FORUM

Current and Future Application of Genetic Toxicity Assays: The Role and Value of In Vitro Mammalian Assays

Rosalie K. Elespuru,*†‡ R. Jagannath, {||†‡||} Dan A. Levy, ||||†‡|| Martha M. Moore, |||†‡|| Yanli Ouyang, ⨂||†‡|| Timothy W. Robison, |.cgi||||†‡|| Rene E. Sotomayor, |||†‡|| Michael C. Cimino,* and Kerry L. Dearfield**

*Office of Science & Engineering Laboratories, U.S. Food and Drug Administration, Center for Devices and Radiological Health, White Oak, Silver Spring, Maryland 20993; †FDA GeneTox Network; ‡CDER Genetic Toxicology Subcommittee; §Office of Pharmaceutical Sciences, U.S. Food and Drug Administration, Center for Drug Evaluation and Research, White Oak, Silver Spring, Maryland 20993; ††Office of New Drugs, U.S. Food and Drug Administration, Center for Drug Evaluation and Research, White Oak, Silver Spring, Maryland 20993; ||Division of Genetic and Reproductive Toxicology, U.S. Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas 72079; |||Office of New Animal Drug Evaluation, U.S. Food and Drug Administration, Center for Veterinary Medicine, Rockville, Maryland 20855; ||||Office of Nutrition, Labeling, and Dietary Supplements, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, Maryland 20740; †††Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, DC 20460; and **Office of Public Health Science, Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, DC 20250

Received October 30, 2008; accepted March 20, 2009

With the advent of new technologies (e.g., genomics, automated analyses, and in vivo monitoring), new regulations (e.g., the reduction of animal tests by the European REACH), and new approaches to toxicology (e.g., Toxicity Testing in the 21st Century, National Research Council), the field of regulatory genetic toxicology is undergoing a serious re-examination. Within this context, Toxicological Sciences has published a series of articles in its Forum Section on the theme, “Genetic Toxicity Assessment: Employing the Best Science for Human Safety Evaluation” (beginning with Goodman et al.). As a contribution to the Forum discussions, we present current methods for evaluating mutagenic/genotoxic risk using standard genotoxicity test batteries, and suggest ways to address and incorporate new technologies. We recognize that the occurrence of positive results in relation to cancer prediction has led to criticism of in vitro mammalian cell genetic toxicity assays. We address criticism of test results related to weak positives, associated only with considerable toxicity, only seen at high concentrations, not accompanied by positive results in the other tests of standard test batteries, and/or not correlating well with rodent carcinogenicity tests. We suggest that the problems pointed out by others with these assays already have been resolved, to a large extent, by international groups working to update assay protocols, and by changes in data interpretation at regulatory agencies. New guidelines at the U.S. Environmental Protection Agency and the U.S. Food and Drug Administration improve data evaluation and help refocus risk assessment. We discuss the results of international groups working together to integrate new technologies and evaluate new tests, including human monitoring. We suggest that strategies for identifying human health risks should naturally change to integrate new technologies; however, changes should be made only when justified by strong scientific evidence of improvement in the risk assessment paradigm.

Toxicological Sciences has published a series of articles in its Forum Section on the theme, “Genetic Toxicity Assessment: Employing the Best Science for Human Safety Evaluation.” As stated in the introductory article (Goodman et al., 2007), “The goal of the Forum Series is to facilitate the communication of ideas and promote discussion and new thinking in order to enhance the scientific basis for evaluating the mutagenic risk that chemicals might pose for people.” It should be clear that mutagenic risk is a surrogate for both somatic (cancer) risk and germ cell (heritable) risk. We provide insight on a central aspect of the Forum discussions, the controversy over the frequency of positive results in the in vitro mammalian cell genetic toxicity assays in relation to cancer prediction (Goodman et al., 2007; Jacobson-Kram and Contrera, 2007; Kirkland et al., 2005, 2006). Within this context, we discuss ongoing, collaborative efforts among governmental, industrial and academic scientists to update the in vitro mammalian assays, modify data interpretation, and integrate new technologies into genetic toxicology safety assessment.

Over the years, we (the authors) have gained extensive experience with the assays that comprise genotoxicity test batteries, in the testing of human and veterinary drugs, food ingredients, environmental chemicals, pesticides, environmental mixtures, and other agents (e.g., Cimino, 2006; Dearfield et al., 1991; Moore et al.,...
2002, 2003, 2006, 2007; Müller et al., 1999). Based on our collective experience, we provide our perspective as follows.

