A SEARCH FOR CHEMICAL AGENTS CAUSING HUMAN CANCER — LESSONS LEARNED FROM RODENT CARCINOGENICITY STUDIES — *

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ABSTRACT — Analysis of classical life-time rodent bioassays conducted over the past 20 years under conditions of Good Laboratory Practice Regulations has led to an improved understanding of possible factors involved in oncogenesis in humans. Improvements in study design coupled with more detailed pathological examinations have led to new insights whereby neoplastic processes can be more clearly identified and understood with confidence in animal models.

Improvements in the ability to identify potential risks through continued animal testing combined with incorporation of advances in genetics and molecular biology in elucidating mechanistic factors will greatly facilitate future research efforts to identify causative agents of human cancer. A great deal of attention will have to be paid to the concept of threshold doses and exposure levels that may be required to achieve pre-neoplastic conditions or the induction of carcinogenic processes.

Future concerns of hazards, risk assessment and the evaluation of carcinogenic potential will involve an amalgamation and understanding of radiological and biological events, especially those of carcinogenic microorganisms, the effects of food and air borne carcinogens, effects of pollution, exposure to xenobiotics and imbalances and disruption of normal biologic functioning of endogenous physiologically active substances, to achieve a better understanding and ultimate prevention of human cancer.

KEY WORDS: Carcinogenicity study, Study design, Rodents, Threshold, Survey, Human carcinogens

INTRODUCTION

Radioactivity, microorganisms, xenobiotics and imbalances in levels of natural endogenous substances are known to cause human cancers. Carcinogenicity testing using animal models and epidemiology studies have been the classical approaches to identify unknown carcinogenic agents, since the occupational and environmental hazards associated with coal tar and chimney sweepers were identified as causative agents as early as the eighteenth century (Hueper and Conway, 1964).

Following the extensive efforts and accomplishment of the U.S. National Toxicology Program (NTP) that started in the U.S.A. (Huff et al., 1991), the Japanese government responded to ever-increasing public concern for increased safety evaluation requirements and pre-market testing of all new chemicals. The first national collaborative program for carcinogen identification began in 1975 consisting of two teams: one for short-term screening, which included Ames tests, rec assay and chromosomal aberrations in vitro, and a second for carcinogenicity testing in mice and rats. Considerable experience was gained from these studies and contributed to the establishment of guidelines to identify both the mutagenic and carcinogenic potential of new chemicals (Hayashi et al., 1986; Ishidate, 1988; Damstra and Kurokawa, 1990).

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Enactment of the Good Laboratory Regulations (GLP’s) in 1977, coupled with the establishment of OECD guidelines in 1981, mandated the use of well-controlled animal testing facilities to assure proper animal handling and husbandry by appropriately trained technical personnel and the standardization of preclinical safety evaluation testing guidelines. Improvements in technical training, safety, health concerns and hygienic practices resulted in higher-quality research animals from reputable animal suppliers and breeders. Consequently, data obtained from studies conducted since 1980 were of higher quality and more reproducible between different testing facilities, resulting in better accuracy in predicting and evaluating potential toxicity and carcinogenicity of chemicals.

Three important outcomes resulted, following the utilization of adequate animal facilities, improvements in training and standardization in testing, as follows:

1. Significant characteristics of neoplastic responses of rodents to the exposure of chemicals were accurately determined from experimental conditions including dose, route of administration, time of dosing and various intrinsic factors including genetic susceptibility (Maronpot, 1985; Huff et al., 1988; Inoue, 1999).

2. Analyses and evaluation of data obtained from short and long-term in vivo studies revealed four classifications in which substances being tested for carcinogenicity may be categorized: genotoxic and non-genotoxic agents for true carcinogens, promoters and non-carcinogens. (Grasso et al., 1991; Enomoto et al., 1996).

3. Compilation of the results of current bioassay data resulted in the availability of readily accessible information on normal growth and aging patterns and the occurrence of natural neoplastic and non-neoplastic changes in rodents (Ward, 1983; Enomoto et al., 1990; Yamamoto et al., 1991; Iwata et al., 1991; Yamamoto et al., 1993; Iwata et al., 1993; Hirouchi et al., 1994; Haseman et al., 1998).

