

# Critical Effective Methods to Detect Genotoxic Carcinogens and Neoplasm-Promoting Agents

by John H. Weisburger\* and Gary M. Williams\*

Neoplasia in fish can result from contamination of waters with carcinogens and promoters. Cancer in fish, therefore, is a possible indicator of cancer risk to man and serves as a guide to the need for preventative approaches involving improved means of waste disposal and environmental hygiene. Moreover, cancer in fish indicates that this important food source may be contaminated. Detection of genotoxic carcinogens to which fish are exposed can be achieved quickly and efficiently by carefully selected batteries of complementary *in vitro* and *in vivo* bioassays. One such battery consists of the Ames test, a reverse mutation assay in prokaryotic *Salmonella typhimurium*, and the Williams test, involving DNA repair in freshly explanted metabolically highly competent liver cells from diverse species, including humans. Determination of DNA-carcinogen adducts by varied techniques, including <sup>32</sup>P- postlabeling, as well as DNA breakage, mammalian cell mutagenicity, chromosome aberrations, sister chromatid exchange, or cell transformation represent additional approaches, each with its own advantages and disadvantages. More research is needed on systems to apprehend neoplasm promoters, but tests to determine interruption of intercellular communications through gap junctions appear promising. Other approaches rely on measurement of enzymes such as ornithine decarboxylase and protein kinase C. Approaches to the definition of risk to fish or humans require characterization of the genotoxic or nongenotoxic properties of a chemical, relative potency data obtained in select, limited rodent bioassays, and knowledge of prevailing environmental concentrations of specific carcinogens.

## Introduction

Specific types of cancer are the major premature killing diseases in many parts of the world (1-5). In the Western World, the high incidence of cancer of the lung, pancreas, kidney, and bladder can be mainly attributed to cigarette smoking. Cancer of the oral cavity and esophagus are associated with tobacco chewing in the Western World and in India where the traditional chewing of a mixture of tobacco and betel nut has led to oral cavity cancer as the major neoplastic disease. In China and Japan, however, the customary intake of salted and pickled food, particularly fish, leads to risk for cancer of the esophagus and stomach, and in part, of the liver. In many parts of Africa, liver cancer is a major problem, with food mycotoxins and the hepatitis B antigen as causative agents. In the Western World, the customary intake of appreciable amounts of fat has been associated with cancers of the breast, colon, ovary, endometrium, and pancreas. Thus, in many parts of the world, changes in lifestyle to avoid defined cancer risks have been recommended.

Historically, however, cancer in man was first documented to be due to an environmental cause through the study of cancer related to specific occupations, such as the scrotal cancers observed by Pott or the bladder cancers recorded by Rehn (3).

With increasing industrialization, especially with the growth of the chemical industry, questions arose as to whether the limited occupational cancers seen by Rehn occurred at an increasing rate due to contamination. Indeed, careless handling of chemicals, with consequent contamination of water with toxic agents, has led to serious adverse effects in sizable numbers of people, such as in the case of Minamata, Japan (6), or more recently, the accidental mixing of polybrominated biphenyls with animal food in Michigan that led to the extensive occurrence of this toxicant in milk and in food reaching humans (7). Incidents of deliberate addition of toxic agents to comestible oils in Spain and Turkey are other examples of undesirable and indeed criminal contamination of the human environment with toxic agents (8). In the U.S., the question of human neoplasia stemming from water has been considered (9-12).

Any toxic effect is the outcome of the occurrence in the human environment of agents at dosages and chronicity of exposure sufficient to lead to the syndromes observed. Concentrations most likely are highest directly at the site of production or use, as for example, in the case of polybrominated biphenyls (PBBs) and congeners (13). Also, a critical review of the literature dealing with the occurrence of angiosarcoma of the liver in factory workers exposed to vinyl chloride has demonstrated that only reactor cleaners exposed chronically to several hundred parts per million or more had a high risk of cancer (14). Workers not exposed to such high concentrations so far have not displayed adverse effects.

\*American Health Foundation, Valhalla, NY 10595.

Address reprint requests to J. H. Weisburger, American Health Foundation, Valhalla, NY 10595.

