DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[Docket No. 97D–0112]

International Conference on Harmonisation; Guidance on Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals; Availability

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is publishing a guidance entitled ''S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals.'' The guidance was prepared under the auspices of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The guidance identifies a standard set of genotoxicity tests that should be conducted for pharmaceutical registration and recommends the extent of confirmatory experimentation in in vitro genotoxicity tests in the standard battery. The guidance complements the ICH guidance ''Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals'' (S2A).

DATES: Effective November 21, 1997. Submit written comments at any time.

ADDRESSES: Submit written comments on the guidance to the Dockets Management Branch (HFA–305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1–23, Rockville, MD 20857. Copies of the guidance are available from the Drug Information Branch (HFD–210), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301–827–4573.

FOR FURTHER INFORMATION CONTACT:

Regarding the guidance: Robert E. Osterberg, Center for Drug Evaluation and Research (HFD–520), Food and Drug Administration, 9201 Corporate Blvd., Rockville, MD 20850, 301–827–2123.

Regarding ICH: Janet J. Showalter, Office of Health Affairs (HFY–20), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301–827–0864.

SUPPLEMENTARY INFORMATION: In recent years many important initiatives have been undertaken by regulatory authorities and industry associations to promote international harmonization of regulatory requirements. FDA has participated in many meetings designed to enhance harmonization and is committed to seeking scientifically based harmonized technical procedures for pharmaceutical development. One of the goals of harmonization is to identify and then reduce differences in technical requirements for drug development among regulatory agencies.

ICH was organized to provide an opportunity for tripartite harmonization initiatives to be developed with input from both regulatory and industry representatives. FDA also seeks input from consumer representatives and others. ICH is concerned with harmonization of technical requirements for the registration of pharmaceutical products among three regions: The European Union, Japan, and the United States. The six ICH sponsors are the European Commission, the European Federation of Pharmaceutical Industries Associations, the Japanese Ministry of Health and Welfare, the Japanese Pharmaceutical Manufacturers Association, the Centers for Drug Evaluation and Research and Biologics Evaluation and Research, FDA, and the Pharmaceutical Research and Manufacturers of America. The ICH Secretariat, which coordinates the preparation of documentation, is provided by the International Federation of Pharmaceutical Manufacturers Associations (IFPMA).

The ICH Steering Committee includes representatives from each of the ICH sponsors and the IFPMA, as well as observers from the World Health Organization, the Canadian Health Protection Branch, and the European Free Trade Area.

In the **Federal Register** of April 3, 1997 (62 FR 16026), FDA published a draft tripartite guideline entitled ''Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals'' (S2B). The notice gave interested persons an opportunity to submit comments by June 2, 1997.

After consideration of the comments received and revisions to the guidance, a final draft of the guidance was submitted to the ICH Steering Committee and endorsed by the three participating regulatory agencies on July 16, 1997.

In accordance with FDA's Good Guidance Practices (62 FR 8961, February 27, 1997), this document has been designated a guidance, rather than a guideline.

Genotoxicity tests are in vitro and in vivo tests designed to detect compounds that induce genetic damage directly or indirectly by various mechanisms. Compounds that are positive in tests that detect such damage have the potential to be human carcinogens and/or mutagens, i.e., may induce cancer and/or heritable defects. The guidance addresses two areas of genotoxicity testing for pharmaceuticals: (1) Identification of a standard set of tests that should be conducted for registration and (2) the extent of confirmatory experimentation in in vitro genotoxicity tests in the standard battery. The guidance is intended to be used together with the ICH S2A guidance entitled ''Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals'' (61 FR 18198, April 24, 1996) as ICH guidance principles for testing pharmaceuticals for potential genotoxicity.

This guidance represents the agency's current thinking on a recommended standard battery for genotoxicity testing of a pharmaceutical. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both.

As with all of FDA's guidances, the public is encouraged to submit written comments with new data or other new information pertinent to this guidance. The comments in the docket will be periodically reviewed, and, where appropriate, the guidance will be amended. The public will be notified of any such amendments through a notice in the **Federal Register**.

Interested persons may, at any time, submit written comments on the guidance to the Dockets Management Branch (address above). Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. The guidance and received comments may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday. An electronic version of this guidance is available on the Internet (http://www.fda.gov/cder/guidance.htm).

