

Implications for Risk Assessment of Suggested Nongenotoxic Mechanisms of Chemical Carcinogenesis

Ronald L. Melnick, Michael C. Kohn, and Christopher J. Portier

Laboratory of Quantitative and Computational Biology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Nongenotoxic carcinogens are chemicals that induce neoplasia without it or its metabolites reacting directly with DNA. Chemicals classified as nongenotoxic carcinogens have been assumed to act as tumor promoters and exhibit threshold tumor dose–responses. This is in contrast to genotoxic carcinogens that are DNA reactive, act as tumor initiators, and are assumed to exhibit proportional responses at low doses. In this perspective, we examine the basic tenets and utility of this classification for evaluating human cancer risk. Two classes of so-called nongenotoxic chemical carcinogens selected for review include cytotoxic agents that induce regenerative hyperplasia (trihalomethanes and inducers of α_2 -microglobulin nephropathy) and agents that act via receptor-mediated mechanisms (peroxisome proliferators and dioxin). Major conclusions of this review include: a) many chemicals considered to be nongenotoxic carcinogens actually possess certain genotoxic activities, and limiting evaluations of carcinogenicity to their nongenotoxic effects can be misleading; b) some nongenotoxic activities may cause oxidative DNA damage and thereby initiate carcinogenesis; c) although cell replication is involved in tumor development, cytotoxicity and mitogenesis do not reliably predict carcinogenesis; d) a threshold tumor response is not an inevitable result of a receptor-mediated mechanism. There are insufficient data on the chemicals reviewed here to justify treating their carcinogenic effects in animals as irrelevant for evaluating human risk. Research findings that characterize the multiple mechanisms of chemical carcinogenesis should be used quantitatively to clarify human dose–response relationships, leading to improved scientifically based public health decisions. Excessive reliance on oversimplified classification schemes that do not consider all potential contributing effects of a toxicant can obscure the actual causal relationships between exposure and cancer outcome. — *Environ Health Perspect* 104(Suppl 1):123–134 (1996)

Keywords: nongenotoxic carcinogenic mechanisms, regenerative hyperplasia, mitogenesis, receptor-mediated carcinogenesis, peroxisome proliferators, α_2 -microglobulin nephropathy

Introduction

The mechanism of carcinogenesis is not fully understood for any chemical, and knowledge of the mechanisms for nongenotoxic carcinogens is substantially less extensive than that for genotoxic carcinogens.

Definitions of nongenotoxic carcinogens are not always consistent in the scientific literature. A nongenotoxic chemical is a chemical that does not form DNA adducts, does not induce DNA repair, and is negative in

in vitro or *in vivo* tests for mutagenicity. Some authors consider a chemical to be nongenotoxic if it is negative in most short-term assays. Thus, genotoxic agents produce chemical alterations in DNA directly, whereas nongenotoxic agents are thought to indirectly stimulate hyperplastic or neoplastic responses. However, this definition does not preclude the possibility that a chemical is both DNA reactive and stimulates cell proliferation.

Classification systems based on labeling chemicals as genotoxic or nongenotoxic and on presumed mechanisms of action for each class lead to ambiguous reconstructions of the carcinogenic process. One motivation for such classification is that nongenotoxic carcinogens are thought to be less hazardous to human health than are genotoxic carcinogens. This view is based on the assumption that nongenotoxic carcinogens act as tumor promoters and exhibit threshold tumor dose–responses, whereas genotoxic carcinogens act as tumor initiators and exhibit proportional responses at low doses. The rationale for this assumption is that, by analogy with ionizing radiation, a single molecule of a genotoxic agent could, in theory, react with a cell's DNA and produce heritable changes in the genome of the affected cell. If an altered gene is involved in cellular differentiation or replication, such heritable changes could result in tumors. In the absence of direct effects of a nongenotoxic agent on DNA, it is assumed that exposure to the chemical leads to production of another substance which stimulates tumor development. Therefore, a minimal dose of the nongenotoxic agent would be required to accumulate a sufficient amount of the proximate carcinogen in the target tissue to produce a response.

In this paper, tumor promotion is used as an operational term referring to the pleiotropic changes in cellular differentiation and proliferation occurring during the clonal expansion of previously initiated cells. Chemicals that effect such changes have been classified as tumor promoters. However, this does not necessarily mean that the chemical affects the carcinogenic process solely through such activities. For example, a strong tumor promoter may elicit weak or indirect genotoxicity and weak tumor-initiating activity.

Animal studies demonstrate that tumor promoters can cause cancer in the absence of an initiating agent, and the existence or

Manuscript received 14 August 1995; manuscript accepted 18 January 1996.

Address correspondence to Dr. Ronald L. Melnick, Laboratory of Quantitative and Computational Biology, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709. Telephone: (919) 541-4142. Fax: (919) 541-1479.

Abbreviations used: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; $\alpha_2\mu$, α_2 -microglobulin; CHO, Chinese hamster ovary; PBPK, physiologically based pharmacokinetics; BDCM, bromodichloromethane; CDBM, chlorodibromomethane; TMP, 2,2,4-trimethylpentane; NBR, NIH Black Reiter; EHEN, *N*-methyl-*N*-hydroxyethylnitrosamine; DEHP, di(2-ethylhexyl)phthalate; DEHA, di(2-ethylhexyl)adipate; PPAR, peroxisome proliferator-activated receptor; Ah, aryl hydrocarbon; arnt, Ah receptor nuclear translocator; EGF, estrogen growth factor; TGF- α , transforming growth factor- α ; TSH, thyroid-stimulating hormone; UGT-1, UDP-glucuronosyltransferase; T₃, 3,5,3'-triiodothyronine; T₄, 3,5,3',5'-tetraiodothyronine.

absence of threshold dose–responses cannot be determined from current knowledge of carcinogenic mechanisms (1). More important, the fact that several non-mutagenic carcinogens have been found to be carcinogenic in experimental animals as well as in humans (e.g., benzene, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD], diethylstilbestrol, asbestos, arsenic) emphasizes the need to consider the nongenotoxic activities of these chemicals in evaluating human risk. Excessive reliance on oversimplified classification schemes to characterize the complex processes involved in chemical carcinogenesis can obscure the actual causal relationships between exposure and cancer outcome.

This review examines the potential role of several nongenotoxic activities suggested to be the critical factors for the carcinogenic effect of certain nongenotoxic carcinogens. Examples of classes of presumed nongenotoxic chemicals selected for review include cytotoxic agents that induce regenerative hyperplasia [trihalomethanes and inducers of α_2 -microglobulin ($\alpha_2\mu$) nephropathy] and agents that act via receptor-mediated mechanisms (peroxisome proliferators and TCDD). Investigations are proposed to help determine whether associations between these nongenotoxic activities and carcinogenicity represent causality and to clarify the nature of their dose–response relationships. Research findings that address assumptions in mechanistic hypotheses of chemical carcinogenesis can aid in reducing uncertainties in predictions of human risk and lead to improved scientifically based public health decisions.

Do “Nongenotoxic Carcinogens” Lack Genotoxic Activity?

As new data are generated on carcinogenic agents, classifications of chemicals into nongenotoxic or genotoxic categories may change. Two examples of liver carcinogens that had been thought to act by nongenotoxic mechanisms are cyproterone acetate and tamoxifen. Recent studies show that it would be misleading to limit evaluations of the carcinogenic potential of these chemicals by simply focusing on their nongenotoxic effects.

The induction of liver tumors in rats by the synthetic steroid cyproterone acetate has been attributed to a tumor-promoting activity because this steroid was not mutagenic in *Salmonella typhimurium* but did induce cell proliferation associated

with increased DNA synthesis and liver growth in rats (2). Recently, Schwarz and co-workers have shown that cyproterone acetate induces DNA repair synthesis in rat hepatocyte cultures, generates DNA adducts in rat hepatocytes after *in vitro* or *in vivo* exposures, and induces a dose-dependent increase in enzyme-altered (ATPase-deficient and γ -glutamyltranspeptidase-positive) hepatic foci in rats (3–5). The latter studies indicate that cyproterone acetate is genotoxic and has tumor-initiating activity. These studies demonstrate the need to understand and evaluate all activities of a chemical that may contribute to the carcinogenic process rather than simply identifying one of the biological effects of the chemical as the sole cause of tumor induction.

Tamoxifen, a nonsteroidal antiestrogen used in the treatment of breast cancer, is a strong liver carcinogen in rats (6,7). Because neoplastic changes were thought to result from hormonal perturbations and because this compound was not mutagenic in several *in vitro* assays [Ames *Salmonella* test, unscheduled DNA synthesis in HeLa cells, Chinese hamster ovary (CHO) cell *hprt* locus assay], tamoxifen was considered to be a rat-specific, nongenotoxic hepatocarcinogen (8). An extensive literature base shows that tamoxifen forms DNA adducts in the livers of rats, mice, and hamsters (9,10); it is activated to form DNA adducts by rat or human liver microsomes (11); it is clastogenic in human lymphoblastoid cells (12); and liver tumors in rats treated with tamoxifen have a high frequency of *p53* mutations (13). These genotoxic activities demonstrate that tamoxifen does not act simply as tumor promoter.

Cytotoxic Agents that Induce Regenerative Hyperplasia

The suggestion that cytotoxic agents may cause tumors due to chronic cell proliferation is based largely on the finding that some chemicals that do not appear to react with DNA cause cytotoxicity and regenerative hyperplasia in the same organ in which tumors develop after long-term chemical exposure (14–16). It has been hypothesized that DNA is more sensitive to damage during cell division and that increased rates of cell replication increase the probability of converting endogenous DNA damage into mutations by reducing the time available for DNA repair.

In this review cell replication is synonymous with cell division. Chemically induced cell proliferation denotes an

increase in the number of a specific type of cell in a treated animal due to an increased rate of cell division relative to the rate of cell loss. Replicative DNA synthesis commonly has been evaluated by measurement of the fraction of cells incorporating bromodeoxyuridine or tritiated thymidine into DNA during S-phase of the cell cycle (S-phase labeling index). It should be noted that the S-phase labeling index would not be identical to the cell division rate when replication of DNA does not progress to formation of two viable daughter cells.