In the context of chemical production, a problem often unfortunately neglected is the disposal of raw or partially processed waste. One improper means of disposal has been to use bodies of water, rivers, lakes, or ocean estuaries. A number of studies in the last 25 years have reported that fish from rivers or harbors contaminated by industrial effluents displayed evidence of cancer, whereas similar fish caught in clean control rivers did not, as reported in detail at this and preceding conferences (15-19). Therefore, fish are indicators of potential problems for humans (20). At this time, in view of our extensive knowledge in toxicology and cancer causation, industries and municipalities must avoid the needless contamination of water with any waste product. Indeed, rational management in industry, supported by wise government actions, will find it not only safer but also profitable to make the investment in recycling waste products and producing valuable new materials. Alternatively, high temperature incineration yielding carbon dioxide, water, and acids such as nitric, sulfuric, and hydrochloric that can be absorbed by bases yielding marketable salts is a proper technique to be used anywhere in the world. Special facilities, including mobile ship-borne facilities have been engineered to accomplish such disposal safely. Governments and political bodies need to encourage such effective means of waste disposal. The world's growing population, generating increasing volumes of wastes, demands urgent effective disposal methods such as high temperature incineration, that in part return cost in the form of energy. The question often bandied about relative to the generation of dangerous dioxins (fortunately not by informed individuals) is not based on documented emissions and harms the effective implementation of reliable destruction of human waste materials. Burial certainly is not sound, as has been shown by the broad contamination of bodies of water.

Monitoring systems need to be established to detect and quantify carcinogens in the effluent from factories and private or public waste treatment plants. Since the limits of sensitivity of biological detection impose restrictions on the ability to prevent contamination of the environment, it will be important to monitor at points where the most concentrated contaminants arise or design means of concentrating potentially harmful products. For example, Hayatsu (21) has developed specific absorbent procedures for certain mutagens and carcinogens.

## Mechanisms of Carcinogenesis and Rational Selection of Bioassay Systems

In the last few decades, it has been established that neoplastic diseases arise through a complex series of steps, beginning with the transformation of normal cells to abnormal cells at the genetic level through specific alterations of DNA (22). Cancer results from a somatic mutation. Rapid *in vitro* and *in vivo* bioassays have been developed to detect chemicals or radiation that can alter DNA and thus act as genotoxic carcinogens (23). Most human cancers due to occupational exposure (a small and declining proportion) and those due to lifestyle (the great majority) are caused by genotoxic carcinogens (24,25). In many instances, however, nongenotoxic epigenetic enhancing or promoting factors play an important role in eliciting invasive, metastatic neoplasms. The overall complex processes are

outlined in Figure 1, and the ensuing logical classification of carcinogens is presented in Table 1, but the reader is referred to more specialized reviews for details (22). Because most human cancers are caused by genotoxic carcinogens, knowing whether carcinogens are present in the environment makes the reliable detection and quantitation of genotoxic carcinogens an essential component of cancer prevention.

Basically, two distinct test systems are available to estimate

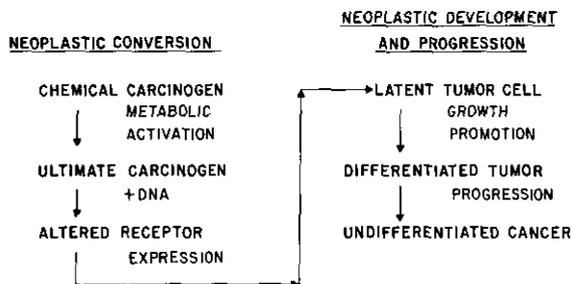


FIGURE 1.

Table 1. Classification of carcinogenic chemicals.

Category and class	Example
DNA-reactive, (genotoxic) carcinogens	
Activation independent	Alkylating agent
Activation dependent	Polycyclic aromatic hydrocarbon, nitrosamine, arylamine
Inorganic*	Specific metals
Epigenetic carcinogens	
Promoter	Organochloride pesticide, phenobarbital
Hormone modifying	Estrogen
Cytotoxic	Nitrotriacetic acid, bile acids
Peroxisome proliferators	Clofibrate, phthalate esters
Immunosuppressor	Purine analog
Solid state	Plastics, asbestos
Unclassified	
Miscellaneous	Dioxane, methapyrilene

\*Some are tentatively categorized as genotoxic because of evidence for damage of DNA; others may operate through epigenetic mechanisms such as alterations in fidelity of DNA polymerases.

