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To: Main Trade Associations, Registrars of Medicine, Registrars of Pharmacy

I am pleased to inform you of the release of the *International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use* (ICH)/Therapeutic Products Programme Guidance, **Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals (ICH Topic S2B)**.

This guidance has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. The ICH Steering Committee has endorsed the final draft and recommended its adoption by the regulatory bodies of the European Union, Japan and USA.

In adopting this ICH guidance, the Therapeutic Products Programme (TPP) endorses the principles and practices described therein. This document should be read in conjunction with this covering letter and with the relevant sections of other applicable Programme guidances.

The Programme recognizes that the scope and subject matter of current TPP guidances may not be entirely consistent with those of the ICH guidances that are being introduced as part of the Programme's commitment to international harmonization and the ICH Process. In such circumstances, the ICH guidances adopted by the TPP take precedence. In this regard , the TPP will be examining mecessary changes to the Programme's 1990 *Toxicological Evaluation Guideline*.

The TPP is committed to eliminating discrepancies through the implementation of a phased-in work plan that will examine the impact associated with the adoption of ICH guidances. This will result in the amendment or, depending on the extent of revisions required, withdrawal of some TPP guidances.

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This and other Guidance documents are available on the Therapeutic Products Programme (TPP) Website (**http://www.hc-sc.gc.ca/hpb-dgps/therapeut**). The availability of printed copies of TPP guidances may be confirmed by consulting the Programme's *Guidelines and Publications Order Forms* (available on the TPP Website) or by contacting the Publications Coordinator[1].

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Disclaimer

The material herein was prepared under the direction of the Therapeutic Products Programme, Health Canada. No changes are permitted.

Health Canada Santé Canada

GUIDANCE FOR INDUSTRY

Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals

ICH Topic S2B

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Therapeutic Products Programme
Guidance Document

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Our mission is to help the people of Canada maintain and improve their health.

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FOREWORD

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In adopting this ICH guidance, the Therapeutic Products Programme (TPP) endorses the principles and practices described therein. This document should be read in conjunction with the accompanying covering letter and with the relevant sections of other applicable Programme guidances.

Guidance documents are meant to provide assistance to industry and health care professionals on **how** to comply with the TPP policies and governing statutes and regulations. They also serve to provide review and compliance guidance to TPP staff, thereby ensuring that the Programme's mandate is implemented in a fair, consistent and effective manner.

Guidance documents are administrative instruments not having force of law and, as such, allow for flexibility in approach. Alternate approaches to the principles and practices described in this document ***may be*** acceptable provided they are supported by adequate scientific justification. Alternate approaches should be discussed in advance with the Programme to avoid the possible finding that applicable statutory or regulatory requirements have not been met.

As a corollary to the above, it is equally important to note that the Programme reserves the right to request information or material, or define conditions not specifically described in this guidance, in order to allow the Programme to adequately assess the safety, efficacy or quality of a therapeutic product. The TPP is committed to ensuring that such requests are justifiable and that decisions are clearly documented.

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1. INTRODUCTION

Two fundamental areas in which harmonisation of genotoxicity testing for pharmaceuticals is considered necessary are the scope of this guidance: 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 harmonisation can be found in the ICH guidance *Notes for Guidance on Specific Aspects of Regulatory Genotoxicity Tests.* **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 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, whilst 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* 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 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 *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 as 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)* 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) depend on the chemical nature, the known reactivity and metabolism data on the structurally alerting compound under question (*see Note 9* and ICH *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. This includes 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 non-genotoxic 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 tumour induction (e.g. liver UDS test, ^{32}P-postlabelling, mutation induction in transgenes, molecular characterisation 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, (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 class 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 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 *tkb* 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*.

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 b clastogens and aneuploidy inducers in the mouse lymphorna 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 include 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 non-genotoxic 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/week/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, sub-lethal 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, (iii) include quantification of mutants in the cytotoxic range. If a compound were not toxic, then mutants should nevertheless be quantified.

(11) A repetition of this 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 metabolising 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 damage 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, either into somatic cells or germ lline cells