DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration [Docket No. 97D-0112]

International Conference on Harmonisation; Draft Guideline 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 draft guideline entitled "Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals." The draft guideline was prepared under the auspices of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The guideline identifies a standard set of genotoxicity tests to be conducted for pharmaceutical registration, and recommends the extent of confirmatory experimentation in in vitro genotoxicity tests in the standard battery. The draft guideline complements the ICH guideline "Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals." The guideline is intended to be used in vitro genotoxicity tests in the standard battery. The draft guideline is intended to be used together with the ICH guideline 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.

Although not required, FDA has in the past provided a 75- or 90-day comment period for draft ICH guidelines. However, the comment period for this guideline has been shortened to 60 days so that comments may be received by FDA in time to be reviewed and then discussed at a July 1997 ICH meeting involving this guideline.

This guideline represents the agency's current thinking on a recommended standard battery for genotoxicity testing of pharmaceuticals. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public or to confer any rights for or on any person.

FOR FURTHER INFORMATION CONTACT: Regarding the guideline: 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 the ICH: Janet J. Showalter, Office of Health Affairs (HFY-20), Food and Drug Administration, 9201 Corporate Blvd., Rockville, MD 20850, 301-827-2123.

Regarding the CDER: The CDER provides a 75- or 90-day comment period for draft ICH guidelines. Comments are to be identified with the docket number found in brackets in the heading of this document. The draft guideline is available via Internet by going to the CDER home page, type "http://www.fda.gov/cder" and go to the "Regulatory Guidance" section.

The text of the draft guideline follows:

Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals

1. Introduction

Two fundamental areas in which harmonization of genotoxicity testing for pharmaceuticals is considered necessary are the scope of this guideline: (I) Identification of a standard set of tests to be conducted for registration, and (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 guideline "Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals," (61 FR 18198, April 24, 1996). The two ICH guidelines 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 which 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 multi-step 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. In addition, the outcome of such tests may be valuable for the interpretation of carcinogenicity studies. Nevertheless, 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.

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 would 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 initially 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 which detect gross chromosomal damage (in vitro tests for chromosomal damage), a system which detects gene mutations and clastogenic effects (mouse lymphoma tk assay), and systems which detect primarily gene mutations (see Notes 1 and 2).

There has been a debate whether in vitro tests for chromosomal damage and the mouse lymphoma tk assay are equivalent for detection of clastogens. Several studies have shown that most of the differences reported are due to differences in the test protocols employed. The scientific information given in Notes 3 and 4 demonstrate that with appropriate test protocols (see section 5) the various in vitro tests for chromosomal damage and the mouse lymphoma tk assay yield results with a high level of congruence. Therefore these systems may be treated as equally sensitive and considered interchangeable for regulatory purposes if these test protocols are used. Consequently, for regulatory purposes, a negative result in an in vitro test with cytogenetic evaluation of chromosomal damage or in a mouse lymphoma tk assay gives additional assurance to the other parts of the standard battery that the compound tested does not induce genetic damage. In any event, the mammalian cells used for genotoxicity evaluation in vitro should be carefully selected taking the specific particulars of the test, the test protocol, and the test compound into account.

(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 rodent hematopoietic cells 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 may be deduced from the considerations mentioned above:

(i) A test for gene mutation in bacteria.
(ii) An in vitro test with cytogenetic evaluation of chromosomal damage with the 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. Compounds giving positive results in the standard test battery may, depending on their therapeutic use, need to be tested more extensively (see ICH “Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals” (60 FR 18198, April 24, 1996)).

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. Only under extremely rare conditions in which one or more tests comprising the standard battery cannot be employed for technical reasons, alternative validated tests can serve as a substitute. 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 numerical chromosome changes, e.g., aneuploidy and polyploidy. However, information on this type of damage should be derived from the cytogenetic evaluation of chromosomal damage in vitro and in vivo.

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 two different endpoints (gene mutation (see Note 1) and chromosomal damage). Nevertheless it is still important to perform the bacterial reverse mutation test, either a full test or a limited (range-finding) test (see section 5).

4.2 Compounds bearing structural alerts for genotoxic activity

Structurally alerting compounds (see Note 6) 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 using induced rat liver S9 for metabolic activation as standard in the in vitro tests and using mouse erythrocyelial cells as standard test cells for the in vivo test may need limited additional testing. The choice of additional test(s) or protocol modification(s) depend on the chemical nature, the known reactivity, and metabolism data on the structurally alerting compound under question (see Note 7).

4.3 New/unique chemical structures/classes

On relatively rare occasions, a completely novel compound in a unique structural or functional (i.e., potentially DNA-reactive) chemical class will be introduced as a pharmaceutical. It may not be easy to categorize such compounds, e.g., with respect to alerting structures, metabolism requirements, or interaction with cell
replication. In order to gain knowledge on the genotoxic potential of such compounds it may be necessary to test them more comprehensively than in the standard 3-test battery, e.g., in a further in vitro test with mammalian cells.