Table 2. Decision-point approach to carcinogen testing.

Stage A. Evaluation of structure-activity relationships
Stage B. Short-term cellular tests
Mammalian cell DNA repair
Bacterial and mammalian cell mutagenesis
Chromosome alterations
Decision point 1: Evaluation of all tests conducted in stages A and B.
Stage C. Tests for promoters
<i>In vitro</i>
<i>In vivo</i>
Decision point 2: Evaluation of results from stages A through C.
Stage D. Limited <i>in vivo</i> bioassays
Altered foci induction in rodent liver
Skin neoplasm induction in mice
Pulmonary neoplasm induction in mice
Breast cancer induction in female rats
Decision point 3: Evaluation of all results from stages A through D and application to health risk analysis. This evaluation may include data from stages A through C to provide the basis for mechanistic considerations.

Table 3. Number of chemicals in each class positive or negative in the hepatocyte primary culture/DNA repair test.<sup>a</sup>

Chemical class	Carcinogen		Noncarcinogen		Unknown	
	+	-	+	-	+	-
Alkylating agents	5					
Polycyclic aromatic hydrocarbons	6			5		
Monocyclic aromatic amines	3	1 <sup>b</sup>				
Polycyclic aromatic amines and amides	8	1 <sup>c</sup>		3	2	
Aminoazo dyes	5					
Nitro-substituted compounds	3	2		2	3	
Aza aromatics	2		2	2	1	
Nitrosamines	7	1 <sup>d</sup>		3		
Mycotoxins	7	1		1		4
Pyrrolizidine alkaloids	4				1	4
Intercalating agents						5
Total	50	6	2	16	7	13

<sup>a</sup>This assay has fewer false negatives or positives than other *in vitro* or *in vivo-in vitro* bioassays.

<sup>b</sup>Aniline, weakly carcinogenic at high dose levels because of slow poisoning of the hematopoietic system.

<sup>c</sup>4-Acetylaminofluorene is unreliably positive in this and also in the Ames test. Carcinogenicity tests negative, but true carcinogenic risk unknown.

<sup>d</sup>Diphenylnitrosamine, considered a classic noncarcinogen, at high dose levels induced a small yield of urinary bladder cancer in rats, through unknown mechanisms.

genotoxic potential: those using prokaryotic organisms and those using eukaryotic cell systems. An initial review of chemical structure (probable activity, or lack thereof) provides important background information and guidance to the selection of bioassay systems (22,23). A systematic decision point approach, providing qualitative and semiquantitative tests of increasing complexity, has been developed (Table 2).

## Prokaryotic Test Systems

The most widely practiced test in prokaryotic organisms is the reverse mutation in several strains of *Salmonella typhimurium* developed by Ames. Previously, Rosenkranz had demonstrated the use of repair-deficient *E. coli* (23). A large number of chemicals has been tested, especially in the Ames test (23). The readily performed standard tests, such as the Ames test, require an exogenous liver cell S-9 fraction to provide for metabolism, since most environmental carcinogens are procarcinogens and promutagens that must be metabolized to the reactive genotoxic product (26). However, the metabolic system of this liver fraction is inherently deficient in detoxification enzymes, which are available *in vivo*. Therefore, the Ames test presents a number of false positives. It is also not uniformly sensitive to all genotoxic agents, again, most likely because of the inadequacy of the S-9 fraction used. Even so, the Ames test is an economic, rapid, and valuable component of screening batteries. It has been used to study the occurrence of Ames-positive mutagens in water or in concentrates of water (27-29). Thus, a positive finding in the Ames test is essentially a warning that a potential, although certainly not an actual, cancer risk is present. Because of the occurrence of false positives, such as the plant component quercetin, which is positive in the Ames test but negative in other tests and negative in all carcinogen bioassays (30), the Ames test is not by itself a predictor of cancer risk but acts as a warning, calling for further exploration.