The debates over how and to what extent cell proliferation influences the carcinogenicity of nongenotoxic chemicals are complicated by the fact that cell replication is an integral component of the carcinogenic process. Indeed, cell division can fix promutagenic DNA damage into heritable mutations, and cell replication occurs during the clonal expansion of premalignant cells. However, it has not been established that the carcinogenic outcome in most tissues is determined by the cell division rate (17–19). The general view at an international symposium on cell proliferation and chemical carcinogenesis was that although cell replication is involved inextricably in the development of cancers, chemically enhanced cell division does not reliably predict carcinogenicity (20).

Several factors influence the predictability of cell proliferation for carcinogenesis. These include *a*) consistency and specificity within a large database of chemical carcinogens and noncarcinogens; *b*) quantitative correspondence between the dose–response curves for cell proliferation and tumor incidence under similar experimental conditions; and *c*) persistence of the proliferative response (21). If sustained cell proliferation is the sole determinant of the carcinogenicity of nongenotoxic chemicals, then equivalent site-specific increases in cell division rate by different chemicals must produce the same tumor response. However, the available data are too sparse to either support or refute this hypothesis.

The importance of a sustained increase in S-phase DNA labeling is illustrated in studies of phenobarbital. Dietary administration of this drug produced a 4- to 5-fold increase in hepatic DNA synthesis in rats after 3 days of treatment, and this rate returned to control levels by day 5 (22). However, this transient response was not sufficient to account for the promoting effect of phenobarbital because prolonged exposure (>100 days) was required to promote 2-acetylaminofluorene-initiated liver

lesions (23). The long-term inhalation studies of tetranitromethane are also instructive with respect to the role of regenerative hyperplasia in chemical carcinogenicity. Tetranitromethane, which is mutagenic in *Salmonella* and induces chromosomal aberrations and sister chromatid exchanges in CHO cells, produced high incidences of benign and malignant lung neoplasms in both sexes of rats and mice at exposure concentrations ranging from 0.5 to 5 ppm (24). These exposures also produced high incidences of hyperplasia of the respiratory epithelium in rats and mice without inducing tumors of the nasal cavity in either species. Thus, even for a potent mutagen and carcinogen, chronic irritation and regenerative hyperplasia are not predictive of a carcinogenic response.

Trihalomethanes

The induction of liver tumors in female mice treated with chloroform by gavage in corn oil was suggested to be due to cytotoxicity and regenerative hyperplasia resulting from the metabolism of this trihalomethane to a reactive toxic intermediate (25). Both doses of chloroform used in the bioassay of this chemical caused centrilobular hepatocyte necrosis, increases in serum activities of the liver enzymes alanine aminotransferase and sorbitol dehydrogenase, and increases in replicative DNA synthesis in the liver.

A 2-year study in female mice exposed to chloroform at doses up to 1800 ppm in drinking water (a similar daily dose as the gavage study) did not induce liver tumors (26). Because these doses did not cause hepatotoxicity or produce an increase in the S-phase labeling index, Larson et al. (25) suggested that the rate of oxidative metabolism of chloroform in the livers of these animals was insufficient to cause regenerative hyperplasia consequent to cytotoxicity. They concluded that lower oxidative rates in the drinking water study, leading to less cell replication, could explain the difference in the cancer responses by the two routes of exposure. Consistent with this hypothesis, the physiologically based pharmacokinetic (PBPK) model of Corley et al. (27) predicted the peak rates of metabolism of chloroform in the liver to be considerably higher in female mice given a single carcinogenic dose of chloroform by gavage than in mice exposed to a comparable daily dose in the drinking water.

Inhalation exposure of female mice to 30 ppm chloroform did not cause

hepatocellular necrosis either, but unlike the drinking water exposure, it did produce a 7-fold increase in the hepatocyte labeling index relative to controls (28). However, the rate of hepatic metabolism of chloroform predicted by the PBPK model in mice exposed to 30 ppm by inhalation was less than the predicted rate of metabolism in mice exposed to 1800 ppm chloroform in the drinking water (J Robert Buchanan, personal communication). The inconsistency between the experimental data and model predictions for the drinking water and inhalation studies demonstrates that either liver metabolism of chloroform does not predict the cell replication rate or the PBPK model is incorrect.

Mutagenicity was not considered to be an important contributing factor in the carcinogenicity of chloroform because the results of the majority of genotoxicity studies on this chemical were negative. Rosenthal (29) contends that there is some evidence of genotoxicity due to chloroform and that many of the short-term tests on this chemical were inconclusive. Chloroform induced sister chromatid exchanges in human lymphocytes *in vitro* and in mouse bone marrow cells *in vivo* (30), gene conversion and mitotic crossover in *Saccharomyces cerevisiae* (31), and elicited low-level binding to calf thymus DNA in the presence of rat liver microsomes (32).

Trihalomethanes as a class are metabolized by a microsomal cytochrome P450-dependent monooxygenase to reactive dihalocarbonyl intermediates (33). The hepatotoxicity of chloroform appears to be related to its metabolism, presumably due to the covalent binding of its metabolite phosgene to cellular macromolecules, leading to cell death (34,35). Pretreatment of rats with phenobarbital enhanced the metabolism and the hepatotoxicity of chloroform, while cysteine treatment was protective against chloroform-induced hepatocellular necrosis.

Because of the large energy difference between C-Br (54 kcal/mol) and C-Cl (78 kcal/mol) bonds (36), bromodichloromethane (BDCM) should be almost exclusively metabolized to the same dihalocarbonyl as that formed from chloroform. Thus, it might be expected that the toxicological effects of BDCM would mimic those of chloroform. When the doses of chloroform, BDCM, or chlorodibromomethane (CDBM) used in the carcinogenicity studies of these trihalomethanes were expressed as mol/kg/day, a composite dose-response plot for liver neoplasms

produced in female B6C3F1 mice by these three chemicals revealed a relationship suggestive of a possible common mechanism of tumor induction (37). Neither BDCM nor CDBM caused hepatocellular necrosis in the 2-year studies or in the 13-week studies at considerably higher doses. Thornton-Manning et al. (38) confirmed the lack of histopathological changes in the livers of female mice exposed to doses of BDCM that produced high incidences of liver tumors in the 2-year study of this chemical. Thus, overt toxicity followed by regenerative hyperplasia is not the sole determinant of the liver tumor response for this group of chemicals. The elucidation of the mechanisms of tumor development by cytotoxic chemicals requires much greater knowledge than that which can be obtained from measurements of S-phase labeling alone.

Inducers of α_2 -Microglobulin Nephropathy and Kidney Cancer in Male Rats

The current hypothesis on the role of $\alpha_2\mu$ in chemically induced kidney cancer is based on the observed accumulation of protein droplets containing $\alpha_2\mu$ in epithelial cells of the proximal convoluted tubules of male rats exposed to hydrocarbons that have been reported to cause kidney cancer in male rats after long-term exposure. $\alpha_2\mu$ is a low molecular weight protein (18.7 kDa) synthesized in the liver of male rats under androgenic control (39). It is not synthesized by hepatocytes of female rats, mice of either sex, or several other species including humans. Hydrocarbons or their metabolites that bind reversibly to $\alpha_2\mu$ do not increase the level of hepatic synthesis of this protein (40).

α_2 -Microglobulin is secreted into the blood, filtered through the glomerulus, and partially reabsorbed (50%) by endocytosis into proximal tubule epithelial cells of the P2 segment (41). The unabsorbed fraction is excreted in the urine while the reabsorbed portion is presumably hydrolyzed to amino acids after fusion of endocytotic vesicles with epithelial cell lysosomes. The accumulation of protein droplets containing $\alpha_2\mu$ was suggested to be due to reversible binding of xenobiotic ligands to this protein, rendering it more resistant to proteolytic degradation (42,43).

The accumulation of $\alpha_2\mu$ is hypothesized to cause lysosomal dysfunction resulting in cell killing (42). The actual cause of cell death is not known. Sloughing of necrotic epithelial cells into the tubule

lumen has been observed, and granular casts of necrotic cellular debris accumulate at the junction of the P3 segment of the proximal tubule and the thin loop of Henle. Regenerative proliferation of epithelial cells in the P2 segment occurs in response to the cell loss (44-46).

Although the mechanistic link between cell proliferation and kidney cancer is unknown, it has been suggested that regenerative hyperplasia causes the tumorigenic response in the male rat kidney by increasing the likelihood of fixing presumed spontaneous cancer-initiating DNA damage into heritable mutations or by promoting the clonal expansion of spontaneously initiated cells (46,47). 2,2,4-Trimethylpentane (TMP), one of the most active nephrotoxic components in unleaded gasoline (48), has been used as a model compound to study the mechanism of $\alpha_2\mu$ nephropathy.

Data that Support the $\alpha_2\mu$ Hypothesis.

Nongenotoxic chemicals that induce $\alpha_2\mu$ accumulation and renal carcinogenesis in male rats have not been shown to induce kidney tumors in animals that lack the ability to synthesize $\alpha_2\mu$ in the liver (e.g., female rats or mice of either sex).

Chemicals that induce $\alpha_2\mu$ accumulation in male F344 or Sprague-Dawley rats do not induce protein droplet nephropathy in male NIH Black Reiter (NBR) rats (49), a strain deficient in hepatic $\alpha_2\mu$ synthesis.

Chemicals (or one of their metabolites) that bind reversibly to $\alpha_2\mu$ induce $\alpha_2\mu$ accumulation in the male rat kidney (50).

In vitro lysosomal degradation of $\alpha_2\mu$ decreased in the presence of chemicals (or their metabolites) that induce $\alpha_2\mu$ accumulation (43).

S-phase labeling index in the P2 segment of renal proximal tubules was increased in male rats exposed to several chemicals or chemical mixtures that induce $\alpha_2\mu$ accumulation. For unleaded gasoline, the dose dependency for the renal epithelial cell labeling index is similar to that of the kidney tumor response (44,45).

Unleaded gasoline and *d*-limonene elicited tumor-promoting activity in the kidney of male F344 rats initiated with *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) (46,47), whereas *d*-limonene did not promote renal carcinogenesis in EHEN-initiated male NBR rats (46). Because 2-year carcinogenicity studies have not been performed with NBR rats, it is not clear that the two strains would respond similarly.

