6. CARCINOGENICITY OF TCDD IN ANIMALS

6.1. INTRODUCTION

Additional scientific information on the use of animal cancer data for estimating human risks from 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has become available since the 1988 health risk assessment for dioxin. Much of the data on tumor incidence in experimental animals available in 1988 demonstrated that TCDD is a carcinogen at multiple sites in both sexes of rats and mice. Some of the cancers occurred following particularly low doses. Since 1988, TCDD has been shown to be a carcinogen in hamsters, and some of the tumor incidence data in rat liver have been reevaluated.

There is considerable evidence that TCDD does not damage DNA directly through the formation of DNA adducts. Mechanisms have been proposed that support the possibility that TCDD might be indirectly genotoxic, either through the induction of oxidative stress or by altering the DNA-damaging potential of some endogenous compounds, including estrogens. In addition, there have been numerous reports on TCDD-induced modifications of growth factor signaling pathways and cytokines in experimental animals and cell systems. Some of the altered systems include those for epidermal growth factor, transforming growth factor alpha, estrogen, glucocorticoids, tumor necrosis factor-alpha, interleukin 1-beta, plasminogen inactivating factor-2, and gastrin. Many of these pathways are involved in cell proliferation and differentiation and provide plausible avenues for researching the mechanisms responsible for the carcinogenic actions of TCDD. These effects are consistent with the generally accepted conclusion that TCDD acts as a “tumor promoter” in multistage models for chemical carcinogenesis and is virtually devoid of initiating activity in these models. It is important to note that “tumor promotion” is an operational and not a mechanistic term and that multiple mechanisms of tumor promotion are likely. Each of these mechanisms may be fundamentally different from the others.

There is a scientific consensus that most, if not all, of the biochemical and toxic effects of TCDD require an initial interaction with its cognate receptor, the aryl hydrocarbon (Ah). The properties of the Ah receptor (AhR) and the mechanisms whereby this receptor regulates gene expression are described in more detail in other chapters. However, formation of the AhR-TCDD complex is only the first of many steps involved in the production of a biochemical and toxic effect. Although there is considerable knowledge of details regarding activation of expression of the TCDD-inducible cytochrome P450 1A1 by the AhR, we still know very little about many components of AhR-mediated responses and their relationship to the development of adverse responses such as cancer. It is clear, however, that tissue- and cell-specific factors other than the AhR must be involved in determining tissue responses once TCDD binds the AhR.

Evaluation of dose-response is one of the more important issues that affect dioxin risk assessments. The focus of this controversy centers on the shape of the dose-response curve, particularly at low doses, and whether the effects of dioxin may exhibit operational thresholds. It now appears that for some responses there is a proportional relationship between receptor occupancy and response, which is evidenced by a linear relationship between target dose and effect over a wide dose range. However, different dose-response relationships are seen for different responses, so it is probably inappropriate to use a single surrogate marker to estimate dioxin's risks. Furthermore, these data reveal there is no unifying dose-response relationship for all AhR-mediated events. A more detailed evaluation of dose-response relationships for TCDD-modulated responses is described in Chapter 8.

Another controversial area in risk assessment is whether experimental animal models are appropriate for estimating human risks. There has been increasing evidence that biochemical and toxic responses resulting from human exposure to TCDD and its structural analogues appear to be similar to responses in experimental animals. However, it may be possible that humans are sensitive or resistant to some responses. There also is increasing awareness that interindividual variations in human responses to dioxin are a complicating factor in risk assessment, as it appears that there are individuals who are responsive and nonresponsive to numerous environmental chemicals, including TCDD.

Much of the controversy surrounding dioxin risk assessment reflects the selection of mathematical models: threshold, linear multistage, or others. We now know considerably more about the mode of action of dioxin, and this knowledge has allowed the construction of biologically based models that may reduce some of the uncertainty in current risk estimates.

These approaches and advances in our understanding of the mechanisms of tumor promotion and dose-response relationships will be discussed in more detail in Chapter 8, Dose-Response Modeling for 2,3,7,8 TCDD.

6.2. ANIMAL BIOASSAYS FOR CANCER

Long-term studies for carcinogenicity of TCDD have been conducted in several species (van Miller et al., 1977; Kociba et al., 1978; NTP, 1982a; Rao et al., 1988; Johnson et al., 1992). All studies have produced positive results. It is clear that TCDD is a multi-site carcinogen in both sexes of rats and mice (U.S. EPA, 1985; Huff et al., 1991; Zeise et al., 1990, IARC 1997). It is a carcinogen in the hamster (Rao et al., 1988), which is considered the most resistant species to the acute toxic effects of TCDD, and a preliminary report indicates that TCDD is also carcinogenic in fish (Johnson et al., 1992). The important studies are summarized in Table 6-1, including information on species, sex, and tumor site.

The 2-year rodent bioassays conducted by Dow Chemical (Kociba et al., 1978) and the National Toxicology Program (NTP, 1982a) studies are the most comprehensive to date and most relevant to risk characterization, and are described in the following paragraphs.