## Eukaryotic Test Systems

Among the systems using eukaryotic cells, a reliable indicator for genotoxic carcinogens rests on the fact that such carcinogens damage DNA, leading to DNA repair. Williams (31) has used the

broad metabolic competence of freshly explanted liver cells from rodent and human livers and the simultaneous presence in the cell of indicator DNA to develop a hepatocyte DNA repair test, using a cell system that metabolically resembles the *in vivo* situation (26,32,33). Thus, this test accurately mimicks the metabolic conditions to detect potential human risk factors (Table 3). A battery composed of the readily performed Ames test and the Williams test is a suitable set of complementary tests to determine whether or not a given chemical or extract is genotoxic and thus may constitute a possible human cancer risk. The Williams test has also been adapted to an *in vivo-in vitro* situation where animals are given a chemical followed by excision of the liver and the determination of DNA repair in such livers (23,34).

Some tests such as the determination of sister-chromatid exchange (SCE) present the advantage that they can be applied to the study of pre-exposed humans. Cell transformation tests, or others such as the lymphoma test, suffer from difficulties in execution and scoring (cell transformation) or lack of accurate responses with genotoxic agents, demonstrating too many false positives or negatives (lymphoma test). Any test system should be evaluated with known carcinogens of various chemical types and related noncarcinogens. Problems with those bioassay approaches and the underlying mechanisms have been critically reviewed (35).

## Promoters and Enhancers

Many types of human cancer including cancer of the lung from cigarette smoking and cancer of the breast or colon in individuals consuming high-fat diets involves not only the action of genotoxic carcinogens but also of nongenotoxic, epigenetic, enhancing, and promoting elements that play crucial roles in the development of important human cancers. For example, tobacco smoke contains an acidic fraction composed of phenolic substances that are not carcinogenic but have enhancing properties. These have significant functions since tobacco smoke contains relatively small amounts of genotoxic carcinogens (36,37). Likewise, in the nutritionally linked cancers, dietary fat translates to metabolic effects such as control of bile acid levels that promote colon cancer or effects on the endocrine system that enhance the risk for breast cancer. Here also, the putative genotoxic carcinogens are present

in small amounts, so that promoting elements are critical (38,39). The mechanism of promotion is only partially understood. Nonetheless, promotion is highly dose dependent and reversible. This is the rationale for the lower lung cancer risk upon cessation of smoking; it is the basis for encouraging Western people to lower their total fat intake to lower their risk for the nutritionally linked diseases.

The occurrence of cancer in fish unquestionably involves the presence of genotoxic carcinogens. For example, the neoplasms in gills may relate to contamination of water and sediments by polycyclic aromatic hydrocarbons and similar products. Aflatoxin B<sub>1</sub> has been the main carcinogen incriminated in causing hepatocellular carcinoma in species such as trout. It is not yet known whether promotion operates in any type of fish or some types of fish, or not at all. Contamination of harbors and estuaries with complex petroleum wastes from ships and other sources may not only be the source of polycyclics but also promoting substances. The effect in fish, however, is not clear. For example, phenobarbital is a good promoter in the development of primary liver cancer in rats, but not in hamsters treated previously with a genotoxic carcinogen such as nitrosodiethylamine or 2-acetylaminofluorene. Future research, therefore, will need to delineate the role of promotion in carcinogenesis in fish. Promotion is often target-organ specific, a fact that needs to be considered in designing appropriate approaches. Such studies are important because a number of the water and especially bottom contaminants such as polychlorinated biphenyls (PCBs), chloroform, other haloalkanes, trichloroethylene, or phenols most likely operate by a promoting mechanism.

Enhancement of carcinogenesis may stem from a cytotoxic action of a given chemical, leading to regeneration. This means there is increased DNA synthesis and mitosis, conditions favoring cancer production in the presence of a genotoxic carcinogen. This type of enhancement should not be defined as promotion but rather co-carcinogenesis due to cytotoxicity. Obviously, dose levels that are not cytotoxic are also not cocarcinogenic.

Promoters can exert their action and therefore can be tested through a number of specific mechanisms, such as those involving membrane effects or through the interruption of cell-to-cell communication via gap junctions (Table 4). The reader is referred to more specialized literature for detailed methods (26,40,41).