Data that Are Inconsistent with the $\alpha_2\mu$ Hypothesis. Gabapentin and lindane

induced $\alpha_2\mu$ accumulation and nephropathy in male rats at doses that did not increase kidney tumor incidences (51-53).

Binding of xenobiotic ligands to $\alpha_2\mu$ alone does not account for the accumulation of this protein or the tumor response in the kidney of male rats because *a*) most of the $\alpha_2\mu$ in the kidney of male rats is not ligand bound (54-57); *b*) the trimethylpentanoic acid metabolites of TMP do not bind to $\alpha_2\mu$, but cause accumulation of this protein in the kidney of male F344 rats (58); *c*) binding affinities vary by 1000-fold for chemicals that induce $\alpha_2\mu$ accumulation (50). Isophorone has a 50-fold higher binding affinity than 1,4-dichlorobenzene or its metabolite 2,5-dichlorophenol; yet isophorone produces a similar dose-dependent carcinogenic response as 1,4-dichlorobenzene in the male rat kidney; *d*) inhibition of lysosomal degradation of $\alpha_2\mu$ was similar for chemicals that have binding affinities for $\alpha_2\mu$ that vary by 2 to 3 orders of magnitude (43).

Foci of chronic progressive nephropathy, renal tubular lesions appearing in control F344 rats by 20 weeks of age, have cellular replication rates that are higher than those of P2 proximal tubule cells in male rats exposed to chemicals that induce $\alpha_2\mu$ accumulation (45). However, the incidence of spontaneous kidney tumors in untreated male rats is low (less than 0.6%) even when held to 146 weeks of age (59). In addition, European high test gasoline increased the renal cell labeling index (60) but did not induce renal carcinogenesis in male rats (Cesare Maltoni, personal communication). Thus, high rates of cell replication alone are not predictive of kidney cancer.

Except for *d*-limonene, the chemicals that induced $\alpha_2\mu$ accumulation and kidney carcinogenesis in male rats also induced cancer at other sites; mouse liver cancer was the most common (57). This finding suggests that other factors are involved in the carcinogenicity of these chemicals.

Although cell replication is a basic component of multistage carcinogenesis, there are no data demonstrating that the carcinogenic outcome in the kidney is determined by the cell division rate (61). There is no adequate database relating level of cell proliferation to renal tumor response in male rats (57).

TMP, the model compound upon which the hypothesis linking $\alpha_2\mu$ accumulation with kidney cancer in the male rat is based, did not produce kidney tumors in male rats after lifetime exposure (Cesare Maltoni, personal communication).

An Alternative Hypothesis. The physiological function of $\alpha_2\mu$ is unknown. Because hydrophobic chemicals bind to this protein, it may serve as a carrier for the urinary excretion of pheromones or other lipophilic ligands. In the liver of male rats where this protein is synthesized, intermediary metabolites of certain nephrotoxic agents may bind to $\alpha_2\mu$ and thereby be shielded from activating and detoxicating reactions (57). The $\alpha_2\mu$ -ligand complex is then transported to the kidney. In female rats there is a greater tendency to form conjugated products of TMP metabolites (54), probably because of the lack of hepatic synthesis of $\alpha_2\mu$. Information on the site of interaction between ligand and $\alpha_2\mu$ and on the transport of this complex to the kidney is needed for mechanistic models that address the role of $\alpha_2\mu$ in chemically induced nephropathy.

Following reabsorption in renal proximal tubule cells in male rats, the ligand (e.g., 2,2,4-trimethylpentanol in rats exposed to TMP) may be released from the $\alpha_2\mu$ xenobiotic complex. That metabolite or a subsequent metabolite, e.g., 2,2,4-trimethylpentanal or 2,4,4-trimethylpentanoic acid, may be cytotoxic to renal tubular epithelial cells. As noted above, the trimethylpentanoic acid metabolites of TMP do not bind to $\alpha_2\mu$ but do cause accumulation of this protein in the kidney of male F344 rats (58). Protein accumulation may be due to inhibition of proteolysis of $\alpha_2\mu$ by one of the metabolites similar to the effect of leupeptin, an inhibitor of lysosomal proteolysis. By this alternative mechanism, $\alpha_2\mu$ is not the primary cause of nephrotoxicity resulting from exposure to chemicals such as TMP; rather, the accumulation of this protein is a result of a chemically induced toxic response in the kidney. Tumor response may be a consequence of the $\alpha_2\mu$ -mediated delivery and concentration of the ligand in the male rat kidney. The ligand or one of its metabolites would then be the actual carcinogenic agent. If $\alpha_2\mu$ influences the delivery of a toxicant to the kidney, then extrapolations across species should adjust for differences in delivered dose (i.e., concentration of unbound ligand) to the target organ instead of dismissing the effects in male rats as irrelevant to humans. Cytotoxic chemicals may also reach the kidney without binding to $\alpha_2\mu$ and cause accumulation of this protein secondary to their cytotoxic effects (e.g., 2,2,4-trimethylpentanoic acid or leupeptin). In these cases, other physiological or metabolic differences

between species may affect the target organ (kidney) dosimetry.

α_2 -Microglobulin is a member of a superfamily of small homologous proteins that appear to serve as carriers for small lipophilic ligands (62). Although Lehman-McKeeman and Caudill (63) did not detect binding of *d*-limonene-1,2-oxide or 2,4,4-trimethyl-2-pentanol to two human-derived proteins in this family, protein-1 and the glycosylated form of α_1 -acid glycoprotein, that study should not be considered an exhaustive search for a ligand-binding human protein that might affect delivery of toxicants to the kidney.

Conclusions on $\alpha_2\mu$ Nephropathy and Kidney Cancer. Mechanisms of hydrocarbon-induced nephropathy and renal carcinogenesis are not well understood. Accumulation of $\alpha_2\mu$ in the kidney of male rats may occur by two different mechanisms: ligand binding to this protein rendering it more resistant to proteolytic degradation (42,43) or direct inhibition of the proteolytic enzymes that degrade this protein (57). Currently available data do not allow discrimination between these possibilities. According to the alternative hypothesis, $\alpha_2\mu$ facilitates the transport of the protease inhibitor or its precursor (e.g., 2,4,4-trimethylpentanoic acid or 2,2,4-trimethylpentanal from 2,2,4-trimethyl-2-pentanol) to the kidney, or protease inhibitors reach the kidney without binding to $\alpha_2\mu$ (e.g., direct administration of 2,2,4-trimethylpentanoic acid or leupeptin).

The hypothesis that kidney tumors in male rats are a direct consequence of accumulation of $\alpha_2\mu$ implies that the male rat kidney response is a poor model for potential human responses to inducers of $\alpha_2\mu$ nephropathy. The alternative hypothesis that $\alpha_2\mu$ merely serves to concentrate the carcinogenic agent or its precursor in the male rat kidney implies that $\alpha_2\mu$ shifts the kidney cancer response of an $\alpha_2\mu$ ligand to lower exposures than those which would produce equivalent tissue doses of the proximate carcinogen in female rats or other species. If, as specified in the alternative hypothesis, ligand binding to hepatic $\alpha_2\mu$ precludes further metabolism, then the unbound chemical in animals that do not synthesize $\alpha_2\mu$ may produce tumors at sites other than the kidney. The finding of mouse liver cancers induced by ligands of $\alpha_2\mu$ supports this concept. Until the mechanism(s) of renal carcinogenesis is more fully understood, it would be inappropriate to accept or reject either hypothesis.

Research addressing the deficiencies and inconsistencies in the hypotheses relating induction of $\alpha_2\mu$ nephropathy with kidney cancer should lead to a better understanding of the processes involved.

Agents that Act via Receptor-mediated Mechanisms

Peroxisome Proliferators

Peroxisomes are subcellular organelles that contain several oxidase enzymes that produce H_2O_2 and catalase, the enzyme that converts this toxic product to water and oxygen. During the past 20 years an increasing number of structurally unrelated compounds, including hypolipidemic drugs and industrial plasticizers, have been shown to produce an increase in liver size, a marked increase in size and proliferation of hepatic peroxisomes, peroxisome enzyme induction, a decrease in serum lipid levels, and increased incidences of hepatocellular neoplasms in rats and mice. The non-neoplastic changes revert back to control levels shortly after exposure to these chemicals ceases.

Correlation with Hepatocarcinogenesis. Based on an apparently strong correlation between peroxisome proliferation and hepatocarcinogenesis, Reddy et al. (64) proposed that hypolipidemic peroxisome proliferators may represent a novel class of chemical carcinogens. Ashby et al. (65) have recently prepared a compilation of the scientific literature on peroxisome proliferators. They found among the chemicals they examined an 80% correlation between peroxisome proliferation and hepatocarcinogenesis in rats and in mice. Such correlations are not proof of a causal relationship between the two responses, and some exceptions have been observed that have not been reconciled in a unified hypothesis. For example, similar levels of peroxisomal induction were observed (66) in rats exposed to di(2-ethylhexyl)phthalate (DEHP) and di(2-ethylhexyl)adipate (DEHA) at doses comparable to those used in the bioassays of these chemicals (67,68). However, DEHP but not DEHA gave a positive liver tumor response in the 2-year studies in rats (67,68).

If peroxisome proliferation alone causes hepatocarcinogenesis, similar levels of peroxisome proliferation should lead to similar liver tumor incidence. However, this is not always the case. At doses of DEHP and Wy-14,643 that produce similar levels of peroxisome proliferation in rats, Wy-14,643 produced an earlier and much greater liver

tumor response than did DEHP (69). In an evaluation of the carcinogenicity of tetrachloroethylene, an expert panel of the International Agency for Research on Cancer concluded that the weak induction of peroxisome proliferation by this chemical in mice was not sufficient to explain the high incidence of liver tumors observed in an inhalation bioassay (70).