6.2.1. Kociba Study

The most cited cancer bioassay for TCDD was published by Kociba et al. (1978). It was a lifetime feeding study of male and female Sprague-Dawley rats using doses of 0, 1, 10, and 100 ng/kg/day. There were 50 males and 50 females in each group. Data derived from these studies have been used as the basis for many risk assessments for TCDD.

The most significant finding was an increase in hepatocellular hyperplastic nodules and hepatocellular carcinomas in female rats. The incidence of hepatocellular carcinomas was significantly elevated above the control incidence at the 100 ng/kg/day dose, whereas increased incidence of hyperplastic nodules was evident in the 10 ng/kg/day dose group.

There have been two reevaluations of slides of liver sections from the Kociba study (Squire, 1980; Sauer, 1990; Goodman and Sauer, 1992). The Squire review was requested by EPA as an independent review of the slides. The Sauer review was carried out using refined criteria for the diagnosis of proliferative hepatocellular lesions (Maronpot et al., 1986, 1989). Liver tumor incidences for the three evaluations are compared in Table 6-2. Although there are some quantitative differences between the evaluations, the lowest detectable effect for liver tumor incidence is consistently observed at 10 ng/kg/day.

In the 10 ng/kg/day dose group, significant increases in the incidence of hyperplastic nodules of the liver were observed in female rats (18/50 in the Kociba evaluation, 27/50 in the Squire evaluation). Two females (2/50) had hepatocellular carcinomas. In the 1990 reevaluation (Sauer, 1990; Goodman and Sauer, 1992), nine females (9/50) were identified with hepatocellular adenomas and none with carcinomas; thus only one-third of the previously observed “tumors” were identified when using the refined diagnostic criteria.

In addition to nodules in the liver, increased incidence of stratified squamous cell carcinoma of the tongue and nasal turbinates/hard palate, and keratinizing squamous cell carcinoma of the lung were also observed in female rats in the 100 ng/kg/day dose group.

There was no detectable increase in liver tumor incidences in male rats in any of the dose groups (Table 6-1). The mechanism responsible for dioxin-mediated sex specificity for hepatocarcinogenesis in rats is not clear, but may involve ovarian hormones (Lucier et al., 1991). This is discussed in Section 6.3 on tumor promotion.

Although there was no increase in liver tumors in male rats in this study, in the 100 ng/kg/day group there was an increased incidence of stratified squamous cell carcinoma of the

hard palate/nasal turbinate, stratified squamous cell carcinoma of the tongue, and adenoma of the adrenal cortex.

Kociba et al. (1978) had reported that chemically related increases in preneoplastic or neoplastic lesions were not found in the 1 ng/kg/day dose group. However, Squire identified two male rats in the 1 ng/kg/day dose group with squamous cell carcinoma of the nasal turbinates/hard palate, and one of these male rats had a squamous cell carcinoma of the tongue. These are both rare tumors in Sprague-Dawley rats, and these sites are targets for TCDD, implying that 1 ng/kg/day may not represent a no-observed-effect level (NOEL). However, no dose-response relationships were evident for tumors at these sites (Huff et al., 1991)

One of the more interesting findings in the Kociba bioassay was a TCDD-induced reduction in the incidence of spontaneous tumors including pituitary adenoma, benign tumor of the uterus, benign mammary neoplasm and mammary carcinoma in female rats, and acinar adenoma of the pancreas and adrenal pheochromocytoma in male rats. For example, carcinomas of the mammary gland occurred in 8 of 86 control female rats, whereas the incidence was 0/49 in the 1 ng/kg/day dose group. However, the incidence of mammary gland carcinomas in the medium- and high-dose groups was similar to that of control rats, suggesting that protection against breast cancer might be a low-dose effect. A relationship between body-weight reduction and spontaneous cancer incidence in rodents has been observed across numerous studies (Rao et al., 1987). This suggest that the reduction in the incidence of the spontaneous tumors by TCDD is likely related to the TCDD-induced reduction in body-weight gain. These findings, coupled with the sex specificity of TCDD-induced liver tumors in rats, highlight that the carcinogenic actions of TCDD may involve a complex interaction of hormonal factors. Moreover, it appears likely that tissue- and cell-specific factors modulate TCDD/hormone actions relevant to cancer.

There is considerable controversy concerning the possibility that TCDD-induced liver tumors are a consequence of cytotoxicity. Goodman and Sauer (1992) have extended the reevaluation of the Kociba slides to include liver toxicity data and have reported a correlation between the presence of overt hepatotoxicity and the development of hepatocellular neoplasms in female rats. With the exception of two tumors in controls and one each in the low- and mid-dose groups, all liver tumors occurred in livers showing clear signs of toxicity. However, male rat livers exhibit cytotoxicity in response to high TCDD doses, yet they do not develop liver tumors. Moreover, both intact and ovariectomized female rats exhibit liver toxicity in response to TCDD, yet TCDD is a more potent promoter in intact but not ovariectomized rats (Lucier et al., 1991). Therefore, if cytotoxicity is playing a role in liver tumorigenesis, other factors must also be involved. Also, there is little information on the role of cytotoxicity in TCDD-mediated cancer at other sites such as the lung and thyroid.