As a result, more accurate predictions of risk assessment and a better understanding of whole-body effects of chemicals and their contribution to the development of cancers in animals followed.

The incorporation of mechanistic and toxicokinetic data of chemicals obtained from rodent bioassays greatly deepened the scientific insight on tumor development (Butterworth, 1990). The search for causative agents of human cancer is a main goal of oncologists and toxicologists involved in bioassay testing for carcinogenicity. The purpose of this overview is to identify probable causative carcinogens for humans based on current and past evaluations obtained from carcinogenicity studies conducted on both synthetic and naturally occurring chemicals.

Analysis of carcinogenicity studies in rodents

Advantages of the current bioassay design for chemical carcinogenicity in animals were reviewed by numerous investigators including Hamn (1985) and Huff (1999). These reviews represent the most accurate means of identifying the carcinogenic potential of unknown agents for human cancer, despite the fact that questions remain concerning the extrapolation of animal data to man. Discussions on such matters were addressed at International Conference on Harmonization (ICH) meetings since 1995 (Contrera et al., 1997). Advantages, disadvantages and limitations of existing testing procedures, resources, finance and the relevance of exaggerated human exposure levels in animal experiments are all factors that must be considered when conducting future studies (Ashby and Morrod, 1991; Huff, 1999). Lastly, the experience and professional judgment of the scientists conducting studies and making decisions cannot be overemphasized (Wachsman et al., 1993).

Improved design of chemical carcinogenesis bioassays

Comparison of current carcinogenicity study designs with those conducted prior to 1980 (Sonig et al., 1976) are shown in Table I. Analysis of the results of these currently completed carcinogenicity studies revealed for the first time dose-levels which had no apparent, treatment-related effects on carcinogenic processes and the concept of threshold doses.

Improved animal study facilities and design, including additional treatment groups with sufficient numbers of animals per group and in particular extensive gross and histological evaluations of all tissues, lesions and masses led to the generation of qualitatively and quantitatively better and reproducible data for more meaningful interpretations. In the past, generally prior to 1980, oncologists and pathologists were primarily concerned with the diagnosis of well-developed tumor masses in carcinogenicity studies. Little or no attention was paid to complete, thorough post mortem examinations or the evaluation of lesser lesions in all tissues. Pathologists could not identify the possibility of tumor induction in the grossly normal animals treated with a low dose level of chemicals.

The compilation, examination and evaluation of
Chemical agents causing human cancer.

data generated from numerous, current, detailed preclinical safety evaluation studies have led to the assumption that threshold and possibly and probably dose-related threshold levels do indeed exist in carcinogenic processes. Evidence of threshold doses for carcinogenicity can be supported by the incorporation and consideration of the following factors in the evaluation of current and future bioassays:

1) 13-week studies on dose-response relationships to establish dose levels to be used in carcinogenicity studies,

2) Detailed evaluation of results of interim sacrifice examinations of animals on Weeks 26, 52 and 78 of the treatment, in chronic toxicity/carcinogenicity bioassays as stipulated in the guidelines issued by the Ministry of Agriculture, Forestry and Fisheries in Japan in 1980.

3) Numerous experimental mechanistic studies including Ito's medium-term assay (Ito et al., 1988; Shirai, 1997) and research on the role of reactive oxygen species in carcinogenesis (Toyokuni, 1999), and

4) Toxicokinetic studies of chemicals in multiple animal species (Sugiyama and Iwatsubo, 1994).

2. Arguments for thresholds

The concept of thresholds for carcinogenesis occurring in various mammalian species has resulted in ongoing debates (Upton, 1977; Kondo, 1988; Melnick et al., 1996; Olivier, 1999). Threshold doses for mutagenesis and carcinogenesis were more recently addressed by Kirsh-Volders et al., (2000). Several researchers (Williams et al., 1998 and 1999) and Fukushima (1998) have reported the results of a series of exposure-response studies of carcinogenesis in rats using genotoxic carcinogens. Several critical parameters were evaluated, including formation of DNA adducts rather than the usual neoplastic or neoplastic histological lesions to provide other possible explanations regarding thresholds for genotoxic carcinogens. This evidence that very low levels of environmental exposure of humans to DNA-reactive carcinogens may pose no risk of cancer (Williams et al., 2000) is convincing. O'Connor et al. (2000) recently demonstrated that efficient DNA repair can effectively protect cells from the deleterious biologic effects of genotoxic carcinogens, including the induction of tumor formation.