PURPOSE OF GENOTOXICITY TEST BATTERIES

Genotoxicity testing batteries were established specifically for hazard identification, the first step in risk assessment. Test batteries have been in use for more than 25 years (e.g., EPA Toxics Test Rule, 1979 [Dearfield et al., 1991]; FDA Redbook, 1982) and are a set of screening tests designed to maximize the chance of detecting genotoxic substances, not to independently predict carcinogenicity. Similar batteries have been adopted for use in a variety of regulatory programs across the world (reviewed in Cimino, 2006). Results from such test batteries firstly provide an indication of whether or not there is genotoxic activity. The results from the battery can be helpful in assessing a possible mutagenic mode of action (mMOA) for cancer, but genetic damage may also be considered in other contexts, including heritable genetic risk. Mammalian in vitro genetic toxicity test results have not been shown to be appropriate for extrapolation to in vivo systems, human or otherwise.

THE ISSUES REGARDING IN VITRO MAMMALIAN CELL ASSAYS

Several articles in this series have addressed the incidence of test compounds that produce positive results only in the in vitro mammalian cell component of test batteries. These results were considered not relevant because they were

- Weakly positive,
- Associated only with considerable toxicity,
- Only seen at high concentrations, and/or
- Not accompanied by positive results in the other tests of the standard test battery.

Others have argued that the in vitro mammalian cell tests and the genetic toxicity testing batteries of which they are a part have turned out to be poor predictors of human risk because responses in them do not correlate well with the results of rodent cancer bioassays (Kirkland et al., 2005, 2006, 2007a,b). A further criticism is the perception that the considerable follow-up work required to assess the genotoxic risk to humans of compounds found to be positive in the in vitro mammalian cell assays (and the associated uncertainty or delays in regulatory approval of new drugs) is unwarranted.

Options proposed to ameliorate the perceived problems of the in vitro mammalian cell genetic toxicity assays include: changing the protocols (e.g., reducing the maximum concentration and cytotoxicity levels) (ICH, 2008; Kirkland et al., 2007a,b); addition or substitution of alternative assays (Lorge et al., 2007a,b; Pfuhler et al., 2007; Witte et al., 2007); changes in the interpretation of genetic toxicity data (Lorge et al., 2007b; Pottenger et al., 2007); and development of a new testing strategy that eliminates the in vitro mammalian cell assays altogether (ICH, 2008; Kirkland et al., 2006, 2007b; Ku et al., 2007; Pfuhler et al., 2007).

PERСПECTIVE ON THE RELEVANCE OF IN VITRO MAMMALIAN CELL GENETIC TOXICITY TESTS

We believe the original rationale for the test battery—to detect the broadest set of genotoxic agents acting via diverse mechanisms, that is, hazard identification—remains a valid approach. Much of the criticism of the in vitro mammalian cell assays is focused on their “failure” at risk assessment, defined as their ability to predict the results of rodent carcinogenicity bioassays. However, genetic toxicity assays are not designed to model the multifactorial process of carcinogenesis. Such an alteration in the goals of genotoxicity testing should be faced and debated directly. Although rodent cancer bioassays have their own limitations as surrogates for human cancer risk, they remain an important indicator. We do not want to miss agents positive in the rodent bioassays (per the discussion in the next section); however, we don’t believe agents negative in rodent bioassays and positive in in vitro tests are necessarily “false positives.” Because there is no “gold standard” for human carcinogenic risk, we support retention of in vitro genetic toxicity tests as sentinels of genotoxic effects that merit further investigation in the context of both genetic and carcinogenic risk.

It is clear that proposed changes (e.g., ICH, 2008 new draft S2, R1) will reduce or eliminate the number of positive in vitro mammalian tests, but we have not seen data demonstrating that these changes improve the identification of compounds with higher genotoxic risk. We argue that further characterization of the compounds identified by this hazard identification screen is both warranted and technically feasible, whereas arbitrary modification of test parameters to reduce the number of positive results without regard to risk is inappropriate.

THE DEBATE

The arguments against the relevance of the in vitro mammalian cell tests (outlined above) are addressed below.

