The Salmonella Mutagenicity Assay: The Stethoscope of Genetic Toxicology for the 21st Century

Larry D. Claxton,1* Gisela de A. Umbuzeiro,2 and David M. DeMarini1

1Genetic and Cellular Toxicology Branch, Integrated Systems Toxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; 2Laboratório de Ecotoxicologia Aquática e Limnologia, Faculdade de Tecnologia, Universidade Estadual de Campinas, Limeira, São Paulo, Brazil

OBJECTIVES: According to the 2007 National Research Council report Toxicology for the Twenty-First Century, modern methods (e.g., “omics,” in vitro assays, high-throughput testing, computational methods) will lead to the emergence of a new approach to toxicology. The Salmonella mammalian microscope mutagenicity assay has been central to the field of genetic toxicology since the 1970s. Here we document the paradigm shifts engendered by the assay, the validation and applications of the assay, and how the assay is a model for future in vitro toxicity assays.

DATA SOURCES: We searched PubMed, Scopus, and Web of Knowledge using key words relevant to the Salmonella assay and additional genotoxicity assays.

DATA EXTRACTION: We merged the citations, removing duplicates, and categorized the papers by year and topic.

DATA SYNTHESIS: The Salmonella assay led to two paradigm shifts: that some carcinogens were mutagens and that some environmental samples (e.g., air, water, soil, food, combustion emissions) were mutagenic. Although there are >10,000 publications on the Salmonella assay, covering tens of thousands of agents, data on even more agents probably exist in unpublished form, largely as proprietary studies by industry. The Salmonella assay is a model for the development of 21st century in vitro toxicity assays in terms of the establishment of standard procedures, ability to test various agents, transferability across laboratories, validation and testing, and structure–activity analysis.

CONCLUSIONS: Similar to a stethoscope as a first-line, inexpensive tool in medicine, the Salmonella assay can serve a similar, indispensable role in the foreseeable future of 21st century toxicology.

KEY WORDS: Ames assay, carcinogenicity, 21st century toxicology, genetic toxicology, high-throughput assays, Salmonella assay, Salmonella mutagenicity assay. Environ Health Perspect 118:1515–1522 (2010). doi:10.1289/ehp.1002336 [Online 2 August 2010]

Every day throughout the world, physicians, nurses, and an array of other health professionals use a stethoscope, which was invented by René Laennec in 1816 (Weinberg 1993). It is a relatively simple instrument whose sounds can indicate a myriad of disease states that can then be confirmed by more sophisticated assessments. It is hard to visualize a physician or imagine medicine without the stethoscope. Similarly, the Salmonella mutagenicity assay, which was developed initially as a spot test (Ames 1971), then as a plate-incorporation test (Ames et al. 1972) using strains of Salmonella bacteria derived from studies by B.N. Ames and P.E. Hartman (Hartman et al. 1986) and rodent liver microsomal activation coupled initially to the assay by H.V. Malling (Malling 1971), is a deceptively simple tool that can be used to detect the mutagenicity of environmental chemicals, environmental mixtures, body fluids, foods, drugs, and physical agents. More complex tests can be applied to confirm and characterize further the mutagenic activity of the agent. Although neither the stethoscope nor the Salmonella assay provides a definitive diagnosis/detection of a disease or a mutagen, respectively, both are indispensable first-line tools in their fields.

There is much unrest in the field of toxicology today because of a variety of scientific developments, including advances in genomic science (Parsons et al. 2008; Wood et al. 2007), improved knowledge of the molecular and mechanistic basis for biological responses to toxicant exposure (Guyton et al. 2009), legislation mandating reduced numbers of animals for toxicity testing (Pfuhler et al. 2009), and environmental direction to incorporate all of the above into a new paradigm for toxicology for the 21st century (National Research Council 2007).

A strict parallel cannot be drawn between a targeted testing assay such as the Salmonella assay, which is used for hazard identification, and a high-throughput screening (HTS) assay such as either the ToxCast program [U.S. Environmental Protection Agency (EPA)] or the combined U.S. EPA/National Institutes of Health (NIH)/National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP) Tox21 program (Kavlock et al. 2009), which can identify specific signaling or biochemical pathways relevant to potential disease development and thus have the possibility of going beyond hazard identification. An assay like the Salmonella assay is a stand-alone screen that requires high accuracy and reproducibility and is correlated with health end points, permitting its use for regulatory purposes. In contrast, HTS assays use emerging technologies and target probes, knowledge of biochemical and disease pathways in rodents and humans, genomics, and other technologies to generate a profile or pattern of effects across a range of chemical classes and biological end points that do not depend greatly on any particular chemical or assay result. As with the Salmonella assay, HTS assays are viewed as a first-line screening tool, with results of interest being followed up by more extensive confirmatory assays.

In the process of developing and adopting new methods, it is important to build on and learn from past paradigm shifts, several of which occurred in the field of genetic toxicology with the introduction of the Salmonella assay. Consequently, the history of the Salmonella assay highlights some of the necessary steps and considerations needed for the development of almost any type of toxicity assay, including some aspects of HTS assays. Our purpose with this review is to (a) describe the paradigm shifts precipitated by the Salmonella assay, including the demonstration of a connection between mutagenicity and carcinogenicity and the ubiquitous nature of mutagens in our environment; (b) document the historic and current applications of the Salmonella assay; and (c) illustrate the lessons learned from the development, validation, testing, assessment, and uses of this in vitro assay that may be applicable to the development of in vitro toxicity assays for the 21st century.

Address correspondence to D.M. DeMarini, B105-03, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 USA. Telephone: (919) 541-1510. Fax: (919) 541-0694. E-mail: demarini.david@epa.gov

*Current address: LDC Scientific Services, 6012 Brass Lantern Court, Raleigh, North Carolina, USA

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Paradigm Shift I: Relating Mutagenic Activity to Carcinogenic Activity

By the middle of the 20th century, there was almost no evidence to support a role for mutation in cancer, and few carcinogens were known to be mutagens (Burdette 1955). However, at this time efforts began to screen carcinogens and other chemicals for mutagenicity in vitro, starting with the use of a base-substitution strain of <i>Escherichia coli</i> by Demerec et al. (1951) and then by Szybalski (1958), who assessed > 400 compounds using filter-paper disks in a spot test with the same strain. This concept was expanded by the development of a set of tester strains that detected different types of gene mutations in <i>Salmonella typhimurium</i> (Whitfield et al. 1966) and in the fungus <i>Neurospora crassa</i> (Malling 1966a). Many in vitro mutagenicity assays were developed throughout this period, including the ad-3 forward-mutation assay in <i>N. crassa</i> (de Serres and Kolmark 1958); cytogenetic assays (Kihlman 1966); Hprt (hypoxanthine-guanine phosphoribosyltransferase) assays in V79 (Chu and Malling 1968) and CHO cells (Hsie et al. 1975); the Tk<sup>−</sup> (thymidine kinase) assay in mouse lymphoma cells (Clive et al. 1972); and assays in yeast (Zimmermann 1971). The development of these and subsequent assays in mammalian cells and <i>in vivo</i> was predicated on the notion that mutagenicity results in these systems would be more relevant to humans than would those from bacteria.