The text of the guidance follows:

S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals[1]

1. Introduction

Two fundamental areas in which harmonization of genotoxicity testing for

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pharmaceuticals is considered necessary are the scope of this guidance: (I) Identification of a standard set of tests that should be conducted for registration. (II) The extent of confirmatory experimentation in in vitro genotoxicity tests in the standard battery. Further issues that were considered necessary for harmonization can be found in the ICH guidance S2A ''Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals.'' *The two ICH guidances on genotoxicity complement each other and therefore should be used together as ICH guidance principles for testing of a pharmaceutical for potential genotoxicity*.

2. General Purpose of Genotoxicity Testing

Genotoxicity tests can be defined as in vitro and in vivo tests designed to detect compounds that induce genetic damage directly or indirectly by various mechanisms. These tests should enable a hazard identification with respect to damage to DNA and its fixation. Fixation of damage to DNA in the form of gene mutations, larger scale chromosomal damage, recombination and numerical chromosome changes is generally considered to be essential for heritable effects and in the multistep process of malignancy, a complex process in which genetic changes may play only a part. Compounds which are positive in tests that detect such kinds of damage have the potential to be human carcinogens and/or mutagens, i.e., may induce cancer and/or heritable defects. Because the relationship between exposure to particular chemicals and carcinogenesis is established for man, while a similar relationship has been difficult to prove for heritable diseases, genotoxicity tests have been used mainly for the prediction of carcinogenicity. Nevertheless, because germ line mutations are clearly associated with human disease, the suspicion that a compound may induce heritable effects is considered to be just as serious as the suspicion that a compound may induce cancer. In addition, the outcome of such tests may be valuable for the interpretation of carcinogenicity studies.

3. The Standard Test Battery for Genotoxicity

Registration of pharmaceuticals requires a comprehensive assessment of their genotoxic potential. It is clear that no single test is capable of detecting all relevant genotoxic agents. Therefore, the usual approach should be to carry out a battery of in vitro and in vivo tests for genotoxicity. Such tests are complementary rather than representing different levels of hierarchy.

The general features of a standard test battery can be outlined as follows:

(i) It is appropriate to assess genotoxicity in a bacterial reverse mutation test. This test has been shown to detect relevant genetic changes and the majority of genotoxic rodent carcinogens.

(ii) DNA damage considered to be relevant for mammalian cells and not adequately measured in bacteria should be evaluated in mammalian cells. Several mammalian cell systems are in use: Systems that detect gross chromosomal damage (in vitro tests for structural and numerical chromosomal aberrations), systems that detect primarily gene mutations (see Note 1), and a system that detects gene mutations and clastogenic effects (mouse lymphoma tk assay) (see Note 2). The information given in Notes 3 and 4 demonstrates that with appropriate test protocols (see section 5 of this document) the various in vitro tests for chromosomal damage and the mouse lymphoma tk assay yield results with a high level of congruence for compounds that are regarded as genotoxic but yield negative results in the bacterial reverse mutation assay. Therefore, these systems are currently considered interchangeable when used together with other genotoxicity tests in a standard battery for genotoxicity testing of pharmaceuticals, if these test protocols are used.

(iii) An in vivo test for genetic damage should usually be a part of the test battery to provide a test model in which additional relevant factors (absorption, distribution, metabolism, excretion) that may influence the genotoxic activity of a compound are included. As a result, in vivo tests permit the detection of some additional genotoxic agents (see Note 5). An in vivo test for chromosomal damage in rodent hematopoietic cells fulfills this need. This in vivo test for chromosomal damage in rodents could be either an analysis of chromosomal aberrations in bone marrow cells or an analysis of micronuclei in bone marrow or peripheral blood erythrocytes.

The following standard test battery is recommended based upon the considerations mentioned above:

(i) A test for gene mutation in bacteria.

(ii) An in vitro test with cytogenetic evaluation of chromosomal damage with mammalian cells *or* an in vitro mouse lymphoma tk assay.

(iii) An in vivo test for chromosomal damage using rodent hematopoietic cells.

For compounds giving negative results, the completion of this 3-test battery, performed and evaluated in accordance with current recommendations, will usually provide a sufficient level of safety to demonstrate the absence of genotoxic activity (see Note 6). Compounds giving positive results in the standard test battery may, depending on their therapeutic use, need to be tested more extensively (see ICH S2A ''Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals'').

