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 draft 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.''

DATES: Written comments by June 2, 1997.

ADDRESSES: Submit written comments on the draft guideline to the Dockets Management Branch (HFA–305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1–23, Rockville, MD 20857. Copies of the draft guideline 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 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, 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 September 1996, the ICH Steering Committee agreed that a draft guideline entitled ''Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals'' should be made available for public comment. The draft guideline is the product of the Safety Expert Working Group of the ICH. Comments about this draft will be considered by FDA and the Safety Expert Working Group.

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 draft guideline addresses two areas of genotoxicity testing for pharmaceuticals: (1) Identification of a standard set of tests to be conducted for registration, and (2) the extent of confirmatory experimentation in 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 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.

Interested persons may, on or before June 2, 1997, submit to the Dockets Management Branch (address above) written comments on the draft guideline. 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 draft guideline 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 guideline is available via Internet by using the World Wide Web (WWW). To connect 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. (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 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. 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 cells, 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 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 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 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. 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 extreme 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 erythropoietic 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 radioimaging 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(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 genotoxic 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, may be considered 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 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 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 9). 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.

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 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 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 in an appropriate dose 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 $tk^{+/-} \rightarrow tk^{-/-}$ mouse lymphoma assay (mouse lymphoma tk assay), the HPRT-tests with CHO-cells, V79-cells, or L5178Y cells, or the GPT-(XPRT) test with AS52 cells, and the human lymphoblastoid TK6 test.

(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. (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 recombinational events (Blazak et al., 1989; El-Tarras et al., 1995). There is some evidence that other loci, such as *hprt* or *gpt* 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., deletion) or numerical 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.

(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, a recently conducted multilaboratory comparison of in vitro tests with cytogenetic evaluation of chromosomal damage gave conclusive evidence that the differences observed are most often 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 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 and the Japanese Pharmaceutical Manufacturers Association, 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 that were thought 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

compounds). These compounds were examined by the microwell method using L5178Y tk$^{+/-}$ 3.7.2C cells or were reexamined in CHL/IU cells for induction of chromosomal aberrations. Various aspects of this study are currently in the process of publication (Matsuoka et al., 1996; Sofuni et al., 1996).

The table below gives the results of this major attempt to compare the results of in vitro tests with cytogenetic evaluation of chromosomal damage in different cells (human lymphocytes, CHO, V79 and CHL cells) and the mouse lymphoma tk assay:

		chromosome damage (CA) mainly structural	chromosome damage (CA) mainly polyploidy	chromosome damage (CA)
		positive	positive	negative
mouse lymphoma tk assay	positive	21[1]	5[1]	2
	inconcl./equiv.	3	2	1
	negative	2	1	3

[1] 7 compounds (colchicine, 2′-deoxycoformycin, dideoxycytidine, phenacetin, p-tert butylphenol, theophylline, thiabendazole) yielded clearly positive results in the MLA when the cells were treated in the absence of S–9 mix for 24 hours instead of 4 hours.

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

Reinvestigation of 9 of 10 mouse lymphoma unique positive carcinogens 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, i.e., at the moment, in the absence of reinvestigation of other NTP reported mouse lymphoma tk uniquely positive compounds, 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 examine the type of genetic damage that was induced.

(4) 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 absence of an exogenous metabolic activation system. Compounds such as colchicine, vincristine, diethylstilbestrol, caffeine, 2′-deoxycoformycin, dideoxycytidine, thiabendazole, theophylline, phenacetin, p-tert butylphenol, and azidothymidine gave negative or only weakly positive results in a standard mouse lymphoma tk assay with 3 or 4 hours of treatment (absence of S–9 mix) but were tested clearly positive with 24 hours of exposure to the test substance. (Azidothymidine and caffeine are the compounds which were tested in the agar version of the mouse lymphoma tk assay whereas the data on 24 hours of treatment on the other compounds are generated with the microwell method.)

(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) Certain structurally alerting molecular entities are recognized as being causally related to the carcinogenic and/or mutagenic potential of chemicals (Ashby and Tennant, 1988; Ashby and Tennant, 1991; Ashby and Paton, 1993). Examples of structural alerts include alkylating electrophilic centers, unstable epoxides, aromatic amines, azo-structures, N-nitroso-groups, aromatic nitro-groups.

(7) 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). Such modifications could form the additional testing needed when the chosen 3-test battery yields negative results for a structurally alerting test compound.

(8) 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; (iii) include quantification of mutants in the cytotoxic range. Even if a compound is not toxic, mutants should nevertheless be quantified.

(9) 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.

7. References to Notes

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Dated: March 29, 1997.

William K. Hubbard,

Associate Commissioner for Policy Coordination.

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