3

Carcinogenicity

James T. MacGregor, Barbara S. Shane, Judson Spalding, and James Huff

The objective of the carcinogenicity and genotoxicity assay working group was to assess the methodology available for predicting and identifying the human carcinogenicity of chemicals that are subject to review and testing under the Toxic Substances Control Act (TSCA), with emphasis on existing chemicals. Specific objectives were to 1) identify existing assays for assessing or predicting the human carcinogenicity of chemicals, 2) indicate which assays are the most reliable for the prediction of human carcinogenicity, and 3) assess the reliability of low cost predictive assays. This mandate included an assessment of genotoxicity assays in the context of their predictive value for carcinogenicity, but did not include an assessment of other health implications of genotoxicity. The objectives are summarized in table 3-1.

ASSAYS IN HUMANS

Epidemiology

Human studies are extremely valuable because they measure the endpoint of concern directly, i.e., induction of human cancer by exposure to chemicals or environmental agents (80, 99). However, such studies have major limitations that restrict their applicability in the context of the TSCA mandate to protect human health by preventing exposure to those chemicals that pose the greatest potential for inducing cancer under the conditions of their actual use. The most significant limitation is that they cannot be used to identify potential carcinogenic agents before exposure occurs, because human epidemiologic studies can be conducted only after sufficiently large populations have been exposed. They are, however, extremely important for assessing the health impacts of *existing* exposures in human populations.

Disadvantages of epidemiologic studies are their relative insensitivity and the difficulty of proving causality. Epidemiologic studies are always subject to uncontrolled factors that can confound the interpretation. They are also expensive and time consuming. Their power is greatest when combined with results from laboratory data that demonstrate similar effects under more rigorously controlled conditions. Finally, such studies can be conducted only when it is possible to identify a reasonably large defined population with a documented exposure to specific agents, and such populations are often difficult or impossible to identify.

Genetic Biomarkers of Cancer

Major strides have been made in determining the molecular basis of human cancer, and this knowledge may soon lead to a greatly improved ability to monitor the induction of cancer in human populations and individuals. Specific molecular alterations in DNA have been associated with certain human cancers (13), including mutations that activate cellular oncogenes or inactivate tumor suppressor genes. Translocations at specific chromosomal sites are also believed to activate or inactivate key genes in the process of cancer development.

As inexpensive methods for monitoring these molecular changes become available and the

The authors appreciate the comments of Dr. Michael Cimino and Dr. Ann Richard, who contributed to the sections on current TSCA testing requirements (MC) and structure-activity analysis (AR). The contributions of these individuals do not represent an official position of the EPA.

- Assess current assays for predicting and identifying human carcinogenicity of chemicals subject to TSCA review
  - Identify the most reliable assays
  - Evaluate low-cost predictive assays and assess their reliability
- Consider the predictive value of genotoxicity assays for carcinogenicity, but not other health effects related to genotoxicity

exact mechanisms of their role in cancer progression become known, studies of these molecular changes in humans should allow more effective monitoring of exposed human populations. At present, however, such methods are not yet practical for studies of chemically induced cancer in humans. The advantages and limitations of direct studies of carcinogenicity in human populations are summarized in table 3-2.

Biomarkers for Nonspecific Genetic Damage

Because genetic alterations in DNA are believed to be involved in the initiation phase of cancer, monitoring genetic damage in "reporter" genes in humans is a useful method of monitoring exposure to genotoxic agents. Demonstration that exposure to chemicals can induce genetic damage in humans is strong evidence that the exposure may pose a significant carcinogenic risk, even when the monitored genetic loci are not necessarily involved directly in the process of carcinogenesis. For those chemicals known to induce cancer via genetic damage (i.e., for genotoxic carcinogens), monitoring genetic damage in humans provides a means of estimating the carcinogenic risk associated with a particular exposure.

Table 3-3 lists biomarkers that have been used to monitor genetic damage in humans. Details of the available methodologies and summaries of their applications have been reviewed (24, 38, 54, 62). These biomarkers provide direct measures of the types of genetic damage believed to be involved in the induction of carcinogenesis, but they are more useful when the mechanism of action of the chemical under study has been elucidated in laboratory studies. When the mechanism of cancer induction by the chemical under study is known, it is possible to estimate risk quantitatively by using these surrogate biomarkers of damage *in lieu* of direct measurements of cancer incidence.

The most widely used of these biomarkers is cytogenetic damage, or the occurrence of chromosomal aberrations. Micronuclei serve as an alternate screen for chromosomal aberrations or cellular chromosome loss. Several methods now

Table 3-2: Direct Assessment of Human Carcinogenicity

- Provides direct evidence of human carcinogenicity
- Restricted to epidemiologic studies, which have important limitations
  - Difficult to prove causality
  - Insensitive
  - Expensive
  - Time consuming
  - Requires a large defined population with documentable exposure
  - Retrospective (damage already incurred)
  - Not practical for screening
- In the near future, assays for unique damage in oncogenes or tumor suppressor genes involved in cancer development may facilitate human studies of cancer induction

Table 3-3: Human Biomarkers of Exposure and Risk

- Available biomarkers
  - Cytogenetic damage (chromosomal aberrations, micronuclei, aneuploidy)
  - Mutations in surrogate tissues(e.g., *hprt,* glycophorin A, HLA-A, T-cell receptor mutations)
- Mutations in oncogenes or tumor suppressor genes
  - DNA adducts
- Uses and Limitations
  - Useful for monitoring exposures to known genotoxic carcinogens
  - Useful for assessing population risk following exposure to genotoxic agents
  - Indirect relationship to carcinogenesis limits interpretation (esp. for individual)
  - Not practical for assessing risk from uncharacterized agents or tumor promotors

allow direct measurement of mutations in human populations, but an important limitation of the mutation and cytogenetic studies in humans is that damage can be measured in only a few tissues – principally cells that can be obtained by blood sampling. Available assays include the measurement of *hprt*, HLA-A, and T-cell receptor mutations, mutations in lymphocytes, and glycophorin A mutations in erythrocytes. The mechanisms of cancer induction are now beginning to be understood at the molecular level, and in a few cases it is already possible to measure mutations in oncogenes and suppressor genes that are believed to be linked directly to carcinogenesis. Unfortunately, the ability to apply these assays to human populations is extremely limited because, in general, it is not possible to obtain samples of the many different tissues in which cancer may arise.

Sensitive new methodologies have been developed for measuring the interaction of the chemical with DNA to form adducts. The most notable are ^{32}P-postlabeling (75) and immunological methods (50, 51) for specific DNA abducts. In general, these assays are also limited to accessible tissues. They are useful for monitoring exposures when the chemical interacts directly with DNA, and they are useful for estimating the risk associated with exposure to agents that have already been characterized as genotoxic carcinogens. The major limitations of these assays are that 1) they are limited to a few tissues, 2) the endpoints are related only indirectly to cancer (cancer is a multistep process and many defense systems can modify the progression of damage that leads to cancer), and 3) certain of the assays are specific to individual chemicals (e.g., immunoassay).

ANIMAL CARCINOGENICITY BIOASSAY

Assessment of cancer risks to humans can, in practice, be conducted only for a small fraction of the chemicals subject to TSCA regulations. Therefore, these assessments are most often based on data from laboratory animal studies (35). Among the available laboratory tests for assessing carcinogenic potential, the rodent cancer bioassay is generally considered the most reliable predictor of human cancer hazard (37). Virtually all the known human carcinogens are carcinogenic in animals, and those characterized as potent DNA-reactive (genotoxic) carcinogens show excellent interspecies concordance (3, 84). These highly reactive genotoxic carcinogens are generally potent multisite carcinogens, and are generally considered to be the most hazardous class of carcinogens. Approximately one-third of the known human carcinogens were first discovered to be carcinogenic in animals and were later shown to be carcinogenic in humans (36, 98).