The suggested standard set of tests does not imply that other genotoxicity tests are generally considered inadequate or inappropriate (e.g., tests for measurement of DNA adducts, DNA strand breaks, DNA repair or recombination). Such tests serve as options in addition to the standard battery for further investigation of genotoxicity test results obtained in the standard battery. Furthermore, molecular techniques to study mechanisms of genotoxicity in the standard battery systems may be useful for risk assessment. Only under extreme conditions in which one or more tests comprising the standard battery cannot be employed for technical reasons, alternative validated tests can serve as substitutes. For this to occur, sufficient scientific justification should be provided to support the argument that a given standard battery test is not appropriate.

The standard battery does not include an independent test designed specifically to test for aneuploidy. However, information on this type of damage may be derived from the tests for chromosomal damage in vitro and in vivo. Elements of the standard protocols that provide such information are elevations in the mitotic index, polyploidy induction and micronucleus evaluation. There is also limited experimental evidence that aneuploidy inducers can be detected in the mouse lymphoma tk assay (see Note 4). In such cases, further testing may be needed.

4. Modifications of the 3-Test Battery

The following sections give situations where the standard 3-test battery may need modification.

4.1 Limitations to the Use of Bacterial Test Organisms

There are circumstances where the performance of the bacterial reverse mutation test does not provide appropriate or sufficient information for the assessment of genotoxicity. This may be the case for compounds that are excessively toxic to bacteria (e.g., some antibiotics) and compounds thought or known to interfere with the mammalian cell replication system (e.g., topoisomerase inhibitors, nucleoside analogues, or inhibitors of DNA metabolism). For these cases, usually two in vitro mammalian cell tests should be performed using two different cell types and of two different endpoints (gene mutation (see Note 1) and chromosomal damage). Nevertheless, it is still important to perform the bacterial reverse mutation test (see Note 7); either a full test or a limited (range-finding) test (see section 5 of this document) may be appropriate.

4.2 Compounds Bearing Structural Alerts for Genotoxic Activity

Structurally alerting compounds (see Note 8) are usually detectable in the standard 3-test battery. However, compounds bearing structural alerts that have given negative results in the standard 3-test battery may necessitate limited additional testing. The choice of additional test(s) or protocol modification(s) depends on the chemical nature, the known reactivity, and metabolism data on the structurally alerting compound under question (see Note 9 and ICH S2A ''Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals'').

4.3 Limitations to the Use of Standard In Vivo Tests

There are compounds for which standard in vivo tests do not provide additional useful information. These include compounds for which data from studies on toxicokinetics or pharmacokinetics indicate that they are not systemically absorbed and therefore are not available for the target tissues in standard in vivo genotoxicity tests. Examples of such compounds are some radioimaging agents, aluminum-based antacids, and some dermally applied pharmaceuticals. In cases where a modification of the route of administration does not provide sufficient target tissue exposure, it may be appropriate to base the evaluation only on in vitro testing.

4.4 Additional Genotoxicity Testing in Relation to the Carcinogenicity Bioassay

4.4.1 Evidence for Tumor Response

Additional genotoxicity testing in appropriate models may be conducted for compounds that were negative in the standard 3-test battery but which have shown effects in carcinogenicity bioassay(s) with no clear evidence for a nongenotoxic mechanism. To help understand the mechanism of action, additional testing can include modified conditions for metabolic activation in in vitro tests or can include in vivo tests measuring genetic damage in target organs of tumor induction (e.g., liver UDS test, 32P-postlabeling, mutation induction in transgenes, molecular characterization of genetic changes in tumor-related genes).

4.4.2 Structurally Unique Chemical Classes

On rare occasions, a completely novel compound in a unique structural chemical class will be introduced as a pharmaceutical. When such a compound will not be tested in chronic rodent carcinogenicity bioassays, further genotoxicity evaluation may be invoked.

5. Standard Procedures for In Vitro Tests

Reproducibility of experimental results is an essential component of research involving novel methods or unexpected findings; however, the routine testing of chemicals with standard, widely used genotoxicity tests need not always be completely replicated. These tests are sufficiently well characterized and have sufficient internal controls that repetition can usually be avoided if protocols with built-in confirmatory elements, such as those outlined below, are used.