Similar to non-genotoxic carcinogens, a number of genotoxic agents induce tumors primarily or only at dose levels which produce tissue damage (Hildebrand et al., 1991; Enomoto et al., 1996). Seventy carcinogenicity studies conducted on 35 chemicals in both rats and mice from 1980 to 1999 at the An-Pyo Center (Enomoto et al., 1996; Inoue, 1999) generally employed the MTD (maximum-tolerated dose) as the highest dose level. Results revealed characteristics of both genotoxic and non-genotoxic carcinogens (Table 2). All three genotoxic chemicals were carcinogenic in both sexes of male and female rats and mice. Non-genotoxic compounds exhibited species and/or sex-related responses to tumor development. Induction of tumors was not evident in the low or low- and mid-dose groups of all studies on both genotoxic or non-genotoxic chemicals.

Analyses of dose-relationships of genotoxic carcinogenic agents have been reported as follows:

1) 2-acetylaminofluorene (Littlefield et al., 1979; Cohen and Ellwein, 1990),

2) A variety of nitroso-compounds (Druckeray et al.,

| Table 1. Improved study quality in recent rodent carcinogenicity studies (1980-1999). |
|---------------------------------|----------------------------------|-------------------------------|
| Improved Points | Recent Studies Conducted from 1980 to 1999 | Studies Conducted prior to 1980 |
| Number of Species | 2 (usually rat and mouse) | One or 2 species |
| Number of Groups | 3 or 4 treatment groups and one control group | One or 2 treatment groups and one control group |
| Duration of Treatment | 104 Weeks (78 Weeks; mouse) | Not determined |
| Histopathological Evaluations | All animals and all organs (Both neoplastic and non-neoplastic findings) | Tumors only (Main organs) |
| Historical Data | Complete | - |
| Quality Assurance | GLP facilities with QAU | - |
| Additional Studies | Interim-sacrifices, Mechanism & Toxicokinetics studies | - |
1963; Lijinsky et al., 1981; Peto et al., 1984; Maekawa et al., 1984; Lijinsky et al., 1988), 3) 1,3-butadiene (Melnick et al., 1990), 4) Aflatoxin B1 (Butler et al., 1969; Wogan, 1973) and 5) Diethylstilbestrol (McLachlyn et al., 1982).

This data appears to suggest that carcinogenic doses generally lie in the range between maximum and minimum levels of doses required to induce tumors (ENOMOTO et al., 1996). The high incidence and often unpredictable prevalence of naturally occurring tumors in aged rodents makes low-dose extrapolations difficult in classical life-time bioassays. Therefore, the importance of a sufficiently large historical control data base cannot be overemphasized in evaluation of results.

Results of the studies listed above revealed that the ratio of the highest dose capable of inducing tumor development to the lowest dose that did not induce tumor development was generally less than a hundred-fold. A few strong carcinogens such as aflatoxin B1 and some nitroso-compounds exhibited a wider range, between 100 to 1000 times greater.

Care must be taken and special consideration given to evaluate and classify substances as carcinogens via environmental routes of exposure in humans. Since most chemical carcinogens, except for some hormones such as diethylstilbestrol, exhibit their potential to induce tumors at dose levels on the order of one microgram (μg) per kilogram of body weight per day or greater, cancer development will not be expected at exposure levels less than 100 parts per billion (ppb) (corresponding to 2.5 μg/kg body weight per day in humans). When human exposure levels are so low or negligible compared to carcinogenic potential, such agents are considered less likely to pose a carcinogenic hazard for humans (Larsen and Richold, 1999; Ashby, 1999).