Despite concerted efforts, few mutagens beyond direct-acting alkylating agents were discovered initially with these assays, and known rodent carcinogens other than direct-acting alkylating agents were largely negative in these assays. However, as reviewed by Brusick (1989), a paradigm shift began when Malling (1966b) used a hydroxylating mixture to activate diethyl- and dimethylnitrosamine, which were not mutagenic <i>in vitro</i>, to metabolites that were mutagenic in <i>N. crassa</i>. Building on this observation, as well as on the work of Miller and Miller (1971) and in consultation with H. Gelboin at NIH/NCI, Malling (1971) then coupled the <i>Salmonella</i> mutagenicity assay with <i>in vitro</i> metabolic activation composed of the supernatant from mouse liver homogenate centrifuged at 30,000 × g (microsomes) plus cofactors. Using this microsomal activation mixture, Malling (1971) showed that dimethylnitrosamine was mutagenic in <i>Salmonella</i> in a liquid-suspension assay, resulting in the first version of what would later be called the <i>Salmonella</i>/mammalian microsome mutagenicity assay. The host-mediated assay provided additional evidence that carcinogens could be mutagens after mammalian metabolism (Legator and Malling 1971). Ames et al. (1972) then showed that DNA-reactive metabolites of known carcinogens were mutagenic (no metabolic activation was used); in that paper, the authors also introduced the plate-incorporation version of the assay, where the bacteria and chemical were combined in the top agar on the Petri plate.

The connection between mutagenesis and carcinogenesis developed further when Ames et al. (1973a) combined their <i>Salmonella</i> tester strains, the test chemical, and the supernatant from a 9,000 × g centrifugation of rat liver homogenate (59 fraction) along with cofactors, as described by Garner et al. (1972), together in the top agar and showed that a variety of heretofore nonmutagenic rodent carcinogens were, in fact, mutagenic after metabolic activation. This plate-incorporation version of the <i>Salmonella</i>/mammalian microsome mutagenicity assay became a standard that is still in use today. Various modifications, including reduced nucleotide-excision repair, enhanced cell-wall permeability (Ames et al. 1973b), and enhanced error-prone repair achieved by the introduction of a plasmid [as suggested by MacPhee (1989)], combined to make for a highly sensitive test system (Maron and Ames 1983). Consequently, a new paradigm emerged within just a few years that led to an entirely new approach to carcinogen prediction and testing. This spurred the use of <i>in vitro</i> assays for mutagenicity in bacteria and mammalian cells as predictors of potential rodent and human carcinogens (Tennant et al. 1987), culminating in the current genetic toxicity test battery (Eastmond et al. 2009).

Decades of research have shown that mutagenesis is a critical component of carcinogenesis, based on a range of evidence including mutational spectra (Dogliotti et al. 1998; Hainaut and Wiman 2009) and genomic sequencing of tumors (Wood et al. 2007; Parsons et al. 2008). Thus, now it is difficult to recall that once it was somewhat bold to propose that there was any direct connection between the two processes (Knudson 1973; Miller and Miller 1971). Prior to 1972, it was not yet clear that the electrophilicity of some chemical carcinogens had a necessary role in the potential mutagenic activity of such compounds or even that DNA, as opposed to protein, was the ultimate target of carcinogens (Miller 1970).

Although sound theoretical reasons existed for proposing that carcinogens might act through a mutagenic mechanism, a compelling demonstration of this connection did not yet exist (Miller and Miller 1971). In fact, mutagenesis shared the stage with other likely mechanisms, including epigenetic changes (Miller 1970; Miller and Miller 1971), altered expression of an integrated viral genome (Tordaro and Huebner 1972), or alteration of immunological factors by carcinogens, permitting the formation and growth of tumors (Baldwin 1973). Of course, time has shown that all of the above mechanisms are important, especially epigenetic mechanisms (Jones and Baylin 2007), which may be particularly relevant for nonmutagenic carcinogens. Given the much broader range of biology that future assays will detect, new paradigm shifts will emerge in other areas of toxicology from 21st century assays.

Paradigm Shift II: Recognition of Ubiquitous Mutagenic Activity in the Environment

When Ames (1971) first introduced the assay, he stated "I will be glad to mail the strains to people desiring them and to serve as a clearinghouse for new and improved bacterial tester strains." Consequently, by the late 1970s, > 2,000 laboratories around the world had requested the <i>Salmonella</i> tester strains to initiate studies in environmental mutagenesis (Ames 1979). The fact that neither Ames nor his employer (University of California-Berkeley) patented the strains and that he made them freely available facilitated their use and dissemination throughout academic, industrial, and government laboratories worldwide—promoting the development of many creative uses and modifications of the assay. Creative uses may also emerge from 21st century assays, especially those developed in the public sector, which would have some probability of being disseminated freely.

The initial uses of the <i>Salmonella</i> assay led to the startling (at the time) recognition that our environment is replete with mutagens, including fungal toxins, combustion emissions, industrial chemicals, and drugs. The <i>Salmonella</i> assay was essential to this effort, providing the means by which researchers discovered for the first time that much of our environment had mutagenic activity, including cigarette smoke (Kier et al. 1974), urban air (Talcott and Wei 1977; Tokiwa et al. 1977), river water (Pelon et al. 1977), drinking water (Loper et al. 1978), food (Sugimura et al. 1977), and soil (Gürgemmel and Spitzauer 1983). The assay was used to show that even people could have systemic mutagenic activity detectable in urine after smoking (Yamasaki and Ames 1977) or after eating fried meat (Baker et al. 1982). Decades of studies have shown that nearly all urban air samples tested (Claxton et al. 2004; Claxton and Woodall 2007), drinking water (Richardson et al. 2007), soil (White and Claxton 2004), and house dust (Maetens et al. 2004) are mutagenic. These reviews document that at least 40–50% of the papers published thus far on the genotoxicity of, for example, air, soil, water, and house dust have used the <i>Salmonella</i> assay, and they show that the vast majority of contemporary studies rely almost exclusively on the <i>Salmonella</i> assay for mutagenicity assessments of environmental media.

