in microbial systems by NCI but the results will not be available for 1-2 years.

In the NIEHS testing program a large number of substances will be tested in a blind study. At present we suggest use of a short-term testing system consisting of microbial tests plus mammalian activation systems (Tier 1), two different Drosophila systems (Tier 2) and four different whole animal systems (Tier 3). The compounds will initially be screened for mutagenicity in Tier 1, and the results obtained in Tier 1 together with what is known about the compound otherwise will dictate the decision whether to continue the test of the compound in Tier 2 and Tier 3.

Over the past decade the field of mutagenesis has experienced widespread growth. This, of course, is a result of the increasing concern over the potential hazard to man of the chemicals in the environment and the concern for the future generations as they may be affected by changes in the genes and chromosomes of the current generation. The majority of this effort has been taking place in the research laboratory where scientists have been at work devising and developing test systems for detecting chemicals with mutagenic potential. The effort in the Laboratory of Environmental Mutagenesis has focused primarily in this area; i.e., developing new methodology ranging from the short-term bacterial tests to whole animal tests which can be used for developing quantitative data in estimating risks to the human population.

The Mutagenesis Testing Program at the National Institute of Environmental Health Sciences is an outgrowth of the research effort developing these new systems; because, certainly for the public health question, there is a need to do large-scale testing for a large number of compounds which are already in the environment as well as those new compounds being added each year. Until recently, mutagenicity testing was done on preselected compounds in a manner in which the testing laboratory knew the identity of the substance under test and the expected results, that is, positive for compounds selected because of their known carcinogenicity and mutagenicity and negative for compounds such as food additives. There is as yet, no completed study in which substances were tested blind by using a preselected standardized protocol. Also very little attention has been placed on reproducibility or variability within and between laboratories utilizing the various test systems which have developed.

Some of these aspects are currently under investigation in microbial systems by the National Cancer Institute used as a preselection for compounds for the *in vivo* bioassay program and by the Food and Drug Administration, Bureau of Foods, primarily for the food additives.

Certainly the main endproduct of the NIEHS Testing Program will be the development of a facility and a set of protocols for testing a large number of chemicals using a tier approach. However, there are other goals of the program which come along the way and these primarily focus on the validity, or some may use the term quality assurance, of the various test systems employed. The NIEHS Testing Program will utilize a large number of substances which will all be tested in a blind study. This will involve the use of a hierarchy of tests sometimes referred to as the tier approach proceeding from rapid screening in simple, highly sensitive microbial systems through *in vitro* mammalian sys-

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tems and insect systems up to the whole mammal tests usually employing mice. Substances which are found to be mutagenic im microbial tests would then be given first priority for additional testing. Those with negative results would be afforded lower priorities. However, factors other than the result of microbial screening will certainly be taken into account in establishing the testing priority of each substance. Because of the magnitude of the task, the eventual goal by 1981 will be to process 1000 compounds a year through the first-stage microbial tests, and because, as previously stated, one of the goals of the program is quality assurance of test systems and laboratories, this program will be conducted totally as a collaborative research contract.

The first and certainly a very important contract will be that for chemical management and analysis. The contractor will be responsible for acquiring, storing, coding, and dispensing the chemicals which will be tested in the program. The contractor, with the assistance of the project officer, will develop a computerized inventory of the compounds including such information as CAS registry number, Wiswesser line notation, lot number, analytical information, as well as all information which is currently available on the toxicity of the material in question. An extensive amount of physical data on the compound will also be stored in the computer for future reference in structure-function analysis. In the initial phases of the program (the first two years) the chemicals selected will not be analyzed for purity. However, subsequent to testing when positive results are obtained (especially when positive results are obtained in the microbial system but are not verified in other systems), chemical analyses will be conducted to identify that fraction of the material tested having the mutagenic properties. That is, a positive result in the microbial test may be due to its extreme sensitivity to low quantities of impurities in the material. Of the three tiers to be employed, only those systems in the first tier have at this time been selected. Testing will be conducted using several of the different Salmonella strains in which reverse mutations are detected in the histidine locus and *E. coli* in which DNA repair is detected.

It is anticipated that two separate contracts will be awarded during this first year to conduct the microbial tests. There will be a certain percentage of overlap of compounds sent to the two laboratories and, additionally, another percentage of compounds will be sent to the same laboratory at two different intervals; hence, we will be able to obtain quality assurance information both within and between laboratories. And, of course, certainly in this first phase of the program a significant percentage of the compounds sent will be known mutagens.

While the systems for the second and third tier have not yet been finally selected, at the present time it seems rather certain that the recessive lethals and heritable translocations as measured in Drosophila will be employed. Also in the second year, mammalian cells in tissue culture will be employed. Exactly which system will be used is not yet certain although the current available information would tend to favor either the Chinese hamster cells or the mouse lymphoma cell system. Both recessive lethals and heritable translocations in Drosophila are tests which have been utilized in research laboratories for many years and their reliability is thought to be high as well as offering relatively inexpensive processing.

The tier 3 or whole-animal tests selections are more in doubt at this point in time. There is a significant amount of work being done in this area and, of course, this part of the program will not commence for probably two more years. Hence, it would be unwise to make a selection at this time. It is, of course, clear to use those tests which measure chromosomal aberrations and tests which measure gene mutations. Currently the heritable translocation test in the mouse seem to offer advantages because as a whole animal test, it is relatively cheap and it provides a clear cut detection of genetic events which are definitely transmitted to the offspring. Chromosome breakage has been demonstrated to be a function of many mutagenic agents, and while the bearers of reciprocal translocations often have a normal phenotype, a high percentage of their offspring are expected to have duplications and deficiences for genetic material. In humans this has been shown to give rise to fetal loss, unbalanced genome effects and trisomes like Down's Syndrome.

The only method which has received wide usage for detecting gene mutations in mammals is the specific locus test first employed by Dr. Russell in radiation mutagenesis some 25 years ago. There are, however, efforts underway in several laboratories to develop additional gene mutation assays which detect mutations at loci which code for various enzymes. One of the advantages of these systems is that they will sample more loci in the genome and hence, it is expected, will give a more reliable estimate of an induced mutation frequency. An additional advantage is that since these are enzymes which are common to a large number of organisms, including humans, while the metabolic differences between the mouse and human still exists, at least the enzyme detection will be at genes of

very similar structure and size.

We will be working very closely with the statisticians and the computer people to provide both for adequate design and for computer capabilities for handling and analyzing all of the data from the various test systems. It is becoming increasingly clear that the management of data for mutagenesis testing is becoming tremendously complex and the only logical way to handle such data is by a computer.

I have described the reasons and the background behind establishing the mutagenesis program at NIEHS and have provided a brief outline of the methods which we intend to employ to establish this program. However, I have failed to mention the one area which to many of you is a question high in your mind; and that is what compounds are going to be tested in the NIEHS Testing Program. At the present time we do not have an answer to that question. However, I can describe to you the system that we intend to utilize to select the compounds.

We will establish a committee at NIEHS in coordination with compound selection committees in the other Federal agencies. This committee will be charged with first establishing a selection of criteria for classes of compounds for testing. The selection criteria could be, for instance, production quantity, level of exposure to man, structural relationship to other mutagens, and various other criteria; and then secondly the committee will select compounds for test upon these criteria. It is anticipated at this time, although not at all definite, that NIEHS will advertise for nominations for compounds to be selected for testing in our program. This mechanism is currently operative at other Federal agencies. Since NIEHS neither has a regulatory function nor is oriented toward a single environmental disease, it is logical to assume that we will be looking at a wide variety of compounds.