Genotoxicity. Peroxisome proliferators, for the most part, lack genotoxic activity. However, when a consistent genotoxic effect is detected for a specific peroxisome proliferator, then that activity cannot be dismissed as unimportant in the carcinogenic process. For example, Wy-14,643, a potent peroxisome proliferator, induced sister chromatid exchanges and micronuclei formation in primary cultures of both rat and human hepatocytes (71), and several peroxisome proliferators induce morphological transformation of Syrian hamster embryo cells (72). Nafenopin and ciprofibrate, but not DEHA, induced sister chromatid exchanges, chromosomal aberrations, and micronuclei in rat hepatocytes (73). Thus, the combination of clastogenicity and/or cell-transforming activity and peroxisomal enzyme induction may contribute to the carcinogenicity of several of the peroxisome proliferators. Hagi et al. (74) reported that the frequency and spectrum of ras gene mutations observed in ciprofibrate-induced liver tumors were different from that in spontaneous liver tumors, indicating that this peroxisome proliferator does not act simply by promoting spontaneous preneoplastic lesions in mice.

Oxidative Stress. The peroxisomal oxidation system has received much attention regarding the mechanism of hepatotoxicity of peroxisome proliferators because the initial step catalyzed by fatty acyl-CoA oxidase produces H_2O_2 by electron transfer to oxygen. In the livers of rats or mice treated with peroxisome proliferators, fatty acyl-CoA oxidase activity is increased 5- to 20-fold, whereas catalase activity is increased by less than 2-fold. Thus, Reddy and Lalwani (75) proposed that the imbalance between production and degradation of H_2O_2 due to enhanced peroxisomal oxidation could lead to an increase in H_2O_2 -mediated oxidative damage and carcinogenesis. Increased levels of hydroxyl radical generated from H_2O_2 may produce tumors due to reactivity of this oxidant with DNA.

In support of this hypothesis, Rao et al. (76) reported that the hepatocarcinogenicity of ciprofibrate was inhibited by simultaneous chronic administration of

either of the anti-oxidants ethoxyquin or 2(3)-*t*-butyl-4-hydroxyanisole. Furthermore, steady-state concentrations of H₂O₂ were increased in liver homogenates prepared from animals treated with peroxisome proliferators, and increased accumulation of lipofuscin in liver parenchymal cells and increased levels of conjugated dienes in hepatic lipid fractions were detected in rats after long-term administration of peroxisome proliferators (77-79). Increases in 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, have been reported in liver DNA of rats after long-term exposure to several peroxisome proliferators (80,81). However, these increases may be limited to extranuclear (mitochondrial) DNA (81), and such lesions may not be directly involved in the carcinogenic process (81).

Cell Proliferation. Increased replicative DNA synthesis and cell division have also been suggested as the mechanisms of peroxisome proliferator-mediated carcinogenesis (69,82). Cell proliferation has been suggested to be causally associated with tumor development by increasing the likelihood of fixing spontaneous cancer-initiating DNA damage into heritable mutations and/or by promoting the clonal expansion of spontaneously initiated cells (14,15,82). Although cell replication is an integral component of chemical carcinogenesis, current scientific data do not substantiate the hypothesis that the induction of cancer by nongenotoxic carcinogens occurs solely by enhancement of cell division (83). Peroxisome proliferators have other effects such as inhibition of apoptosis, increased oxidative stress, and expression of oncogenes (83). It is likely that these effects make important contributions to the carcinogenic process.

S-phase-labeling indices are markedly increased in the livers of rats and mice during the first 1 to 2 weeks of treatment with all peroxisome proliferators. Subsequently, the rate of replicative DNA synthesis returned to control levels in rats chronically treated with DEHP, but the rate remained elevated for 1 year in rats treated with Wy-14,643 (69). The sustained increase in hepatocyte replication corresponded empirically to the more potent carcinogenicity of Wy-14,643 in this species. Persistent increased replicative DNA synthesis was not detected with nafenopin, clofibrate, DEHP, or ciprofibrate (84-86). With these agents, replication rates returned to control levels within 10 to 30 days of continuous treatment.

Thus, the sustained cell replication rate due to treatment with Wy-14,643 does not apply for all peroxisome proliferators, including compounds that are potent liver carcinogens and potent peroxisome proliferators. This issue is important because transient stimulation of hepatocyte proliferation by nongenotoxic carcinogens is not sufficient to induce cancer or promote liver tumor development (23). The finding that enzyme-altered hepatic foci were not induced in rats fed Wy-14,643 for 3 weeks followed by partial hepatectomy (87) indicates that early high levels of replicative DNA synthesis and peroxisome proliferation are not sufficient activities for initiation of hepatocarcinogenesis.

The Peroxisome Proliferator-Activated Receptor. The discovery of the peroxisome proliferator-activated receptor (PPAR) (88), a ligand-activated intracellular transcription factor, provides a mechanistic basis for understanding how peroxisome proliferators modulate gene expression leading to induction of peroxisomal enzymes. Ligand binding activates this receptor, which subsequently forms a heterodimer with the retinoid X receptor. It is this ternary complex which binds to specific DNA response elements, causing transcriptional activation of genes coding for peroxisomal enzymes (89,90). Humans possess PPAR subtypes, including one that shows high homology with rodent PPAR- α and that can be activated by peroxisome proliferators (91). It is not known whether a peroxisome proliferator (or one of its metabolites) binds directly to the receptor or whether receptor activation is mediated by changes in cellular levels of an endogenous ligand (e.g., fatty acid or fatty acyl-CoA). Further research is needed on binding of exogenous and endogenous ligands to PPAR subtypes in rodent and human hepatocytes, dose-response comparisons of the transcriptional activation of peroxisomal genes in rodent and human hepatocytes, regulation of PPAR activity, and interindividual variability of PPAR in human populations.

Effects in Humans. The fact that hypolipidemia, one of the pleiotropic effects of peroxisome proliferators in rodents, is also induced by these drugs in humans demonstrates that humans are responsive to these chemicals. Moderate increases in peroxisome number or volume density have been observed in patients taking clofibrate or ciprofibrate (65). Induction of peroxisome proliferation in human hepatocyte cultures could not be

demonstrated. This difference between *in vivo* and *in vitro* behavior may be related to culturing conditions, as insulin inhibits and dexamethasone stimulates fatty acid-induced transcription of PPAR and peroxisomal enzymes in rat hepatocytes both *in vivo* and *in vitro* (92). Effects of these factors in human hepatocytes need to be investigated.

No adequate epidemiological studies have been reported on the potential carcinogenicity of hypolipidemic peroxisome proliferators in humans. Because of the greater sensitivity of biochemical assays compared to epidemiological studies, the variability in human response, and the rapid regressive changes that occur once treatment is stopped, a detailed study is needed on changes in human hepatic peroxisomal enzyme activities before and during treatment with hypolipidemic peroxisome proliferators. Comparison of such information with the effectiveness of these agents in lowering serum lipid levels could provide a better measure of the sensitivity of humans to these chemicals.

Conclusions on Peroxisome Proliferation and Liver Cancer. The mechanism by which peroxisome proliferators induce liver cancer is not understood; however, several hypotheses have been advanced. Because peroxisome proliferation is one of several changes produced by these chemicals, it is not possible to conclude that peroxisome proliferation alone is the cause of liver cancer. In fact, there may not even be a unifying mechanism for this group of chemicals, i.e., the dose-dependent carcinogenic outcomes may involve contributions from several activities of which peroxisome proliferation represents one possible factor. The differential induction of peroxisomal enzyme activities may simply shift or alter the shape of the cancer dose-response curves for these chemicals. Further research is needed to identify the contribution made by peroxisome proliferation to hepatocarcinogenesis.

Dioxin and the Ah Receptor

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin has been implicated in the etiology of soft-tissue cancers at several sites in a number of species, including humans (93). There is no convincing evidence that TCDD has genotoxic activity (93); rather, it has long been thought that TCDD acts solely as a tumor promoter (94). Biological effects of TCDD, its congeners, and other polychlorinated aromatic hydrocarbons are mediated by binding to and activating the Ah

(aryl hydrocarbon) receptor. The activated Ah receptor forms a heterodimer with another transcription factor (Ah receptor nuclear translocator, arnt), and this ternary complex binds to regulatory sequences on DNA and alters the expression of several proteins (95). Some of these proteins may be involved in the carcinogenic response. It has been suggested (96) that the dose-response curve for tumor incidence consequent to exposure to TCDD may show appreciable sigmoidicity, owing to insufficient occupancy of the Ah receptor at low doses. In that case, TCDD-induced cancers might exhibit a threshold below which no effects of dioxin would occur (97). The large amount of data available on responses of laboratory animals to treatment with dioxin provides an opportunity to test the hypotheses that TCDD is purely a tumor promoter and that the Ah receptor-mediated tumor dose-response exhibits threshold behavior.

In order to identify conditions under which threshold responses to TCDD are possible, several PBPK models of its disposition in the rat have been constructed (e.g., 98,99). These models include absorption of TCDD from the gut, its distribution to tissues, its metabolic clearance from the liver, and alterations in the concentrations of several hepatic proteins which are candidates for biomarkers of TCDD's effects. The rates of induction of the proteins were assumed to follow saturation kinetics with respect to the concentration of the Ah-TCDD complex. A dose-response curve whose slope approaches zero as the dose approaches zero was assumed to be evidence of a threshold. Such behavior might provide a rationale for deviating from linear extrapolations of cancer risk from low-dose TCDD exposures. The following discussion is based on the PBPK model of Kohn et al. (98), as it is the only one of the existing TCDD models to extend beyond dosimetry and propose carcinogenic mechanisms.

Enzyme Induction. The model of Kohn et al. reproduced the measured concentrations of dioxin in the liver and blood after 31 weeks of biweekly oral dosing with TCDD (100) and matched the liver and fat concentrations for a number of other dosing scenarios. The model also reproduced the observed hepatic concentrations of cytochromes P4501A1 and P4501A2 (CYP1A1 and CYP1A2) and the Ah, estrogen, and epidermal growth factor (EGF) receptors after 31 weeks of exposure. The computed response of each of

these proteins was proportional to administered TCDD at dose rates up to 10 ng/kg/day. Because CYP1A1 is constitutively expressed in liver only at very low levels and TCDD induces this protein by about 200-fold, this response is a good biomarker of dioxin exposure. The proportional response of CYP1A1 at low doses argues against the existence of a threshold for effects mediated by the Ah receptor. Allowing for sigmoidicity in the rate of expression of this protein, which would produce a threshold response, did not improve the fit to the data. This indicates that sigmoidal kinetics is not required to reproduce the observed dose-response.