### Identification of carcinogens

The data of many carcinogenicity studies conducted prior to 1980 lack the necessary, detailed complete gross postmortem examinations of necropsied animals other than observed numbers of tumors and nodules.

| Table 2. Carcinogenicity results of 35 chemical studies in rodents (An-Pyo center; 1980-1999)1). |
|---|---|---|---|---|---|
| Proportion of positive studies | < Rats > | < Mice > | Number of chemicals | Subtotals (%) |
|   | Male | Female | Male | Female |   |
| 4/4 | + | + | + | + | 3 | 3 (8.6) | All genotoxic |
| 3/4 | + | + | - | + | 1 | 1 |
|     | + | - | + | + | 0 | 0 |
|     | + | - | + | + | 1 |
| 2/4 | + | + | - | - | 1 | 1 |
|     | + | - | + | - | 0 | 0 |
|     | - | + | - | + | 0 | 0 |
|     | - | + | + | + | 3 | 3 (14.3) | All non-genotoxic |
| 1/4 | + | - | - | - | 2 |
|     | - | + | - | - | 0 | 0 |
|     | - | - | - | + | 1 | 1 |
| 0/4 | - | - | - | - | 21 | 21 (60.0) |
| Number of positive chemicals | 9 | 7 | 8 | 10 |
| One species positive | Rats (3) | Mice (4) | Both Rats & Mice Positive (7) |

(Ref.: ENOMOTO, M. et al., 1996 and INOUE, H., 1999)

1) 3 studies on genotoxic chemicals (one industrial compound and two anti-neoplastic drugs), and 11 studies on non-genotoxic compounds (4 agricultural products; herbicides, germicide and plant growth regulator and 7 pharmaceuticals; two antihypertensive drugs, two anti-ulcer drugs, one anti-asthmatic, one central nervous stimulant and one α-blocker).
There is a paucity of historical control data, especially those on spontaneously occurring tumors, in addition to a lack of data on low-dose effects of the chemicals or data on time-to-tumor development. It is of paramount importance that such factors be taken into consideration before classifying substances as carcinogens.

1. Importance of carcinogenicity data on natural crude materials in search of causative agents for human cancer

Soot and coal tar were among the first materials to be identified as causative agents of human cancer. Carcinogenic studies conducted on natural materials revealed that contaminants in food such as aflatoxin B1 and mycotoxins, including sterigmatocystin and luteoskyrin (Wogan, 1973; Enomoto and Saito, 1972; Enomoto and Ueno, 1974) and paquilloside were also carcinogenic (Saito et al., 1975; Hirono, 1987). Feeding experiments with moldy rice infested with the mycotoxin-producing fungi and diets containing bracken-fern powder revealed a carcinogenic potential of natural crude materials. Subsequent isolation and identification of the causative carcinogenic agents followed. However, the only positive evidence of carcinogenicity in animals at high doses of the purified extracts from natural crude materials may not be indicative of a carcinogenic effect in humans. Many or most potential carcinogens contained in crude materials are either very weak carcinogens and/or are present only at extremely low concentrations. Generally, intakes of far greater than one kilogram per day of food containing substances which were classified as carcinogenic in rodent bioassays are required to induce tumors in man.

Industrial chemicals that require concentrations of more than 500 mg/kg body weight/day to induce cancer in experimental animals are not generally classified as carcinogens because of the unrealistic high human exposure levels that would be difficult or impossible to achieve (ACGIH, 1984).

Traditionally, over the last half-century, chemically related human cancers were limited to occupational and/or accidental exposure to high concentrations of reactive industrial chemicals. Many genotoxic carcinogens have been identified since this time based on better understandings of mechanistic processes in species and organ-specific cancers, detailed pathological examinations and advances in molecular biology (Table 3). However, many are not suspect agents for human cancer, because of the very low levels of environmental exposure to humans.

2. Causative agents for human cancer

Agents that are known to be capable of inducing tumors in humans and experimental animals, from clinical and/or animal carcinogenicity studies are presented in Table 4.

Many occupational carcinogens were readily identified in the past, probably due to the lack of safety and preventive procedures for workers (Hueper and Conway, 1964). Some pharmaceutical products, especially antineoplastic and immuno-suppressive agents, can cause cancer when used at high dose levels therapeutically (Fraser, 1991; Dedrick and Morrison, 1992).

