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The GENE-TOX Program: Genetic Activity Evaluation†

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Received July 31, 1980

The GENE-TOX program, a two-phase evaluation from the existing literature of selected bioassays for detecting mutagenicity and presumptive carcinogenicity, is described. Sponsored and directed by the Office of Testing and Evaluation within the U.S. Environmental Protection Agency's (EPA) Office of Pesticides and Toxic Substances, this program will aid EPA in establishing standard genetic testing and evaluation procedures for the regulation of toxic substances and determining the direction of research and development in the area of genetic toxicology.

INTRODUCTION

The GENE-TOX program (evaluation of current status of bioassays in genetic toxicology) is a systematic scientific evaluation of selected bioassays currently used for detecting mutagenicity and presumptive carcinogenicity of chemicals. This program is sponsored and directed by the Office of Testing and Evaluation (OTE) within the Office of Pesticides and Toxic Substances (OPTS), U.S. Environmental Protection Agency (EPA). Its primary purpose is to aid EPA in establishing standard genetic testing and evaluation procedures for the regulation of toxic substances. On a broader scale, the data compiled through this program and its appraisals of existing methodology for determining mutagenicity and presumptive carcinogenicity will be invaluable to scientists working in genetic toxicology and related areas.

In regulating the release of toxic substances into the environment, EPA and other agencies attempt to determine the mutagenic or carcinogenic potentials of a wide variety of environmental chemicals. The more than 100 inexpensive short-term bioassays now in use, while not yielding conclusive evidence, can indicate which chemicals should be more thoroughly investigated through long-term, whole-animal studies. However, the results of such assays for a given chemical or class of chemicals are frequently incomplete, inconclusive, or conflicting. Discrepancies arise from differences among the bioassays in their sensitivity and applicability to various classes of chemicals and differences among investigators in experimental protocols and techniques of data analysis and interpretation. EPA thus frequently lacks the necessary information to permit definitive conclusions concerning a chemical's possible mutagenicity or carcinogenicity.

It is desirable that mutagenicity and related testing required under the Toxic Substances Control Act (TSCA), the Federal Insecticide, Fungicide, and Rodenticide Act, and other Federal legislation be standardized and regulatory personnel be provided objective criteria by which to evaluate test results. Furthermore, it is essential that existing literature be made available in a readily usable form. Through the GENE-TOX program, selected literature on the most useful and relevant bioassays is being assembled, evaluated, and placed in a computer file for rapid retrieval and analysis.

The assays to be evaluated were selected on the basis of their genetic end points (e.g., primary DNA damage, gene mutation, or chromosome aberration) and the number and kinds of chemical compounds for which the assays have been used. Although some of the bioassays have been used with relatively few chemicals, they have been included because of their unique roles in the evaluation of genetic effects; for instance, an in vivo system might be a primary tool in risk assessment, or a certain test might be especially sensitive in detecting a particular type of genetic damage. The performances of the assays are to be compared on a chemical-by-chemical and class-by-class basis. This information will be used by EPA in designating batteries of tests for mutagenicity evaluation of chemicals by means of a sequential testing scheme. It will also bring to light areas in which additional test system development and validation are most urgently needed.

ORGANIZATION AND OBJECTIVES

The GENE-TOX program includes two phases: (1) work group evaluation of individual bioassays and (2) summary evaluation and assessment, which will address a series of

† Presented on March 24, 1980, as part of the Symposium on Development and Use of Quantitative Structure–Activity Relationships (QSARs) during the 179th National Meeting of the American Chemical Society, Houston, TX, and on April 23, 1980, as part of the Symposium on Development and Use of Quantitative Structure–Activity Relationships (QSARs) during the 14th Middle Atlantic Regional Meeting of the American Chemical Society, King of Prussia, PA.

Table I. Work Groups To Evaluate Individual Bioassays

Gene Mutation Tests	Chromosomal Effects
Salmonella typhimurium	plant cytogenetics[a]
Escherichia coli WP2	*Drosophila melanogaster*
mouse lymphoma cells L5178Y	chromosomal aberrations
Chinese hamster lung cells V79	*Saccharomyces cerevisiae* and
Chinese hamster ovary cells	*Schizosaccharomyces pombe*
Drosophila melanogaster	mammalian cytogenetics
sex-linked recessive lethal	sister chromatid exchange
Neurospora crassa	micronucleus test
Aspergillus nidulans	dominant lethal test
higher plants[a]	heritable translocation test
mouse specific locus	
(including spot test)	Oncogenic Transformation
	chemical enhancement of viral
Primary DNA Damage	transformation
Escherichia coli Pol A and	transformation using cell lines
Bacillus subtilis rec	transformation using cell strains
unscheduled DNA synthesis	
DNA repair	Ancillary Tests
	host-mediated assay
	sperm morphology

[a] These tests are evaluated by the same Work Group.

fundamental questions concerning tests for mutagenesis and presumptive carcinogenesis. The organizational units of the program are the Steering Committee, the Work Groups, and the Assessment Panel. The composition and objectives of each of these groups are as follows:

Steering Committee. The Steering Committee's 11 members and 7 consultants are affiliated primarily with EPA, the National Institute of Environmental Health Sciences, and Oak Ridge National Laboratory (ORNL). The Steering Committee coordinates the entire program, provides direct guidance to the Work Group leaders, and monitors Work Group progress. The assays considered under the GENE-TOX program were selected by the Steering Committee as described before and are listed in Table I.