6.2.2. NTP Study

The NTP study was conducted using Osborne-Mendel rats and B6C3F1 mice (NTP, 1982a). Groups of 50 male rats, 50 female rats, and 50 male mice received TCDD as a suspension in corn oil:acteone (9:1) by gavage twice each week (Tuesday and Friday) to achieve doses of 0, 10, 50, or 500 ng TCDD/kg/week for 2 years; groups of 50 female mice were treated similarly to achieve doses of 0, 40, 200, or 2,000 ng/kg/week. These exposures correspond to daily averaged doses of 1.4, 7.1, or 71 ng/kg/day for rats and male mice and to doses of 5.7, 28.6, or 286 ng/kg/day for female mice, so the doses were comparable to those used in the Kociba feeding study. There were no statistically significant dose-related decreases in survival in any sex-species group.

Tumor data in the NTP bioassay are summarized in Tables 6-3 and 6-4. TCDD-induced malignant liver tumors occurred in the high-dose female rats and in male and female mice. These can be considered to result from TCDD exposure because they are relatively uncommon lesions in control Osborne-Mendel rats (male, 1/208; female, 3/208), are seen in female rats and mice of both sexes, and their increasing incidence with increasing dose is statistically significant (Cochran-Armitage trend test, p=0.004). Because liver tumors were increased in both sexes of mice, this effect is not female-specific as was observed in rats. Interestingly, liver tumor incidences were decreased in female rats in both the NTP and Kociba low doses (not statistically significant compared with controls). For example, the combined control incidence data were 11/161 (7%) compared with 4/99 (4%) in the low-dose group.

The incidences of thyroid gland (follicular cell) tumors were increased in all three dose groups in male rats. Because the responses in the two highest dose groups are highly significant, the statistically significant elevation of incidence in the lowest dose group (Fisher exact p-value=0.042) is considered to be caused by exposure to TCDD. Thus, for this study the lowest-observed-effect level (LOEL) is 1.4 ng/kg/day and a NOEL was not achieved within the specified dose range, suggesting that thyroid tumor incidence may be the most sensitive site for TCDD-mediated carcinogenesis. Because 71 ng/kg/day is above the maximum tolerated dose (MTD) (Huff et al., 1991), thyroid tumors occur at doses more than 50 times lower than the MTD.

TCDD-induced neoplasms of the adrenal gland were observed in the 7.1 ng/kg/day/dose group in male rats and in high-dose female rats. Fibrosarcomas of the subcutaneous tissue were significantly elevated in high-dose female mice and female rats. One additional tumor type, lymphoma, was seen in high-dose female mice. Lung tumors were elevated in high-dose female mice; the increase was not statistically significant when compared with concurrent controls, but the increase was dose related (Cochran-Armitage trend test, p=0.004).

Therefore, TCDD is a multisite complete carcinogen (Huff, 1992) and induced neoplasms in rats and mice of both sexes. As was observed in the Kociba study (Kociba et al., 1978), liver tumors were observed with greater frequency in treated female rats, but in male rats the thyroid appears to be the most sensitive (increased tumor incidence at doses as low as 1.4 ng/kg/day).

6.2.3. Syrian Golden Hamster

Groups of 10 to 24 male Syrian Golden hamsters were given two to six intraperitoneal or subcutaneous injections of TCDD over a 4-week period at doses of 0, 50, or 100 μg TCDD/kg in dioxane (Rao et al., 1988). The experiments were terminated after 12 to 13 months. The 100 μg/kg groups (total dose of 600 μg/kg) from both injection routes developed squamous cell carcinomas of the skin in the facial region: 4/18 (22%) from the intraperitoneal injection and 3/14 (21%) from the subcutaneous injection. The lesions were large (1.5 to 3 cm) with extensive necrosis, and some metastasized to the lung. The earliest neoplasms were detectable 8 months after the initial injection. Similar lesions were not seen in hamsters receiving two intraperitoneal injections of 100 μg/kg TCDD or six subcutaneous injections of dioxane vehicle, and none have been reported over the past 10 years in this laboratory. An extensive study by Pour et al. (1976) identified only 1 skin papilloma in 533 control Syrian hamsters. This report demonstrates that the hamster, a nonresponsive species for acute toxic effects, is susceptible to the carcinogenic actions of TCDD at doses well below the MTD.

6.2.4. B6C3 and B6C Mice

In a study by Della Porta et al. (1987), TCDD was administered intraperitoneally in corn oil at doses of 0, 1, 30, and 60 μg/kg to groups of 89 to 186 B6C3 and B6C mice of both sexes once weekly for 5 weeks starting at day 10 of life, and the animals were observed until 78 weeks of age. Histopathological observations were limited to the liver, kidney, and organs with apparent or suspected pathological changes. Thymic lymphomas were induced at the 60 μg/kg level in both sexes of both hybrids and at 30 μg/kg in all but female B6C3 mice. Neoplasms of the liver occurred in male B6C3 mice at 30 μg/kg and female B6C3 mice at 60 μg/kg. In a separate experiment, groups of 42 to 50 B6C3 mice were exposed to 0, 2.5, and 5.0 μg/kg TCDD in corn oil by gavage once weekly for 52 weeks starting at 6 weeks of age. The study was stopped at 110 weeks. Increased incidences of liver tumors were related to TCDD exposure at both dose levels.

