General Summary and Recommendations for Workshop on The Evaluation of Chemical Mutagenicity Data in Relation to Population Risk

by Alexander Hollaender*

For a number of years I have spent a great deal of time trying to convince people of the significance of chemically produced mutations. There was at first almost no response. It was then pointed out to me that the Drug Research Board of the National Academy of Sciences-National Research Council might be interested. The chairman of this board was Dr. W. S. Middleton, a pathologist who was formerly Dean at the University of Wisconsin Medical School, whom I had met many years ago. Dr. Middleton was responsive, and I presented my ideas to the Drug Research Board. At that time the toxicologists and pharmacologists thought I was a fanatic and paid little attention to my ideas. Of course we now know that chemically produced mutations may be even more important than those produced by radiation. With my usual persistence (or stubbornness, if you will) I continued to press for investigations on chemical mutagenesis, and gradually the pharmacologists, and the toxicologists too, began to listen. Dr. Middleton also felt there might be something in what I was saying. Subsequently, several round table discussions were held, the Environmental Mutagen Society was established, and a number of workshops were cooperatively organized by the EMS and the Drug Research Board.

This brings me back to something that happened almost twenty years ago. In 1953 the BEAR (Biological Effects of Atomic Radiation) Committee was appointed by the National Academy of Sciences. This committee was under the chairmanship of Warren Weaver, then at the Rockefeller Foundation. Dr. Weaver is a mathematician, physicist, and biometrician with an excellent feeling for the basic problems of biology, but with no training in the field. The BEAR Committee met many times and in 1955 came out with their first report, which is still one of the best I have seen on this subject. Warren Weaver did a first-class job of presenting scientifically correct material in a nontechnical manner. This and a similar British report led to the formation of UNSCEAR (The United Nations Scientific Committee on the Effect of Atomic Radiation) which helped to set the limits on permissible exposure to radiation.

We are today at about the same stage con-

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cerning chemical mutagenesis as we were with radiation studies 40 years ago. Hopefully, some of the methods we have heard discussed here will lead to the establishment of similar standards and limitations for chemical mutagenic compounds.

Before proceeding further, I should reemphasize the varying degrees of sophistication concerning the known methods of testing for chemical mutagens. A great deal of confusion concerning these tests exists in the minds of people who are not directly involved in basic research in mutagens. This confusion has at least partly been created by our failure to state clearly which of these methods can be used under practical conditions. For instance, what is the background necessary for an individual to work with these methods? Can a laboratory technician handle some of them, or should they be supervised by a geneticist? I agree it is not easy to give clearcut statements at the present time, but if we want our methods to be used generally these statements have to be made even if it is necessary to redefine them later. I think one reason the Food and Drug Administration has not gone further in recommending or requiring testing of all compounds before release for public use is because we have not provided sufficient recommendations. There are, of course, other complicated reasons for this delay. Twenty-five years ago we were not sure about radiation, but we learned how to describe our methods in such a way that their limitations could be understood.

Each workshop such as this one, the ones held at Brown University and Zurich, the round-table discussions held in Washington, etc. brings out new ideas, more questions, and some progress. This is as it should be.

Let us review the testing methods that have been discussed at this workshop.

The dominant lethal method can be very useful, but unfortunately some industrial groups have used it exclusively in testing. This is a mistake. It is necessary to use a battery of methods to catch as many mutations as possible. Even today we have not found a 100% reliable method for detecting radiation effects. The dominant-lethal method for testing for chemical mutagens seems to be in good enough shape to be recommended if it is used in conjunction with other methods.

The translocation method is promising, but we have not had sufficient experience under practical conditions. Two chemicals have been used as described by Dr. Generoso, but this is not adequate. I am reminded of the tests Cattanach ran in Oak Ridge using triethylenemelamine and the specific locus method. He got a highly significant increase in mutations, especially on certain loci that were different from the loci most affected by radiation. We were very enthusiastic and thought we were really on our way as to how to use the specific locus method for detecting chemical mutagens, but when other chemicals were used many of these tests were negative. Slowly we are learning more about why they were negative at that time. We must be wary of overenthusiasm about any particular method until enough chemicals have been tested. I like the translocation method, but feel we must do further testing before we recommend it for practical use. Drs. Generoso and Cumming in Russell's group in Oak Ridge have demonstrated that it is a very versatile method of testing, and they have also helped to point out the importance of chemical mutagenesis.

The host-mediated assay method has been thoroughly discussed at this meeting, and I will not go into it further.

I am most impressed with the cytogenetic methods that have been developed, and I regret that we did not have more discussion on the studies by Dr. Miller on micronucleii. There are much more data available than were brought out, and I think the micronucleii studies will become an excellent tool for detecting mutagenic chemicals by chromosome aberrations.

Several good electrophoresis systems are now available as discussed here. I am especially acquainted with the methods Neel is using in attempts to follow the serum proteins in human populations to determine whether there is an increase in the muta-

tion rate. The difficulty in these methods is the low mutation rate, as a consequence of which the resultant numbers tested are large and the procedure is difficult. It is even more complicated than the specific locus method. Anderson, of the MAN Program in Oak Ridge, is working in close cooperation with Neel to develop automated methods for electrophoresis systems. This field is expanding so rapidly that it will be worthwhile having it discussed at a future meeting. A chapter in the forthcoming Volume 3 of *Chemical Mutagens: Principles and Methods for Their Detection* will include a chapter by Neel and Anderson concerning this.

