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# Guidance for Industry Recommended Approaches to Integration of Genetic Toxicology Study Results

## ***DRAFT GUIDANCE***

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For questions regarding this draft document contact (CDER) David Jacobson-Kram 301-443-5346.

**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)**

**November 2004  
Pharmacology and Toxicology**

# Guidance for Industry Recommended Approaches to Integration of Genetic Toxicology Study Results

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Center for Drug Evaluation and Research  
Food and Drug Administration  
5600 Fishers Lane  
Rockville, MD 20857  
(Tel) 301-827-4573  
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**U.S. Department of Health and Human Services  
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*Contains Nonbinding Recommendations*

*Draft — Not for Implementation*

1 **Guidance for Industry<sup>1</sup>**  
2 **Recommended Approaches to Integration of Genetic Toxicology**  
3 **Study Results**  
4  
5  
6

7  
8 This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current  
9 thinking on this topic. It does not create or confer any rights for or on any person and does not operate to  
10 bind FDA or the public. You can use an alternative approach if it satisfies the requirements of the  
11 applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff  
12 responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the  
13 appropriate number listed on the title page of this guidance.  
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18 **I. INTRODUCTION**  
19

20 This guidance is intended to inform industry on how the Center for Drug Evaluation and  
21 Research (CDER) views positive findings in genetic toxicology assays during drug development.  
22 The guidance provides recommendations on how to proceed with clinical studies while ensuring  
23 the safety of study participants when results in genotoxicity studies suggest a potential cancer or  
24 genetic hazard. Regulatory decisions involving both single- and repeat-dose clinical studies are  
25 discussed. This guidance pertains to pharmaceuticals administered through oral, intravenous,  
26 topical, and other routes, as appropriate.  
27

28 FDA's guidance documents, including this guidance, do not establish legally enforceable  
29 responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should  
30 be viewed only as recommendations, unless specific regulatory or statutory requirements are  
31 cited. The use of the word *should* in Agency guidances means that something is suggested or  
32 recommended, but not required.  
33

34  
35 **II. BACKGROUND**  
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37 For the purpose of this guidance, a *single-dose clinical study* is defined as a study involving a  
38 single administration or up to 24 hours of an intravenous infusion of a drug product. *Repeat-*  
39 *dose studies* are studies involving multiple administrations or infusions of more than 24 hours  
40 duration. Administration of sustained-release preparations or agents with an in vivo half-life of  
41 greater than 12 hours can result in systemic exposure for greater than 24 hours.  
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<sup>1</sup> This guidance has been prepared by the Pharmacology Toxicology Coordinating Committee (PTCC) in the Office of New Drugs (OND) in the Center for Drug Evaluation and Research (CDER) of the Food and Drug Administration.

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43 The timing and conduct of genetic toxicology studies have been described in the ICH guidelines  
44 M3, S2A, and S2B.<sup>2</sup> We recommend that these guidances be consulted and that this document  
45 be considered an adjunct guidance.

46  
47 Risk for carcinogenesis is usually determined in rodent assays, either 2-year studies or shorter-  
48 term studies using alternative models.<sup>3</sup> A core battery of genetic toxicology studies has been  
49 accepted by industry and regulators through the International Conference on Harmonisation  
50 (ICH) consultative process. These studies, which are designed to identify genotoxic hazard,  
51 include:

- 52 • A test for gene mutation in bacteria;
- 53 • An in vitro assessment of chromosomal damage using mammalian cells or an in vitro  
54 mouse lymphoma tk<sup>+/−</sup> assay; and
- 55 • An in vivo test for chromosomal damage using rodent hematopoietic cells.

56  
57 The following discussion is based on current guidance documents.<sup>4</sup> We recommend that results  
58 from in vitro genetic toxicology studies be available prior to the initiation of Phase 1 trials.

59  
60

### **III. INTEGRATION OF GENETIC TOXICITY STUDY RESULTS**

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63 When translating hazard identification into possible risk, we recommend considering the  
64 following factors, including mechanism of action (MOA) for genotoxicity.

- 65 • Pharmaceuticals that give positive results in genetic toxicology assays but do not directly  
66 interact with DNA do not always present a significant in vivo risk. We recommend that  
67 evidence for the mechanism of genotoxicity and relevance of the mechanism to  
68 anticipated in vivo exposure be provided in such cases.
- 69 • Drugs known to directly damage DNA may be permitted to be used in patients with  
70 debilitating or life-threatening disease, such as cancer, but should not be administered to  
71 healthy subjects.<sup>5</sup>

72

73 The Agency recommends that the decision of whether to begin a clinical trial when there are  
74 positive genetic toxicity study results be based on a weight-of-evidence (WOE) approach that  
75 includes consideration of the results of all genetic toxicology tests and the nature of the proposed  
76 trial. If the results of the genetic toxicology tests indicate a lack of genotoxic potential, then

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<sup>2</sup> ICH guidance for industry *M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals*, ICH guidance for industry *S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals*, and ICH guidance for industry *S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals*. (<http://www.fda.gov/cder/guidance/index.htm>)

<sup>3</sup> ICH guidance for industry *S1B Testing for Carcinogenicity of Pharmaceuticals*. (<http://www.fda.gov/cder/guidance/index.htm>)

<sup>4</sup> We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance Web page at <http://www.fda.gov/cder/guidance/index.htm>.