The realization that much of the environment had mutagenic activity was unanticipated.
by most researchers and posed a challenge to environmental scientists, public health authorities, and regulators. As 21st century toxicology proceeds, previously unrecognized, ubiquitous toxicities in our environment may be discovered—beyond findings of mutagenicity and potential carcinogenicity—and a new paradigm of toxicity effects may emerge (Boekelheide and Campion 2010). Regulators and public health authorities may have to expand or reconsider their approaches based on the results from such assays.

How the Salmonella Assay Has Been Used

Published data. We searched three publication databases [PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Scopus (http://www.scopus.com/home.url), and Web of Knowledge (http://apps.isiknowledge.com/)], and we found 10,169 unique publications dealing with the Salmonella assay. This was accomplished by searching each database for “Ames test OR Salmonella mutagen.” This gave 11,064 responses in PubMed, 13,694 in Scopus, and 3,453 in the Web of Knowledge.

Although it is likely that not all references were found in this search, the number of references retrieved should give a good sampling of trends. We merged the citations into an EndNote (Thomson Reuters, New York, NY) database, and we deleted duplicates based on the same first author name, journal name, journal year, volume, and page number. We examined the remaining information individually to eliminate additional duplicates, non-Salmonella mutagenicity papers, abstracts, and papers that seemed to refer to the assay only tangentially. Then we categorized papers by key words/phrases that reflected how the assay was used or discussed within the context of the paper. The final database had 10,169 publications sorted into 7 major categories and 20 subcategories. A publication was often included in more than one category/subcategory based on the nature of that publication. The reference database is available in Supplemental Material (doi:10.1289/ehp.1002336).

Figure 1A shows the numbers of publications per year that have used the Salmonella assay as well as the other gene-mutation assays developed near the same time, including those in mammalian cells. The number of publications using the Salmonella assay rose dramatically, peaking at approximately 500 papers/year in the early 1980s, but has declined gradually to a rather constant level of approximately 200 papers/year during the past decade. Other assays rose to approximately 10–20 papers/year, with the mouse lymphoma Tk<sup>-/-</sup> assay remaining at that level today.

Subsequently, newer genotoxicity assays became popular, and the number of publications for these are shown in Figure 1B. By far, the comet assay has the highest surge in usage and is just now starting to plateau. The micronucleus assays also are prominent, with approximately 100 papers/year being published consistently for the past 20 years. The publication frequency for papers using micronucleus assays has surpassed those using in vitro chromosome aberration assays, which peaked in the mid-1980s (data not shown).

With regard to the Salmonella assay, the papers documenting the testing of agents associated with environmental samples (Figure 1C), as well as commerce, metabolism, or personal exposure (Figure 1D), peaked in the 1980s but still continue at a steady rate. A closer look at the number of papers published on various types of environmental samples (Figure 1C) shows that a) relatively few publications have been associated with soil and sediment samples; b) papers looking at air samples follow the overall declining trend seen since 1983; and c) publications dealing with water reached a plateau starting in 1980 and have remained stable. However, reports dealing with natural substances have increased since the mid-1990s. This increase is due largely to a search for and analysis of antimutagens, mainly from plant extracts. Figure 1D shows a decline in the number of publications on mechanism and metabolism; although there was a rise in the personal-exposures subcategories until the late 1980s (Figure 1D), the number has since declined.

Unpublished data. For a variety of reasons, little toxicological data have either been generated or are available publically for a large proportion of compounds in commercial use. For example, toxicological data are available for only 7% of high-production-volume chemicals (> 1 million pounds/year) (Guth et al. 2007) and for only a fraction of regulated industrial chemicals (Schwarzman and Wilson 2009; Wilson and Schwarzman 2009). The few publications dealing with commercial substances (Figure 1D) likely reflect the fact that such data are proprietary. In the U.S. EPA New Chemicals Program, approximately 50,000 premanufacturing notice (PMN) cases have been received since 1979 when the program began; however, only 10% (4,997) have mutagenicity data, with 87% of these (4,351) having Salmonella assay data (Cimino MC, personal communication). Thus, only 8.7% of the 50,000 PMNs submitted during the past 30 years have Salmonella mutagenicity data, almost none of which are available publically, and approximately 2% of pre-1979 PMNs have been reviewed for the need for toxicological data (Guth et al. 2007).

To estimate the percentage of commercial chemicals that are mutagens, Zeiger and Margolin (2000) assembled randomly 100 chemicals in commercial use, which
included 46 organics in highest production in the United States (inorganic and elemental compounds were not included among the 100 chemicals), and evaluated them for mutagenicity in the Salmonella assay. They found that 22% of the total 100 compounds were mutagenic, and 20% of the subset of 46 high-production compounds were mutagenic. In the absence of required testing and reporting (Guth et al. 2007; Schwarzen and Wilson 2009), these data are the best estimates available regarding the proportions of mutagens among organic compounds in current commercial use. Improved estimates may emerge after potential changes to the Toxic Substances Control Act (TSCA) (Birnbaum 2010; U.S. EPA 2010b; Wilson and Schwarzen 2009).

The U.S. Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) program does not keep cumulative data for each assay submitted, largely because each submission is usually considered solely on the basis of the information within it (Benz RD, personal communication). It must be assumed, however, that the pharmaceutical industry also has tested thousands of substances in the Salmonella assay. In an analysis using the Physicians’ Desk Reference from 1999 through 2008, Snyder (2009) compiled the mutagenicity of > 500 marketed drugs, excluding the cytotoxic anticancer and antivirals, nucleosides, steroids, and biologicals. He found that approximately 7% (38/525) of these drugs were mutagenic in bacterial assays (data from either E. coli or Salmonella assays); this small percentage is likely due to the extensive early screening in the Salmonella assay to eliminate mutagenic molecules from further development.

There are a few reports of environmental monitoring programs using the Salmonella assay, such as the 20-year program on surface-water mutagenicity in Brazil (Umbuzeiro et al. 2001). However, such monitoring is rarely done and almost never reported in the peer-reviewed literature, although the Brazilian data are available online from the Companhia Ambiental do Estado de São Paulo (CETESB) (2010). Therefore, the large number of agents whose test results in the Salmonella assay have been published may not reflect either the equally large—or larger—number of proprietary chemicals tested by the pharmaceutical and chemical industries or environmental monitoring data, which are not published.