6.2.5. Fish

A preliminary study, reported in abstract form only, examined the carcinogenicity of TCDD in medaka (*Oryzias latipes*) immersed in 2,3,7,8 TCDD-treated water for 28 days, followed by immersion in clean water for up to 8 months (Johnson et al., 1992). Exposure to 33.9 ppq TCDD led to an increase in tumors at multiple sites including gills, thyroid, and swim bladder. Total body burden of TCDD in these fish was 2 ppb (Johnson et al., 1992).

6.2.6. Carcinogenicity of Related Compounds

A mixture of two isomers of hexachlorodibenzo-*p*-dioxin (HCDD) (1,2,3,6,7,8 and 1,2,3,7,8,9) was given by gavage twice weekly for 2 years to Osborne-Mendel rats and B6C3F1 mice (NTP, 1980). The doses of HCDD were 0, 1.25, 2.5, or 5 μg/kg/week in rats and male mice. Doses for female mice were 0, 2.5, 5, and 10 μg/kg/week. There was no effect of administration of HCDD on survival of either sex of rats or mice (NTP, 1980). Results revealed that HCDD increased liver tumors in both sexes of rats and mice, although female rats seemed to be more sensitive than male rats (significant increases detected in female rats in the 1.25 μg/kg/week dose group, equivalent to 180 ng/kg/day). Therefore, HCDD is approximately 1/20 as potent a liver carcinogen as TCDD.

Dermal applications of the HCDD mixture described above (NTP, 1982b) were given to Swiss Webster mice for 104 weeks (three times per week). For the first 16 weeks, doses of 5 ng/application were used. Thereafter, doses of 10 ng/application were used. No HCDD-exposure-related carcinogenic responses were noted.

Dibenzo-*p*-dioxin given in the diet for 2 years at concentrations of 0, 5,000, and 10,000 ppm did not increase carcinogenic responses in Osborne-Mendel rats or B6C3F1 mice (NCI, 1979a). 2,7-Dichlorodibenzo-*p*-dioxin (DCDD) in the diet of Osborne-Mendel rats for 110 weeks or B6C3F1 mice for 90 weeks at levels of 0, 5,000, or 10,000 ppm did not increase neoplasms in male or female rats or in female mice. In male mice, increased incidences of lymphoma or hemangiosarcoma were observed in the low-dose group and neoplasms of the liver were observed in both dose groups (NCI, 1979b). The more highly chlorinated dibenzo-*p*-dioxins (CDDs) and dibenzofurans (CDFs) have not been studied in long-term animal cancer bioassays. Many of the CDDs and CDFs bioaccumulate and exhibit toxicities similar to those of TCDD and are considered to be carcinogens (EPA Science Advisory Board, 1989).

There are no carcinogenicity data on individual congeners of coplanar (dioxin-like) polychlorinated biphenyls. However, laboratory studies found statistically significant increased incidences of liver tumors in rats ingesting Aroclor 1260 or Clophen A60. Significant increases in gastric cancer, leukemia, and lymphoma were found in rats ingesting Aroclor 1254. Partial lifetime studies found precancerous liver lesions in rats and mice ingesting PCB mixtures of high or low chlorine content. More recent studies have compared the carcinogenicity of several

Aroclor mixtures (Mayes et al., 1998). The Aroclor 1254 mixture contains the highest level of dioxin-like coplanar PCBs of these mixtures. All Aroclors tested, 1016, 1242, 1254, and 1260, resulted in an increased incidence of liver neoplasms in female rats. However, only Aroclor 1260 at high doses was carcinogenic in males. In addition, Aroclor, 1242, 1254, and 1260 induced thyroid tumors in male rats. Analysis of liver levels of specific PCB congeners suggests that in males the induction of tumors is dependent on total PCB content, whereas the liver tumor incidence in females is dependent upon the total TCDD toxic equivalents level (TEQ) as a result of accumulation of dioxin-like PCBs from the Aroclor mixture (Silkworth et al., 1997).

With regard to studies of the carcinogenicity of dioxin-like compounds, including PCBs, the National Toxicology Program is currently conducting 2-year carcinogenicity bioassays of multiple dioxin-like compounds and mixtures in female Sprague-Dawley rats (van Birgelen et al., 1997). Compounds under study include TCDD 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB 126, a coplanar dioxin-like PCB), 2,3',4,4'5 -pentachlorobiphenyl (a mono-ortho PCB, PCB 118), 2,2',4,4',5,5'-hexachlorobiphenyl (a non-dioxin-like PCB, PCB 153), a binary mixture of a non-dioxin-like (PCB 153) and a dioxin-like PCB (PCB 126), and a mixture of dioxin-like compounds (TCDD, PeCDF, and PCB126).