<sup>5</sup> ICH guidance for industry *S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals*. (<http://www.fda.gov/cder/guidance/index.htm>)

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77 single-dose or short-term repeat-dose trials can generally be undertaken in healthy subjects or  
78 patient populations with the proposed medical indication, provided that these results are  
79 supported by other appropriate nonclinical pharmacology or toxicology studies.  
80

81 In general, single-dose studies can proceed regardless of results in genetic toxicity studies, and  
82 any positive results are included in the investigator's brochure and informed consent form. If  
83 either of the in vitro genetic toxicity study results is equivocal, then we recommend repeating the  
84 equivocal study prior to or concurrently with the single-dose studies. If any of the three assays in  
85 the ICH genotoxicity standard battery is positive, then we recommend completing the fourth test  
86 in the ICH battery. If a positive response is seen in one or more assays, sponsors should consider  
87 choosing from the following options.  
88

### **A. Weight-of-Evidence Approach**

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91 In some instances, after evaluation of all available data, the WOE suggests a lack of genotoxic  
92 hazard. For example, a positive response is observed in one exposure regimen of an in vitro  
93 cytogenetics assay. The positive result is seen only at the high dose, and the increase is within or  
94 just outside the range for historical control values for the solvent and cell line employed. The  
95 WOE approach could indicate that although the result is statistically significant, it lacks  
96 biological relevance. Contributing considerations could include (1) the level of cytotoxicity at  
97 which the response was seen, and (2) corroborating data from the same or complementary  
98 assays. For example, a positive response seen in a short-term exposure without metabolic  
99 activation but not corroborated in the longer exposure at comparable levels of cytotoxicities  
100 would argue against the biological significance of the positive result. Similarly, such a positive  
101 finding that is not corroborated by the matching exposure regimen of the mouse lymphoma assay  
102 could also call into question the significance of the positive finding. If the WOE approach  
103 indicates a lack of genotoxic hazard, the repeat-dose clinical studies could proceed provided the  
104 positive response is described in the investigator's brochure and the informed consent form.  
105

### **B. Mechanism of Action**

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107  
108 Positive results are sometimes satisfactorily explained by knowledge of the mechanism of action.  
109 For example, it has been demonstrated that in vitro clastogenic effects can result from  
110 excessively high osmolarity or low pH. Positive responses elicited under such nonphysiologic  
111 exposure conditions are not relevant to human risk. In addition, certain genotoxic responses are  
112 thought to have thresholds below which a hazard does not exist. Agents that induce effects by  
113 indirect mechanisms, such as interference with metabolism of nucleotides and their precursors,  
114 damage to spindle proteins, or inhibition of topoisomerase, may have thresholds for genotoxic  
115 effects. In such cases, we recommend presenting direct evidence of the existence of a threshold  
116 that would not be attained during the proposed clinical exposure. Positive responses that are  
117 satisfactorily explained by an MOA may allow repeat-dose studies to proceed without additional  
118 studies.  
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### **C. Additional Supportive Studies**

On occasion, results in in vitro studies demonstrate dose-responsive and reproducible positive responses. Results from the bone marrow cytogenetics studies are frequently negative, even for those compounds giving positive results in in vitro genetic toxicity assays. This discrepancy can result from a number of differences between cultured cells and intact animals: differing metabolic pathways occurring in vitro and in vivo, metabolic inactivation in the intact animal, failure of the parent compound or active metabolite to reach the target cell, or simply, an inability to achieve plasma levels in vivo comparable to concentrations that generated positive responses in the in vitro assays.

Additional in vivo assays can be useful in clarifying in vitro positive results. For example, peripheral blood smears from repeat-dose toxicity studies in mice can be evaluated for micronucleus induction, and peripheral blood lymphocytes from repeat-dose studies in rats or monkeys can be cultured and assessed for chromosome damage in metaphase spreads. DNA damage can be assessed in potential target tissues (e.g., DNA adducts or comet assays), or transgenic rats or mice can be used to assess mutagenicity in potential target tissues.<sup>6</sup>

The Syrian hamster embryo (SHE) transformation assay has been suggested as a follow-up assay in the face of positive in vitro genotoxicity results. Data in the literature suggest that the SHE assay correlates well with rodent carcinogenicity results for chemicals in general (Isfort et al. 1996). Results from an International Life Sciences Institute (ILSI) validation effort on human pharmaceuticals, although smaller in scope, suggest that the SHE assay is less predictive for human carcinogenic risk (Mauthe et al. 2001). With respect to human pharmaceuticals, the ILSI study found that the SHE assay had high sensitivity (83 percent) for detection of human carcinogens. However, its low specificity (15 percent) for prediction of *putative human noncarcinogens* led to a poor overall concordance of 37 percent. Nevertheless, transformation assays measure endpoints more akin to the health effect of concern (cancer) and may be useful in making a WOE judgment.

In the last several years, a number of transgenic mouse strains have become available for use in short-term carcinogenicity studies. The p53 haplo insufficient mouse has been found to be useful in the identification of mutagenic carcinogens (MacDonald et al. 2004). Negative results in a p53 carcinogenicity study are considered evidence that a genotoxic agent does not present a carcinogenic hazard to humans through a p53-mediated mechanism.

Supportive studies contribute to the WOE determination as to whether a drug giving a positive response in one of the ICH-specified assays presents a risk of genetic damage to subjects involved in clinical trials.

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<sup>6</sup> ICH guidance for industry *S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals*. (<http://www.fda.gov/cder/guidance/index.htm>)

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