When data on CYP1A1 mRNA levels in TCDD-treated rats became available (101), the model was extended to include two steps in expression: transcription of the gene and translation of the message into protein (102). Several mechanistic models were compared with the experimental data (101). The model which best fit the data included high-affinity and low-affinity binding sites for the Ah-TCDD-arnt complex; occupancy of both sites was required for transcriptional activation of the *CYP1A1* gene. The model predicted a response of message production that was sublinear at low doses and a response of protein synthesis that was supralinear at low message level, indicating that the proportional expression of *CYP1A1* is the net response of these two processes. These results show that a threshold response is not an inevitable result of a receptor-mediated mechanism. Even if the initial response does show a threshold, subsequent events leading to the final outcome can compensate for this sublinearity.

It could be argued that the computed behavior for CYP1A1 induction is an artifact of the choice of model specification; a different mathematical representation may lead to a different predicted dose-response. The model of Kohn et al. (98) included the increase in ligand binding capacity of the hepatic Ah receptor observed with increasing dose of dioxin (103). Because dioxin increases the Ah receptor binding capacity in liver, the concentration of the Ah-TCDD complex is predicted to rise more rapidly with dose than would be predicted by models that neglect this effect. The PBPK model of Andersen et al. (99) ignored this effect and found that sigmoidal kinetics best described the relationship between the concentration of the Ah-TCDD complex and the observed production of CYP1A1. Sigmoidal kinetics predicts

a steeper rise in protein production with increasing dose than does a model with hyperbolic response, and such steep kinetics imitates the effect of increases in Ah receptor binding capacity. Models that do not represent all of the pertinent biological events may give unreliable results.

Induction of a Growth Factor. Because there is no evidence that CYP1A1 is involved in the carcinogenic action of TCDD, the hypothesis that production of liver tumors in female rats by TCDD is due to promotion mediated by an induced hepatic growth factor was examined with the model. TCDD down-regulates the hepatic plasma membrane EGF receptor without altering the transcription of its gene into mRNA *in vivo* (104). Binding of peptide ligand to the EGF receptor causes its internalization. The internalized receptor's tyrosine kinase activity initiates a cascade of events leading to increased cell replication (105).

The liver does not produce EGF, but it does produce transforming growth factor- α (TGF- α), another ligand of the EGF receptor (106). This peptide was treated as the induced growth factor in the model of Kohn et al. (98). TCDD induces TGF- α in tissues such as keratinocytes (107), but it does not increase mRNA for TGF- α in rat liver (100). This result suggests that either the proposed increase in TGF- α is mediated by post-transcriptional events, a different EGF-like peptide is the induced ligand of the EGF receptor in liver, or a growth factor ligand of the hepatic EGF receptor is not involved in production of liver tumors by TCDD.

The model predicts concentrations of TGF- α in the extracellular fluid comparable to those observed in cell cultures (107). It predicts internalization of EGF receptors consequent to ligand binding which accounts for loss in plasma membrane receptor activity (108). The computed loss of EGF binding capacity is proportional to dose in the low-dose region, arguing against a threshold for a response that may be mechanistically linked to cell proliferation caused by dioxin.

Oxidative DNA Damage. The model also reproduces the observed induction of CYP1A2 by TCDD, and the computed response is also proportional to dose at low doses. This enzyme converts estradiol to an A-ring hydroquinone (109). Oxygen can convert the hydroquinone to a semiquinone free radical, forming superoxide radicals (110), and also to a benzoquinone. These materials can cause DNA damage

(111,112). The PBPK model includes estrogen metabolism and predicts considerable hydroxylation of estradiol by CYP1A2, suggesting that TCDD may also induce DNA damage. A stochastic clonal growth model of the dose-response of the size distribution of enzyme-altered focal lesions in livers of TCDD-treated rats (113) is consistent with secondary mutagenic activity following TCDD exposure. Thus, substances that are considered to be purely tumor promoters may also act as initiators by indirect mechanisms. Separation of carcinogens into classes of genotoxic initiators and nongenotoxic promoters may be an inappropriate and misleading simplification of the complex processes involved in chemical carcinogenesis.

Promotion of Thyroid Tumors.

Another hypothesis explored is that induction of thyroid tumors in mice and rats by TCDD is due to promotion by chronic overstimulation of the thyroid by thyrotropin (thyroid-stimulating hormone, TSH). Dioxin, like other Ah receptor agonists, induces an isoform of UDP-glucuronosyltransferase (UGT-1) by an Ah receptor-dependent mechanism (114). This enzyme conjugates thyroxine (3,5,3',5'-tetraiodothyronine, T_4), leading to its clearance. Metabolism of T_4 and its consequent depletion from the blood relieves inhibition of TSH release from the pituitary by circulating T_4 and causes the serum TSH concentration to rise. As this mechanism commences subsequent to binding of TCDD to the Ah receptor, alterations in serum hormone levels by dioxin should exhibit threshold behavior if a minimal number of receptors must be occupied to evoke the responses of the hormones.

Increases in UGT-1 mRNA and alterations in T_4 and TSH as described above have been observed in rats given biweekly oral doses of TCDD for 31 weeks (115). Such treatment results in increased serum TSH levels and in concomitant hypertrophy of thyroid follicular cells and thyroid hyperplasia (115). Other Ah agonists have similar effects as dioxin (116,117). Goitrogenic compounds that depress serum T_4 by other mechanisms (118) also cause thyroid tumors. The PBPK model described above was extended to include release of thyroid hormones into the blood, their uptake by peripheral tissues, binding of T_4 to cytosolic receptors and of 3,5,3'-triiodothyronine (T_3) to nuclear receptors, metabolism of thyroid hormones, and induction of UGT-1 by the Ah-TCDD complex (119,120). The model also

includes complex regulation of TSH release from the pituitary by effects of serum T_4 on the hypothalamic peptides thyrotropin-releasing hormone (TRH), which stimulates TSH release, and somatostatin, which antagonizes the effect of TRH. The model reproduces the effects of chronic exposure to TCDD on serum T_3 , T_4 , and TSH concentrations (115). The computed dose-response curve for TSH exhibits proportional response at low doses.

The model also reproduces data for induction of UGT-1 (both mRNA and enzymatic activity) by dioxin for several dosing scenarios. Because the concentrations of T_3 , T_4 , and TSH in blood are highly variable among individuals and vary with diet and time of day, they are not likely to be useful as biomarkers of effects of dioxin exposure. Because fewer factors influence UGT-1 activity, induction of this enzyme in an individual known to have been exposed to TCDD is more likely to reflect effects of that xenobiotic agent. The computed dose-response curve for UGT-1 induction is approximately linear at low doses. The low-dose linear responses of TSH and UGT-1 suggest the absence of a threshold for dioxin's effects on the thyroid. This model is consistent with induction of thyroid tumors by chronic overstimulation of the gland by elevated TSH consequent to induction of UGT-1. However, it is not known if TCDD has effects on the thyroid in addition to enhanced cell proliferation.

When Can Receptor-mediated Mechanisms Lead to Threshold Responses?

The PBPK model of dioxin action is consistent with a large number of observed responses under several dosing scenarios. The model's predictions do not support the hypothesis that mediation of dioxin's effects by the Ah receptor imposes threshold dose-response behavior. The model of Portier et al. (121) showed that the predicted response of CYP1A1 in rat liver to low doses of TCDD depends on whether its constitutive expression is due to an endogenous ligand of the Ah receptor (their "additive" mechanism) or due to a mechanism that is independent of the receptor. The parameter values optimized for the additive mechanism, which gave the best fit to the experimental data, predicted proportional response at low doses. The parameter values optimized for the independent mechanism predicted a dose-response curve that was concave upwards at

low dose, suggestive of a threshold for induction of this protein. The difference in the dose-response curve shape can have significant consequences for estimating risks of adverse health effects from exposure to dioxin if CYP1A1 is used as a biomarker for effects of TCDD.

To identify conditions under which receptor mediated responses could exhibit thresholds, Kohn and Portier (122) constructed a theoretical model of receptor mediated gene expression. This model included binding of endogenous and xenobiotic ligands to the receptor, binding of the ligand-receptor complex to DNA and induction of a protein, proteolysis of the gene product, and metabolism of the xenobiotic inducer. This model also included constitutive expression of the protein by a mechanism that is independent of the receptor. Parameter values were varied systematically to cover a wide range of combinations of additive and independent routes of induction of the protein. The model's equations were solved for a series of bolus doses of the xenobiotic ligand up to the time point where a pseudo-steady state of the protein was achieved for all doses.

This model predicted threshold behavior in net protein production only when binding of ligand to the receptor or binding of the liganded receptor to DNA exhibited positive cooperativity (i.e., sigmoidal binding kinetics) and all other effects followed hyperbolic kinetics. The independent mechanism of Portier et al. (121) is consistent with positive cooperativity, whereas their additive model predicted a low-dose linear response and produced a better fit to the data. The PBPK model, which included an endogenous ligand of the Ah receptor, did not require cooperative binding in order to reproduce the observed responses. Noncooperative ligand binding may partly explain the proportional response predicted by the model. The theoretical model shows that a threshold response is possible for receptor-mediated carcinogens, but it is not obligatory. Every carcinogen thought to exert its effects by such a mechanism should be studied individually to determine its low-dose response.

Conclusions

This critical review examined the observed effects of carcinogens that increase cell replication rates by regenerative hyperplasia consequent to cytotoxicity or that modulate gene expression by binding to and activating transcription factors. Depending on available data, the relevance to humans of

carcinogenic effects observed in rodents or the predicted shape of dose–response curves were discussed. Some chemicals that have been classified as nongenotoxic carcinogens fall into both categories and may even possess a genotoxic component. Therefore, attributing a chemical's carcinogenicity solely to its ability to induce one effect (e.g., $\alpha_2\mu$ accumulation, peroxisome proliferation, enzyme induction) may obscure important contributions to its carcinogenic mechanism.