6.3. INITIATION/PROMOTION STUDIES

The multistage nature of chemical carcinogenesis is being defined by an increasing understanding of the discrete steps required to produce a genetically altered cell that is clonally expanded and ultimately progresses to a tumor (IARC, 1992; Barrett and Wiseman, 1987; Swenberg et al., 1987; Barrett, 1992) (Figure 6-1). Briefly, the process involves damage to a specific site on DNA, a round of cell replication to fix that damage into the genome, clonal expansion of the genetically altered cells (tumor promotion), and additional genetic damage and rounds of cell replication (tumor progression). Figure 6-1 schematizes the multistage nature of cancer. The birth and death rates of genetically altered cells compared with normal cells are the centerpiece of risk assessment models that recognize the multistage nature of chemical carcinogenesis (Moolgavkar and Knudson, 1981; Portier, 1987).

The roles of proto-oncogene activation and tumor suppression gene inactivation have provided clues in attempts to discern discrete steps in carcinogenesis. It is also clear that cell proliferation is an essential component of chemical carcinogenesis, for without it, DNA damage would not be fixed into the genome and clonal expansion of genetically altered cells would not occur.

Concurrent with our increased understanding of the mechanistic underpinnings of chemical carcinogenesis, multistage models have been developed to identify the particular stage or stages in which carcinogens act to increase tumor incidence.

There is a wealth of information on liver initiation/promotion protocols in the scientific literature (Pitot and Sirica, 1980; Farber, 1984; Pitot and Campbell, 1987). These protocols frequently employ a single initiating dose of a chemical that damages DNA, followed by enhancement of cell replication (partial hepatectomy or cytotoxicity) to fix that damage into the genome (initiation), and then chronic exposure to a chemical that produces clonal expansion of the genetically altered cells (promotion). Increased tumor incidence is produced by chemicals that act at either stage. It is important to note that "initiation" and "promotion" are operational and not mechanistic terms because both stages are likely to be composed of multiple steps, and the mechanisms are not mutually exclusive. Nevertheless, the protocols have provided valuable information in our attempts to understand chemical carcinogenesis. Detailed descriptions of initiation/promotion protocols in liver and skin are provided elsewhere (Pitot and Campbell, 1987; Dragan et al., 1991; Pitot et al., 1987; Farber, 1984; Slaga et al., 1982; Peraino et al., 1981; Ito et al., 1980).

6.3.1. TCDD Is Not a Direct Genotoxic Agent

There is substantial evidence that TCDD is not a direct genotoxic agent. Because "genotoxic" and "nongenotoxic" are controversial and often misused terms, it is prudent to describe accurately the scientific criteria used to call a chemical "genotoxic" or "nongenotoxic" (IARC, 1992). Some of the criteria for designating TCDD a nongenotoxic agent are that it does not bind covalently to DNA (does not form DNA adducts). Although one study detected radioactivity associated with crude DNA preparations after in vivo exposure, no study that has rigorously looked for TCDD-DNA adducts has been positive. TCDD is negative in short-term tests for genotoxicity and is a potent promoter and weak initiator in multistage models for chemical carcinogenesis. In a another study (Turteltaub et al., 1990) using accelerator mass spectrometry, DNA adducts were not detected in rodent tissue following exposure to TCDD. This method is extraordinarily sensitive, being capable of detecting one adduct in 10^{12} normal nucleotides. Randerath et al. (1988) were unable to detect TCDD-related DNA adducts by the sensitive ^{32}P postlabeling method (limit of detection of one adduct in 10^{9} normal nucleotides). For comparison, approximately one adduct in 10^{6} normal nucleotides is found in rodent tissues following carcinogenic doses of benzo(a)pyrene (7,8-diol-9,10 epoxide deoxyguanosine DNA adduct) or methylnitrosourea (O^{6} methylguanine).

Another criterion for designating TCDD a "nongenotoxic carcinogen" is that numerous studies have demonstrated that TCDD is negative in the *Salmonella*/Ames test in the presence or absence of a mixed-function oxidase (MFO) activating system. These negative studies have encompassed 13 different bacterial strains with tests performed in 9 laboratories (Wassom et al., 1977; Kociba, 1984; IARC, 1982; Giri, 1987; Shu et al., 1987). Using its battery of tests for

genetic toxicity, the NTP (1984) concluded that TCDD was nonmutagenic. Additionally, several scientific panels have stated that false negatives for TCDD genetic toxicity are highly unlikely (EPA Science Advisory Board, 1984). TCDD has been found to promote the transformation of C3H/10T1/2 cells; it was concluded that this response did not reflect TCDD's ability to directly damage DNA (Abernethy et al., 1985). In human populations accidentally or occupationally exposed to TCDD, there is no consistent evidence for increased frequencies of chromosomal aberrations in workers exposed to TCDD (Shu et al., 1987).

However, Yang et al. (1992) demonstrated that immortalized human keratinocytes cultured with TCDD were neoplastically transformed, as evidenced by tumorigenic activity of those cells in nude mice. This response is characteristic of genotoxic carcinogens and occurred at a low TCDD concentration (0.1 nM). For comparison, induction of CYP1A2 in these same cells was not detected until a dose of 3 nM was used (Yang et al., 1992).