Weak In Vitro Positives are not Relevant

Potency in in vitro assays may not reflect potency in animal or human responses. For example, weak mutagens are not necessarily weak rodent carcinogens and vise versa (Fetterman et al., 1997; Sanner and Dybing, 2005; Schildcrout et al., 1999). Sanner and Dybing showed that mutagenic and carcinogenic potencies generally correlated across log-log scales, but many compounds were off the scale in one direction or the other, some by an order of magnitude (Figures 1 and 2 of Sanner and Dybing, 2005). In addition, mutagenic and carcinogenic potencies of structurally different carcinogenic compounds from a single chemical class did not correlate well
A series of three direct-acting N-nitroso compounds (N-methyl-N-nitrosourea, N-methyl-N'-nitro-N-nitrosoguanidine, and nitroso-carbaryl), generating the same methylating intermediate, produced potency differences of several orders of magnitude for bacterial mutagenesis (Elespuru, 1979), and the order of mutagenic potency did not correlate with their carcinogenic potency in rats. Although DNA interaction was a focus, the major determinant for mutagenicity appeared to be uptake into the bacteria (measured by radioactive labeling), which correlated with the lipophilicity of the compounds.

Extensive studies have been undertaken with aromatic amine carcinogens in an effort to understand potency determinants for mutagenicity and carcinogenicity. In one study, quantitative structure-activity relationship modeling of metabolic activation differences accounted for 20% of the 100-fold potency differences among a set of food-derived heterocyclic amines (Felton et al., 2007). The studies of Hatch (e.g., Hatch et al., 2001) and others have suggested that hydrophobicity is a major determinant of activity, followed by electronic stability of the nitrenium ion. Felton (op. cit) suggested that determinants of potency likely include binding affinity for P450 enzymes, metabolic intermediate stability, DNA adduct stability, DNA repair, and fixation of the mutation through cellular processes. These factors are likely to vary among cells, systems, and species.

Extrapolation of mutagenic and carcinogenic potency differences, either weak or strong, from one system to another is not supported by theoretical considerations or the evidence. Therefore, weakly positive in vitro genotoxicity responses should not be considered as necessarily signaling lower human carcinogenic risk; similarly, strong in vitro positives may not indicate a major in vivo risk.

Results Associated with Toxicity Should be Discounted

Toxicity is an associated response of some genotoxic agents, including many positive controls. Understanding of toxicity-related artifacts in in vitro mammalian assays (e.g., concentrations causing high osmolarity) has resulted in protocol modifications designed to eliminate them. However, it is not clear to what extent toxic mechanisms lead to genotoxic responses. Genotoxic agents are capable of interactions with cell components other than DNA (e.g., proteins; Melikian et al., 1996 on benzo[a]pyrene interactions with DNA and hemoglobin) and thus may be cytotoxic via mechanisms independent of their genetic toxicity.

Arbitrarily lowering the cytotoxicity limits, as suggested by some, would likely eliminate detection of several carcinogens. For example, by changing the limit toxicity in the mouse lymphoma assay (MLA) from a Relative Total Growth (RTG) of 10% to 20% (as has been proposed), the following carcinogens would be missed: (Criticism of the seemingly higher cytotoxicity limit in the MLA compared with other in vitro assays may be a product of misunderstanding of cytotoxicity measurements. It is a fundamentally different measure in the MLA, that is, the RTG is calculated as the product of the test culture’s relative growth as compared with the negative control in the expression [suspension growth] phase of the assay and the relative plating efficiency during mutant selection. This takes into account the growth of cells post-treatment and their ability to form colonies. The cytotoxicity measure for the cytogenetic assays is related to the relative number of cells shortly after treatment and does not incorporate a measure of viability. Reevaluating the level of RTG in the MLA is a worthwhile endeavor; however, it is scientifically unjustifiable to alter the limit without basing it on improved biological relevance.) 4-chloro-o-toluidine (17% RTG), piperonyl butoxide (17%), and 2,4,6-trichlorophenol (11%) (Kirkland et al., 2006). Rather than an arbitrary reduction of the limit toxicity, a better understanding of the positive result at the toxicity level seen should be sought.

Results Associated with High Concentrations are Irrelevant

Testing at high concentrations is a basic principle of safety evaluation to assure the identification of a potential hazard, and also to compensate for short exposure times, statistically small numbers of samples in the tests, and sensitive populations. For example, most of the short-term tests cannot measure cumulative genetic damage; they only measure damage incurred in the hours or days before completion of the test. A proposed 10-fold reduction of the limit concentration to 1 mM for the in vitro mammalian cell assays (ICH, 2008; Kirkland et al., 2006) runs the risk of eliminating the detection of genotoxic agents in the hazard identification stage. Several well-characterized carcinogens, positive only in the MLA, would likely be missed at a limit concentration of 1 mM: malonaldehyde sodium salt (5.6 mM), and toluene (2.4 mM). Other carcinogens, although not detected solely in the MLA, would be missed at 1 mM: for example, glycidamide (2 mM) (Mei et al., 2008). Several carcinogens, for example, trimethyliourea and acrylamide, are already missed at a cut-off of 10 mM (acrylamide limit of detection: 12 mM) (Mei et al., 2008). Again, preferable to a reduction of the top concentration, which has served us well, is a better analysis of the positive result in the in vitro test at higher test concentrations.