One of the major advantages of direct animal cancer bioassays over other shorter term predictive assays is that the animal model is closely related to the human in terms of anatomy, absorption, metabolism, uptake, and pharmacokinetics as well as in the histology of the tumors in various tissues. Thus, it is possible in this

Table 3-4: Advantages and Limitations of the Rodent Carcinogenicity Bioassay

Advantages

- Most reliable method of predicting human carcinogenicity
- Most known human carcinogens are carcinogenic in animals
- Interspecies concordance is good, especially for multisite carcinogens
- Approximately 3070 of human chemical carcinogens first identified in animals
- Can relate effect to exposure for quantitative extrapolation to humans (if dose-response determined)
- Biologic model closely related to human (absorption, metabolism, uptake, pharmacokinetics)

Limitations

- Expensive and lengthy (5 years and >$1.0 M/rodent species)
- Metabolism, pharmacokinetics, tissue defenses, and DNA repair responses may differ quantitatively from human
- Impractical to evaluate low-doses or multiple exposure routes (high-dose data maybe misleading)
- Quantitative extrapolation to humans (and other species) is imprecise
- High-dose bioassay can cause cancer by species - and/or tissue-specific mechanisms not relevant to humans (e.g., -2-globulin nephropathy, saccharin-induced bladder tumors, halogenated organics-induced mouse liver tumors)

model to make quantitative dose response predictions. Extrapolation from rodent cancer bioassay data is the method used by most regulatory agencies, including the EPA, to quantitatively assess human risk. The mathematical model and assumptions involved in such extrapolations have been summarized recently by Fan and Howd (18).

The rodent cancer bioassay also has some important limitations, principally time and expense. A rodent bioassay requires approximately 5 years and costs $500,000-700,000 even with straightforward exposure regimens in a single species. Specialized expertise and laboratory space are also required.

Although the rodent provides a relatively reasonable model for the processes of metabolism, pharmacokinetics, tissue defense, and tumor development, there are often significant quantitative differences in these parameters between the human and the rodent. Metabolic pathways and kinetics often differ between humans and rodents. These important differences may influence the potency of the carcinogen and the shape of the dose response curve (47).

Expense and time restraints make it impractical to. evaluate such important factors as the total shape of the dose response curve (in particular, the low dose portion of the response curve) and limit the range and type of exposures and other experimental variables that can be evaluated. Consequently, the quantitative extrapolations of risk to humans made from bioassays are often imprecise.

Additionally, cancer development in animal models can involve mechanisms that are unlikely to occur in humans. For example, the genotype of the rodent strain can influence the chemical effect, or the high dose regimens used in animal studies can lead to an increased tumor frequency due to enhanced cell proliferation (1, 34, 61, 91, 92, 102). Some well known examples include alpha-globulin nephropathy (unique to male rat kidney) (90), saccharin-induced bladder tumors (due to crystallization in the bladder at very high doses) (14), and the high incidence of liver tumors observed in mouse liver after treatment with certain agents (such as organochlorine compounds) (57, 60). The major advantages and limitations of the rodent cancer bioassay are summarized in table 3-4.

PREDICTIVE METHODS AND MODELS

The rodent assays may provide the most reliable prediction of human carcinogenic hazard, but time and cost factors limit the number of chemicals that can be evaluated in these systems. Therefore, much effort has been devoted to developing low-cost short-term assays that can be

Table 3-5: Predictive Methods and Models

- Chemical structure-activity analysis and mechanism-based inference
- *In vitro* genotoxicity and cell transformation assays
- Short- or mid-term animal models
  - Accelerated tumor development models
    - Strain A mouse
    - TG.AC mouse
    - p53+/- mouse
    - DNA repair-deficient mice
    - Initiated short-term model
  - Biomarkers of preneoplastic tissue growth
    - e.g., -glutamyltranspeptidase-positive preneoplastic foci
- *In vivo* genotoxicity assays
  - Endogenous or transgenic reporter genes for mutation detection
  - Chromosomal aberrations and aneuploidies/micronuclei
  - Unscheduled DNA synthesis (UDS)
  - DNA adducts
  - DNA strand-breaks
  - DNA damage-inducible genes

used to predict carcinogenic potential. Table 3-5 summarizes the major types of predictive methods and provides examples of some important assays.

Structure-Activity Analysis and Mechanism-Based Inference

Structure-based models for predicting chemical carcinogenicity attempt to take advantage of the currently available rodent bioassay carcinogenicity data, which represent hundreds of millions of dollars in testing investment. Carcinogenicity is one of the most complex, yet most widely studied toxicity endpoints from the perspective of structure-activity relationships (SAR) and structure-based mechanism inference. The unifying mechanistic paradigm underlying much of this work is the electrophilic theory of chemical carcinogenesis, which proposes that genotoxic chemical carcinogens form reactive electrophilic intermediates that intercalated, adduct, or otherwise alter or damage DNA (63).

Several models for predicting carcinogenicity based on chemical properties or biochemical indicators have been developed for noncongeneric chemicals, i.e., diverse chemical structures. One published prediction method is based on an experimentally measured, biochemical indicator of electrophilicity, i.e., an electron attachment rate constant (7). COMPACT, a computer-based prediction method, models oxidative P-450 metabolism of a chemical in terms of calculated structural and electronic features as a presumed condition for formation of a reactive electrophile (46). Other computerized prediction programs rely on statistical "discovery" of chemical features significantly associated with carcinogenicity, where model predictions are based on the presence or absence of chemical fragments (CASE) and/or values of calculated molecular properties (TOPKAT, ADAPT) [for reviews, see 22,45, 76].

Ashby (2) has formulated a list of "structural alerts" for use in predicting chemical carcinogenicity, i.e., structural features that are likely to be associated with formation of electrophilic intermediates and whose presence in a molecule provides an alert to potential carcinogenicity. Tennant et al. (95) used such alerts, in conjunction with available short-term test data, subchronic toxicity data, and organ pathology data from the rodent bioassay, as the basis for an "expert intuition" approach to carcinogenicity prediction.

In a recent National Toxicology Program prospective prediction exercise (NIT-44), this approach had an accuracy of >80% in predicting the outcome of the NTP rodent bioassay of 44 previously untested chemicals—an accuracy better than many short-term bioassays (4). This performance was judged significantly better than the 55%-70% accuracy achieved by the computerized SAR models that participated in the exercise, e.g. TOPKAT, CASE and COMPACT (32, 101). However, there were some important distinctions between these approaches: the "expert intuition" approach was neither automated nor easily applied by "non-experts" and required biological test data, whereas the statistical approaches were fully automated and based on information pertaining only to chemical structure.

The special problems and issues surrounding the application of statistical SAR approaches to the modeling of noncongeneric carcinogenicity data bases have been discussed (2, 76). Computerized SAR models such as CASE, TOPKAT, and COMPACT have the advantages of wide applicability, no requirement for availability of the chemical or biological testing, and the potential for generating insight into mechanisms of carcinogenicity. However, due to the dissociation of the results from mechanistic interpretation and the large uncertainties associated with prospective predictions, such methods are not currently in use by EPA for TSCA screening, and the use of such methods in isolation from expert judgment and oversight is not recommended.

On the other hand, SAR and mechanism inference have contributed greatly to understanding of the molecular basis for chemical carcinogenicity when applied to more narrowly defined classes of carcinogens, such as PAHs, nitroaromatics, or certain PCBs. Chemical class, mechanism-based SARs applied with expert judgment are relied on heavily within EPA for carcinogenicity screening of a wide range of new and existing TSCA chemicals (107).