6.3.2. Two-Stage Models of Liver Tumor Promotion by TCDD

TCDD is designated as a nongenotoxic carcinogen because it is negative in most assays for DNA damaging potential, a potent tumor promoter, and a weak initiator or noninitiator in two-stage models for liver (Pitot et al., 1980; Graham et al., 1988; Lucier et al., 1991; Clark et al., 1991a; Flodstrom and Ahlborg, 1991) and skin (Poland et al., 1982).

Pitot et al. (1980) were the first to report that TCDD was a potent liver tumor promoter in female rats. Animals were initiated with a single dose of diethylnitrosamine (DEN)(10 mg DEN/kg) 24 hours following a 2/3 hepatectomy, followed by chronic TCDD exposure (0.14 and 1.4 μg/kg subcutaneously once every 2 weeks for 7 months). When expressed as a daily averaged dose, these doses are equivalent to 10 and 100 ng TCDD/kg/day (the medium and high dose in the Kociba bioassay). Histological evaluation revealed that five of seven animals that had received DEN and 100 ng TCDD/kg/day had hepatocellular carcinomas. No liver tumors were evident in rats receiving DEN only, DEN/low-dose TCDD, or TCDD only (high or low dose). Altered hepatocellular foci (AHF) exhibiting altered expression of the marker enzymes glucose-6-phosphatase, canalicular ATPase, and gamma glutamyl transpeptidase were also evaluated in this study. AHF are considered to represent preneoplastic lesions because increases in AHF are associated with liver cancer in rodents (Maronpot et al., 1989; Popp and Goldsworthy, 1989; Pitot et al., 1989; Williams, 1989). The AHF data were consistent with the tumor data in that a large proportion of the liver was occupied by AHF (43%) in animals initiated with DEN and the high dose of TCDD. A much smaller proportion of the liver was occupied by AHF in the other groups. This work provides strong evidence that TCDD is a potent tumor promoter in liver.

A second set of studies (Graham et al., 1988; Lucier et al., 1991; Clark et al., 1991a; Dragan et al., 1992) confirmed and extended Pitot's findings, including data suggesting a mechanistic basis for TCDD's tumor-promoting effects in rat liver. These DEN studies, using a necrogenic dose of DEN (200 mg/kg) as the initiator, have demonstrated that the effect of TCDDs on the promotion of AHF are reduced following ovariectomy. This finding is consistent with 2-year bioassays showing that TCDD is a hepatocarcinogen in female rats but not in male rats. In the tumor-promoting studies (Graham et al., 1988; Lucier et al., 1991), DEN was used as the initiating agent and TCDD (biweekly doses of 1.4 μg TCDD/kg, equivalent to 100 ng/kg/day for 30 weeks) was used as the promoter. There were four groups of intact female rats (controls, TCDD only, DEN only, and DEN/TCDD). The same four groups were used following ovariectomy. Data revealed that TCDD was a weaker liver tumor promoter in ovariectomized rats (Table 6-5). For example, there were 387 gamma glutamyl transpeptidase (GGT) positive AHF/cm^3 in intact rats compared with 80 in ovariectomized rats in the DEN/TCDD groups. Corresponding differences were evident in the proportion of liver occupied by GGT positive AHF: 0.37% in DEN/TCDD intact rats compared with 0.08% in DEN/TCDD ovariectomized rats. Few or no AHF were found in the control or TCDD-only groups. Placental glutathione S-transferase (PGST) is being used increasingly as a phenotypic marker of AHF (Ito et al., 1989), and results with this marker of preneoplasia were similar to those for GGT in that ovariectomy reduced the liver tumor-promoting actions of TCDD. The influence of ovariectomy on liver tumor incidence was evaluated in a parallel experiment using the same treatment groups in which TCDD was administered for 60 weeks. In the intact DEN/TCDD rats, liver tumor incidence was 13/37, with a total of 32 tumors compared with 7/39 (11 total tumors) in DEN/TCDD ovariectomized rats. Both hepatocellular adenomas and carcinomas were evident, along with a smaller incidence of hepatocholangiomas and hepatocholangiocarcinomas.

The mechanisms responsible for the protective effect of ovariectomy are not clear, but ovarian influences on liver TCDD retention do not seem to be involved; liver TCDD concentrations were ~20 ppb in both intact and ovariectomized rats (Lucier et al., 1991), which is similar to liver concentrations reported by Kociba et al. (1978) using the same dose of TCDD (100 ng/kg/day) but for 2 years rather than 60 weeks. One plausible mechanism may be related to cell proliferation. Another possible mechanism for the influence of the ovaries is that TCDD induces cytochrome P4501A2, which could lead to DNA-reactive metabolites of 17-beta-estradiol, the naturally occurring estrogen. P4501A2 catalyzes the formation of catechol estrogens that are carcinogens in hamsters and are considered by some to be DNA-reactive precursors (Metzler, 1984; Li and Li, 1990, Yager and Liehr, 1996).