Singular Results with No Positives in Other Assays Should be Discounted

The principle of the test battery was developed when it became clear that no single test could identify all known genotoxic activity or genotoxic mechanisms (reviewed in Cimino, 2006). Therefore, single positive results have value. We think it is important to note that in vitro mammalian cell assays detect classes of genotoxic agents not identified in bacteria (e.g., antibiotics and chromosomal damaging agents). Furthermore, it is well established that the MLA detects a full array of genetic damage, including point mutations, chromosome deletions, translocations, mitotic recombination, and at least some aneugens (Applegate et al., 1990). Therefore, it should be expected that some chemicals will be negative in bacterial assays and positive in in vitro mammalian assays. When new molecular entities are
being generated with novel mechanisms of action, we believe it is prudent to employ tests capable of detecting the broadest range of genotoxic mechanisms.

In Vitro Assays Lack Value Because of a Lack of Correlation with Rodent Bioassay Results

This issue is the major argument presented against the use of in vitro mammalian genetic toxicity assays. However, the low specificity and low overall concordance between National Toxicology Program of the U.S. Department of Health and Human Services carcinogenesis assays and short-term genetic toxicity tests (e.g., Tennant and Zeiger, 1993) were known long before the discussions leading to the development of the present ICH test battery (Müller et al., 1999). Even so, there was general consensus on the value of a genotoxicity test battery that included in vitro mammalian assays. That value remains today. Recent re-analyses of the correlation with rodent bioassay results (Kirkland et al., 2005, 2006, 2007a,b) rely on databases heavily populated with data that were questioned in the past (e.g. Mitchell et al., 1997) and even if updated may not reflect recent evaluation criteria. In addition, as previously mentioned, there are generally recognized problems with limiting human risk assessment considerations to rodent bioassay results.

Thus, we do not agree with the rationales for discounting weak, toxicity associated, or singular positive results in mammalian cell genetic toxicity tests. We are concerned that the complete elimination of in vitro mammalian cell assays from a test battery (as suggested by Ku et al. 2007, as well as in a current draft revision of the ICH guideline) would limit our capability to detect some genotoxic agents in the hazard identification stage, including those that are not detected in the tissue(s) monitored in vivo in rodents.

ALTERNATIVE APPROACHES FOR ADDRESSING “PROBLEMS” WITH THE CURRENT TEST BATTERIES

Regulatory Approaches

There are ongoing efforts to better interpret data from existing tests, develop more consistent follow-up strategies, and integrate new technologies as they are shown to add value to current regulatory structures.


Efforts have been made to train reviewers in the scientific background of the assays, to achieve consistency and scientific validity in assay interpretation. FDA/CDER has generated a Weight of Evidence (WOE) guidance document on the interpretation of genotoxicity testing results in the context of regulatory action (http://www.fda.gov/cder/guidance/index.htm). This was designed to deal with a positive result in the standard battery of genetic toxicity tests through the use of a WOE argument with more structured, mechanistically based follow-up genetic toxicity tests. This document appears to minimize or remove the need for significant modifications of the standard genetic toxicity test battery such as changes in protocols and/or removal of the in vitro mammalian genetic toxicity tests.

2) Environmental Protection Agency (EPA) requires a genotoxicity testing battery to evaluate many chemicals (e.g., pesticides, toxic substances, environmental pollutants) in its various regulatory offices (i.e., Office of Pesticide Programs, Office of Pollution Prevention and Toxics, Office of Air and Radiation, Office of Solid Waste and Emergency Response, and Office of Water) (Cimino, 2006). Because the battery is used for a wide variety of chemicals and there is a wide variety of possible genotoxic activities that can occur, no single test is able to detect the entire spectrum of induced genotoxicity. Accordingly, assays and test batteries have been developed to assess effects on three major endpoints of genotoxicity associated with human disease: gene mutation (i.e., point mutations that affect single genes or blocks of genes), clastogenicity (i.e., structural chromosome aberrations), and aneuploidy (i.e., numerical chromosome aberrations) (Auletta et al., 1993; Cimino, 2006; Dearfield et al., 1991). EPA uses such data as part of its WOE approach for evaluating heritable risk to humans and for evaluating risk for adverse health outcomes such as cancer (Auletta et al., 1993).