ONCOLOGIC is a recently developed, computerized expert system for carcinogenicity prediction that represents an ambitious attempt to reproduce the cancer prediction expertise of the Structure-Activity Team within EPA. For the chemical classes covered in ONCOLOGIC, early indications are that this method accurately reproduces such expertise.

Hybrid approaches to modeling chemical carcinogenicity data are now being used more extensively in an effort to improve prospective prediction accuracy (see e.g. 6, 12). Inclusion of short-term bioassay data or subchronic toxicity data, when available, provides elements of the complex biological interaction that may be difficult to model by structure alone. In the absence of such data, approaches such as ONCOLOGIC that combine elements of successful SARs, current knowledge of mechanisms, and human judgment appear most promising. The accuracy of such models is expected to improve over time with increased understanding of mechanisms of carcinogenesis and with SAR model refinement resulting from additional data and prospective prediction exercises. See the chapter on SAR/modeling in this volume for a more in-depth discussion of the general requirements and limitations of SAR modeling for use in toxicity prediction.

In Vitro Genotoxicity and Cc// Transformation Assays

In the early 1970s there was great enthusiasm that *in vitro* assays for genetic damage would be an effective and inexpensive means of identifying the carcinogenic potential of chemicals. This confidence was spurred by reports that mutagenicity in *in vitro* assays that incorporated mammalian metabolic enzymes for the activation of metabolically dependent electrophilic carcinogens was an excellent predictor of carcinogenicity *in vivo (59).* Unfortunately, later studies that incorporated larger numbers of chemicals showed that the overall concordance (agreement between the tests in both positive and negative results) between carcinogenicity *in vivo* and mutagenicity *in vitro* was considerably less than that observed in the more limited early studies, i.e., approximately 59%-66% rather than the 90°/0 implied by earlier studies (94, 109). However, the *predictivity* (percentage of agents positive in mutagenicity

assays that are carcinogenic) of positive responses in assays such as the Ames *Salmonella* mutation assay has been found to be quite good, approximately 89°/0. Thus, agents found to be mutagenic in multiple short-term assays are quite likely to prove carcinogenic, but a lack of mutagenicity in *in vitro* assays does not provide strong assurance of noncarcinogenicity (93).

Nonetheless, those carcinogens that are inherently DNA-reactive and are potent multisite carcinogens *in vivo* (those believed to be the greatest carcinogenic hazard) are generally mutagenic in *in vitro* genotoxicity assays. *In vitro* assays are therefore an extremely useful means of identifying potential carcinogens, but there is a substantial risk of misclassification.

In addition, induction of cancer is mechanistically complex, and *in vivo* factors such as metabolism, pharmacokinetics, and tissue specific defenses and proliferation rates often result in marked tissue specificity of carcinogenesis *in vivo. The* complexity of these *in vivo* factors generally makes it impossible to obtain reliable quantitative estimates of the human carcinogenic risk based solely on data from *in vitro* assays (52, 55). These assays therefore are best used as screening assays to provide an initial qualitative assessment of potential carcinogenic hazard.

In vitro mammalian cell transformation assays have undergone extensive study for screening chemicals for potential carcinogenic activity. Among transformation assays, the primary Syrian hamster embryo (SHE) cell assay and the BALB/c 3T3 mouse embryo cell line have been the most extensively used for identifying the potential carcinogenic activity of chemicals.

A change in morphological phenotype is the measured endpoint for chemical activity in both assays, although the transformed phenotype must ultimately be related to the ability of the cells to produce neoplastic growth in suitable recipient animals. The relationship between cellular transformation and genetic alterations is now becoming clear, and suggests an important role for such assays (9, 10, 1 1). Retrospective studies that evaluated the activity of carcinogens and noncarcinogens that had previously been identified in the National Toxicology Program's standard two-year rodent bioassay indicated that positive activity in the transformation assays was most highly correlated with electrophilic and/or mutagenic activity (42, 58).

Although the mammalian cell transformation assays are able to correctly identify nonelectrophilic carcinogens, more work is required to. develop protocols that can correctly discriminate between nonelectrophilic carcinogens and noncarcinogens. The report by Matthews et al. (58) indicated that the BALB/c 3T3 transformation assay was able to discriminate between nonmutagenic *(Salmonella-* negative) carcinogens and noncarcinogens and thus complement the *Salmonella* mutagenicity assay. Similar results were reported for the Syrian hamster embryo assay (23,74). All these results support the conclusion of Swierenga and Yamasaki (91) that cell transformation assays appear to respond to both genotoxic and nongenotoxic carcinogens. The SHE cell transformation system has been used successfully to detect several chemical carcinogens that are not typically identified in short-term assays and often have been considered to be nongenotoxic. These include diethylstilbestrol, 17-estradiol, asbestos, amitrole, arsenic, and reserpine.

However, for the purposes of screening, additional validation studies would be required before these assays could be recommended.

Accelerated Tumor Development Models

The development of tumors in humans and other animal species is the culmination of a multistage process. It is believed that the contributing components of this process are multiple gene mutations in cellular protooncogenes, loss of tumor suppressor gene function, alterations in the regulation of gene expression, and the "time factor", which can be one-half to two-thirds of the human/animal lifespan. The sequence of these events in the development of specific tumors is unknown.

Mutations in the family of cellular ras protooncogenes and mutations or loss of function

in the tumor suppressor p53 gene are associated with a wide range of human tumor types and chemically induced or spontaneous tumors in animal models. The TG.AC transgenic mouse line, which carries inactivated v-Ha-ras gene, has the properties of genetically initiated skin, and skin papillomas are readily induced by promoting agents as well as mutagenic and nonmutagenic carcinogens (43, 88, 89). A mouse line deficient in the p53 gene has been shown to be sensitive to mutagenic carcinogens, and chemically induced tumors are detected as early as 20-26 weeks of treatment (15, 16, 21). Another approach is to "initiate" the animals by pretreating with multisite carcinogens (27). The results from the first phase of a validation study performed in the TG.AC and p53 (+/-) mouse models have been summarized recently (96). These validation studies in the TG.AC transgenic and heterozygous p53 (+/-) knockout mouse lines are being extended by the NTP to determine their ability to discriminate between known rodent carcinogens and noncarcinogens.

The advantage of using transgenic or gene deficient animal (mouse) models to evaluate chemicals for their potential carcinogenic activity is that the presence of an altered gene (oncogene) or absence of a specific tumor suppressor gene can significantly reduce the "time factor" required to observe a tumorigenic effect (from 72-96 weeks to 20-26 weeks). With these models, fewer animals are required per treatment group, and the cost and time required to determine the tumor endpoint are reduced significantly.

Although models of this type have the potential to shorten both the time required and the expense of the traditional rodent cancer bioassay, these models are not sufficiently validated and cannot replace the traditional bioassay at this time.

Biomarkers of Preneoplastic Tissue Growth

A number of biochemical markers are presently being used to monitor neoplastic disease in humans. One example is the prostate specific antigen (PSA). An elevation in the level of this antigen in the blood indicates cell growth that may lead to prostate cancer. As far as is known, this marker is specific for prostate cancer (44, 108). In contrast, three other detectable markers are released from various tumors. Carcinoembryonic antigen (CEA) is elevated in patients with cancer of the breast (82), lung, kidney, pancreas, stomach, and colon (8, 105); CA 125 indicates ovarian, pancreatic, breast, and lung cancer (83), and -fetoprotein (ALF) is indicative of liver and lung cancer (19) and, to a lesser extent, ovarian and testicular cancer. However, CEA can be increased in the serum of patients with benign tumors (79) and varies widely in the human population, thus resulting in a high incidence of false positives.

More recently, attention has focused on the p53 gene, which is involved in restricting cell replication following DNA damage (28, 29, 33, 87). Mutations in p53 alter its normal function and can result in uncontrolled replication of damaged cells. Although, p53 is mutated in 50°/0 of tumors from various tissues, it does not indicate a specific type of cancer (77). This marker may have more relevance in monitoring the prognosis of cancer because mutations in p53 have been correlated with metastatic cancer (48).