Work Groups. The function of each Work Group is to evaluate the current usefulness of a selected mutagenicity or related assay system. There are 23 Work Groups of 5–10 scientists each, for a total of about 150 scientists involved in this phase of the GENE-TOX program. The members of the Work Groups were chosen by their chairmen, in conjunction with the Steering Committee, on the basis of their knowledge of and familiarity with the bioassays to be evaluated. A balance among academic, government, industry, and commercial testing laboratories was sought in selecting scientists to serve in Work Groups. The activities of the Work Groups are coordinated through ORNL and the Environmental Mutagen Information Center (EMIC) of ORNL. EMIC provides all literature to the Work Groups and designs and edits all data sheets used to record the Work Groups's evaluations of published reports.

In the first phase of the process, the goals of each Work Group are

(1) evaluation of the assay's ability to discriminate between mutagens and nonmutagens and/or carcinogens and noncarcinogens;

(2) evaluation of the system's performance with chemicals of various classes and identification of chemicals whose effects are not adequately detected;

(3) formation of generalized protocols and criteria for data evaluation and interpretation;

(4) identification of areas requiring additional research or further development and validation;

(5) publication of an evaluation of the assay in the open literature.

It is generally recognized that any given mutagenicity bioassay is more effective for testing some chemicals or classes of chemicals than others. Each Work Group's objective is to determine, through an evaluation of the existing literature, the validity of a particular system, the chemical(s) for which it is best suited, the proper test protocol, and the appropriate techniques of data analysis, interpretation, and presentation. In addition, the Work Group recommends specific research needed for development or validation of the test system.

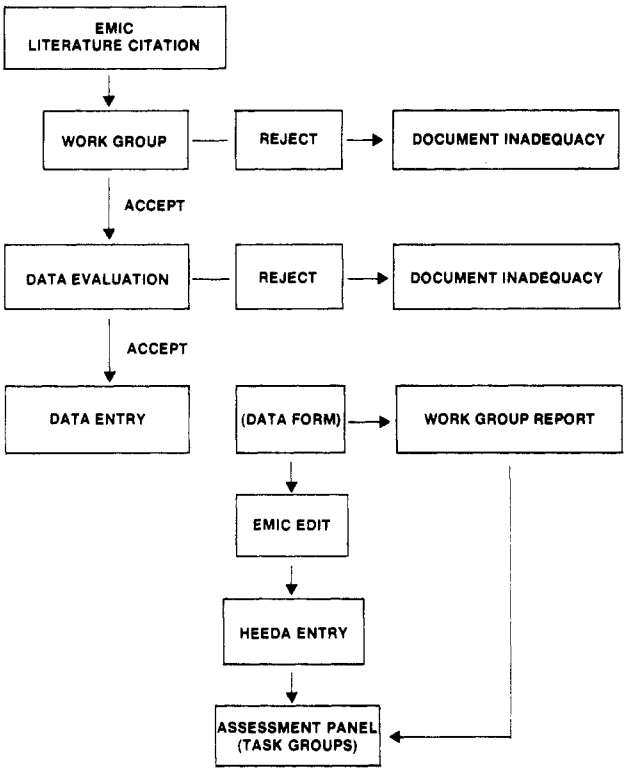

Figure 1. Flow chart of the GENE-TOX bioassay evaluation process.

Assessment Panel. The Assessment Panel, also selected by the Steering Committee, consists of scientists with broad knowledge and experience in genetic toxicology. The panel is organized into small task groups, each charged with critically evaluating the reports of the Work Groups to satisfy the following requirements:

(1) determination of groups of tests that most effectively and efficiently detect a broad range of a particular class of mutagens;

(2) determination of groups of tests that detect the relevant types of genetic damage;

(3) determination of groups of tests that most effectively and efficiently detect presumptive carcinogens or provide information concerning correlation between mutagenicity and carcinogenicity;

(4) determination of the desirable or essential degrees of redundancy in testing for given objectives;

(5) determination of the feasibility of estimating levels of effect by comparing results in whole-animal systems with those in short-term bioassays;

(6) identification of research needs for development or validation of tests.