The Salmonella Assay as a Model for 21st Century Toxicology Assays

Because of its simplicity, cost effectiveness, flexibility, and large validated database, the Salmonella assay is an ideal model to consider in the development of equally reliable in vitro toxicity assays for the 21st century. The predictivity, specificity, and sensitivity of the Salmonella assay have been validated against selected other mutagenicity assays and rodent carcinogenicity data (Tennant et al. 1987). Likewise, new HTS assays will need to be validated against something (Hartung 2009a), and one possibility is to measure some end points against the Salmonella assay (Schoonen et al. 2009). As outlined by Zeiger (2003), there are fundamental procedures to consider when developing, validating, and ultimately accepting new assays, and below we highlight some ways in which the Salmonella assay serves as a model for this process.

**Standard procedures, quality assurance, and statistical assessment.** Soon after the introduction and widespread use of the Salmonella assay, researchers recognized the need for standardized procedures. Consequently, Ames published methods papers (Ames et al. 1975; Maron and Ames 1983), and the procedures were quickly adopted by the mutagenesis community. Procedures included the use of positive and negative controls, standard procedures for performing the assay, preparation of 59 mix, checking the tester strains for genetic and physiological stability, and evaluating the results statistically (Bernstein et al. 1982; Claxton et al. 1984, 1987; Kim and Margolin 1999; Margolin et al. 1981; McCann et al. 1984; Stead et al. 1981). Although positive controls and metabolic activation were generally missing from some first-generation HTS assays, these and other issues are being considered and corrected in current and future iterations of the ToxCast and Tox21 assays (Hartung 2009a, 2009b; Huang et al. 2009; Kavlock et al. 2008; Westerink et al. 2010), as well as for toxicogenomic assays (Ellinger-Ziegelbauer et al. 2009). As noted above, even the early versions of the Salmonella assay did not incorporate metabolic activation (because it had not yet been developed). Despite these limitations, initial analyses of data from ToxCast Phase 1 have identified those chemicals able to induce oxidative stress as evidenced by Nrf2 activity (Martin et al. 2010).

**Structure–activity analysis (SAR).** Data from the Salmonella assay were used by Ashby (1985) to identify structural alerts for potential carcinogenicity, providing critical data for the development of computerized structure–activity methods for carcinogenicity prediction (Richard 1998). These methods are still used widely within the chemical, pharmaceutical, and regulatory communities (Benfenati et al. 2009). Claxton et al. (1988) examined Salmonella assay data in the peer-reviewed literature for individual chemicals, classified the chemicals by an International Union of Pure and Applied Chemistry chemical class scheme, and found that mutagenicity in the Salmonella assay was highly predictive of rodent carcinogenicity for some chemical classes, such as aromatic amines, polycyclic aromatic hydrocarbons, and nitroarenes, but was less predictive for others, such as chlorinated organics. Ashby and Tennant (1988) noted that for 222 chemicals evaluated by the NTP, data from the Salmonella assay, combined with structural alerts and a more limited protocol for the rodent cancer bioassay, permitted the detection of trans-species/multiple-site rodent carcinogens, which are likely human carcinogens (Ashby and Paton 1993; Tennant 1993).

Building on this past success, current efforts still rely on Salmonella assay data and are extending the analyses using newly developed computational methods and structural features. For example, Hansen et al. (2009) assembled a benchmark database containing 6,500 chemicals with Salmonella assay data along with structural information [Simplified Molecular Input Line Entry Specifications (SMILES)] to develop a prediction model that outperforms a variety of commercial predictive tools. Yang et al. (2008) compiled a group of 2,428 compounds, each of which has structural information and data for six mutagenicity tests, and showed that the percentage of industrial chemicals that were mutagenic was greater than that of chemicals used as drugs or food ingredients. The incorporation of chemical structure into the DSSTox EPA ToxCast continues to grow (Houck et al. 2008), and this structural and toxicology database will enable data from HTS assays to be used for SAR as Salmonella assay data have been used for decades.

**Reproducibility and transferability of the assay across laboratories.** High reproducibility of an assay allows results to be compared not only within the same laboratory over time but also among laboratories. To address this issue, a set of international, collaborative testing programs was established to evaluate the Salmonella assay as well as several other mutagenicity assays using coded chemicals from the same lot (Ashby et al. 1985, 1988; de Serres et al. 1981) and standard protocols (Dunkel et al. 1984, 1985; Margolin et al. 1984; Piegorsch and Zeiger 1991). These comparative studies paved the way for the establishment of standard methods and procedures for selected mutagenicity assays that are still largely in place. A similar international effort was established for the evaluation of standards of complex mixtures in the Salmonella assay (Claxton et al. 1992; Lewtas et al. 1992).

Concurrently, the establishment of the U.S. EPA GENE-TOX program (Ray et al. 1987; Waters and Auletta 1981) provided, to our knowledge, the first self-assessment of the literature in any field of toxicology—in this case, genetic toxicology. This enormous effort (Waters 1994) involved 196 scientists who critically read all of the papers published on each of 23 assays, resulting in 41 comprehensive, published reviews. The consequence
of this effort was that out of nearly 200 assays, the mutagenesis community agreed on the general use of a subset for routine use, including, for example, the protocols, publication requirements, and use of positive and negative controls, much of which is reflected in the current genotoxicity test battery (Eastmond et al. 2009).

As a plethora of new assays emerge over the coming years, a similar self-assessment being organized by the Transatlantic Think Tank of Toxicology (Hartung 2009a) will be invaluable. Just as with the self-assessment by the GENE-TOX program, it will likely result in the acceptance of just a few assays, as well as the establishment of the standards, protocols, interpretation, and publication requirements for those assays, which will provide a test battery that will serve the regulatory community well in the coming years.