In Vivo Genotoxicity Assays

During the last decade, major advances have been achieved in the technology of measuring mutations, chromosomal interchanges, and aneuploidy in tissues *in vivo.* These advances have provided sensitive and rapid methods of measuring the key genetic endpoints associated with neoplastic development in any tissue of interest in laboratory animals. These methods include development and validation of transgenic and endogenous reporter genes that allow direct measurement of mutation in tissues from animals (reviews by 25, 54, 64), improved methodologies for measuring *stable* (balanced) chromosomal aberrations and aneuploides (55, 78, 100), improved methods for surrogate markers of DNA damage such as micronucleus induction (53, 56) or DNA strand breakage (97), and damage-specific inducible responses to DNA damage or intracellular oxidative damage (20, 31, 81).

Table 3-6: Correlation Between Mutagenicity and Carcinogenicity in Tissues of B6C3F1 Mice and F344 Rats

			CARCINOGENICITY		MUTAGENICITY *IN VIVO*		
Agent	**Tissue**	**Sex/Species**	**Dose**	**Care. Activ.[a]**	**Dose**	**Muta. Activ.[a]**	**Refs.**
2-AAF	liver	fem. mouse	60 ppm	2-3	75 ppm	2.7	**49, 72**
1,3-Butadiene	bone marrow	male mouse	625 ppm	C	1250 ppm	4.8	**26,69, 71**
Aflatoxin B1	liver	rat	15 ppb	C	0.25 mg/kg	18.3	**73,** 104
Aflatoxin B1	liver	mouse	1000 ppb	NC or WC	2.5 mg/kg	NM	73
Dimethylnitrosamine	liver	male mouse	4 mg/kg	C	4 mg/kg	10	39,40,65
Methylmethane-sulfonate	liver	male mouse	30 mg/kg	NC	20 mg/kg	NM	65
2,4-Diaminotoluene	liver	male mouse	200 ppm	NC/WC	1000 ppm	2	17, 67
2,6-Diaminotoluene	liver	male mouse	200 ppm	NC	1000 ppm	NM	17,68
Benzene	spleen, lymphoma	male mouse	100 mg/kg	C	750 mg/kg	1.7	30, 70
Benzene	lung	male mouse	100 mg/kg	C	750 mg/kg	1.2 (NM)	30
o-Anisidine	liver	mouse	5000 ppm	NC	750 mg/kg	NM	66, 86
o-Anisidine	bladder	mouse	5000 ppm	C	750 mg/kg	2.1	41, 86

[a]Carcinogenic and mutagenic activity given as increase over control or qualitatively: C = Carcinogenic; NC = Not carcinogenic; WC = Weakly carcinogenic; and NM = Not mutagenic.

The development of labeled hybridization probes that are specific for individual chromosomes or regions of chromosomes has made possible the development of assays that can detect stable chromosomal aberrations and aneuploidy. These same methods have also increased the sophistication of *in vivo* micronucleus assays by allowing determination of whether micronuclei arise from chromosome breakage or loss of whole chromosomes (55, 56.). Although data using these assays are limited, they do suggest that these new *in vivo* assays can predict the carcinogenic activity of chemicals more effectively than has been possible with *in vitro* assays.

One major advance is the development of transgenic animal models with "reporter" genes that allow the measurement of mutations in essentially all tissues of the animal. This advance is of major importance because the systems for measuring mutations *in vivo* were previously limited to one or a few tissues, whereas cancer induction is known to be highly tissue specific. These new transgenic models provide the first opportunity to determine if mutations in specific target tissues are correlated with the development of tumors in those same tissues (a necessary feature if the predictive model is to be used in risk assessment).

The potential of one of these new transgenic mutagenesis assays [the "Big Blue" mouse, with a *lacI* reporter gene; (85)] to predict tumorigenesis is illustrated by the data in table 3-6. This table compares the induction of mutations in a "neutral" reporter gene *(lacI)* with induction of cancer in specific target tissues. Excellent quantitative correlation between the induction of mutations and development of tumors is shown when the comparison is made for specific target tissues,

Table 3-7: In Vitro and In Vivo Genotoxicity Assays

- *In vitro assays*
  - Advantages
    - Useful prescreen for potent genotoxic (DNA-reactive) carcinogens (esp. direct-acting)
    - Rapid
    - Relatively inexpensive
  - Disadvantages
    - Overall predictivity for carcinogenicity of nonelectrophilic agents is poor
    - Predictivity not quantitative
    - Does not model uptake, metabolism, distribution, pharmacokinetics *in vivo*
- *In vivo* genotoxicity **assays**
  - Potentially very useful, but not yet sufficiently evaluated for predictivity of carcinogenesis
  - Tissue specificity of carcinogens limits utility of single-tissue assays (e.g., conventional cytogenetics, micronucleus)
  - General models of initiation/promotion paradigm of carcinogenicity are not established

but only a limited number of agents has been tested to date. The agents included in this table show marked selectivity in tissue site and species sensitivities to carcinogenicity, and similar selectivity is observed for mutation induction in these same target tissues.

In contrast, *in vitro* models fail to predict these quantitative selectivities. For example, MMS and DMN both methylate liver DNA to a similar extent at the doses used, yet only the hepatocarcinogen (DMN) induces mutations in liver at carcinogenic doses. This difference is attributed to a differential spectrum of methylated adducts and a markedly higher stimulation of cellular proliferation by DMN, which facilitates fixation of mutations (65). Currently used *in vitro* testing schemes do not adequately predict these types of *in vivo* differences. Thus, these *in vivo* transgenic assays have a strong potential to provide low-cost predictivity of carcinogenicity by genotoxic chemicals. Evaluation of the predictive value of these assays, using a wide range of classes of chemical carcinogens and noncarcinogens, should be given a high priority by funding agencies.

In addition to the above methods already established *in vivo,* it is now known that there are many different mechanisms of repair and control of DNA damage, and that many of the genes that control these responses are inducible. Simple assays to assess many of these responses *in vitro are* already available, and *in vivo* methodologies are being developed. When available, these methods will provide additional indicators of genotoxic damage *in vivo* and will expand our understanding of the nature of genetic damage and repair by carcinogens (56).

Table 3-7 summarizes the advantages and limitations of currently available assays for genotoxic damage *in vitro* and *in vivo.*

In summary, the advantages of *in vitro* assays are their low cost and speed of performance; they have proved useful as screening assays for ranking hazards and are particularly effective at identifying potent DNA-reactive (genotoxic) carcinogens, especially those not requiring metabolic activation. The major disadvantage is that the *in vitro* systems do not model uptake, metabolism, distribution, and pharmacokinetics *in vivo, so* that it is not possible to make quantitative predictions based on them.

The *in vivo* genotoxicity assays are potentially very useful, but these assays have not yet been evaluated systematically to determine their overall predictability for a wide range of chemical classes. Our working group recommends that such a systematic evaluation be undertaken. Assays that are not restricted to specific tissues are expected to be the most valuable, because those that are restricted to specific tissues, such as

the micronucleus assay and conventional cytogenetic analysis, cannot be expected to serve as a surrogate for all the tissues in which cancer can occur.

A major need is the development of models that predict the potential to induce cancer via nongenotoxic mechanisms. As specific genes involved in the carcinogenic process continue to be elucidated, models for evaluating the factors that modify progression of initiated cells into metastatic tumors will be developed. One example of such a model that has already proved to be useful is the TG.AC mouse model discussed above.