In addition to these initial studies, a large number of studies have addressed the effect of dioxins on the development of preneoplastic AHF in the rat liver. These studies are summarized

in Tables 6-5, 6-6, and 6-7. These studies, while using different rat strains, different initiation protocols, and different dosing regimens, are consistent in showing that the induction of AHF by TCDD in the female Sprague-Dawley rat liver is dose-dependent (Maronpot et al., 1993; Teeguarden et al., 1999) (Table 6-8), exposure duration-dependent (Dragan et al., 1992; Walker et al., 2000; Teeguarden et al., 1999), and reversible after cessation of treatment (Dragan et al., 1992; Tritscher et al., 1995; Walker et al., 2000).

Other studies indicate that the capacity to induce the development of AHF in the liver by compounds structurally related to TCDD, such as the polychlorinated dibenzo-dioxins and polychlorinated dibenzo-furans, exhibits a rank-order potency similar to that for the induction of CYP1A1 activity (Flodstrom and Ahlborg, 1992; Waern et al., 1991; Schrenk et al., 1994). Although these data suggest that the potency of different dioxin-like compounds cannot be predicted solely on the basis of their potency for induction of CYP1A1, they provide evidence that liver tumor promotion likely requires an initial interaction with the AhR. Studies also demonstrate that the non-ortho-substituted (dioxin-like) polychlorinated biphenyls (PCBs) that induce the development of AHF exhibit a similar potency to that required to induce CYP1A1 activity (Hemming et al., 1995; van der Plas, 1999). Furthermore, when PCBs are administered in combination with TCDD, the effects on AHF development are additive, suggesting that tumor promotion by dioxins and dioxin-like PCBs likely acts through similar mechanisms.

6.3.3. Lung

Because the lung and respiratory tract seem to be target sites for TCDD carcinogenesis in humans (Fingerhut et al., 1991), it is of interest to evaluate whether TCDD is a tumor promoter in rodent lung. There are few published reports on the promotion of lung tumors in rats. Clark et al. used DEN as the initiating agent and TCDD (100 ng/kg/day for 60 weeks) as the promoting agent (Clark et al., 1991a) in both intact and ovariectomized rats. In contrast to liver tumor promotion, lung tumors were seen only in DEN/TCDD ovariectomized rats (4/37). No lung tumors were present in DEN/TCDD intact rats, in DEN only/TCDD only, or in control rats with or without ovariectomy. The background incidence of lung tumors in rats is very low, so the lack of tumors in controls was not unexpected (Haseman et al., 1984). The four tumors in DEN/TCDD intact rats were two squamous cell carcinomas and two adenocarcinomas.

More recently, the induction of lung lesions was examined in DEN-initiated female rats exposed biweekly to 1,750 ng TCDD/kg for up to 61 weeks. Although there was no significant effect on the development of lung tumors, TCDD exposure was associated with an increase in alveo-bronchiolar metaplasia and bronchiolar hyperplasia (Tritscher et al., 1999).

There is only a single report of the effect of TCDD on promotion of lung tumors in mice (Beebe et al., 1995). Three weeks following a single initiating dose of 25 mg NDMA/kg, male

Swiss mice were administered a single dose ranging from 0.05 μg up to 48 μg TCDD/kg, or were treated with 50 ng TCDD/kg per week for 20 weeks. The incidence of lung tumors (alveolar adenomas and carcinomas) in the initiated animals that received vehicle alone was 100%, but treatment with TCDD, either as a single dose of 1.6 μg/kg or as 50 ng/kg/week, resulted in a significant increase in tumor multiplicity. Single doses of TCDD greater than 1.6 μg/kg had no effect on tumor multiplicity, although the authors note that this may have been due to observed pulmonary toxicity.

The rodent tumorigenicity data provide clues to the complex hormonal interactions that produce site-specific carcinogenic actions of TCDD. Liver tumors are ovarian dependent, whereas the ovaries appear to protect against TCDD-mediated tumor promotion in lung. Therefore, the rat tumor data are of interest because recent epidemiologic studies (Chapter 7) have shown that TCDD exposure is associated with an increase in respiratory tract tumors.

6.3.4. Mouse Skin

Initiation/promotion studies on skin have demonstrated that TCDD is a potent tumor promoter in mouse skin as well as rat liver. Poland et al. (1982) administered a single dermal initiating dose of *N*-methyl-*N*-nitrosoguanidine (MNNG) to HRS/J hairless mice followed by twice-weekly doses of TCDD (3.75, 7.5, 15, or 30 ng) or TPA (1 or 3 μg) for 20 weeks. TCDD promoted the development of papillomas at all doses, and the response was dose dependent (100% of the animals in the high-dose TCDD group had tumors). Control animals or animals receiving only MNNG or TCDD exhibited a low incidence of tumors. These studies demonstrate that TCDD is at least two orders of magnitude more potent an agent than tetradecanoyl phorbol acetate (TPA) in mouse skin (Poland et al., 1982). On the basis of structure activity and genetic studies, it appears that the skin tumor-promoting actions of TCDD are AhR dependent. Moreover, tumorigenic responses segregate with the *hr* locus, and biochemical responses such as CYP1A1 induction can occur without carcinogenesis (Poland and Knutson, 1982; Poland et al., 1982).