## Rodent Bioassays

The traditional chronic bioassay in rodents is an important tool to examine whether or not a given chemical represents a cancer risk for man (42,43). However, results need to be interpreted cautiously. A chronic bioassay, indeed, displays positive responses, at least in mice and especially in mouse liver, with chemicals such as chloroform or trichloroethylene that are not genotoxic for mouse liver. Rather, the occurrence of hepatomas

in the treated animals represents the promotion phenomenon on an organ that already has the cellular genetic structure typical of a transformed cell documented by genetic analyses (44,45). Thus, even chronic bioassays in rodents need to be analyzed carefully as to underlying mechanisms. This in turn requires *in vitro* bioassays through the Ames and Williams test to assign genotoxicity or absence thereof. More fundamental, precise studies on DNA binding, DNA lability, and chromosomal changes aid in defining the genotoxic properties of a given chemical (46-50). Thus, the *in vitro* tests and biochemical studies necessarily precede a chronic rodent bioassay so as to be in a position to design the bioassay in the light of the findings made (51). The chronic bioassay would serve to provide semiquantitative information on the potency of a given agent, once it has been established to be genotoxic. This is important, for in the absence of genotoxicity, quantitative risk assessment needs totally different parameters, including the question of dose-response relationships, the probable existence of a threshold with nongenotoxic agents, and above all, the reversibility of effects of such agents (22,51,52).

In relation to the question of neoplasms found in fish growing and living in waste-contaminated waters, discussed at this conference, bioassays in specific types of fish are of great relevance. Several previous recent reports (15-19,53-55) have dealt with the problem, as well as with the necessary species-related and controlled biochemical activation of procarcinogens to reactive genotoxins through metabolism, demonstrating that types of fish studied differ from rodents and humans in this respect. Anders and associates (56) have provided interesting new concepts as to gene rearrangements and amplification in neoplasia through their detailed study of hybrids of *Xiphophorus*, a tropical fish originally found in Central America that develops melanomas and other neoplasms.

## Conclusions

In summary, in the overall context of cancer prevention, it is important to adjust lifestyle to avoid conditions with demonstrated adverse effects such as that of tobacco or excessive fat intake, obesity, or the relative deficiency of cereal fiber and vegetable consumption. Also, methods have been developed, based on sound knowledge of the mechanisms of carcinogenesis, that rapidly and accurately give qualitative information as to whether or not a given environmental chemical or mixture is genotoxic or has promoting potential. This permits improved control measures to be instituted and also effective designs for chronic animal studies that will provide the basis for risk assessment and risk control.

In the context of this conference, it is also important to realize that among the sources of protein available to man, fresh or salt water fish represent one of the best nutritional resources available to humans (Table 5) (57-60). Another reason, documented in the last 15 years, is that the type of fat present in seafood itself, namely, omega-3 fatty acids, is highly beneficial in maintaining desirable plasma cholesterol levels and thus avoiding heart disease risk, high blood pressure and stroke, and controlling the clotting process and avoiding emboli. It behooves all concerned to avoid contamination of rivers, lakes, and oceans with chemicals that would adversely affect such a valuable food resource and make it potentially hazardous to humans. Health

Table 4. Select properties of tumor promoters.

Ornithine decarboxylase
Protein kinase C
Anchorage independence
Surface attachment
Intercellular communication
Enhancement of cell transformation
Increased Epstein-Barr virus in lymphoblasts

**Table 5. Benefits of fish in health promotion.**

High protein food
Desirable micronutrients
Controls serum cholesterol
Controls blood pressure
Lowers risk of emboli
Lowers risk of nutrition-linked neoplasms colon, breast, possibly pancreas, ovary, endometrium, prostate

promotion not only requires accurate knowledge of environmental carcinogens, cocarcinogens, and promoters affecting fish and man, but also appropriate recycling and disposal of human and animal wastes, not by burial and water disposal, but by effective high temperature combustion and simultaneous use of heat generated for electricity production, and recovering of valuable metal and glass. Medical and engineering research has provided sound facts and methods. It is essential and urgent that current knowledge be translated to a cleaner, more wholesome environment to ensure man's survival.

This investigation was supported by US PHS grants CA-17613, CA-24217, CA-42381, and CA-45720. We are grateful to C. Horn for excellent editorial assistance.