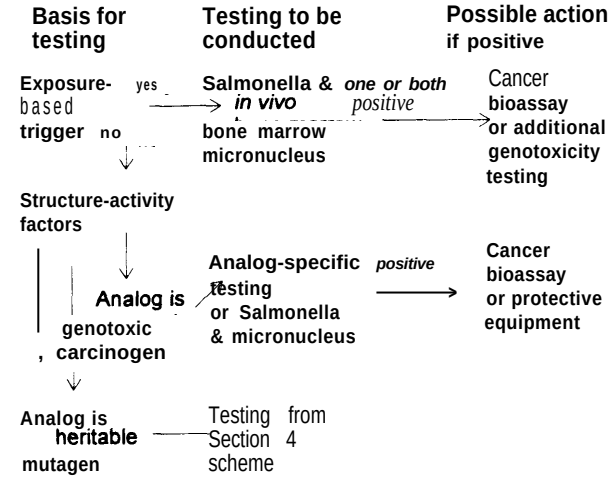
CURRENT TSCA GENOTOXICITY AND CARCINOGENICITY TESTING REQUIREMENTS

The current genotoxicity and carcinogenicity testing schemes for chemicals subject to regulation under TSCA are summarized in figure 3-1 (for new chemicals) and figure 3-2 (for existing chemicals). In each case, the weight of available evidence is considered by the EPA; the arrows in figures 3-1 and 3-2 therefore indicate the steps that are considered under the circumstances indicated rather than a mandate to perform the indicated assays. These testing schemes have been discussed by Auletta et al. (5).

New chemicals that meet specified volume and exposure criteria require testing in two short-term genotoxicity assays (in addition to short-term toxicity and ecological effects testing). The two genotoxicity tests are the Ames *Salmonella* mutagenicity assay and an *in vivo* bone marrow micronucleus test (see figure 3-1). If positive response(s) are obtained, additional genotoxicity testing and/or a cancer bioassay may be required. If both are negative, then a cancer bioassay is unlikely to be required by EPA unless strong evidence (such as chemical structural alerts) suggest carcinogenic potential.

New chemicals may require testing under two other conditions, both depending on structure-activity considerations. If there is a weight-of-evidence argument that the chemical may be a

Figure 3-1: EPA's Mutagenicity Test Scheme for New Chemicals under TSCA

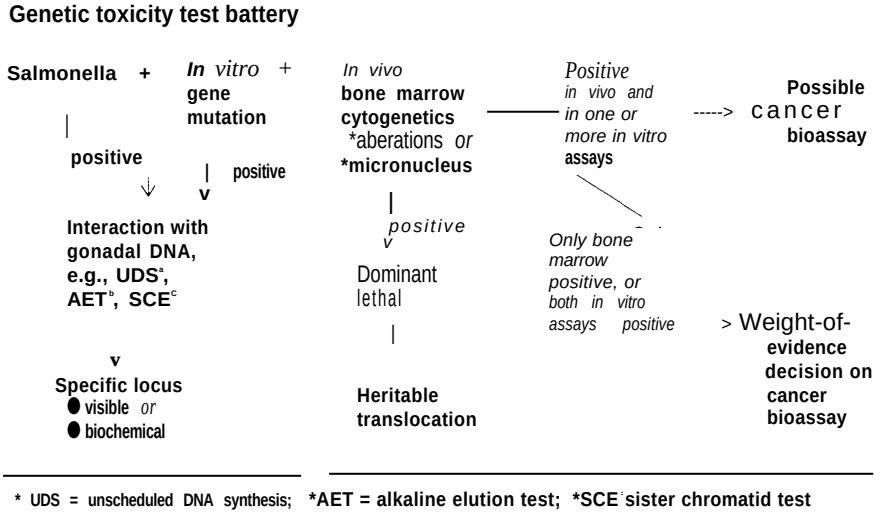


potential carcinogen, genotoxicity testing may be required in assay systems for which positive genotoxicity data on analogs exist. Positive responses for the new chemical generally require a cancer bioassay and/or use of protective equipment to limit exposure.

The third situation for testing of new chemicals involves agents for which data on analogs indicate the potential for heritable mutagenicity, but there is minimal concern for potential carcinogenicity. Such chemicals may require testing similar to that shown in the three-tier scheme for existing chemicals (see discussion below and figure 3-2).

For existing chemicals, the priorities for testing are set by the Interagency Testing Committee. If genotoxicity testing is deemed appropriate, a battery of three short-term genotoxicity tests are required: the Ames *Salmonella* mutagenicity assay, an assay for mutagenicity in mammalian cells *in vitro,* and an *in vivo* bone marrow cytogenetics assay (either a micronucleus or a chromosomal aberration test; see the first row of figure 3-2). If the *in vivo* assay and a minimum of one of the *in vitro* assays are positive, then a rodent cancer bioassay may be required. If both *in vitro* assays or any one assay is positive, then all available data, including test results from other toxicity endpoints, structure-activity relation

Figure 3-2: EPA's Mutagenicity Test Scheme for Existing Chemicals under TSCA



ships, production volume and/or exposure, are reviewed to determine if a cancer bioassay is warranted. If all assays are negative, then a cancer bioassay is unlikely to be required by EPA unless other available data suggest a cause for concern. Positive results in the first tier of mutagenicity testing may trigger additional genotoxicity testing in the second and third tiers for potential heritable mutagenicity (see second and third rows of figure *3-2).*

In view of current knowledge, these testing schemes are considered by the Working Group to be reasonable requirements. They allow reasonable protection against exposure to carcinogens, given the cost constraints of evaluating the many thousands of chemicals subject to TSCA regulations. However, as the test methodologies described above become better validated as predictors of carcinogenic potential during the next few years, strong consideration should be given to including them in the decision tree for carcinogenicity evaluation.

CONCLUSIONS

The principal conclusions of the Working Group are as follows:

- The current OPPT testing scheme is reasonable in view of currently available technology.
- Human epidemiological studies provide the only available direct measure of human carcinogenicity. They provide information that is extremely valuable, but are relatively insensitive, expensive, lengthy, and usually retrospective.
- The chronic rodent carcinogenesis bioassay appears to be the best available assay for predicting human carcinogenicity, but it is expensive and lengthy and therefore practical only for agents with widespread high exposure potential.
- SAR methods provide a highly cost-effective approach to identifying agents with carcinogenic potential, especially if the agents are related to structural analogs with known carcinogenic activity.
- In vitro assays are useful for identifying DNA-reactive carcinogens, especially those that are direct-acting, at relatively low cost. The overall concordance between carcinogenicity assays and in vitro geno-

toxicity is relatively poor and not quantitative.

- In vivo mutagenesis assays applicable to multiple tissues are relatively new and data are still limited. They are potentially very useful but require "validation" as predictors of carcinogenesis. Existing data suggest that predictivity for DNA-reactive carcinogens may be very good.
- Rapid tumor development models are potentially useful. Current data suggest the utility of this type of assay for specific types of carcinogen (e.g., skin cancer in TG.AC mouse). Such assays require further development and "validation" with a variety of classes of carcinogens and noncarcinogens.

The following technologies have a high potential for more cost-effective prediction of carcinogenic potential of chemicals in the near future:

- Reporter genes for mutagenicity in vivo (e.g., Big Blue, MutaMouse, MutaMetrix Mouse).
- Models with rapid tumor development (such as defense knockout [p53, DNA repair], activated oncogenes [TG.AC, pim mouse], etc.).
- Animals with specific oncogene targets.
- Reporters of cell-system- or damage-specific response.
- Markers of cell proliferation and/or tumor growth (CDKs, tumor markers, PCNA).
- Probes that facilitate chromosomal aberration or aneuploidy screening in vivo.
- Transgenic animals with human-like metabolic capacity.
- Improved structure-activity predictions based on improved modeling and more reliable databases.