Most of the lung and pleural cancers have shown a steady increase in incidence in humans living in Civilized Countries from the beginning of the 20th Century. They most likely owe their effects to air pollution caused by exhaust fumes from coal, petroleum and diesel fuels or from particulates of asphalt and asbestos. Risks of cancer in the kidney and urinary bladder also seem to be dependent on exposure to chemicals including polycyclic aromatic hydrocarbons (Hueper, 1969; Mastrangelo et al., 1996).

Diesel exhaust was shown to induce lung cancers in rats in extensive inhalation studies conducted in the U.S.A., Europe and Japan (Kuwabara et al., 1986; Mohr and Dungworth, 1988). Diesel and gasoline are known to contain numerous carcinogenic constituents (Heinrich et al., 1986; Caprino and Togna, 1988). The results of these inhalation studies are quite significant, since the rodents were exposed to the diesel exhaust in inhalation chambers which simulated conditions of human exposure.

Asbestos is another important cause of pulmonary

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**Table 3.** Classical chemical carcinogens useful in the study of experimental carcinogenesis.

<table>
<thead>
<tr>
<th>Nitroso Compounds:</th>
<th>DMN, DENA, MNU, ENU, EHEN, MNNG, BBN, &amp; N-nitrosomorpholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterocyclic Amines:</td>
<td>Trp-P-1, Glu-P-1, Glu-P-2, MelQx</td>
</tr>
<tr>
<td>Miscellaneous Agents:</td>
<td>3-MC, B(a)P, DMBA, 2-AAF, PCB, 4-NQO, HAQQ</td>
</tr>
<tr>
<td>Carbon tetrachloride, 1,3-Butadiene, MNH</td>
<td></td>
</tr>
<tr>
<td>Urethane, Potassium Bromate, Cathecol</td>
<td></td>
</tr>
<tr>
<td>Thiourea, Aminotriazole, Azaserine</td>
<td></td>
</tr>
<tr>
<td>MAM, DMH, Aflatoxin B1</td>
<td></td>
</tr>
<tr>
<td>Hormones:</td>
<td>Diethylstilbestrol, Estrogens, &amp; Androgens</td>
</tr>
</tbody>
</table>
and pleural cancer (mesothelioma), although the mechanisms by which asbestos causes cancer is not fully understood (Selikoff and Seidman, 1991; Churg and Green, 1995). The findings of Anttila et al. (1993) suggest that the ability of single, small asbestos fibers to induce mesotheliomas, independent of fibrosis, in the lower lobe of human lungs following single or very low exposure levels for short periods of time are worth further investigation for this possible unique mechanism of carcinogenesis.

Food-borne carcinogens including many natural products of plant origin have been demonstrated to be carcinogenic based on animal carcinogenicity studies (Table 5). However, there has not been any clear or direct evidence of human cancer induction by any of these agents, with few exceptions, such as alcoholic beverages or betel nuts which exhibited only indirect or epidemiological evidence of carcinogenicity (IARC, 1987). The carcinogenic potentials of food-borne carcinogens are generally comparable to those of synthetic substances (Ames et al., 1995). Although food-borne carcinogens are present in nature at very low concentrations, generally less than one ppm, human exposure is continuous and may be high or additive due to normal dietary habits and requirements. Consequently, their role in human carcinogenesis represents natural unavoidable exposures unlike exposure to man-made chemicals such as pesticide residues, which are present at low levels in the environment at concentrations generally less than one ppb.

Table 4. Known or probable human carcinogens based on clinical and animal studies1).