The Assessment Panel task groups will prepare reports for publication in the open literature. In addition, the Assessment Panel is reponsible for convening one or more conferences at which reports of Work Groups and the Assessment Panel task groups will be presented and for having the conference proceedings published.

PROTOCOL

This section outlines the steps by which the goals of the GENE-TOX program are to be accomplished, through Work

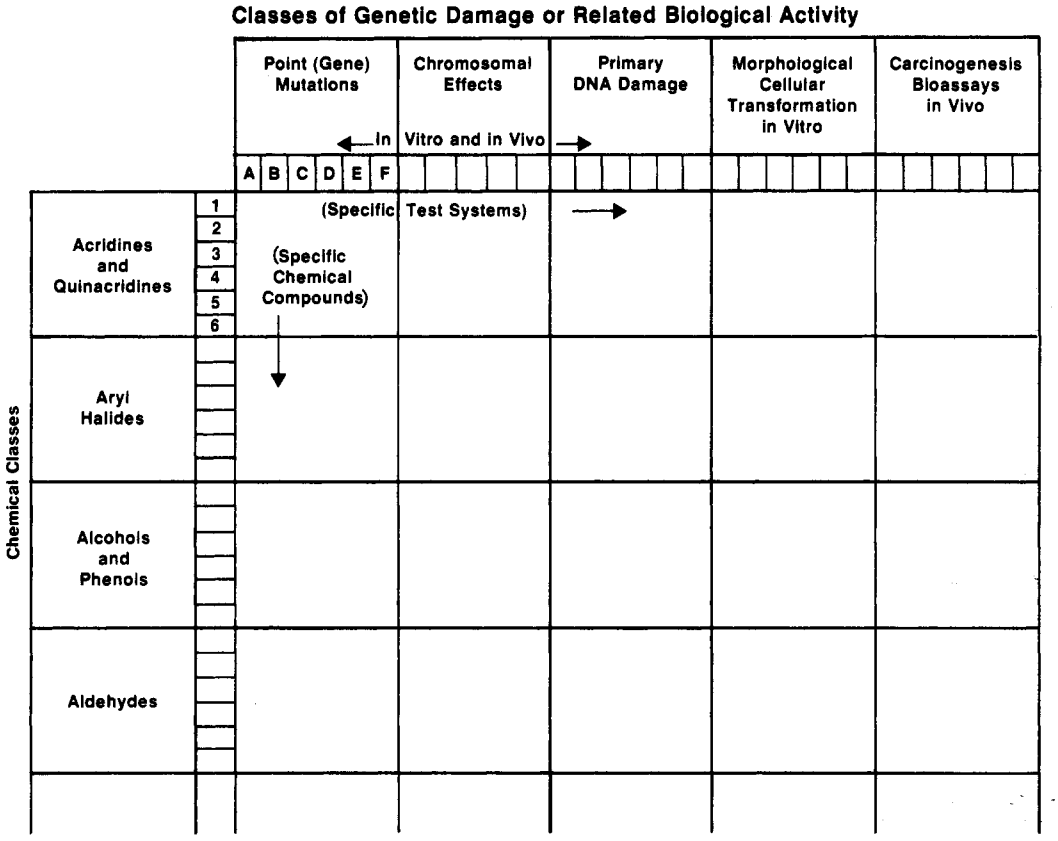

Figure 2. Generalized matrix for summarizing qualitative results from approximately 39 test systems (within 5 classes of damage) and approximately 2800 chemicals (grouped according to chemical class).

Group evaluations, establishment of a computerized data file, and summary evaluation and assessment (see Figure 1).

Work Group Evaluation. Each Work Group is provided a list of all EMIC citations for its assigned bioassay. Any published report of which a Work Group is aware that is not cited by EMIC is included and added to the EMIC file. The Work Group's data base is drawn from this literature pool through a two-step screening process. First, publications are selected from the list of EMIC citations based on general guidelines established in conjunction with the Steering Committee and data requirements for the evaluation process. Work Group evaluation is limited to published primary papers and contract final reports that have entered the public domain through deposition at EMIC or with the National Technical Information Service in Springfield, VA. Abstracts and review papers with sufficient appropriate data are included by exception. Reasons for rejection of any EMIC citation are documented and annotated in the computer file.

After the first cut of the literature, the Work Group evaluates the remaining papers on a chemical-by-chemical basis, selecting those studies that meet the following criteria:

(1) proper experimental design;
(2) proper use of positive and negative controls;
(3) proper selection of solvents and vehicles;
(4) acceptable spontaneous background mutation frequency or rate;
(5) provision of metabolic activation systems, if necessary;
(6) use of appropriate criteria for positive, negative, or inconclusive results;
(7) provision of dose–response information (not critical if all other criteria are met).