REFERENCES

1. Ames, B.N. and Gold, L. S., "Too Many Carcinogens: Mitogenesis Increases Mutagenesis," *Science* 249:970-971, *1990.*
2. Ashby, J., "Two Million Rodent Carcinogens? The Role of SAR and QSAR in their Detection," *Mutat. Res.* 305:3-12, *1994.*
3. Ashby, J. and Tennant, R. W., "Definitive Relationships Among Chemical Structure, Carcinogenicity and Mutagenicity for 301 Chemicals Tested by the US NTP," *Mut. Res.,* 257:220-306, 1991.
4. Ashby, J. and Tennant, R. W., "Prediction of Rodent Carcinogenicity for 44 Chemicals: Results," *Mutagenesis* 9:7-15, *1994.*
5. Auletta, A. E., Dearfield, K.L., and Cimino, M. C., "Mutagenicity Test Schemes and Guidelines: U.S. EPA, Office of Pollution Prevention and Toxics and Office of Pesticide Programs," *Environ. Mol. Mutagen.* 21:38-45, *1993.*
6. Bahler, D., and Bristol, D. W., The Induction of Rules for Predicting Chemical Carcinogenesis in Rodents," *Intelligent Systems for Molecular Biology,* L Hunter, J Shavlik, and D Searls (eds.), (Menlo Park, CA: AAAI/MIT Press, 1993).
7. Bakale, G. and McCreary, R. D., "A Physico-Chemical Screening Test for Chemical Carcinogens: the k_e Test," *Carcinogenesis* 8:253-264, *1987.*
8. Ballesta, A. M., et al., "Carcinoembryonic Antigen in Staging and Follow-up of Patients with Solid Tumors," *Tumor Biol.* 16:32-41, *1995.*
9. Barrett, J. C., "Relationship Between Mutagenesis and Carcinogenesis," *Mechanisms of Environmental Carcinogenesis: Role of Genetic and Epigenetic Changes, 1:129-142,* J. Barrett (cd.), (Boco Raton, FL: CRC Press, 1987).
10. Barrett, J. C., Tsutsui, T., and Ts'o P., "Neoplastic Transformation Induced by a Direct Perturbation of DNA," *Nature 274: 229-232, 1987.*
11. Barrett, J.C. and Wiseman, R. W., "Molecular Carcinogenesis in Humans and Rodents," *Prog. Clin. Biol. Res.* 376:1-30, *1992.*
12. Benigni, R., et al., "Electrophilicity as Measured by Ke: Molecular Determinants, Relationship with Other Physical-Chemical and Quantum Mechanical Parameters, and Ability to Predict Rodent Carcinogenicity," *Carcinogenesis* 13:547-553, 1992.

13. Brugge, J., et al. *Origins of Human Cancer: A Comprehensive Review,* (Plainview, NY: Cold Spring Harbor Laboratory Press, 1991).
14. Cohen, S.M., and Ellwein, L.B. "Cell Proliferation in Carcinogenesis," *Science 249: 1007-1011, 1990.*
15. Donehower, L.A., *FASEB J., 5: 225-229, 1993.*
16. Donehower, L. A., et al., "Mice Deficient for P53 are Developmentally Normal but Susceptible To Spontaneous Tumors," *Nature* 356:215-221, *1992.*
17. Dycaico, M.J., Rogers, B.J., and Provost, G. S., "The Species Specific Difference of Mutation Sensitivity of Transgenic lambda/laci Rats," *Environ. Molec. Mutagen. 25,* Suppl. 25:13, 1995.
18. Fan, A. and Howd, R., "Risk Assessment of Environmental Chemicals," *Ann. Rev. Pharmacol. Toxicol.* 35:341-368, *1995.*
19. Ferrigno, D., Buccheri, G. and Biggi, A., "Serum Tumor Markers in Lung Cancer: History, Biology and Clinical Applications," *Eur. Respir. J.* 7:186-197, *1994.*
20. Fornace, A. J., et al., "Genotoxic-Stress-Response Genes and Growth-Arrest Genes," *Ann. NY Acad. Sci.,* 663:139-53, *1992.*
21. French, J. E., et al., "Short Term Carcinogenesis and Mutagenesis Studies with p53 Deficient (+/-) and/or F1 lambda Liz a1pha:p53 Deficient (+/–) Mice," *Environ. Molec. Mutagen. 25,* Suppl. 25:16, 1995.
22. Frierson, M. R., Klopman, G., and Rosenkranz, H. S., "Structure-Activity Relationships SARs among Mutagens and Carcinogens: A Review," *Environ. Mutagenesis* 8:283-327, *1986.*
23. Gibson, D. P., et al., "Detection of Aneuploidy-Inducing Carcinogens in the Syrian Hamster Embryo (SHE) Cell Transformation Assay," *Mutat. Res.* 343:7-24, 1995.
24. Gledhill, B. L., and Mauro, F. New *Horizons in Biological Dosimetry, (New* York, NY: Wiley-Liss, 1991).
25. Gorelick, N.J., "Overview of Mutation Assays in Transgenic Mice for Routine Testing," *Environ. Molec. Mutagen.* 25:218-230, 1995.
26. Gunz, D., Shephard, S. E., and Lutz, W. K., "Can Nongenotoxic Carcinogens be Detected with the lacI Transgenic Mouse Mutation Assay?," *Environ. Molec. Mutagen.* 21:209-211, *1993.*
27. Hagiwara A., et al., "Correlation Between Medium-term Multi-organ Carcinogenesis Bioassay Data and Long-term Observation Results in Rats," *Japan J. Cancer Res.* 84(3):237-245, *1993.*
28. Harris, C., "Chemical and Physical Carcinogenesis: Advances and Perspectives," *Cancer Res.* 51:5023s-5044s, *1991.*
29. Harris, C.C. and Hollstein, M., "Clinical Implications of the p53 Tumor-suppressor Gene," N. *Engl. J. Med.* 329:1318-1327, *1993.*
30. Hayward, J.J., et al., "Differential in vivo Mutagenicity of the Carcinogen-Noncarcinogen Pair 2,4-and 2,6-Diaminotoluene," *Carcinogenesis* (in press, 1995).
31. Herrlich, P., Angel, P., and Rahmsdorf, J. H., "The Mammalian Genetic Stress Response," *Adv. Enzyme Regul.* 25:485-504, *1986.*
32. Hileman, B. "'Expert Intuition' Tops in Test of Carcinogenicity Prediction," *Chem. & Engin. News 71(25):35-38, 1993.*
33. Hollstein, M., et al., "p53 Mutations in Human Cancers," *Science* 253:49-53, 1991.
34. Huff, J. E., "Chemical Toxicity & Chemical Carcinogenesis. Is There a Causal Connection? A Comparative Morphological Evaluation of 1500 Experiments," *Mechanisms of Carcinogenesis in Risk Identification,* 116:437-475, H. Vainio, et al., (eds) (Lyon, France: IARC Sci. Pub., 1992).
35. Huff, J. E., "Chemicals and Cancer in Humans: First Evidence in Experimental Animals," *Environ. Health Perspect.* 100:201-210, 1993a.
36. Huff, J.E. "Issues and Controversies Surrounding Qualitative Strategies for Identifying and Forecasting Cancer Causing Agents in the Human Environment," *Pharmacol. Toxicol.* 72(1):12-27, 1993b.
37. Huff, J. E., Haseman, J.K. and Rail, D.P. "Scientific Concepts, Value, and Significance

of Chemical Carcinogenesis Studies," *Ann. Rev. Pharmacol. Toxicol.* 31:621-652, *1991.*

38. Hulka, B. S., Wilcosky, T. C., and Griffith, J.D., *Biological Markers in Epidemiology,* (Oxford, U.K.: University Press, 1990).

39. International Agency for Research on Cancer, *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 7, Some Anti-thyroid and Related Substances, Nitrofurans, and Industrial Chemicals,* (Lyon, France: IARC Sci. Pub., 1974).