A. Physical Factors:
X-rays, Radium, Thorium, Radioisotopes2)

B. Microorganisms:
*Helicobacter pylori*, Pathogenic Viruses (Causing Diseases: T-cell Leukemia, Malignant Lymphoma, Nasopharyngeal Carcinoma, Hepatitis C, Hepatitis B, Cervical Carcinoma)

C. Chemical Factors:
1. Metals and Air Pollutants:
   Arsenic, Chromates, Nickel, Beryllium, Mineral Oil
   Asbestos, PAHs3): Diesel-&Gasoline-Exhaust, Benzene4), Soot, Tar:1,3,4-Benzpyrene, 1,3-Butadien5)

2. Natural Compounds:
   a. Plants
      Bracken Fern (Ptaquiloside6), Betel Nut (Arecoline7)
   b. Food Processing Products, Contaminants and Additives
      Nitroso Compounds8) [N-nitrosodimethylamine etc.], Alcoholic Beverages7), Urethane4)
      Mycotoxins5) [Aflatoxins Sterigmatocystin etc.] Potassium bromate9)

3. Industrial Chemicals
   Aromatic Hydrocarbons and Amines including β-Naphthylamine, Benzene4), Aniline, Benzidine, 4-Aminobiphenyl, & Vinyl Chloride Monomer
   Bis (chloroethyl) ether
   Pharmaceuticals: Cyclophosphamide, Melphalan, Chlorophosphazine, Phenacetin
   Diethylstilbestrol, Estrogens, Contraceptives etc.

   [M.Enomoto, An-Pyo, 2000]

1) IARC, 1987; Enomoto et al., 1990; Pitot and Dragan, 1996; Gold et al., 1998; Inoue, 1999.
2) Bold letter means positive for human evidence.
3) PAHs: polycyclic aromatic hydrocarbons.
5) Melnick et al., 1990.
9) Kurokawa et al., 1982; DeAngelo, 1998.
3. Future programs for detection of possible human carcinogens

Risk assessment should be based on the evaluation of state-of-the-art scientific evidence coupled with sound epidemiology studies and not speculation. High priority should be given to future research programs to identify and understand the mechanisms of human cancers. Five approaches could be considered in our quest to find new carcinogenic agents:

1) Clarification of pathogenic mechanisms of carcinogenic microorganisms would lead to prevention or better treatments and prevention of cancers of digestive organs in humans. Such biological agents include hepatitis C and B virus for hepatocellular cancer, Helicobacter pylori for stomach lymphoma or cancer and liver flukes for cholangiocellular cancer. Clinical experts on liver diseases in Japan consider the hepatitis virus to be the major cause for liver cancer in humans. The remainder, less than 10% of all liver cancers, arises from the consumption of alcoholic beverages. Cases of human liver cancer caused by man-made chemicals are extremely rare by agents such as thorotrast, oral contraceptives and vinyl chloride. Conversely, the main target organ for chemical carcinogens in animals is the liver, because it is the major organ of metabolism and/or activation and promotion of a number of chemical carcinogens. The reason for this difference between humans and experimental animals seems to be low chemical exposure of humans to environmental carcinogens (Choy, 1999). Effects of summation of environmental exposure levels seem to be far below doses needed to induce cancer (Berger et al., 1990).

2) Since there is little or no exposure data in humans on potent food-borne carcinogens that were investigated in animals, attention and further research should be geared to examine potent carcinogens of plant-origin including ptaquiloside, pyrrolizidine alkaloids (Hirono, 1993), argaritine (Shephard et al., 1995) and allyl isothiocyanate (NTP Technical Report, 1982), and for urethane (Imai et al., 1991; Waddel, 1993), potassium bromate (Kurokawa et al., 1982), and potent carcinogenic mycotoxins contaminating a variety of foods, including aflatoxin B1, sterigmatocystin and fumonisin B1 (Howard et al., 1999; Ueno, 2000).

3) Hormones including estrogens are well-known human carcinogens for cancers of the breast, endocrine and other reproductive organs (IARC, 1987). Therefore, more attention should be directed to their use in pharmaceuticals, since extremely high-dose levels (more than a million times the endogenous levels as compared with those of environmental contaminants) of these agents are being used routinely for therapy of human diseases. Human exposure to therapeutic hormones far