#### REFERENCES

- Wynder, E. L., and Gori, G. B. Contribution of the environment to cancer incidence: an epidemiologic exercise. *J. Natl. Cancer Inst.* 58: 825-832 (1977).
- Doll, R., and Peto, R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* 66: 1191-1308 (1981).
- Schottenfeld, D., and Fraumeni, J. F., Jr. *Cancer Epidemiology and Prevention*. W. B. Saunders Co., Philadelphia, PA, 1982.
- Vessey, M. P., and Grey, M., Ed. *Cancer Risks and Prevention*. Oxford University Press, Oxford, 1985.
- Bannasch, R., Ed. *Cancer Risks. Strategies for Elimination*. Springer-Verlag, Berlin, 1986.
- Tollefson, L., and Cordle, F. Methylmercury in fish: a review of residue levels, fish consumption and regulatory action in the United States. *Environ. Health Perspect.* 68: 203-208 (1986).
- Aust, S. Workshop on scientific aspects of polybrominated biphenyls. *Environ. Health Perspect.* 23: 1-350 (1978).
- Posada, M., Castro, M., Kilbourne, E. M., Diaz de Rojas, F., Abaitua, I., Tabuenca, J. M., and Vioque, A. Toxic-oil syndrome: case reports associated with the ITH oil refinery in Sevilla. *Food Chem. Toxicol.* 25: 87-90 (1987).
- Cantor, K. P., Kopfler, F. C., Hoover, R. N., and Strasser, P. H. Cancer epidemiology as related to chemicals in drinking water. *Toxicol. Environ. Chem. Rev.* 4: 47-65 (1981).
- Crump, K. A., and Guess, H. A. Drinking water and cancer: review of recent epidemiological findings and assessment of risk. *Annu. Rev. Public Health* 3: 339-357 (1982).
- Stara, J. F., Mukerjee, D., McGaughy, R., Durkin, P., and Dourson, M. L. The current use of studies on promoters and cocarcinogens in quantitative risk assessment. *Environ. Health Perspect.* 50: 359-368 (1983).
- Shy, C. M. Chemical contamination of water supplies. *Environ. Health Perspect.* 62: 399-406 (1985).
- Hesse, J. L., and Powers, R. A. Polybrominated biphenyl (PBB) contamination of the Pine River, Gratiot, and Midland Counties, Michigan. *Environ. Health Perspect.* 23: 19-25 (1978).
- Williams, G. M., Reiss, B., and Weisburger, J. H. A comparison of the animal and human carcinogenicity of environmental, occupational and therapeutic chemicals. In: *Advances in Modern Environmental Toxicology*, Vol. XII (W. G. Flamm and R. J. Lorentzen, Eds.), Princeton Science Publishers, Princeton, NJ, 1985, pp. 207-248.
- Dawe, C. J., and Harshbarger, J. C., Eds. *Neoplasms and related disorders of invertebrate and lower vertebrate animals*. *Natl. Cancer Inst. Monogr.* 31: 1-769 (1969).
- Kraybill, H. F., Dawe, C. J., Harshbarger, J. C., and Tardiff, R. G., Eds. *Aquatic Pollutants and Biologic Effects with Emphasis on Neoplasia*. *Ann. N.Y. Acad. Sci.* 298: 1-604 (1977).
- Dawe, C. J., Scarpelli, D. G., Wellings, S. R., and Homburger, F., Eds. *Tumors in Aquatic Animals*. *Prog. Exp. Tumor Res.* 20: 3-411 (1976).
- Dawe, C. J., Harshbarger, J. C., Kondo, S., Sugimura, T., and Takayama, S., Eds. *Phyletic Approaches to Cancer*. Japan Scientific Press, Tokyo, 1981.
- Pritchard, J. B., Ed. *Mechanisms of Pollutant Action in Aquatic Organisms*. *Environ. Health Perspect.* 71: 3-190 (1987).
- Dawe, C. J. Implications of aquatic animal health for human health. *Environ. Health Perspect.* 86: 245-255 (1990).