Use of genotoxicity data is further outlined in the EPA’s Guidelines for Carcinogen Risk Assessment and Supplemental Guidance for Assessing Cancer Susceptibility from Early-life Exposure to Carcinogens, which address if a chemical is a carcinogen working via a mMOA (EPA, 2005a,b). A draft mMOA framework proposed by EPA considers all relevant evidence (e.g., genotoxicity data, structural alert information, pharmacokinetic data) to determine if a chemical or its metabolite causes cancer via a mMOA (EPA, 2007). The important aspect of this framework is in the interpretive analysis of all the data, including in vitro mammalian tests, to discern a possible mMOA for cancer. Of particular importance in this evaluation of the dose response data and the differential susceptibility of various populations of individuals is the potentially increased susceptibility of newborns and of children to genotoxic insult compared with adults (Dearfield and Moore, 2005; EPA, 2005b; Keshava et al., 2005; McCarroll et al., 2008).

Recent Scientific Advances

Several laboratories have made significant progress in the resolution of issues with current assays or in developing new assays. One example is the use of flow cytometry to better characterize cell populations, cytotoxicity, and chromosomal damage endpoints following treatment with genotoxins (Muehlbauer and Schuler, 2003; Muehlbauer et al., 2008). These experiments provided resolution of cytotoxicity artifacts
Joint International Efforts

There are ongoing efforts by international consortia of regulatory, academic, and industry scientists to better interpret data from existing tests, develop more consistent follow-up strategies, and bring new technologies into regulatory use. There is also a long-standing effort at protocol alteration to refine genetic toxicity tests for better execution and generation of results. Protocol revisions are being organized by the International Life Sciences Institute (ILSI), International Workshop on Genotoxicity Testing (IWGT), and Organisation for Economic Cooperation and Development (OECD), among others. In each case, comments and input from experts around the world are solicited and welcomed. These efforts are working toward a consensus-based resolution that seeks additional value from in vitro test results.

- The ILSI/Health and Environmental Sciences Institute (HESI), a consortium of interested parties from industry, academia, and government, has a Project Committee on In vitro Genetic Toxicity Testing to study the Relevance and Follow-up of Positive Results in In Vitro Genetic Toxicity Testing (Thybaud et al., 2007b). The most recent workshop was held in February, 2009. This effort is organized around three focal points: (1) A review of current tests and new tests (e.g., in silico, toxico-genomic, combination, humanized, and targeted approaches, e.g., skin). Tests were subjected to an analysis concerning their state of validation, relevance, strengths, weaknesses, and particular utility in a test battery or testing scheme. This also included an analysis of gaps in existing technologies/tools and efforts to bridge these gaps. (2) A decision-tree framework was created for follow-up testing when positive in vitro genotoxicity results are obtained which emphasized the value of the in vitro result when followed up with additional, mechanism-based in vitro and in vivo testing. (3) An investigation of quantitative relationships between in vitro and in vivo endpoints, with a potential goal of developing more predictive data from in vitro assays.

- Still another ILSI/HESI initiative is investigating the potential role of genomic (gene expression) profiling in differentiating genotoxic mechanisms, particularly DNA-reactive versus nonreactive agents. Coordinated studies in nine laboratories evaluated the gene expression profile of TK6 cells treated with three genotoxic agents acting via different mechanisms: The DNA cross-linking agent Cisplatin, topoisomerase inhibitor Etoposide, and mitotic spindle poison Taxol. Sodium chloride, which can test positive for chromosomal damage at high concentrations, was a non-DNA-interactive control. A targeted reverse transcription–polymerase chain reaction approach was based on a set of 47 preselected genes. Chemical dosimetry, sample timing for gene expression analysis, and interlaboratory results were addressed in the studies. Results were found to be promising for differentiation of mechanisms and interlab reproducibility. Results of the studies will be published soon.

- The IWGT (e.g., Kirkland et al., 2007c; Thybaud et al., 2007a) is another forum within which experts from the international community address issues related to the evaluation of specific tests. For example, acceptance criteria and improved data interpretation for the MLA have been recommended by an IWGT expert group (most recently Bigger et al., 2008; Moore et al., 2007). These recommendations reduced the incidence of in vitro mammalian cell positive results considered to lack relevance to human risk. Also, the IWGT strategy for genotoxicity testing is helpful for evaluating such positive results (Thybaud et al., 2007a). This includes an analysis of positive results in test batteries, coupled with follow-up options and an incorporation of mode of action for the induction of mutation information into the decision-making process.