Table 5. Food-borne carcinogens. -Positive in animal carcinogenicity studies-

<table>
<thead>
<tr>
<th>Carcinogens of Plant-origin:</th>
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<tbody>
<tr>
<td>Bracken Fern: Warabi (Ptauquiloside)</td>
</tr>
<tr>
<td>Cycasin (Methylazoxymethanol-β-D-glucoside)</td>
</tr>
<tr>
<td>Pyrrolidine Alkaloids [Petasites japonicus Maxim:] Fuki-no-toh (Petasitenine), Colt's foot: Kab-to-ka (Senkirkin) etc. ]</td>
</tr>
<tr>
<td>Betel Nuts (Arecoline)</td>
</tr>
<tr>
<td>Saffrole (1'-Hydroxysafrole), Sesamol</td>
</tr>
<tr>
<td>Carrageenan, Caffeic Acid, Mustard (Allyl Isothiocyanate)</td>
</tr>
<tr>
<td>Mustroom: Agaritine (4-Hydroxymethylphenylhydrazine)</td>
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<tr>
<th>Carcinogenic Food Processing Products:</th>
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<tbody>
<tr>
<td>N-nitrosodimethylamine, N-nitrosopyrrolidine, N-nitrosopiperidine</td>
</tr>
<tr>
<td>Trp-P-1, Glu-P-1, Glu-P-2, MelQx</td>
</tr>
<tr>
<td>Alcoholic Beverages, Urethane (Ethyl Carbamate), Furfural</td>
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<tr>
<th>Carcinogenic Food Contaminants:</th>
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<tr>
<td>Mycotoxins: Aflatoxins, Sterigmatocystin, Ochratoxin A, Luteoskyrin, Ruglosin, Fumonisin B1</td>
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<tr>
<th>Carcinogenic Food Additives:</th>
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<tbody>
<tr>
<td>AF-2 (Furylfuramide), Saccharin Na, Auramine-O</td>
</tr>
<tr>
<td>Catechol 3R, Potassium Bromate</td>
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Vol. 25 No. 5

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M. ENOMOTO

exceeds, by order of magnitude, any such risk from environmental exposure to endocrine disrupters, of which there is much current concern, controversy and a great deal of allocation of limited resources. Awareness should also be made of the presence of estrogenic mycotoxins synthesized by *Fusarium* (Mirocha and Christensen, 1974) and phytoestrogens (Barrett, 1996) ubiquitously present in nature, before making rash judgements and generalizations on the severe effects of endocrine disruption and human health hazards of trace amounts of man-made, industrial endocrine disrupters.

4) Recent understanding of key principles that are fundamental to cancer induction and development are revealing complex, multi-step processes of tumor formation in altered control cells. Factors involved may include cellular proliferation, generation of reactive oxygen species, breakage of gap junctions, resistance to apoptosis, activation of telomerase, immunosuppressive effects, and other homeostatic failures of cellular integrity. Farber (1996) made the significant observation that the effects of dimethyl nitrosamine were quantitatively and qualitatively dissimilar in that it can act as a genotoxic carcinogen in the rat liver, in methylation of various macromolecules, induction of DNA damage and the induction of liver cell necrosis. Dose-related liver hypertrophy, mitogenesis, enzyme induction, and tumor formation were also clearly demonstrated in mechanistic studies on non-genotoxic carcinogens (Grasso and Hinton, 1991). Future challenges will involve additional efforts and approaches necessary to assimilate and integrate the advances in evolving technologies to elucidate the causation and ultimate prevention of human cancer as suggested by Weitzman and Yaniv (1999).

5) Detailed observations of experimental animals provide substantial evidence that heredity and environmental factors contribute to the development of tumors. However, the occurrence of spontaneous lesions seen in experimental animals must be understood and considered when making risk assessments of chemicals (Enomoto *et al.*, 1994). Nodular pulmonary lesions in dogs (Hajdu and Rona, 1965) and endometriosis in primates (Suzuki and Goto, 1993) is occasionally mis-diagnosed as induced lesions in analysis of the data obtained at low dose levels. Additional, more sophisticated approaches to understand the development mechanisms of the spontaneously occurring tumors in animals (Floyd, 1990; Maronpot *et al.*, 1995; Nakae, 1999; Iida, *et al.*, 2000), will be extremely useful in elucidating the occurrence of endogenous cancers of humans.

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Chemical agents causing human cancer.


M. ENOMOTO

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Vol. 25 No. 5

Vol. 25 No. 5
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392

M. ENOMOTO

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