- Hayatsu, H., Oka, T., Wakata, A., Ohara, Y., Hayatsu, T., Kobayashi, H., and Arimoto, S. Absorption of mutagens to cotton bearing covalently bound trisulfo-copper-phthalocyanine. *Mutat. Res.* 119: 233-238 (1983).
- Williams, G. M., and Weisburger, J. H. Chemical carcinogens. In: *Casarett and Doull's Toxicology. The Basic Science of Poisons* (C. D. Klaassen, M. O. Amdur, and J. Doull, Eds.), Macmillan, New York, 1986, pp. 99-173.
- Rosenkranz, H. S., Ed. *Strategies for the deployment of batteries of short-term tests*. *Mutat. Res.* 205: 1-426 (1988).
- Cartwright, R. A. Cancer epidemiology. In: *Chemical Carcinogens*, Vol. 1, ACS Monograph 182 (C. E. Searle, Ed.), American Chemical Society, Washington, DC, 1984, pp. 1-40.
- IARC. *Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7. International Agency for Research on Cancer, Lyon, 1987.
- Milman, H. A., and Weisburger, E. K. *Handbook of Carcinogen Testing*. Noyes Publications, Park Ridge, NJ, 1985.
- Loper, J. C. Mutagenic effects of organic compounds in drinking water. *Mutat. Res.* 76: 241-268 (1980).
- Heartlein, M. W., DeMarini, D. M., Katz, A. J., Means, J. C., Plecwa, M. J., and Brockman, H. E. Mutagenicity of municipal water obtained from an agricultural area. *Environ. Mutagen.* 3: 519-530 (1981).
- Zoeteman, C. J., Hrubec, J., de Greef, E., and Kool, H. J. Mutagenic activity associated with byproducts of drinking water disinfection by chlorine, chlorine dioxide, ozone, and UV-irradiation. *Environ. Health Perspect.* 46: 197-205 (1982).
- Hirono, I. Carcinogenicity of plant constituents: pyrrolizidine alkaloids, flavonoids, bracken fern. In: *Genetic Toxicology of the Diet* (I. Knudsen, Ed.), Alan R. Liss, New York, 1986, pp. 45-54.
- Williams, G. M., Laspia, M. F., and Dunkel, V. C. Reliability of the hepatocyte primary culture/DNA repair test in testing of coded carcinogens and noncarcinogens. *Mutat. Res.* 97: 359-370 (1977).
- Williams, G. M., Mori, H., and McQueen, C. A. Structure activity relationships in the hepatocyte DNA repair test for 300 chemicals. *Mutat. Res.* 221: 263-286 (1989).
- Casciano, D. A., Ed. *Unscheduled DNA synthesis: workshop overview*. *Cell Biol. Toxicol.* 3: 109-218 (1987).
- Naismith, R. W., Ed. *Guidelines for minimal criteria of acceptability for selected short-term assays for genotoxicity*. *Mutat. Res.* 189: 81-175 (1987).
- Williams, G. M. Methods for evaluating chemical genotoxicity. *Annu. Rev. Pharmacol. Toxicol.* 29: 189-211 (1989).
- Hoffmann, D., and Hecht, S. S., Eds. *Mechanisms in Tobacco Carcinogenesis*, Banbury Report 23. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986.
- IARC. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Tobacco Smoking, Vol. 38. International Agency for Research on Cancer, Lyon, 1986.
- Weisburger, J. H. Quantitative aspects of the causes of the main human cancers in the light of initiating and promoting mechanisms. *Cancer Lett.* 26: 89-95 (1985).
- Weisburger, J. H. Mechanism of nutritional carcinogenesis associated with specific human cancers. *ISI At. Sci. Pharmacol.* 1: 162-167 (1987).
- Yamasaki, H., Enomoto, T., and Martel, N. Intercellular communication, cell differentiation and tumour promotion. *IARC Sci. Publ.* 56: 217-238 (1984).
- Milman, H. A., and Elmore, E., Eds. *Biochemical Mechanisms and Regulations of Intercellular Communication*. Princeton Scientific Publishing Co., Inc., Princeton, NJ, 1987.