Testing. As reviewed by Zeiger (2004), many factors led to the initial effort of the U.S. government, in particular, M. Legator at the FDA, to sponsor mutagenicity testing in 1971, followed by numerous contracts in the ensuing years. Ames himself published an extensive testing and validation study early on in which he used his assay to assess the mutagenicity of 300 compounds (McCann et al. 1975; McCann and Ames 1976). This effort was followed soon by other screening studies involving the Salmonella and other assays (Bruce and Heddle 1979; Ishidate and Odashima 1977; Nagao et al. 1978; Purchase et al. 1978; Rinkus and Legator 1979). The NIEHS/ NTP mounted the most comprehensive effort in testing, involving the comparison of four mutagenicity assays along with rodent carcinogenicity data (Tennant et al. 1987). This effort and subsequent analyses (Kirkland et al. 2005; Zeiger 1998) have shown that the Salmonella assay alone, in the absence of a test battery, is reasonably predictive of rodent carcinogenicity. Among a group of chemicals of mixed chemical class, a greater percentage of the compounds that are mutagenic in the Salmonella assay are likely to be rodent carcinogens compared with the percentage of nonmutagens likely to be noncarcinogens (Kirkland et al. 2005; Zeiger 1998). At present, there are no reliable methods to assess Salmonella-negative compounds for potential carcinogenicity. This conclusion has prompted discussion, pro and con, regarding the option of eliminating the mammalian cell assays from the genotoxicity test battery or the inclusion of other assays (Elespuru et al. 2009; Kirkland et al. 2007).

This development is ironic, as efforts proceed swiftly to develop high-throughput assays in mammalian cells (Kavlock et al. 2008; Westerink et al. 2010). Despite the theoretical and scientific relevance of mammalian cell assays, their prognostic value may, in fact, be limited. For example, the Salmonella assay is less susceptible than mammalian cell assays to artifacts resulting from high toxicity, pH shifts, and osmotic effects (Kirkland et al. 2007). Nonetheless, Zhu et al. (2008) showed that using HTS cell viability data for 1,408 compounds greatly improved quantitative structure–activity relationship (QSAR) predictions for rodent carcinogenicity. They suggest that an approach using improved models, coupled with HTS assay data and structural features of the compounds, might partially replace in vivo toxicity testing. Even some in vivo assays may be of little or no added value, as indicated by the inability of the mouse bone-marrow micronucleus assay to improve carcinogen prediction beyond that of the Salmonella assay alone (Zeiger 1998).

The history of genetic toxicity demonstrates that only assays that can be adopted by many laboratories and validated through extensive testing are of value for regulatory purposes. Consequently, based on the testing efforts described above, testing schemes were put into law for testing new chemicals (U.S. EPA 2010b), pesticides (U.S. EPA 2010a), and new pharmaceuticals (FDA 2010). Recent discussions have explored how new types of assay data might have an impact on the regulation of genotoxic compounds (Elespuru et al. 2009; Ge et al. 2007; Guyton et al. 2009; Hartung 2009a, 2009b; Hartung and Daston 2009; Hartung and Rovida 2009; Hoppin and Clapp 2005; Krewski et al. 2009; Meek and Doull 2009; National Research Council 2007; Service 2009). Many such issues will need to be settled before legislation of the type above could ever be instituted for 21st century assays.

Assay flexibility. The flexibility of the Salmonella assay has allowed the assay to be used in a variety of protocols with a variety of agents, including complex mixtures, gases, and radiation. Current HTS assays use nonvolatile, single agents that are soluble in dimethyl sulfoxide, but agents with other characteristics (e.g., water-soluble compounds, gases) will need to be tested (Kavlock et al. 2008; Tice RR, personal communication). Over the years, this recognition for the Salmonella assay resulted in a plethora of modifications that have enabled the assay to be used in an almost infinite variety of ways. These include modifications permitting a) the use of small amounts of sample (Diefel et al. 2000; Flamand et al. 2001; Green et al. 1977; Houk et al. 1989; Kado et al. 1983) in semi–high-throughput modes involving colorimetric analysis (Kamber et al. 2009; Umbuzeiro et al. 2010) and fluorescent assays (Aubrechet et al. 2007; Cariello et al. 1998); b) the testing of volatiles and gases (Baden et al. 1976; Hughes et al. 1987); c) the testing of body fluids, including urine (Cerná and Pastorková 2002), feces (de Kok and Van Maanen 2000), breast milk (Phillips et al. 2002; Thompson et al. 2002), breast nipple aspirates (Klein et al. 2001), and cervical mucus (Holly et al. 1993); d) the testing of all types of complex mixtures, including air, soil, water, house dust, and combustion emissions (see “Paradigm Shift II” above), and fried meat (Knize and Felton 2005); e) molecules (DeMarini 2000; Koch et al. 1994) and genomic analyses (Porwollik et al. 2001; Ward et al. 2010); and f) the evaluation of mutagenicity inside the International Space Station (Rabbow et al. 2003). This flexibility has permitted the Salmonella assay to be used for almost every conceivable type of environmental and molecular epidemiology study.

In addition, numerous modifications of the tester strains or testing conditions have permitted researchers to explore the role of metabolism and to detect the mutagenicity of specific chemical classes of substances (Claxton and Barnes 1981; Gee et al. 1994; Hagiwara et al. 1993; Hayashida et al. 1976; Houk and Claxton 1986; Houk et al. 1989; Josephy 2002; Prival and Mitchell 1982; Reid et al. 1984; Rosenkranz and Mermelstein 1983; Watanabe et al. 1990). Whether it has been in the development of commercial products (Zeiger and Margolin 2000), the evaluation of industrial products and wastes (Aguayo et al. 2004; Bessi et al. 1992; Brooks et al. 1998; Claxton et al. 1998; Ohe et al. 2004), or substances known to contaminate the environment (Chen and White 2004; Claxton et al. 2004; Claxton and Woodall 2007; White and Claxton 2004), the Salmonella assay has been the screening test of choice in genetic toxicology for nearly four decades. Perhaps a new assay will emerge in the coming years that can assess a comprehensive set of predictive biological changes and also have the range of flexibility exhibited by the Salmonella assay.

Standardization of sample preparation. The flexibility of the Salmonella assay prompted the development of methods to prepare environmental samples for the assay (Hewitt and Marvin 2005; Marvin and Hewitt 2007). This included solvents and materials for the delivery of substances to the assay, preparation of environmental and epidemiological samples, and methods for the concentration and determination of doses for testing gases. The coupling of chemical methods with the Salmonella assay enabled extensive use of the assay for bioassay-directed chemical fractionation to identify chemical classes of mutagens or individual mutagens (Austin et al. 1985; Brooks et al. 1998; Lewtas 1993; Lewtas et al. 1990; Oliveira et al. 2006), permitting the discovery of many environmental mutagens, such as PBTA (2-phenylbenzotriazole) in surface waters (Nukaya et al. 1997), MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2-(5H)-furane) in drinking water (Hemming et al. 1986), and PhIP (2-aminomethyl-6-phenylimidazo[4,5-b]pyridine) in fried meat.
References

Agusov S, Munoz MJ, de la Torre A, Aguayo S, de la Torre A, Roset J, de la Pena E, Morita M, Clayton E, Rouleau A, Carney S, Brooks LR, Zuniga E. 2007. The detection of chemical mutagens with Salmonella typhimurium. Mutat Res 638:235–242.