The hypothesis that cell proliferation causes cancer is based on the notion that if replicative DNA synthesis and cell division occur before repair of damaged DNA, then promutagenic lesions could become fixed into heritable mutations and contribute to the genetic changes that lead to neoplastic transformation. Fortunately, progression through the cell cycle is highly regulated to permit repair of DNA damage before cells undergo replicative DNA synthesis or mitosis. Furthermore, responses to DNA damage are inducible. There are no data for the classes of chemicals reviewed here demonstrating compromise of cell cycle controls during regenerative hyperplasia. Increases in labeling indices may indicate that more cells are actively cycling; however, this does not signify that rates of transit through cell cycle checkpoints are reduced. Thus, the hypothesis that mitogenesis can lead to mutation and that carcinogenicity is simply a regenerative response to cytotoxicity remains unproven.

A threshold for a response to a carcinogen has been defined in absolute biological terms as the dose below which no response occurs. In practice, an apparent threshold

is detected statistically as that dose below which the activity of a biomarker for the response in treated subjects is indistinguishable from that in controls. However, attribution of a threshold in such circumstances may be an artifact. Because of measurement errors and interindividual variability, it is always possible to find a dose which satisfies this criterion even for genotoxic chemicals, which have been assumed to exhibit linear cancer dose–responses. The more sensitive and repeatable is the measurement of the biomarker, the lower such an apparent threshold would seem. Thus, categorization of a response as exhibiting a threshold is limited by the nature of the end point being measured and by the accuracy of that measurement. Assessment of risks of adverse health effects consequent to exposure to a chemical should be based on the shape of the dose–response curve obtained from experimental data by the best available mathematical techniques.

Mechanistic studies in chemical carcinogenesis have greatly added to our understanding of the steps involved in the carcinogenic process. However, there is still much uncertainty on the nature of the complex interacting processes that occur between exposure to nongenotoxic carcinogens and tumor development. Use of mechanistic data in risk assessments requires scientific judgment and should not rely on overly speculative hypotheses. The application of new research findings to public health decisions should proceed with caution to ensure adequate validation and proper interpretation of the data. Several critical questions must be examined.

Is the mechanism biologically plausible?

Are the data of sufficient quality to reasonably link the specific mechanistic process to the cancer outcome?

Are competing explanations valid?

Is the particular mechanism (mode of action) the determinant of the carcinogenic effect or are multiple processes possibly involved?

Over-simplified classification systems add uncertainty and inaccuracy to evaluations of human risk. Evaluations of carcinogenicity by chemicals that act via “nongenotoxic” mechanisms should not be limited to promotion nor should the response be assumed to exhibit a threshold. Cancer is a complex multistep process and chemicals may affect the carcinogenic outcome by producing changes that affect one or several steps.

Research is needed to identify the multiple factors that contribute to the carcinogenicity of both genotoxic and nongenotoxic carcinogens and to quantify their contributions to the cancer dose–response curve. Integrating this information into cancer dose–response models would permit prediction of the shape of the dose–response curve instead of having to rely on default assumptions. Such an approach should provide a more realistic, hence more credible, means of estimating human low-dose risk. Until a better understanding of the mechanistic processes involved in the carcinogenic response is available, the prudent policy for protecting public health is the one that considers the dose–response of all potential contributing effects of each specific chemical.

REFERENCES

- Perera FP. Perspectives on the risk assessment for non-genotoxic carcinogens and tumor promoters. *Environ Health Perspect* 94:231–235 (1991).
- Schulte-Hermann R, Timmermann-Trosiener I, Schuppler J. Promotion of spontaneous preneoplastic cells in rat liver as a possible explanation of tumor production by nonmutagenic compounds. *Cancer Res* 43:839–844 (1983).
- Neumann I, Thierau D, Andrae U, Greim H, Schwarz LR. Cyproterone acetate induces DNA damage in cultured rat hepatocytes and preferentially stimulates DNA synthesis in glutamyltranspeptidase-positive cells. *Carcinogenesis* 13:373–378 (1992).
- Topinka J, Andrae U, Schwarz LR, Wolff T. Cyproterone acetate generates DNA adducts in rat liver and in primary rat hepatocyte cultures. *Carcinogenesis* 14:423–427 (1993).
- Demi E, Schwarz LR, Oesterle D. Initiation of enzyme-altered foci by the synthetic steroid cyproterone acetate in rat liver foci bioassay. *Carcinogenesis* 14:1229–1231 (1993).
- Williams GM, Iatropoulos MJ, Djordjevic MV, Kaltengberg OP. The triphenylethylene drug tamoxifen is a strong liver carcinogen in the rat. *Carcinogenesis* 14:315–317 (1993).
- Greaves P, Goonetilleke R, Nunn G, Topham J, Orton T. Two-year carcinogenicity study of tamoxifen in Alderley Park Wistar-derived rats. *Cancer Res* 53:3919–3924 (1993).
- Tucker MJ, Adam HK, Patterson JS. Tamoxifen. In: *Safety Testing of New Drugs. Laboratory Predictions and Clinical Performance* (Laurence DR, McLean AEM, Wetherall M, eds). London:Academic Press, 1984;125–161.
- Han XL, Liehr JG. Induction of covalent DNA adducts in rodents by tamoxifen. *Cancer Res* 52:1360–1363 (1992).
- Randerath K, Moorthy B, Mabon N, Sriram P. Tamoxifen: evidence by ^{32}P -postlabeling and use of metabolic inhibitors for two distinct pathways leading to mouse hepatic DNA adduct formation and identification of 4-hydroxytamoxifen as a proximate metabolite. *Carcinogenesis* 15:2087–2094 (1994).
- Pathak DN, Bodell WJ. DNA adduct formation by tamoxifen with rat and human liver microsomal activation systems. *Carcinogenesis* 15:529–532 (1994).