40. International Agency for Research on Cancer, *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 17, Some N-nitroso Compounds,* (Lyon, France: IARC Sci. Pub., 1978).

41. International Agency for Research on Cancer, *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Supplement 7. Overall Evaluation of Carcinogenicity: An Updating of IARC Monographs. Volumes 1-42,* (Lyon, France: IARC Sci. Pub., 1987).

42. Jones, C. A., et al., "An Interlaboratory Evaluation of the Syrian Hamster Embryo Transformation Assay Using Eighteen Coded Chemicals," *Toxicology In Vitro* 2:103-116, *1988.*

43. Leder, A., et al., "v-Ha-ras Transgene Abrogates the Initiation Step in Mouse Skin Tumorigenesis: Effects of Phorbol Esters and Retinoic Acid," *Proct. Natl. Acad. Sci. USA* 87:9178-9182, *1990.*

44. Lee, W. R., Giantonio, B., and Hanks, G. E., "Prostate Cancer," *Curr. Probl. Cancer* 18:295-357, *1994.*

45. Lewis, D. F. V., "Computer-assisted Methods in the Evaluation of Chemical Toxicity," *Reviews in Computational Chemistry,* K.B. Lipkowitz and D.B. Boyd (eds.), (New York, NY: VCH Publishers, Inc., 1992).

46. Lewis, D. F. V., Ioannides, C., and Parke, D. "A Retrospective Evaluation of COMPACT Predictions of the Outcome of NTP Rodent Carcinogenicity Testing," *Environ. Health Perspect.* 103:178-84, *1995.*

47. Lijinsky, W., "Species Differences in Carcinogenesis, " *in vivo* 7:65-72, *1993.*

48. Lipponen, P, et al., "p53 Protein Expression in Breast Cancer as Related to Histopathological Characteristics and Prognosis," *Int. J. Cancer 55:51-56, 1993.*

49. Littlefield, N.A., et al., "Effects of Dose and Time in a Long-term Low-dose Carcinogenicity Study," *J. Environ. Pathol. Toxicol.* 3:17-34, *1979.*

50. Lehman, P.H.M., Laauwerys, R., and Sorsa, M. "Methods of Monitoring Human Exposure to Carcinogenic and Mutagenic Agents," *Monitoring Human Exposure to Carcinogenic and Mutagenic Agents, 59: 423-427,* A. Berlin, et al. (eds.) (Lyon, France: IARC Sci. Pub., 1984).

51. Lehman, P. H. M., et al., *Molecular Dosimetry of Genotoxic Damage: Biochemical and Immunochemical Methods to Detect DNA-damage in vitro and in vivo. TIPS-FEST Supplement, (New* York, NY: Elsevier, 1985).

52. MacGregor, J. T., "Environmental Mutagenesis: Past and Future Directions," *Environ Molec Mutagen,* 23(suppl.24):73-77, *1994.*

53. MacGregor, J. T., et al., "The *in vivo* Erythrocyte Micronucleus Test: Measurement at Steady State Increases Assay Efficiency and Permits Integration with Toxicity Studies," *Fund. Appl. Toxicol.* 14:513-522, *1990.*

54. MacGregor, J. T., et al., "Monitoring Environmental Genotoxicants," *Methods for Genetic Risk Assessment,* D. Brusick, (cd.) (Boca Raton, FL: Lewis Publishers, 1994).

55. MacGregor, J. T., et al., "Integration of Cytogenetic Assays with Toxicology Studies," *Environ Molec Mutagen.* 25:328-337, 1995a.

56. MacGregor, J. T., et al., "New Molecular Endpoints and Methods for Routine Toxicity Testing," *Fund. Appl. Toxicol.* 26:156-173, 1995b.

57. Maronpot, R.R, et al., "Liver Lesions in B6C3F1 Mice: The National Toxicology Program Experience and Position," *Arch. Toxicol. Supl.* 10:10-26, *1987.*

58. Matthews, E.J., Spalding, J. W., and Tennant, R. W., "Transformation of Balb/c-3T3 cells:

V. Transformation Responses of 168 Chemicals Compared with Mutagenicity in *Salmonella* and Carcinogenicity in Rodent Bioassay," *Environ Health Perspect Suppl.* 101(2):347-482, *1993.*

59. McCann, J., et al., "Detection of Carcinogens as Mutagens in the *Salmonella/Microsome* Test: Assay of 300 Chemicals," *Proc. Natl. Acad. Sci. USA* 72:5135-5139, *1975.*

60. Melnick, R.L., and Huff, J.E., "Liver Carcinogenesis is not a Predicted Outcome of Chemically Induced Hepatocyte Proliferation," *Toxicol. Indust. Health* 9:415-438, *1993.*

61. Melnick, R. L., et al., "Cell Proliferation and Chemical Carcinogenesis: a Symposium Overview," *Mol. Carcinog.* 7:135-138, *1993.*

62. Mendelssohn, M. L., Peeters, J. P., and Normandy, M.J., *Biomarkers and Occupational Health,* (Washington, DC: Joseph Henry Press, 1995).

63. Miller, J.A. and Miller, E. C., "Ultimate Chemical Carcinogens as Reactive Mutagenic Electrophiles," *Origins of Human Cancer,* H.H. Hiatt, H.D. Watson and J.A. Winsten (eds), (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1977).

64. Mirsalis, J.C., Monforte, J.A., and Winegar, R. A., "Transgenic Animal Models for Measuring Mutations *in vivo," Crit Rev Toxicol 24: 255-280, 1994.*

65. Mirsalis, J. C., et al., "Induction of Hepatic Mutations in lacI Transgenic Mice," *Mutagenesis* 8:265-271, 1993.

66. National Cancer Institute (NCI), *Bioassay of O-Anisidine Hydrochloride Dihydrochloride for Possible Carcinogenicity (CAS No. 134-29-0), NCI* Tr. No. 89, (Bethesda, MD: National Cancer Institute, 1978).

67. National Cancer Institute (NCI), *Bioassay of 2,4-Diaminotoluene Dihydrochloride for Possible Carcinogenicity (CAS No. 95-80-7), NCI* Tr. No. 162. (Bethesda, MD: National Cancer Institute, 1980a).

68. National Cancer Institute (NCI), *Bioassay of 2, 6- Toluenediamine Dihydrochloride for Possible Carcinogenicity (CAS No. 15481-70-6).* NCI Tr. No. 200, (Bethesda, MD: National Cancer Institute, 1980b).

69. National Toxicology Program (NTP), *Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No. 106-99-0) in B6C3F1 Mice (Inhalation Studies),* NTP Tr. No. 288, (Research Triangle Park, NC: National Toxicology Program, 1984).

70. National Toxicology Program (NTP), *Toxicology and Carcinogenesis Studies of Benzene (CAS No. 71-43-2) in F344/n Rats and B6C3F1 Mice (Gavage Studies),* NTP Tr. No. 289, (Research Triangle Park, NC: National Toxicology Program, 1986).

71. National Toxicology Program (NTP), *Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No. 106-99-0) in B6C3F1 Mice (Inhalation Studies),* NTP Tr. No. 434, (Research Triangle Park, NC: National Toxicology Program, 1993).

72. Provost, G. S., et al., "Evaluation of Mutagenic and Non-mutagenic Compounds Using lacI Transgenic Rodents," *Environ Molec. Mutagen. 23(* Suppl. *23): 55, 1994.*

73. Provost, G. S., et al., "Validation Studies of the lambda/lacI Transgenic Mouse Assay," *The Toxicologist* 15:174, 1995.

74. Przygoda, R.T., McKee, R.H. and Traul, K.A., "The Use of Short Term Assays in the Evaluation of the Dermal Carcinogenic Potential of Petroleum-Derived Materials," *Environ. Molec. Mutagen.* 19:51, 1992.