Ashby J, Paton D. 1993. The influence of chemical structure on the extent and sites of carcinogenesis for 52 rodent carcinogens and 55 different human carcinogen exposures. Carcinogenesis 14:257–264.

Ashby J, Tennant RW. 1988. Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 252 chemicals tested in rodent bioassays by the National Toxicology Program (NTP) and the U.S. Environmental Protection Agency (EPA). Cancer 61:129–134.

Baden JM, Brinkenhoff WH, Hitt BA, Simmon VF, de Serres FJ, Kølmark HG. 1958. A direct method for determining inactivation of genotoxic agents in water. Nature 181:314–315.

Baker R, Arlukirk J, Angus D. 1982. Detection of mutagenic activity in human urine following fried pork or bacon meals. Cancer Lett 18:81–89.

Baldwin RW. 1953. Immunological aspects of chemical carcinogenesis. Adv Cancer Res 1:1–27.

Benfanti E, Benigni R, DeMarini DM, Helma C, Kirkland D, Martin TM, et al. 2009. Predictive models for carcinogenicity and mutagenicity: frameworks, state-of-the-art and perspectives. J Environ Sci Health C Environ Carcinog Ecotolog Rev 27:57–80.

Bernstein L, Kaldor J, McCann J, Pike MC. 1982. An empirical approach to the statistical analysis of mutagenesis data from the Salmonella assay. Mutat Res 97:267–281.

Bessi H, Ferard JF, Vasseur P, Colin F, Belkhadir E. 1992. Evaluation of short-term tests for carcinogenicity: a screen for detecting mutagenicity with high throughput attributes. Mutagenesis 22:335–342.

Bennett AJ, Taylor MG, Aspinall OA, Paul MW. 1983. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat Res 31:347–364.

Birch J. 1985. Fundamental structural alerts to potential carcinogens. Cancer Res 45:1191–1196.

Claxton et al. 1991. A simplistic system for the detection and classification of mutagens and carcinogens: Report of the IPCS Collaborative Study on in Vivo Assays. Amsterdam:Elsevier.

Claxton LD, Woodall MG Jr. 2007. A review of the mutagenicity and carcinogenicity assessments of industrial chemicals in the EU. Mutat Res Genet Toxicol Environ Mutagen 66:211–221.

Deng S, et al. 2009. Characterization and interlaboratory comparison of a gene expression signature for differentiating human colon cancer from normal colon tissue. Cancer Res 69:12390–12398.

Dingli E, Hajnal P, Hernandez T, Erroco M, DeMarini DM. 1998. Mutation spectra resulting from carcinogenic exposure: from model systems to cancer-related genes. Recent Results Cancer Res 154:97–124.

Dunke VC, Zeiger E, Brusick DJ, McCoy D, McGregor D, Mortelmans K, et al. 2007. Reproducibility of microbial mutagenicity assays: I. Tests with Salmonella typhimurium and Escherichia coli using a standardized protocol. Environ Mutagen 6:21–25.

Eastmond DA, Hartwig A, Anderson D, Anwar WA, Cimino MC, Dobrev I, et al. 2009. Mutagenicity testing for chemical risk assessment: update of the WHO/IPCS harmonized scheme. Food and Drug Administration (FDA). 2010. Regulatory Information. Available: http://www.fda.gov/RegulatoryInformation/Legislation/default.htm [accessed 25 March 2010].

Feltow DS, Knize MG, Schaid DJ, Burow RE, Izenberg AD, Happe J, et al. 1988. The isolation and identification of a new mutagen from fried ground beef: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Carcinogenesis 9:1081–1086.

Flamand N, Meunier JR, Meunier P-A, Agapakis-Caussé C, Peroues F. 2001. Microtubule mutagenicity: a miniaturized version of the Ames test used in a prescreening assay for point mutation assessment. Toxicol In Vitro 15:105–114.
Garner RC, Miller EC, Miller JA. 1972. Liver microsomal metabolism and mutation testing in a reactive derivative toxic to Salmonella typhimurium TA1530. Cancer Res 32:2508–2506.

Ge Y, Preston RJ, Russell DO. 2007. Toxicoproteomics and its application to human health risk assessment. Proteomics 7:1813–1833.

Gee P, Maron DM, Ames BN. 1994. Detection and classification of mutagens: a set of basic-specific Salmonella tester strains. Proc Natl Acad Sci USA 91:11666–11610.

Göppel-Juhasz B. 1992. Mutagenic activity in agricultural soils. In: Carcinogens and Mutagens in the Environment: 3. Naturally Occurring Compounds: Epidemiology and Distribution (Stich HF, ed). Boca Raton, FL: Lewis Publishers 445–484.

Green MLH, Bridges BA, Rogers AM, Horvitz M, Urie JW, Bridges JW, et al. 1977. Mutagen screening by a simplified bacterial fluctuation test: use of microbial preparations and whole liver cells for metabolic activation. Mutat Res 48:287–294.

Guth JH, Denison RA, Sass J. 2007. Require comprehensive safety data for all chemicals. New Solut 17:233–258.

Guyton KZ, Kyle AD, Aurbach J, Cogliano VJ, Eastmond DA, Bridges JW, et al. 1977. Mutagen screening by a simplified bacterial fluctuation test: use of microbial preparations and whole liver cells for metabolic activation. Mutat Res 48:287–294.

Hartung T. 2009b. Toxicology for the twenty-first century. In: Chemical Mutagens: Principles and Methods for Their Detection, Vol. I (Hollander A, ed). New York: Plenum Press.

Hartman PE, Ames BN, Roth JR, Barnes WM, Levin DE. 1988. Target sequences for mutagenesis in Salmonella histidine-requiring survivors: use of target Mutations 1631–641.

Hartung T. 2009a. A toxicity for the 21st century—mapping the road ahead. Toxicol Sci 109:183–219.

Hartung T. 2009b. Toxicology for the twenty-first century. Nature 460:208–212.

Hartung T, Daston G. 2009. Are in vitro tests suitable for regulatory use? Toxicon Sci 111:223–237.

Hartwig T, Rovida C. 2009. Chemical regulators have overreached. Nature 460:1080–1081.

Hayashida S, Wang CY, Bryan GT. 1976. A simple method for determination of the Salmonella liquid-incipient assay. Increased sensitivity for detecting mutants in human urine. Mutat Res 121:25–32.