12. Styles JA, Davies A, Lim CK, De Matteis F, Stanley LA, White INH, Yuan ZX, Smith LI. Genotoxicity of tamoxifen, tamoxifen epoxide and toremifene in human lymphoblastoid cells containing human cytochrome P450s. *Carcinogenesis* 15:5-9 (1994).
13. Vancutsem PM, Lazarus P, Williams GM. Frequent and specific mutations of the rat *p53* gene in hepatocarcinomas induced by tamoxifen. *Cancer Res* 54:3864-3867 (1994).
14. Stott WT, Reitz RH, Schumann AM, Watanabe PG. Genetic and nongenetic events in neoplasia. *Food Cosmet Toxicol* 19:567-576 (1981).
15. Ames BN, Gold LS. Too many rodent carcinogens: mitogenesis increases mutagenesis. *Science* 249:970-971 (1990).
16. Cohen SM, Ellwein LB. Cell proliferation in carcinogenesis. *Science* 249:1007-1011 (1990).
17. Farber E. Sequential events in chemical carcinogenesis. In: *Cancer: A Comprehensive Treatise* (Becker FF, ed) Vol 1, 2d ed. New York:Plenum, 1982;485-506.
18. Weinstein, IB. Mitogenesis is only one factor in carcinogenesis. *Science* 251:387-388 (1991).
19. Tomatis L. Cell proliferation and carcinogenesis: a brief historical background and current view based on an IARC workshop report. *Environ Health Perspect* 101 (Suppl 5):149-152 (1993).
20. Melnick RL, Huff J, Barrett JC, Maronpot RR, Lucier G, Portier CJ. Cell proliferation and chemical carcinogenesis: symposium overview. *Environ Health Perspect* 101 (Suppl 5):3-8 (1993).
21. Melnick RL Huff JE. Liver carcinogenesis is not a predicted outcome of chemically induced hepatocyte proliferation. *Toxicol Ind Health* 9:415-438 (1993).
22. Peraino C, Fry RJM, Staffeldt E, Christopher JP. Enhancing effects of phenobarbitone and butylated hydroxytoluene on 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat. *Food Cosmet Toxicol* 15:93-96 (1977).
23. Peraino C, Fry RJM, Staffeldt E, Kisieleski WE. Effects of varying the exposure to phenobarbital on its enhancement of 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat. *Cancer Res* 33:2701-2705 (1973).
24. Bucher JR, Huff JE, Jokinen MP, Haseman JK, Stedham M, and Cholakis JM. Inhalation of tetranitromethane causes nasal passage irritation and pulmonary carcinogenesis in rodents. *Cancer Lett* 57:95-101 (1991).
25. Larson JL, Wolf DC, Butterworth BE. Induced cytotoxicity and cell proliferation in the hepatocarcinogenicity of chloroform in female B6C3F1 mice: comparison of administration by gavage in corn oil vs ad libitum in drinking water. *Fundam Appl Toxicol* 22:90-102 (1994).
26. Jorgenson TA, Meierhenry EF, Rushbrook CJ, Bull RJ, Robinson M. Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice. *Fundam Appl Toxicol* 5:760-769 (1985).
27. Corley RA, Mendrala AL, Smith FA, Staats DA, Gargas ML, Conolly RB, Andersen ME, Reitz RH. Development of a physiologically based pharmacokinetic model for chloroform. *Toxicol Appl Pharmacol* 103:512-527 (1990).
28. Larson JL, Wolf DC, Morgan KT, Mery S, Butterworth BE. The toxicity of 1-week exposures to inhaled chloroform in female B6C3F1 mice and male F-344 rats. *Fundam Appl Toxicol* 22:431-446 (1994).
29. Rosenthal SL. A review of the mutagenicity of chloroform. *Environ Mol Mutagen* 10:211-226 (1987).
30. Morimoto K and Koizumi A. Trihalomethanes induce sister chromatid exchanges in human lymphocytes *in vitro* and mouse bone marrow cells *in vivo*. *Environ Res* 32:72-79 (1983).
31. Callen DF, Wolf CR, Philpot RM. Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in *Saccharomyces cerevisiae*. *Mutat Res* 77:55-63 (1980).
32. DiRenzo AB, Gandolfi AJ, Sipes IG. Microsomal bioactivation and covalent binding of aliphatic halides to DNA. *Toxicol Lett* 11:243-252 (1982).
33. Ahmed AE, Kubic VL, and Anders MW. Metabolism of haloforms to carbon monoxide. I. *In vitro* studies. *Drug Metab Dispos* 5:198-204 (1977).
34. Docks EL, Krishna G. The role of glutathione in chloroform-induced hepatotoxicity. *Exp Mol Pathol* 24:13-22 (1976).
35. Pohl LR, Martin JL, Taburet AM, George JW. Oxidative bioactivation of haloforms into hepatotoxins. In: *Microsomes, Drug Oxidations, and Chemical Carcinogenesis*, Vol II (Coon MJ, Conney AH, Estabrook RW, Gelboin HV, Gillette JR, O'Brein PJ, eds), Academic Press, New York;881-884 (1980).
36. Pitzer KS. *Quantum Chemistry*. Englewood Cliffs, NJ:Prentice-Hall, 1953;170.
37. Dunnick JK, Melnick RL. Assessment of the carcinogenic potential of chlorinated water: experimental studies of chlorine, chloramine, and trihalomethanes. *J Natl Cancer Inst* 85:817-822 (1993).
38. Thornton-Manning JR, Seely JC, Pegram RA. Toxicity of bromodichloromethane in female rats and mice after repeated oral dosing. *Toxicology* 94:3-18 (1994).
39. Roy AK, Neuhaus OW. Androgenic control of a sex-dependent protein in the rat. *Nature* 214:618-620 (1967).
40. Olson MJ, Garg BD, Murty CVR, Roy AK. Accumulation of $\alpha_2\mu$ -globulin in the renal proximal tubules of male rats exposed to unleaded gasoline. *Toxicol Appl Pharmacol* 90:43-51 (1987).
41. Neuhaus OW, Flory W, Biswas N, Hollerman CE. Urinary excretion of $\alpha_2\mu$ -globulin and albumin by adult male rats following treatment with nephrotoxic agents. *Nephron* 28:133-140 (1981).
42. Swenberg JA, Short B, Borghoff S, Strasser J, Charbonneau M. The comparative pathobiology of $\alpha_2\mu$ -globulin nephropathy. *Toxicol Appl Pharmacol* 97:35-46 (1989).
43. Lehman-McKeeman LD, Rivera-Torres MI, Caudill D. Lysosomal degradation of $\alpha_2\mu$ -globulin and $\alpha_2\mu$ -globulin-xenobiotic conjugates. *Toxicol Appl Pharmacol* 103:539-548 (1990).
44. Short BG, Burnett VL, Cox MG, Bus JS, Swenberg JA. Site-specific renal cytotoxicity and cell proliferation in male rats exposed to petroleum hydrocarbons. *Lab Invest* 57:564-577 (1987).
45. Short BG, Burnett VL, Swenberg JA. Elevated proliferation of proximal tubule cells and localization of accumulated $\alpha_2\mu$ -globulin in F344 rats during chronic exposure to unleaded gasoline or 2,2,4-trimethylpentane. *Toxicol Appl Pharmacol* 101:414-431 (1989).
46. Dietrich DR, Swenberg JA. The presence of $\alpha_2\mu$ -globulin is necessary for *d*-limonene promotion of male rat kidney tumors. *Cancer Res* 51:3512-3521 (1991).
47. Short BG, Steinhagen WH, Swenberg JA. Promoting effects of unleaded gasoline and 2,2,4-trimethylpentane on the development of atypical cell foci and renal tubular cell tumors in rats exposed to *N*-ethyl-*N*-hydroxyethylnitrosamine. *Cancer Res* 49:6369-6378 (1989).
48. Halder CA, Holdsworth CE, Cockrell BY, Piccirillo VJ. Hydrocarbon nephropathy in male rats: identification of the nephrotoxic components of unleaded gasoline. *Toxicol IndHealth* 1:67-87 (1985).
49. Dietrich DR, Swenberg JA. NCI-Black-Reiter (NBR) rats fail to develop renal disease following exposure to agents that induce $\alpha_2\mu$ -globulin ($\alpha_2\mu$) nephropathy. *Fundam Appl Toxicol* 16:749-762 (1991).
50. Borghoff SJ, Miller AB, Bowen JP, Swenberg JA. Characteristics of chemical binding to $\alpha_2\mu$ -globulin *in vitro*-evaluating structure-activity relationships. *Toxicol Appl Pharmacol* 107:228-238 (1991).
51. Dominick MA, Robertson DG, Bleavins MR, Sigler RE, Bobrowski WF, Gough AW. $\alpha_2\mu$ -Globulin nephropathy without nephrocarcinogenesis in male Wistar rats administered 1-(aminomethyl)cyclohexanecetic acid. *Toxicol Appl Pharmacol* 111:375-387 (1991).
52. Dietrich DR, Swenberg JA. Lindane induces nephropathy and renal accumulation of $\alpha_2\mu$ -globulin in male but not female Fischer 344 rats or male NBR rats. *Toxicol Lett* 53:179-181 (1990).

53. National Cancer Institute. Bioassay of lindane for possible carcinogenicity (CAS No. 58-89-9). NCI Carcinogenesis Technical Rpt No 14. Bethesda, MD:National Institutes of Health, 1977.
54. Charbonneau M, Lock EA, Strasser J, Cox MG, Turner MJ, Bus JS. 2,2,4-Trimethylpentane-induced nephrotoxicity. I. Metabolic disposition of TMP in male and female Fischer 344 rats. *Toxicol Appl Pharmacol* 91:171-181 (1987).
55. Lock EA, Charbonneau M, Strasser J, Swenberg JA, Bus JS. 2,2,4-Trimethylpentane-induced nephrotoxicity. II: The reversible binding of a TMP metabolite to a renal protein fraction containing $\alpha_2\mu$ -globulin. *Toxicol Appl Pharmacol* 91:182-192 (1987).
56. Lehman-McKeeman LD, Rodriguez PA, Takigiku R, Caudill D, Fey, ML. *d*-Limonene-induced male rat-specific nephrotoxicity: Evaluation of the association between *d*-limonene and $\alpha_2\mu$ -globulin. *Toxicol Appl Pharmacol* 99:250-259 (1989).
57. Melnick RL. An alternative hypothesis on the role of chemically induced protein droplet ($\alpha_2\mu$ -globulin) nephropathy in renal carcinogenesis. *Regul Toxicol Pharmacol* 16:111-125 (1992).
58. Charbonneau M, Lock EA, Strasser J, Short BG, Bus JS. Nephrotoxicity of 2,2,4-trimethylpentane (TMP) metabolites in male Fischer 344 rats. *Toxicologist* 7:89 (1987).
59. Solleveld HA, Haseman JK, McConnell EE. Natural history of body weight gain, survival, and neoplasia in the F344 rat. *J Natl Cancer Inst* 72:929-940 (1984).
60. Borghoff SJ, Youtsey NL, Swenberg JA. A comparison of European high test gasoline and PS-6 unleaded gasoline in their abilities to induce $\alpha_2\mu$ -globulin nephropathy and renal cell proliferation. *Toxicol Lett* 63:21-33 (1992).
61. Barrett JC, Huff JE. Cellular and molecular mechanisms of chemically induced renal carcinogenesis. *Renal Failure* 13:211-225 (1991).
62. Pevsner J, Reed RR, Feinstein PG, Snyder SH. Molecular cloning of odorant-binding protein: member of a ligand carrier family. *Science* 241:336-339 (1988).
63. Lehman-McKeeman LD, Caudill D. $\alpha_2\mu$ -Globulin is the only member of the lipocalin protein superfamily that binds to hyaline droplet inducing agents. *Toxicol Appl Pharmacol* 116:170-176 (1992).
64. Reddy JK, Azarnoff DL, Hignite CE. Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* 283:397-398 (1980).
65. Ashby J, Brady CR, Elcombe CR, Elliott BM, Ishmael J, Odum J, Tugwood JD, Kettle S, Purchase IFH. Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Human Exptl Toxicol* 13:S1-S117 (1994).
66. Reddy JK, Reddy MK, Usman, MI, Lalwani ND, Rao MS. Comparison of hepatic peroxisome proliferative effect and its implication for hepatocarcinogenicity of phthalate esters, di(2-ethylhexyl) phthalate, and di(2-ethylhexyl) adipate with a hypolipidemic drug. *Environ Health Perspect* 65:317-327 (1986).
67. National Toxicology Program. Carcinogenesis bioassay of di(2-ethylhexyl)phthalate (CAS No. 117-81-7) in F344 rats and B6C3F1 mice (feed study). NTP Technical Rpt 217. Bethesda, MD:National Institutes of Health, 1982.
68. National Toxicology Program. Carcinogenesis bioassay of di(2-ethylhexyl)adipate (CAS No. 103-23-1). NTP Technical Rpt 212. Bethesda, MD:National Institutes of Health, 1981.
69. Marsman DS, Cattley RC, Conway JG, Popp JA. Relationship of hepatic peroxisome proliferation and replicative DNA synthesis to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]-acetic acid (Wy-14643) in rats. *Carcinogenesis* 48:6739-6744 (1988).
70. IARC. Tetrachloroethylene. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Dry-Cleaning, Some Solvents and Other Industrial Chemicals, Vol 63. Lyon:International Agency for Research on Cancer, in press.
71. Hwang JJ, Hsia MTS, Jirtle RL. Induction of sister chromatid exchange and micronuclei in primary cultures of rat and human hepatocytes by the peroxisome proliferator, Wy-14,643. *Mutat Res* 286:123-133 (1993).
72. Tsutsui T, Watanabe E, Barrett JC. Ability of peroxisome proliferators to induce cell transformation, chromosome aberrations and peroxisome proliferation in cultured Syrian hamster embryo cells. *Carcinogenesis* 14:611-618 (1993).
73. Reisenbichler H, Eckl PM. Genotoxic effects of selected peroxisome proliferators. *Mutat Res* 286:135-144 (1993).
74. Hegi ME, Fox TR, Belinsky SA, Devereux TR, Anderson MW. Analysis of activated protooncogenes in B6C3F1 mouse liver tumors induced by ciprofibrate, a potent peroxisome proliferator. *Carcinogenesis* 14:145-149 (1993).
75. Reddy JK, Lalwani ND. Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit Rev Toxicol* 12:1-58 (1983).
76. Rao MS, Lalwani ND, Watanabe TK, Reddy JK. Inhibitory effect of antioxidants ethoxyquin and 2(3)-*tert*-butyl-4-hydroxyanisole on hepatic tumorigenesis in rats fed ciprofibrate, a peroxisome proliferator. *Cancer Res* 44:1072-1076 (1984).
77. Tomaszewski KE, Agarwal DK, Melnick RL. *In vitro* steady-state levels of hydrogen peroxide after exposure of male F344 rats and female B6C3F1 mice to hepatic peroxisome proliferators. *Carcinogenesis* 7:1871-1876 (1986).
78. Reddy JK, Lalwani ND, Reddy MK, Qureshi SA. Excessive accumulation of autofluorescent lipofuscin in the liver during hepatocarcinogenesis by methyl clofenapate and other hypolipidemic peroxisome proliferators. *Cancer Res* 42:259-266 (1982).
79. Conway JG, Tomaszewski KE, Olson MJ, Cattley RC, Marsman DS, Popp JA. Relationship of oxidative damage to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and Wy-14,643. *Carcinogenesis* 10:513-519 (1989).
80. Takagi A, Sai K, Umemura T, Hasegawa R, Kurokawa Y. Relationship between hepatic peroxisome proliferation and 8-hydroxydeoxyguanosine formation in liver DNA of rats following long-term exposure to three peroxisome proliferators; di(2-ethylhexyl) phthalate, aluminum clofibrate and simifibrate. *Cancer Lett* 53:33-38 (1990).
81. Cattley RC, Glover SE. Elevated 8-hydroxydeoxyguanosine in hepatic DNA of rats following exposure to peroxisome proliferators: relationship to carcinogenesis and nuclear localization. *Carcinogenesis* 14:2495-2499 (1993).
82. Butterworth BE. Consideration of both genotoxic and nongenotoxic mechanisms in predicting carcinogenic potential. *Mutat Res* 239:117-132 (1990).
83. Melnick RL. Does chemically induced hepatocyte proliferation predict liver carcinogenesis? *FASEB J* 6:2698-2706 (1992).
84. Eacho PI, Lanier TL, Brodhecker CA. Hepatocellular DNA synthesis in rats given peroxisome proliferating agents: comparison of WY-14,643 to clofibrate, nafenopin and LY171883. *Carcinogenesis* 13:409-415 (1992).
85. Yeldandi AV, Milano M, Subbarao V, Reddy JK, Rao MS. Evaluation of liver cell proliferation during ciprofibrate-induced hepatocarcinogenesis. *Cancer Lett* 47:21-27, (1989).
86. Smith-Oliver T, Butterworth BE. Correlation of the carcinogenic potential of di(2-ethylhexyl)phthalate (DEHP) with induced hyperplasia rather than with genotoxic activity. *Mutat Res* 188:21-28 (1987).
87. Cattley RC, Marsman DS, Popp JA. Failure of the peroxisome proliferator WY-14,643 to initiate growth-selectable foci in rat liver. *Toxicology* 56:1-7 (1989).
88. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 347:645-650 (1990).
89. Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RA. Convergence of 9-*cis* retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature* 358:771-774 (1992).