42. Huff, J. E., McConnell, E. E., Haseman, J. K., Boorman, G. A., Eusits, S. L., Schwetz, B. A., Rao, G. N., Jameson, C. W., Hart, L. A., and Rall, D. P. Carcinogenesis studies: results of 398 experiments on 104 chemical from the U.S. National Toxicology Program. *Ann. N.Y. Acad. Sci.* 534: 1-30 (1988).
43. Huff, J., Bucher, J., and Yang, R. Carcinogenesis studies in rodents for evaluating risks associated with chemical carcinogens in aquatic food animals. *Environ. Health Perspect.* 90: 127-132 (1991).
44. Fox, P. G., and Watanabe, P. G. Detection of a cellular oncogene in spontaneous liver tumors of B6C3F1 mice. *Science* 228: 596-597 (1985).
45. Reynolds, S. H., Stowers, S. J., Maronpot, R. R., Anderson, M. W., and Aaronson, S. A. Detection and identification of activated oncogenes in spontaneously occurring benign and malignant hepatocellular tumors of the B6C3F1 mouse. *Proc. Natl. Acad. Sci. USA* 83: 33-37 (1986).
46. Bridges, B. A., Butterworth, B. E., and Weinstein, I. B., Eds. Indicators of Genotoxic Exposure, Banbury Report 13. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982.
47. Randerath, K., Randerath, E., Danna, T. F., van Golen, K. L., and Putnam, K. L. A new sensitive <sup>32</sup>P-postlabeling assay based on the specific enzymatic conversion of bulky DNA lesions to radiolabeled dinucleotides and nucleosides 5'-monophosphates. *Carcinogenesis* 10: 1231-1239 (1989).
48. Farmer, P. B., Neumann, H. -G., and Henschler, D. Estimation of exposure of man to substances reacting covalently with macromolecules. *Arch. Toxicol.* 60: 251-260 (1987).
49. Harris, C. C., Weston, A., Willey, J. C., Trivers, G. E., and Mann, D. L. Biochemical and molecular epidemiology of human cancer: indicators of carcinogen exposure, DNA damage, and genetic predisposition. *Environ. Health Perspect.* 75: 109-119 (1987).
50. Anderson, D., Ed. Human monitoring. *Mutat. Res.* 204: 353-551 (1988).
51. Weisburger, J. H., and Williams, G. M. Bioassay of carcinogens: in vitro and in vivo tests. In: *Chemical Carcinogens*, Vol. 2, 2nd ed., ACS Monogr. 182 (C. E. Searle, Ed.), American Chemical Society, Washington, DC, 1984, pp. 1323-1373.
52. Fujiki, H., Hecker, E., Moore, R. E., Sugimura, T., and Weinstein, I. B., Eds. *Cellular Interactions by Environmental Tumor Promoters*. Japan Scientific Societies Press, Tokyo, and VNO Science Press BV, Utrecht, The Netherlands, 1984.
53. Ishikawa, T., Masahito, P., and Takayama, S. Usefulness of the Medaka, *Oryzias latipes*, as a test animal: DNA repair processes in Medaka exposed to carcinogens. *Natl. Cancer Inst. Monogr.* 65: 35-43 (1984).
54. Masahito, P., Ishikawa, T., Sugano, H., Uchida, H., Yasuda, T., Inaba, T., Hirotsaki, Y., and Kasuga, A. Spontaneous hepatocellular carcinomas in lungfish. *J. Natl. Cancer Inst.* 77: 291-298 (1986).
55. Masahito, P., Ishikawa, T., and Sugano, H. Fish tumors and their importance in cancer research. *Jpn. J. Cancer Res.* 79: 545-555 (1988).
56. Anders, F., Schartl, M., Barnekow, A., and Anders, A. *Xiphophorus* as an in vivo model for studies on normal and defective control of oncogenes. *Adv. Cancer Res.* 42: 191-275 (1984).
57. Lands, W. E. M. *Fish and Human Health*. Academic Press, New York, 1986.
58. Karmali, R. A. Eicosanoids in neoplasia. *Prev. Med.* 16: 493-502 (1987).
59. McGiff, J. C. Arachidonic acid metabolism. *Prev. Med.* 16: 503-509 (1987).
60. Weisburger, J. H. Comparison of nutrition as customary in the Western World, the Orient, and northern populations (Eskimos) in relation to specific disease risks. *Arctic Med. Res.* 47(Suppl. 1): 110-120 (1988).