Kirkland D, Pfuhler S, Tweats D, Aardema M, Darroudi F, Marvin CH, Hewitt LM. 2007. Analytical methods in bioassay-directed investigations of mutagenicity of air particulate material. Mutat Res 638:4–35.

Kolaczek A, Oehme K, Perriard ZC, Bartsch H. 2009. Weighted feature significance: a simple interpretable model of compound toxicity based on the statistical enrichment of mutagenic activity from a set of strong mutagens. Mutat Res 675:151–158.

Kloosterman JD, Claxton LD. 1987. Dimethylnitrosamine and diethylnitrosamine, in.: Chemical Mutagens: Principles and Methods for Their Detection, Vol. I (Hollander A, ed). New York: Plenum Press.

Kloosterman JD, Claxton LD. 1987. Mutagen screening by a simplified bacterial fluctuation test; use of microbial preparations and whole liver cells for metabolic activation. Mutat Res 48:287–294.

Kondo NY, Langley D, Eisenstadt E. 1983. A simple modification of the Salmonella histidine-requiring survivors: use of target Mutations 1631–641.

Jones PA, Baylin SB. 2007. A simple modification of the Salmonella histidine-requiring survivors: use of target Mutations 1631–641.

Josephy PO. 2002. Genetically-engineered bacteria expressing human enzymes and their use in the study of mutagens and mutagenesis. Toxicology 181:182–255–6.

Kado NY, Langley D, Eisenstadt E. 1983. A simple modification of the Salmonella histidine-requiring survivors: use of target Mutations 1631–641.

Kavlock RJ, Austin CP, Tice RR. 2009. Toxicity testing in the 21st century: implications for human health risk assessment. Risk Anal 29:736–746.

Kier LD, Yamazaki E, Ames BN. 1974. Detection of mutagenic activity in cigarette smoke condensates. Proc Natl Acad Sci USA 71:4143–4149.

Kilman BA. 2005. Databases of Chemicals on Dividing Cells. Englewood Cliffs, NJ:Prentice-Hall.

Kim BS, Margolin BH. 1999. Statistical methods for the Ames Salmonella assay: a review. Mutat Res 436:113–122.

Kirkland D, Aardema M. 2005. Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens - I. Specificity, sensitivity and relative predictivity. Mutat Res 563:281–296.

Kirkland D, Plopper C, Suetos D, Aardema M, Corvi R, Darroudi F, et al. 2007. How to reduce false positive results when undertaking in vitro genotoxicity testing and thus avoid unnecessary follow-up animal tests: report of an ECAM workshop. Mutat Res 628:31–55.

Klein P, Glaser E, Grogan L, Keane M, Lipkowitz S, Sobol E, et al. 2001. Biomarker assays in niple aspirate fluid. Breast J 7:387–391.

Knize MG, Felton JS. 2005. Formation and human risk of carcinogenic heterocyclic amines formed from natural precursors. Environ Mol Mutagen 43:387–388.

Knudson MA, et al. 1995. Mutagens in surface water show increased sensitivity in the Salmonella/microsome test. Mutat Res 340:129–136.

Kloosterman JD, Claxton LD. 1987. Mutagen screening by a simplified bacterial fluctuation test: use of microbial preparations and whole liver cells for metabolic activation. Mutat Res 48:287–294.

Kloosterman JD, Claxton LD. 1987. Mutagen screening by a simplified bacterial fluctuation test; use of microbial preparations and whole liver cells for metabolic activation. Mutat Res 48:287–294.

Kloosterman JD, Claxton LD. 1987. Mutagen screening by a simplified bacterial fluctuation test; use of microbial preparations and whole liver cells for metabolic activation. Mutat Res 48:287–294.

Kloosterman JD, Claxton LD. 1987. Mutagen screening by a simplified bacterial fluctuation test; use of microbial preparations and whole liver cells for metabolic activation. Mutat Res 48:287–294.
et al. 2002. Mutagens in human breast lipid and milk: the search for environmental agents that initiate breast cancer. Environ Mol Mutagen 39:143–149.

Piegerseh WW, Zeiger E. 1991. Measuring intra-assay agreement for the Ames Salmonella assay. In: Statistical Methods in Toxicology (Riemhult O, Lindberg DAB, eds). Heidelberg:Springer-Verlag, 35–41.

Porvolik S, Wong RM, Sims SH, Schaepfer RM, DeMarini DM, Mccollard M. 2001. The Auvé mutations in the Ames strains of Salmonella span 15 to 119 genes. Mutat Res 483:1–11.

Poth A, Jaeger M. 2007. Alternative testing—the intelligent way to REACH compliance. AATEX 14:799–803.

Prival MJ, Mitchell VD. 1982. Analysis of a method for testing azo dyes for mutagenic activity in Salmonella typhimurium in the presence of flavin mononucleotide and hamster liver S9. Mutat Res 97:103–116.

Purchase IFH, Longstaff E, Ashby J, Styles JA, Anderson D, Lefevre PA, et al. 1978. An evaluation of 6 short-term tests for detecting organic chemical carcinogens. Br J Cancer 37:873–903.

Rabbob E, Retberg P, Baumstark-Khan C, Horneck G. 2003. The SOS-LUX-LAC-FLUORO-Toxicity-test on the International Space Station (ISS). Adv Space Res 31:1513–1524.

Ray VA, Kier LD, Kannan KL, Hasa RT, Aulette AE, Wasson JS, et al. 1987. An approach to identify specialized batteries of bioassays for specific classes of chemicals: class analysis using molecular systems and phylogenetic relationships and discordance patterns. 1. Composition and analysis of the overall data base. A report of phase II of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 185:197–241.

Reid TM, Morko WC, Wang CY, King CM. 1984. Mutagenicity of azo dyes following metabolism by different reductive/oxidative systems. Environ Mutagen 6:705–717.

Richard AM. 1998. Structure-based methods for predicting mutagenicity and carcinogenicity: are we there yet? Mutat Res 400:498–507.

Richardson SD, Pleva MJ, Wagner ED, Schoeny R, DeMarini DM. 2007. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. Mutat Res 636:178–242.

Rinkus SJ, Legator MS. 1979. Chemical characterization of 465 known or suspected carcinogens and their correlation with mutagenic activity in the Salmonella typhimurium system. Cancer Res 39:2289–3318.

Rosenkranz HS, Mermelstein R. 1983. Mutagenicity and genotoxicity of nitroaromatics. All nitro-containing chemicals were not created equal. Mutat Res 118:217–267.