90. Issemann I, Prince RA, Tugwood JD, Green S. The retinoid X receptor enhances the function of the peroxisome proliferator activated receptor. *Biochimie* 75:251-256 (1993).
91. Sher T, Yi HF, McBride OW, Gonzalez FJ. cDNA cloning, chromosomal mapping, and functional characterisation of human peroxisome proliferator activated receptor. *Biochemistry* 32:5598-5604 (1993).
92. Steineger HH, Sorensen HN, Tugwood JD, Skrede S, Spyevoold O, Gautvik, KM. Dexamethasone and insulin demonstrate marked and opposite regulation of the steady-state mRNA level of the peroxisomal proliferator-activated receptor (PPAR) in hepatic cells. Hormonal modulation of fatty acid-induced transcription. *Eur J Biochem* 225:967-974 (1994).
93. Huff JE, Salmon AG, Hooper NK, Zeise L. Long-term carcinogenesis studies on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and hexachlorodibenzo-*p*-dioxins. *Cell Biol Toxicol* 7:67-94 (1991).
94. Poland A, Knutson JC. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu Rev Pharmacol Toxicol* 22:517-554 (1982).
95. Whitlock JP. Mechanistic aspects of dioxin action. *Chem Res Toxicol* 6:754-763 (1993).
96. Roberts L. Dioxin risks revisited. *Science* 251: 624-626 (1991).
97. Johnson E. A partnership between the dioxin receptor and a basic helix-loop-helix protein. *Science* 252:924-925 (1991).
98. Kohn MC, Lucier GW, Clark GC, Sewall C, Tritscher AM, Portier CJ. A mechanistic model of effects of dioxin on gene expression in the rat liver. *Toxicol Appl Pharmacol* 120:138-154 (1993).
99. Andersen ME, Mills JJ, Gargas ML, Kedderis L, Birnbaum LS, Neubert D, Greenlee WF. Modeling receptor-mediated processes with dioxin: implications for pharmacokinetics and risk assessment. *Risk Anal* 13: 25-36 (1993).
100. Tritscher AM, Goldstein JA, Portier CJ, McCoy Z, Clark GC, Lucier GW. Dose-response relationships for chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a rat tumor promotion model: quantification and immunolocalization of CYP1A1 and CYP1A2 in the liver. *Cancer Res* 52:3436-3442 (1992).
101. Vanden Heuvel JP, Clark GC, Kohn MC, Tritscher AM, Greenlee WF, Lucier GW, Bell DA. Dioxin-responsive genes: examination of dose-response relationships using quantitative reverse transcriptase-polymerase chain reaction. *Cancer Res* 54: 62-68 (1994).
102. Kohn MC, Lucier GW, Portier CJ. The importance of biological realism in dioxin risk assessment models. *Risk Anal* 14:993-1000 (1994).
103. Sloop TC, Lucier GW. Dose-dependent elevation of Ah receptor binding by TCDD in rat liver. *Toxicol Appl Pharmacol* 88:329-337 (1987).
104. Lin FH, Clark G, Birnbaum LS, Lucier GW, Goldstein JA. Influence of the Ah locus on the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the hepatic epidermal growth factor receptor. *Mol Pharmacol* 39: 307-313 (1991).
105. Schlessinger J, Schreiber AB, Levi A, Lax I, Liberman T, Yarden Y. Regulation of cell proliferation by epidermal growth factor. *CRC Crit Rev Biochem* 14:93-111 (1983).
106. Mead JE, and Fausto N. Transforming growth factor- α may be a physiological regulator of liver regeneration by means of an autocrine mechanism. *Proc Natl Acad Sci USA* 86:1558-1562 (1989).
107. Choi EJ, Toscano DG, Ryan JA, Riedel N, Toscano WA. Dioxin induces transforming growth factor- α in human keratinocytes. *J Biol Chem* 266:9591-9597 (1991).
108. Sewall C, Lucier G, Tritscher A, Clark G. TCDD-mediated changes in hepatic EGF receptor may be a critical event in the hepatocarcinogenic action of TCDD. *Carcinogenesis* 14:1885-1893 (1993).
109. Li SA, Klicka JK, Li JJ. Estrogen 2- and 4-hydroxylase activity, catechol estrogen formation, and implications for estrogen carcinogenesis in the hamster kidney. *Cancer Res* 45:181-185 (1985).
110. Bagchi M, and Stohs S.J. *In vitro* induction of reactive oxygen species by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, endrin, and lindane in rat peritoneal macrophages and hepatic mitochondria and microsomes. *Free Radic Biol Med* 14:11-18 (1993).
111. Tsibris JCM, and McGuire P. Microsomal activation of estrogens and binding to rat liver DNA *in vivo* by selective induction of microsomal and nuclear aryl hydrocarbon hydroxylase activity. *Cancer Res* 38:4640-4644 (1977).
112. Cerutti P. Repairable damage in DNA. In: DNA Repair Mechanisms (Hanawalt P, Friedberg E, Fox C, eds). New York:Academic Press,1978;1-14.
113. Portier CJ, Kohn M, Sherman CD, and Lucier G. Modeling the number and size of hepatic focal lesions following exposure to 2,3,7,8-TCDD, *Organohalogen Compounds* 21:393-397 (1994).
114. Bock KW. Roles of UDP-glucuronosyltransferases in chemical-carcinogenesis. *Crit Rev Biochem Mol Biol* 26:129-150 (1991).
115. Sewall CH, Flagler N, Vanden Heuvel JP, Lucier GW. Alterations in thyroid function in female Sprague-Dawley rats following chronic treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Appl Pharmacol* 132:237-244 (1995).
116. Saito K, Kaneko H, Sato K, Yoshitake A, Yamada H. Hepatic UDP-glucuronyltransferase(s) activity toward thyroid hormones in rats: induction and effects on serum thyroid hormone levels following treatment with various enzyme inducers. *Toxicol Appl Pharmacol* 111: 99-106 (1991).
117. Barter RA, Klaassen CD. UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. *Toxicol Appl Pharmacol* 113:36-42 (1992).
118. Andrae U, Greim H. Initiation and promotion in thyroid carcinogenesis. In: *Tissue Specific Toxicity: Biochemical Mechanisms*. New York:Academic Press,1992;71-93.
119. Kohn MC. Computer simulation of effects of dioxin on thyroid hormones in the rat. In: *Health Sciences, Physiological and Pharmacological Simulation Studies* (Anderson JG, Katzper M, eds). La Jolla, CA:Society for Computer Simulation, 1995;117-122.
120. Kohn MC, Sewall CH, Lucier, GW, Portier CJ. A mechanistic model of effects of dioxin on thyroid hormones in the rat. *Toxicol Appl Pharmacol* (in press).
121. Portier C, Tritscher A, Kohn M, Sewall C, Clark G, Edler L, Hoel D, Lucier G. Ligand/receptor binding for 2,3,7,8-TCDD: Implications for risk assessment. *Fundam Appl Toxicol* 20:48-56 (1992).
122. Kohn MC, Portier CJ. Effects of the mechanism of receptor-mediated gene expression on the shape of the dose-response curve. *Risk Anal* 13:565-572 (1993).