- The OECD has facilitated the development of Detailed Review Papers or new test guidelines for genotoxicity assays, including transgenic rodent mutagenicity assays, cellular transformation assays, the single cell gel electrophoresis (Comet) assay, and the in vitro micronucleus assay. Within this context are discussions related to protocols, validation, cytotoxicity limits relevant to false positive generation, and regulatory applications.

- The U.N. World Health Organization (WHO)/International Programme on Chemical Safety; WHO, 2007) document “Mutagenicity testing for chemical risk assessment” outlines a strategy for genotoxicity testing, interpretation of test results, and follow-up testing. This is generally consistent with the standard ICH test battery involving in vitro bacterial and mammalian cell mutagenicity assays. In the WHO document, however, in vivo assays are assigned a follow-up role to positive in vitro tests, unless there is concern related to a particular structure or high levels of human exposure.

- A major consortium of international groups is working toward alternatives to animal testing (Interagency Coordinating
Committee on the Validation of Alternative Methods, European Centre for the Validation of Alternative Methods, Japanese Centre for the Validation of Alternative Methods, National Academy of Sciences/EPA Toxicology for the 21st century report; see Collins et al., 2008). These groups support new testing paradigms that move toward greater use of in vitro test systems and less reliance on animal tests.

**ALTERNATIVES TO CURRENT IN VITRO MAMMALIAN CELL GENOTOXICITY ASSAYS**

New test batteries have been proposed that incorporate the in vitro micronucleus assay and the in vivo Comet assay (Lorge et al., 2007a,b). A new draft for the ICH test battery includes two options: the standard test battery option or an option that eliminates in vitro mammalian cell genotoxicity tests, instead incorporating a second unspecified in vivo assay that could be integrated into ongoing toxicology studies (ICH S2(R) Guidance Step 2 Version dated March 6, 2008). In addition, other assays have been proposed instead of a test battery (Ku et al., 2007). In our view, there is insufficient experience with these approaches for regulatory screening and there are no generally accepted, standardized, or validated protocols for these tests. Although the in vitro micronucleus assay has a draft OECD guidance, no standard protocols have been established for the in vitro or in vivo Comet assays or the rodent transgenic mutation assays.

International validation efforts are underway for both the in vitro micronucleus assay and the Comet assay. We support and are participating in these efforts to better understand the appropriate parameters for conducting these assays.

**CONCLUSIONS**

Existing strategies for identifying human health risks should be under continual review and modified to improve their performance, but changes should be made only when justified by strong scientific evidence. All interested parties should continue to support international efforts to validate new genetic toxicity tests, including those appropriate for follow-up testing, and to improve the ability of current tests to assess the potential human health risks of new products prior to their entry into the food supply, the health system, or the marketplace. Thus it is prudent to employ the most sensitive assays, such as the in vitro mammalian cell assays, for this task. We suggest that it is preferable to improve the assays and their interpretation, rather than to eliminate them, or alter them to the point that they are no longer useful as sentinels for genotoxic activity.

There is a great need for new approaches to the assessment of human cancer risk. However, because there is no mechanism for assessing cancer risk in humans postmarket, there is no good standard against which a testing paradigm, old or new, may be verified. The exposure now to agents that might increase cancer risk is unlikely to be traced 20 years hence. Thus, it is appropriate to apply a prudent approach to risk assessment, maintaining current testing standards that are working properly until others have proven superior by rigorous scientific evidence and widespread agreement (e.g., the ICH S2A and S2B test guidelines; Müller et al., 1999). The potential for the development of technologies capable of assessing genotoxic effects in humans directly, for example, in the PIG-A assay (Albertini, 2008; Dertinger et al., 2007), is encouraging, but only a first step.

**ACKNOWLEDGMENTS**

The views and opinions expressed are solely those of the authors and are not official policy of the affiliated institutions.

**REFERENCES**


Kirkland, D. J., Aardema, M., Bandhun, N., Carmichael, P., Fautz, R., Meunier, J.-R., and Pfühler, S. (2007b). In vitro approaches to develop weight of evidence (WoE) and mode of action (MoA) discussions with positive in vitro genotoxicity results. Mutagenesis 22, 161–175.