4.4 Genotoxicity testing of pharmaceuticals using solely in vitro tests

There are compounds for which conventional in vivo tests do not provide additional useful information. These include compounds that are not systemically absorbed and therefore are not available for the target tissues in in vivo genotoxicity tests (i.e., bone marrow or liver). Examples of such compounds are some radiomimetic agents, aluminum-based antacids, and some dermally applied pharmaceuticals. In these cases, a test battery composed solely of in vitro test models is acceptable which should consist of a bacterial gene mutation assay, a gene mutation assay with mammalian cells (see Note 1), and a test for chromosomal damage with mammalian cells.

4.5 Considerations for additional genotoxicity testing in relation to the carcinogenicity bioassay

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(ies) 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 for mutagenic damage in target organs of tumor induction (e.g., liver UDS test, 32P-postlabeling, mutation induction in transgenes).

5. Standard Procedures for In Vitro Tests in the Standard Battery

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 outlined below are used.

Complete repetition of gene mutation tests is usually not necessary if the protocol includes a range-finding test that supplies sufficient data to provide reassurance that the reported result is the correct one. For example, 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 (see Note 8). For both bacterial and mammalian cell gene mutation tests, the results of the range-finding test should guide the selection of concentrations to be used in the definitive mutagenicity test.

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 cell cycles is needed in case of a negative result for the short treatment period without metabolic activation. If severe cell cycle delay is noted, a prolonged treatment or sampling time is needed. Negative results in the presence of a metabolic activation system may need confirmation on a case-by-case basis (see Note 4). Disappearance of the ploidy status should be obtained by recording the incidence of polyploid cells as a percentage of the number of metaphase cells.

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 24 hours is advisable in case of a negative result for the short-term treatment with 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 9). In any case, the conduct of a mouse lymphoma tk assay involves 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 define test results as clearly negative or clearly positive. But test results sometimes do not fit into the criteria for a positive or negative call and therefore have to be defined as "equivocal." In these circumstances, the application of statistical methods can aid in data interpretation. Since the use of statistical methods is not always satisfying for some of the standard genotoxicity tests, adequate biological interpretation is of critical importance. The criteria for declaration of a test result as positive or negative must in part be based on the experience and standards of the laboratory carrying out the test. Equivocality then, for example, encompasses test results which lack a dose-related increase of the effect of the test article range and/or test results which exceed the concurrent negative control values but may lie within historical negative control data. Further testing is usually indicated in the case of results that have to be called equivocal even if the results are obtained with protocols such as outlined above.

6. Notes

(1) Test systems seen currently as appropriate for the assessment of mammalian cell gene mutation include the L5178Y tk+/−→tk−/− mouse lymphoma assay (mouse lymphoma tk assay), the HPRT-tests with CHO-cells, V79-cells, or L5178Y cells, or the GFT-(X)PRT test with A552 cells, and the human lymphoblastoid cell test.

(2) The molecular dissection of mutants induced at the tk locus shows a range of genetic events including point mutations, deletions, translocations, recombinations, etc. (e.g., Applegate et al., 1990). Small colony mutants have been shown to predominantly lack the tkb allele as a consequence of structural or numerical alterations or recombination events (Blazak et al., 1989; El-Tarra et al., 1995). There is some evidence that other loci, such as hprt or get are also sensitive to large deletion events (Glatt, 1994; Kinashi et al., 1995). However, due to the X-chromosomal origin of the hprt gene which is probably flanked by essential genes, large scale chromosomal damage (e.g., deletional) alterations often do not give rise to mutant colonies, thus limiting the sensitivity of this test. Therefore, the mouse lymphoma tk assay has advantages in comparison to other gene mutation assays and it may be recommended to conduct the mouse lymphoma tk assay as the gene mutation test. A positive result in the mouse lymphoma tk assay may constitute a case for further investigation of the type and/or mechanism of genetic damage involved.

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, a recently conducted multi-laboratory comparison of in vitro tests with cytogenetic evaluation of chromosomal damage gave conclusive evidence that the differences observed are mostly due to protocol differences (Galloway et al., 1996).