For both bacterial and mammalian cell gene mutation tests, the results of a range-finding test can be used to guide the selection of concentrations to be used in the definitive mutagenicity test. By these means, a range-finding test may supply sufficient data to provide reassurance that the reported result is the correct one. In bacterial mutagenicity tests, preliminary range-finding tests performed on all bacterial strains, with and without metabolic activation, with appropriate positive and negative controls, and with quantification of mutants, may be considered a sufficient replication of a subsequent complete test. Similarly, a range-finding test may also be a satisfactory substitute for a complete repeat of a test in gene mutation tests with mammalian cells other than the mouse lymphoma tk assay (see below) if the range-finding test is performed with and without metabolic activation, with appropriate positive and negative controls, and with quantification of mutants (see Note 10).

For the cytogenetic evaluation of chromosomal damage in vitro, the test protocol includes the conduct of tests with and without metabolic activation, with appropriate positive and negative controls, where the exposure to the test articles is 3 to 6 hours and a sampling time of approximately 1.5 normal cell cycles from the beginning of the treatment. A continuous treatment without metabolic activation up to the sampling time of approximately 1.5 normal cell cycles is needed in case of a negative result for the short treatment period without metabolic activation. Certain chemicals may be more readily detected by longer treatment or delayed sampling times, e.g., some nucleoside analogues or some nitrosamines. Negative results in the presence of a metabolic activation system may need confirmation on a case-by-case basis (see Note 11). In any case, information on the ploidy status should be obtained by recording the incidence of polyploid cells as a percentage of the number of metaphase cells. An elevated mitotic index or an increased incidence of polyploid cells may give an indication of the potential of a compound to induce aneuploidy. In such cases, further testing may be needed.

For the mouse lymphoma tk assay, the test protocol includes the conduct of tests with and without metabolic activation, with appropriate positive and negative controls, where the exposure to the test articles is 3 to 4 hours. A continuous treatment without metabolic activation for approximately 24 hours is needed in case of a negative result for the short treatment without metabolic activation (see Note 4). Negative results in the presence of a metabolic activation system may need confirmation on a case by case basis (see Note 11). In any case, an acceptable mouse lymphoma tk assay includes (i) the incorporation of positive controls, which induces mainly small colonies and (ii) colony sizing for positive controls, solvent controls, and at least one positive test compound dose (should any exist), including the culture that gave the greatest mutant frequency.

Following such testing, further confirmatory testing in the case of clearly negative or positive test results is not usually needed.

Ideally, it should be possible to declare test results as clearly negative or clearly positive. However, test results sometimes do not fit the predetermined criteria for a positive or negative call and therefore are declared ''equivocal.'' The application of statistical methods aids in data interpretation, however, adequate biological interpretation is of critical importance. Nonetheless, further testing is usually indicated for equivocal results.

6. Notes

(1) Test approaches currently accepted for the assessment of mammalian cell *gene* mutation involve the *tk* locus using mouse lymphoma L5178Y cells or human lymphoblastoid TK6 cells, the *hprt* locus using CHO cells, V79 cells, or L5178Y cells, or the *gpt* locus using AS52 cells.

(2) The molecular dissection of mutants induced at the *tk* locus shows a broad range of genetic events including point mutations, deletions, translocations, recombinations, etc. Small colony mutants have been shown to predominantly lack the tk_b allele as a consequence of structural or numerical alterations or recombinational events. There is some evidence that other loci, such as *hprt* or *gpt* are also sensitive to large deletion events. However, due to the X-chromosomal origin of the *hprt* gene which is probably flanked by essential genes, large scale deletion events or numerical alterations often do not give rise to mutant colonies, thus limiting the sensitivity of this genetic locus relative to the *tk* locus for the detection of a wide range of genetic changes.

(3) With respect to the cytogenetic evaluation of chromosomal damage, it is not uncommon for the systems currently in use, i.e., several systems with permanent mammalian cells in culture and human lymphocytes either isolated or in whole blood, to give different results for the same test compound. However, there is evidence that some of the differences observed have been due to protocol differences. This may be minimized by using the procedures described in section 5 of this document.