The Work Group may adopt additional criteria specific to a given assay. Again, reasons for rejection of any data are documented and will become part of the EMIC data file for each published report evaluated by the Work Groups. Each Work Group will report its findings in *Mutation Research—Reviews in Genetic Toxicology*. The prescribed outline for this report is given in Table II.

Computerized Data File. Because the data from the papers selected for evaluation is to be entered in the EPA OPTS Health Effects and Environmental Data Analysis (HEEDA) system, EMIC has aided in the design of data extraction forms for use by the Work Groups. All of the data from reports judged acceptable by the Work Groups criteria will become part of the HEEDA data base. The information will then be retrievable by Chemical Abstracts Service chemical number, chemical class, chemical structure, organism and specific test system, detected end points and obtained results, and bibliographic information. In addition, EMIC will edit and process the data in order to categorize each citation's results as positive, negative or inconclusive. This information will be added to EMIC's files and will permit information display in the form of a two-dimensional matrix: for each combination of test system and chemical, the numbers of positive, negative, and inconclusive findings will be given as the elements of the matrix. Figure 2 is a generalized matrix for the display of qualitative test results.

Summary Evaluation and Assessment. After the Work Group evaluations are complete, the Assessment Panel task groups will use the Work Group reports and the computerized data file to address the questions listed above. The Assessment Panel can then use the answers to these questions in proposing recommended batteries of tests for specific purposes (e.g., detecting particular kinds of genetic damage by certain classes of chemicals, assessing correlations between mutagenicity and carcinogenicity, extrapolating from in vivo test results to human risk, and so forth). The panel will also be well equipped to recommend whether a test requires further development before it can be used for routine screening and to assign priorities for such developmental research.

One or more conferences will be held for open discussion

Table II. Work Group Report Outline

Introduction
A general description and brief historical review of the assay system, including key references and the criteria for literature selection and rejection
Test Description
A detailed discussion of the system which includes, but is not necessarily limited to, the points listed below
Genetic basis of effect detected
Description of strains and uses, with procedures for genotype confirmation
Suggested testing protocol, with modifications required for specific chemical classes or physical states
(1) standard treatment conditions (e.g., time, temperature, pH, buffer)
(2) positive and negative controls
(3) vehicles or solvents
(4) acceptable background frequencies or rates
(5) dosage selection and numbers of doses
(6) collection of raw data
Interpretation of Data
Presentation of data
(1) dose-response curves
(2) data transformations
(3) various units of expression
Criteria for acceptability of data
Statistical evaluation
Criteria for positive/negative/inconclusive results
Applicability of results to hazard evaluation
Test Performance
Number of chemicals and chemical classes tested
Chemicals or chemical classes that give anomalous results, and reasons or speculation as to cause
Chemicals or chemical classes for which the test is particularly well suited
Conclusions
Strengths and weaknesses of the assay
Recommendations for research, development, and validation of the system
Bibliography
List of the references evaluated by the Work Group

of the findings concerning the individual bioassays and the conclusions of the Assessment Panel. Reports of the Assessment Panel task groups will be published in *Environmental Mutagenesis*.

USE OF THE GENE-TOX DATA BASE

It is anticipated that GENE-TOX evaluation will be repeated in the future as sufficient new data concerning any of these test systems accumulates or when new test systems are developed that warrant evaluation.

The data compiled and the evaluations made through this program will be available to the general community and are potentially of great value to scientists in the field of genetic toxicology, both as a summary of existing information on the major mutagenesis bioassays and as a guide to productive areas for future research. The program will facilitate identification of chemical compounds that are highly mutagenic or that pose special testing problems, and it will spotlight areas in which more data are needed for test system assessment.

Evaluations carried out under the GENE-TOX program will be used as a basis for regulatory decision making. The GENE-TOX program will provide objective information for prescribing batteries of tests to determine mutagenicity or presumptive carcinogenicity of chemicals under TSCA and other regulatory programs. In constructing test batteries for gene or chromosomal mutation and related biological activity, it is desirable to minimize false negative and false positive results. It is important to identify test systems that are highly sensitive and specific for particular classes of mutagens, reproducible, and easily and inexpensively performed. It is also important to be able to compare dose–response relationships among the various test systems, for use in genetic risk assessment. The GENE-TOX program will enable EPA to determine the most appropriate and practical tests, prescribe uniform protocols and data-reporting procedures, and direct efforts toward the filling of data gaps.

The information generated through the GENE-TOX program will also be used by the international community. Committee I of the International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) will carry out a survey similar to the GENE-TOX program, although more limited in scope. The ICPEMC survey considers only those chemicals for which multitest data exist; its purpose is to determine concordance among test results for this relatively small group of chemicals and use this information in drafting guidelines for uniform approaches to testing chemicals for mutagenicity. As part of this effort, ICPEMC Committee I will have access to the information developed through the GENE-TOX program.