75. Randerath, K, et al., "Postlabeling Methods for Carcinogen-DNA Adduct Analysis," *Environ. Health Perspect.* 62:57-65, 1985.

76. Richard, A. M., "Application of SAR Methods to Non-congeneric Data Bases Associated with Carcinogenicity and Mutagenicity: Issues and Approaches," *Mutation Research* 305:73-97, *1994.*

77. Riou, G., et al., "The p53 and mdm Genes in Human Testicular Germ-cell Tumors," *Molec. Carcinog.* 12:124-131, *1995.*

78. Robbins, W. A., et al., "Detection of Aneuploid Sperm by Fluorescence *in situ* Hybridization: Evidence for a Donor Difference in Frequency of Sperm Disomic

for Chromosomes 1 and Y," *Am J Human Genet* 52:799-807, *1993.*

79. Ruiball-Morell, A., "CEA Levels in Non-neoplastic Disease," *Int J Biol Markers* 7: *160-166, 1992.*

80. Sankaranarayanan, R., Wahrendorf, J., and Demaret, E., *Directory of On-Going Research in Epidemiology 1994,* IARC Sci. Pub. No. 130 (Lyon, France: IARC Sci. Pub., 1994).

81. Sarasin, A., "SOS Response in Mammalian Cells," *Cancer Invest.* 3:163-174, *1985.*

82. Schmitt, F. C., and Andrade, L., "Spectrum of Carcinoembryonic Antigen Immunoreactivity from Isolated Ductal Hyperplasias to Atypical Hyperplasia Associated with Infiltrating Ductal Breast Cancer," *J Clin Pathol 48:53-56, 1995.*

83. Shabana, A., and Onsrud, M., "Tissue Polypepetide-specific Antigen and CA 125 as Serum Tumor Markers in Ovarian Carcinoma," *Tumor Biol* 15:361-367, *1994.*

84. Shelby, M.D., "The Genetic Toxicity of Human Carcinogens and its Implications," *Mutat. Res.* 204:3-15, *1988.*

85. Short, J. M., et al., "The Use of lambda Phase Shuttle Vectors in Transgenic Mice for Development of a Short Term Mutagenicity," *Mutation and the Environment, Part A, New* York, NY: Wiley-Liss, 1990).

86. Sisk, S. C., et al., "Molecular Analysis of lacI Mutants from Bone Marrow of B6C3F1 Transgenic Mice Following Inhalation Exposure to l,3-Butadiene," *Carcinogenesis* 15:471-477, *1994.*

87. Smith, M. L., et al., "Involvement of the p53 Tumor Suppresor in Repair of U.V. Type DNA Damage," *Oncogene* 10:1053-1059, 1995.

88. Spalding, J. W., et al., "Chemically-induced Skin Carcinogenesis in a Transgenic Mouse Line (TG.AC) Carrying a v-Ha-ras Gene," *Carcinogenesis* 14:1335-1341, *1993.*

89. Spalding, J. W., et al., "The TG.AC Transgenic Mouse Line: An Important *in vivo* Short Term Test Model for Identifying Nongenotoxic Carcinogens," *Environ. Molec. Mutagen. 25,* Suppl. 25:50, 1995.

90. Swenberg, J.A. "Alpha 2 u-globulin Nephropathy: Review of the Cellular and Molecular Mechanisms Involved and their Implications for Human Risk Assessment," *Environ. Health Perspect.* 101, Suppl. 6:39-44, 1993.

91. Swierenga, S. H. H., and Yamasaki, H., "Performance of Tests for Cell Transformation and Gap-junction Intercellular Communication for Detecting Nongenotoxic Carcinogenic Activity," *Mechanisms of Carcinogenesis in Risk Identification,* H. Vaino, et al. (eds.) (Lyon, France: IARC Sci. Pub., 1992).

92. Tennant, R. W., "Stratification of Rodent Carcinogenicity Bioassay Results to Reflect Relative Human Hazard," *Mutat. Res.* 286:111-118, 1993.

93. Tennant, R. W., and Zeiger, E., "Genetic Toxicology: The Current Status of Methods of Carcinogen Identification," *Environ. Health Perspect.* 100:307-315, *1993.*

94. Tennant, R. W., et al., "Prediction of Chemical Carcinogenicity in Rodents from *in vitro* Genetic Toxicity Assays," *Science 236:933-941,* 1987.

95. Tennant, R. W., et al., "Prediction of the Outcome of Rodent Carcinogenicity Bioassays Currently Being Conducted on 44 Chemicals by the National Toxicology Program," *Mutagenesis* 5:3-14, *1990.*

96. Tennant, R. W., French, J. E., and Spalding, J. W., "Identification of Chemical Carcinogens and Assessing Potential Risk in Short Term Bioassays using Transgenic Mouse Models," *Environ. Health Perspect. 103(10)* (in press), 1995.

97. Tice, R.R., et al., "The Single Cell Gel (SCG) Assay: an Electrophoretic Technique for the Detection of DNA Damage in Individual Cells," *Biological Reactive Intermediates. IV. Molecular and Cellular Effects and Their Impact on Human Health,* Witmer, C. R., et al., (eds.) (New York, NY: Plenum, 1991).

98. Tomatis, L., "The Predictive Value of Rodent Carcinogenicity Tests in the Evaluation of Human Risks," *Ann. Rev. Pharmacol. Toxicol. 19:51 1-530, 1979.*

99. Tomatis, L., *Cancer: Causes, Occurrence and Control.* IARC Sci. Pub. No. 100, (Lyon, France: IARC Sci. Pub., 1990).

100. Tucker, J., et al., "Validation of Chromosome Painting as a Biodosimeter in Human Peripheral Blood Lymphocytes Following Acute Exposure to Ionizing Radiation *in vitro," Int. J. Radiat. Biol.* 64:27-37, *1993.*

101. Wachsman, J.T., et al., "Predicting Chemical Carcinogenesis in Rodents," *Environ. Health Perspec.* 101:444-445, *1993.*

102. Weinstein, I.B. "Mitogenesis is Only One Factor in Carcinogenesis," *Science 251:387-388, 1991.*

103. Weinstein, I.B., "Toxicity, Cell Proliferation, and Carcinogenesis. *Mol. Carcinog.* 5:2-3, *1992.*

104. Wogan, G.N., and Newberne, P.M. "Dose Response Characteristics of Aflatoxin B, Carcinogenesis in the Rat," *Cancer Res.* 27:2370-2376, *1967.*

105. Wolmark, N., et al., "The Prognostic Significance of Preoperative Carcinoembryonic Antigen Levels in Colorectal Cells. Results from NSABP (National Surgery Adjuvant Breast and Bowel Project) Clinical Trials," *Annals Surgery* 199:375-382, 1984.

106. Woo, Y-T., et al., *Chemical Induction of Cancer: Structural Bases and Biological Mechanisms,* Vols. 11, III, (New York, NY: Academic Press, Inc., 1985).

107. Woo, Y-T., et al. "Development of Structure Activity Relationship Rules for Predicting Carcinogenic Potential of Chemicals. Proceedings of ATSDR Sponsored Workshop on Screening Technologies," *Toxicology Letters 78:* (in press), 1995.

108. Zagars, G. K., et al., "The Source of Pretreatment Serum Prostate Specific Antigen in Clinically Localized Prostate Cancer," *Int. J. Radiat. Oncol. Biol. Phys.* 32:21-32, 1995.

109. Zeiger, E., et al., "Evaluation of Four *in vitro* Genetic Toxicology Tests for Predicting Rodent Carcinogenicity: Confirmation of Earlier Results with 41 Additional Chemicals," *Environ. Molec. Mutagen. 16,* Suppl. 18:1-14, 1990.