Schoonen WGEJ, Westerink WMA, Horbach GJ. 2009. High-throughput screening for analysis of intravitamin toxicity. In: Molecular, Clinical and Environmental Toxicology (Luch A, ed). Basel, Switzerland:Birkhäuser, 1–35.

Schwarzman MR, Wilson MP. 2009. New science for chemicals policy. Science 326:1065–1066.

Service RF. 2009. A new wave of chemical regulations just ahead? Science 325:692–693.

Snyder RD. 2009. An update on the genotoxicity and carcinogenicity of nitroaryl pharamaceuticals with reference to in silico predictivity. Environ Mol Mutagen 50:435–450.

Steag AD, Hasselblad V, Creason JP, Claxton LD. 1981. Modeling the Ames test. Mutat Res 85:13–27.

Supigura T, Naoe M, Kawachi T, Honda M, Hayagi T, Seino Y, et al. 1977. Mutagenic carcinogens in foods, with special reference to highly mutagenic pyrrolid products in broiled foods. In: Origins of Human Cancer (Hiatt HH, Watson JD, Winston JA, eds). Cold Spring Harbor, NY:Cold Spring Harbor Laboratory, 1561–1577.

Szybalski W. 1958. Special microbiological systems. II. Observations on chemical mutagenesis in microorganisms. Ann NY Acad Sci 76:475–489.

Talcott R, Wei E. 1977. Airborne mutagens bioassayed in Salmonella typhimurium. J Natl Cancer Inst 58:449–451.

Tennant RW. 1993. Stratiﬁcation of rodent carcinogenicity bioassay results to relate speciﬁc human hazard. Mutat Res 286:111–118.

Tennant RW, Margolin BH, Shelby MD, Zeiger E, Haseman JK, Spalding J, et al. 1987. Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. Science 269:333–341.

Thompson PA, DeMarini DM, Kadiub FF, McClure GY, Brooks LR, Green BL, et al. 2002. Evidence for the presence of mutagenic arylamines in human breast milk and DNA adduct formation. Mutat Res 527:237–248.

Tordjaj GJ, Hueberer RJ. 1972. N.A.S. symposium: new evidence as the basis for increased efforts in cancer research. The viral oncogene hypothesis: new evidence. Proc Natl Acad Sci USA 69:1009–1015.

Umbuzeiro Gde A, Rech CM, Correia S, Bergamasco AM, Cardenete GHL, Flückiger-Iserl S, et al. 2010. Comparison of the Salmonella/microsome microsuspension assay with the new microplate ﬂuctuation protocol for testing the mutagenicity of environmental samples. Environ Mol Mutagen 51:31–38.

Umbuzeiro Gde A, Roubicke DA, Sanchez PS, Sato MI. 2001. The Salmonella mutagenicity assay in in vivo quality monitoring program based on a 20-year survey. Mutat Res 491:119–126.

U.S. EPA (U.S. Environmental Protection Agency). 2010a. Pesticides: Regulating Pesticides. Available: http://www.epa.gov/pesticides/regulating [accessed 25 March 2010].

U.S. EPA (U.S. Environmental Protection Agency). 2010b. Summary of the Toxic Substances Control Act. Available: http://www.epa.gov/registiy/laws/tmsca.html [accessed 25 March 2010].

Ward WD, Swartz CD, Hanley NM, Whitaker JW, Franzén R, Waters MD, Auletta A. 2001. The GENE-TOX program: genetic activity evaluation. J Chem Inf Comput Sci 21:35–38.

Weinberg F. 1993. The history of the stethoscope. Can Fam Physician 39:2223–2224.

Westenik WMA, Stevenson JCR, Horbach GJ, Schoonen WGEJ. 2010. The development of RAD51C, cysatin a, p53 and Nrf2 luciferase-reporter assays in metabolite competent HepG2 cells for the assessment of mechanism-based genotoxicity and of oxidative stress in the early research phase of drug development. Mutat Res 696:21–40.

White PA, Claxton LD. 2004. Mutagens in contaminated soil: a review. Mutat Res 567:227–346.

Whitfield HJ, Martin RG, Ames BN. 1966. Classiﬁcation of antimutagenic compounds (G gene) mutants in the histidine operon. J Mol Biol 21:335–350.

Wilson MP, Schwarzman MR. 2009. Toward a new U.S. chemicals policy: rebuilding the foundation to advance new science, green chemistry, and environmental health. Environ Health Perspect 117:1202–1209.

Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, et al. 2007. The genomic landscapes of human breast and colorectal cancers. Science 318:1108–1113.

Yamakasi A, Ames BN. 1977. Concentration of mutagens from urine by absorption with the nonpolymer resin XAD-2 cigarette smokers have mutagenic urine. Proc Natl Acad Sci USA 74:3595–3599.

Yang C, Hasselgren CH, Bayer S, Arvidson K, Aveston S, Dierkes P, et al. 2008. Understanding genetic toxicity through data mining: the process of building knowledge by integrating multiple genetic toxicity databases. Toxicol Mech Methods 18:277–295.

Zeiger E. 1998. Identiﬁcation of rodent carcinogens and noncarcinogens using genetic toxicity tests: premises, promises, and performance. Regul Toxicol Pharmacol 28:85–95.

Zeiger E. 2002. Validation and acceptance of new and revised tests: a ﬂexible but transparent process. Toxicol Lett 140–141:31–35.

Zeiger E. 2004. History and rationale of genetic toxicity testing: an impersonal, and sometimes personal, view. Environ Mol Mutagen 44:363–371.

Zeiger E, Margolin BH. 2000. The proportions of mutagens among chemicals in commerce. Regul Toxicol Pharmacol 32:219–225.

Zhu H, Rusyn I, Richard A, Tropsha A. 2008. Use of cell viability assays to detect mutagenic nitroarenes and aromatic amines: new derivatives of Salmonella typhimurium tester strains possessing elevated O-acetylation transferase levels. Mutat Res 234:337–348.

Watanabe M, Ishidate M Jr, Nohrni T. 1990. Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: new derivatives of Salmonella typhimurium tester strains possessing elevated O-acetylation transferase levels. Mutat Res 234:337–348.

Waters MD. 1994. Development and impact of the Gene-Tox program, genetic activity proﬁles, and their computerized data bases. Environ Mol Mutagen 23:67–72.

Waters MD, Auletta A. 1981. The GENE-TOX program: genetic activity evaluation. J Chem Inf Comput Sci 21:35–38.

Weinberg F. 1993. The history of the stethoscope. Can Fam Physician 39:2223–2224.