I was pleased that Dr. Shaw discussed the banding techniques since we are now beginning to understand the basic structure of chromosomes so that they can be recognized on the basis of banding. I have been told that attempts are being made to get even more detailed structure in regard to banding and recognizing different parts of the chromosome. Many more cytogeneticists who are using chromosome aberrations for recognizing the mutation effect of chemicals should get involved in this method.

In the beginning we were criticized for not involving more pharmacologists in the mutation studies. It was pointed out that we were using chemicals, and the job of the pharmacologists is to study what happens to chemicals in the body. This was a valid criticism, and as we have heard in some of the talks this morning, we have made a great deal of progress in this direction.

An area that I am surprised we have not touched, especially in regard to the dominant-lethal method, is the physiological and genetic aspect of reproduction. A symposium on this subject will be held in Salvador, Bahia, Brazil this year. For the first time, geneticists such as Lyon, Oakberg, and others will be involved in this area. We hope to bring about closer contact between the geneticists and the reproductive physiologists. The Latin American symposium will be quite general in approach, but the program has been so enthusiastically received that another dealing with the female reproductive physiology is being planned for 1975 in New Delhi, India. Again, many geneticists will be included, and we hope for closer contact between them and the people working on chemical mutation.

I started out in the mutation field in microbiology, so I am always enthusiastic about methods that can be used to acquire statistical information with a relatively small amount of work. I am as lazy as the next fellow, and I like to get as much data as possible with as little work as necessary, but I don't think we should fool ourselves that the data we collect are adequate to judge for mammalian studies. This work must be extended into areas where the data can be applied.

In the final analysis, the mutations will have to be recognized in human populations. I mentioned Neel's attempts in this area, and we should think about other approaches to this problem. Embryology studies will help, and there must be better ways of following changes in populations since we have so many recognized inherited diseases. Crow mentioned several hundred that appear in McKusick's book. I think this is an area which warrants great effort.

We have heard some discussion about using the substructure of the cell for recognizing mutations. There are such elegant methods available for the separation of the different parts of the cell that there must be improved ways for following the effect of chemicals on them. A good part of these chemical changes affect the cell wall, and since the nucleii often attach themselves to or become an integral part of the cell wall, permeability studies should be encouraged. Cell permeability in relation to genetics will become very important in the future, especially in regard to chemicals: whether they get into the cell or not, what form they are in when they get into the cell, and how they change in the process of penetrating the cell wall, and under what practical circumstances will the cell wall be changed.

We will not get very far in the develop-

ment of this entire field until more competent scientific investigators are trained to do the work. The Federal government, the Food and Drug Administration, and the Cancer Institute give contracts to commercial testing houses to check on mutagenesis and carcinogenesis, but at this time there are not sufficient qualified people to prepare adequate critical evaluations. The tremendous amounts of money being spent through these contracts could better be used to train more individuals to do the work competently. In the long run, this testing should be done by the commercial houses, but we should assume the responsibility for making certain that they have the proper background, are critical enough in evaluating their tests, and that they have qualified geneticists on their staffs. This need makes it even more surprising that it is so difficult to get funds for setting up training courses. We have been trying for the past couple of years, and so far have not been able to break the bottleneck of each government agency thinking someone else should do it. It is especially shortsighted for the FDA not to favor the training of individuals who would be qualified at least to understand the rudimentary aspects of testing and to know the three most accepted methods. This could be accomplished in a six to eight-week training course. The interest of the basic research laboratories is very intense right now, but there is not enough support from the commercial houses. I hope that we will have some discussion on this.

Other aspects of the problem of chemical mutagenesis are being developed elsewhere. The Federal Environmental Protection Agency is interested in the field. The new Environmental Agency of the United Nations has asked me to outline the organization of an international registry of potentially toxic compounds. The intent is not to eliminate national registries, but rather to set up additional ones with all feeding into a central agency, thus allowing more rapid recognition of toxic chemicals, and faster dissemination of this information. Again, I think the success of this program depends greatly on bringing more well-trained people into this area.

Dr. Crow mentioned that it is urgent to set some numerical value to the danger of exposure to chemicals. An attempt was made several years ago to compare radiation effects and chemical mutagens. During the war it became mandatory to set a standard limitation on the amount of radiation to which humans could safely be exposed. The value set at that time was much too high, and has been reduced by the BEAR Committee and again by the Radiation Protection and UNSCEAR Committees. It is this sort of standard we are attempting to set up for chemical hazards. It will no doubt be a crude measurement and quite limited in its application, but it will at least be a "statement" and a starting point that can be altered and adjusted as more data become available. I think the time has come for the National Academy or some similar group to establish a committee to consider the problems of chemical mutagenesis. It is to be hoped that the United Nations will respond to the survey and organize a registry on an international basis.

If all this takes place, we will have to call on you to help because this must be a cooperative effort. The people in industry, in the medical profession, in government laboratories, and investigators in research laboratories and universities must work together. Otherwise we won't get anywhere.