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 assay results are in agreement. A recently conducted mouse lymphoma tk collaborative study reinforced this view. Under cooperation of the Japanese Ministry of Health and Welfare, a collaborative study on the mouse lymphoma tk assay (MLA) was conducted by 45 Japanese and 7 other laboratories in order to clarify how well the MLA can detect in vitro clastogens and polyploidy (aneuploidy) inducers and how well the in vitro tests with cytogenetic evaluation of chromosomal damage can detect compounds expected to act exclusively in the MLA. On the basis of published data, 40 compounds were selected, which were negative in bacterial reverse mutation assays, but positive either in in vitro tests with cytogenetic evaluation of chromosomal damage (30 compounds) or in the MLA (9...
includes a 24 hour treatment regimen in the

detect some clastogens/aneuploidy inducers

ability of the mouse lymphoma tk assay to
different compounds give evidence that the
lymphoma tk assay may need to be
in the mouse lymphoma tk assay (see Note
type of damage induced in mutant colonies
of chromosomal damage in vitro. Colony
aspect of safety testing for pharmaceuticals,
conclusion, it is perceived that, from the
literature (Garriott et al., 1995). In
treatment can be found in the published
HPRT assay in which large-scale DNA
CA positive compounds were negative in the
lymphoma tk uniquely positive compounds,
i.e., at the moment, in the absence of
positive compounds may be quite limited,
indicate that the number of MLA unique
and cinnamylanthranilate). These data
MLA study and two of the three CA-negative
compounds were reexamined in the present
outlined in section 5. The same nine
compounds were reexamined in the present
Zeiger et al., 1990) were reported by the NTP (National
compound, the MLA positive results in the MLA when the cells were treated in the absence of S–9 mix for 24 hours instead of 4 hours.
(8) The dose range-finding study should: (i)

Of 34 CA (carcinogen) positive chemicals, 3 (9 percent) were negative in the MLA. These results suggest that while the MLA
can detect most clastogens and polyploidy
inducers, there may be some that cannot detect (bromodichloromethane, isophorone, tetrachloroethane). Tetrachloroethane
induced polyploidy only, whereas bromodichloromethane and isophorone were only weakly clastogenic.

Reinvestigation of 9 of 10 mouse
lymphoma uniquely positive compounds that were reported by the NTP (National
Toxicology Program) (Zeiger et al., 1990) showed that only 3 were negative in CHL/IU cells using the comprehensive protocol as
outlined in section 5. The same nine
compounds were reexamined in the present
MLA study and two of the three CA-negative
compounds were positive (trichloroethylene and cinnamylanthranilate). These data
indicate that the number of MLA unique
positive compounds may be quite limited,
indicate that the number of MLA unique
positive compounds may be quite limited,
only trichloroethylene and
cinnamylanthranilate are known.

Comparison with published data and data in
regulatory files show that many MLA and CA
positive compounds were negative in the
HPRT assay in which large-scale DNA
rearrangements could not be detected.

Only a few more clastogenic compounds
giving negative results in the usual mouse
lymphoma tk assay with 3 to 4 hours of
treatment can be found in the published
literature (Garriott et al., 1995). In

conclusion, it is perceived that, from the
aspect of safety testing for pharmaceuticals,
the mouse lymphoma tk assay is an
acceptable alternative for the direct analysis of
chromosomal damage in vitro. Colony
sizing gives only limited information on the
type of damage induced in mutant colonies in
the mouse lymphoma tk assay (see Note 2).
Therefore, a positive result in a mouse
lymphoma tk assay may need to be
investigated further to determine the type of
genetic damage that was induced.

Recent results from a number of
different compounds give evidence that the
ability of the mouse lymphoma tk assay to
detect some clastogens/aneuploidy inducers is
enhanced when the treatment protocol
includes a 24 hour treatment regimen in the


dose-response curve if the test compound
exhibits toxicity; (ii) include highly toxic
concentrations; (iii) include quantification of
mutants in the cytotoxic range. Even if a
compound is not toxic, mutants should
nevertheless be quantified.

A repetition of a test using the identical
source and concentration of the metabolic
activation system is usually not necessary.
However, 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 involve the
use of an external metabolizing system which
is known to be competent for the metabolism/activation of the class of
compound under test.

References to Notes

Ashby, J., and R. W. Tennant, “Chemical
structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of
genotoxic carcinogenesis among 222

Ashby, J., and R. W. Tennant, “Definitive
relationships among chemical structure,
carcinogenicity and mutagenicity for 301
chemicals tested by the U.S. NTP,” Mutation

Ashby, J., and D. Paton, “The influence of
chemical structure on the extent and sites of
carcinogenesis of 522 rodent carcinogens
and 55 different human carcinogen exposures,”


Blazak, W. F., F. J. Los, C. J. Rudd, and W.
J. Caspary, “Chromosome analysis of small
and large L5178Y mouse lymphoma cell
colonies. Comparison of trifluorothymidine
resistant and unselected cell colonies from
mutagen-treated and control cultures,”


El-Tarras, A. J., S. Dubins, J. Warner, C.
Hoffman, and R. R. Cobb, “Molecular
analysis of the TK locus in L5178Y large
and small colony mouse lymphoma cell mutants
induced by hydantoin methanesulfonate,”


William K. Hubbard,
Associate Commissioner for Policy Coordination.
[FR Doc. 97–8554 Filed 4–2–97; 8:45 am]