For the great majority of presumptive genotoxic compounds that were negative in a bacterial reverse mutation assay, the data on chromosomal damage in vitro and mouse lymphoma tk results are in agreement. Several reliable studies indicate that the mouse lymphoma tk assay is able to detect compounds that induce structural and numerical chromosomal damage. For safety testing of pharmaceuticals, the mouse lymphoma tk assay is considered an acceptable alternative to the direct analysis of chromosomal damage in vitro. Although colony sizing is an essential element of the mouse lymphoma tk assay test protocol, it gives only limited information on the type of damage induced in mutant colonies. Further mechanistic investigations may be used to assess the nature of cytogenetic changes induced by clastogens and aneuploidy inducers in the mouse lymphoma tk assay. Such information could be provided by studies to demonstrate the loss of the *tk* gene or the loss of the chromosome carrying the *tk* gene.

(4) The detection of a number of different nucleoside analogues and base analogues is enhanced for the mouse lymphoma tk assay when the treatment protocol for both agar and microtitre methods includes a 24-hour treatment regimen in the absence of an exogenous metabolic activation system. Similarly, the detection of aneuploidy inducers is enhanced if a 24-hour treatment regimen is used with the microtitre method. Currently, there is no evidence to support this conclusion for the soft agar method. The specificity of the test protocol, i.e., to obtain correct test results for presumptive nongenotoxic compounds, does not change significantly using a 24-hour treatment in the microtitre method. For the soft agar method, there appears to be a reduction in specificity under the same treatment regimen. Based on this information, the microtitre method is recommended for use in the standard battery.

(5) There are a small but significant number of genotoxic carcinogens that are reliably detected by the bone marrow tests for chromosomal damage that have yielded negative/weak/conflicting results in the pairs of in vitro tests outlined in the standard battery options, e.g., bacterial reverse mutation plus one of a selection of possible tests with cytogenetic evaluation of chromosomal damage or bacterial mutation plus the mouse lymphoma tk assay. Carcinogens such as procarbazine, hydroquinone, urethane and benzene fall into this category.

(6) The continuing evolution of short-term tests and test methodologies will afford new,

more sensitive, more practical, more expeditious, and more economical techniques for detection of genotoxic compounds. Some of these may ultimately replace the genotoxicity tests used for regulatory purposes. Among the more promising tests, the in vitro micronucleus test appears to offer potential for screening purposes.

(7) Some antibacterial agents, albeit highly toxic to the tester strains, are detected as genotoxic at very low, sublethal concentrations in the bacterial reverse mutation test (e.g., nitrofuran antibiotics).

(8) Certain structurally alerting molecular entities are recognized as being causally related to the carcinogenic and/or mutagenic potential of chemicals. Examples of structural alerts include alkylating electrophilic centers, unstable epoxides, aromatic amines, azo-structures, N-nitroso-groups, aromatic nitro-groups.

(9) For some classes of compounds with specific structural alerts, it is established that specific protocol modifications/additional tests are necessary for optimum detection of genotoxicity (e.g., molecules containing an azo-group, glycosides, compounds such as nitroimidazoles requiring nitroreduction for activation, compounds such as phenacetin requiring another rodent S9 for metabolic activation). The additional testing needed when the chosen 3-test battery yields negative results for a structurally alerting test compound could consist of such modifications.

(10) The dose range-finding study should: (i) Give information on the shape of the toxicity dose-response curve if the test compound exhibits toxicity, (ii) include highly toxic concentrations, and (iii) include quantification of mutants in the cytotoxic range. If a compound is not toxic, then mutants should nevertheless be quantified.

(11) A repetition of a test using the identical source and concentration of the metabolic activation system is usually not necessary. A modification of the metabolic activation system may be indicated for certain chemical classes where knowledge is available on specific requirements of metabolism. This would usually invoke the use of an external metabolizing system which is known to be competent for the metabolism/activation of the class of compound under test.

7. Glossary

Cytogenetic evaluation: Chromosome structure analysis in mitosis or meiosis by light microscopy.

DNA adduct: (Covalent) binding of chemicals to DNA.

DNA repair: Reconstitution of damaged DNA sequence.

DNA strand breaks: Single or double strand scissions in the DNA.

Numerical chromosome changes: Chromosome numbers different from the original haploid or diploid set of chromosomes; for cell lines, chromosome numbers different from the modal chromosome set.

Recombination: Breakage and balanced or unbalanced rejoining of DNA.

Transgene: An exogenous or foreign gene inserted into the host genome, into either somatic cells or germ line cells.

Dated: November 15, 1997.

William K. Hubbard,

Associate Commissioner for Policy Coordination.

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