Other studies have tested TCDD as an initiator and TPA as a promoter in CD-1 mice (DiGiovanni et al., 1977). Results revealed that TCDD had weak or no initiating activity in this system. To better understand the possible influence of TCDD-mediated induction of cytochrome P450 on the carcinogenicity of PAHs, TCDD was coadministered with benzo(a)pyrene or dimethylbenzanthracene to mice, followed by promotion with TPA (Cohen et al., 1979). Results revealed that TCDD decreased tumor incidence of both PAHs compared with controls. However, coadministration of TCDD with 3-methylcholanthrene to mice produced tumor incidences similar to those produced by 3-methylcholanthrene alone (Kouri et al., 1978). These results are

consistent with the findings that TCDD induction of drug-metabolizing enzymes is associated with both metabolic activation deactivation of PAHs (Lucier et al., 1979).

The relative toxicity and tumor-promoting capacity of two CDFs (2,3,4,7,8-CDF and 1,2,3,4,7,8-CDF) have been investigated in hairless mice (Hebert et al., 1990). These studies used a treatment protocol similar to that of Poland et al. (1982), including the use of MNNG as the initiating agent and varying doses of TCDD, 2,3,4,7,8-CDF, or 1,2,3,4,7,8-CDF for 20 weeks. Proliferative lesions (squamous cell papilloma, squamous cell carcinoma, or hyperproliferative nodules) were quantified. Results demonstrated that 2,3,4,7,8-CDF was 0.2 to 0.4 times as potent as TCDD and that 1,2,3,4,7,8-CDF was 0.08 to 0.16 times as potent as TCDD. These data suggest that the tumor-promoting potencies of structural analogues of TCDD, like the promotion of liver tumors, reflect relative binding properties to the AhR as well as pharmacokinetic parameters.

Taken together, results on initiation/promotion protocols indicate that TCDD is an extraordinarily potent promoter of liver and skin tumors (Pitot et al., 1987), and the results provide strong evidence that the carcinogenic actions are AhR mediated. A summary of studies on tumor promotion by TCDD or the polychlorinated dibenzofurans is given in Table 6-6. Plausible mechanisms responsible for the tumor-promoting actions of TCDD and the impact of these mechanisms on dose-response relationships are presented in Section 6.4.

6.3.5. Transgenic Models

Studies on the effect of TCDD on tumor promotion in rat liver and mouse skin require the use of an exogenous initiating agent such as diethylnitrosamine or *N*-methyl-*N*-nitrosoguanidine. Recently transgenic models for classifying the mechanism of action of carcinogens have been used to examine the mechanism of carcinogenicity of TCDD in mice (Eastin et al., 1998). These are the Tg.AC transgenic mouse, which harbors an activated mouse v-Ha-ras oncogene, and the p53 +/- transgenic mouse models, which are heterozygous for the wild-type tumor suppressor p53. Dermal application of tumor promoters such as phorbol esters results in the development of epidermal papillomas in the Tg.AC. Topical application of 166 ng TCDD/kg in acetone three times per week for 24 weeks led to a significant increase in the incidence of squamous cell papillomas in both male Tg.AC mice (8/15 TCDD-treated vs. 1/15 controls) and female Tg.AC mice (10/15 TCDD-treated vs. 1/15 controls) (Eastin et al., 1998). Treatment of p53 +/- mice by gavage with 250 ng/kg (males) or 1,000 ng/kg (females) twice a week for 24 weeks did not result in any neoplastic lesions.

Subsequent studies showed that the induction of papillomas by dermal application of TCDD to hemizygous Tg.AC mice is dose dependent over a dose range of 0-760 ng TCDD/kg 3 times per week for 26 weeks, with the lowest observed effect occurring in the 17 ng/kg dose

group (7.3 ng/kg/day) (van Birgelen et al., 1999, Dunson et al., 2000). In addition, the induction of skin papillomas in this model occurs when administration is at a site distant to the site of administration. Treatment of Tg.AC mice for 26 weeks by oral gavage with 0, 105, 450, or 1,250 ng TCDD/kg led to an increase in skin papillomas in the 1,250 ng/kg dose group only (5/20 TCDD-treated vs. 0/18 in controls) (van Birgelen et al., 1999). These data provide further support for the potent tumor-promoting action of TCDD.

6.4. MECHANISMS OF TCDD CARCINOGENICITY

6.4.1. Indirect DNA Damage

Although TCDD is negative in genetic toxicity tests, high doses of TCDD (50 to 100 μg/kg) induce single-strand breaks in Sprague-Dawley rats, presumably as a consequence of increased lipid peroxidation (Wahba et al., 1988, 1989). In addition, though TCDD may not be directly genotoxic, it has been suggested that it may be indirectly genotoxic through the formation of potentially DNA reactive oxygen species. This may result from cytochrome P450 induction by TCDD (Park et al., 1996), through the induction of oxidative stress (Slezak et al., 1999), or through the formation of catechol estrogens (Graham et al., 1988; Spink et al., 1992; Yager et al., 1996). Indeed, higher levels of oxidative DNA damage (8-OH-dG adducts) have been observed in chronically exposed female rats (Tritscher et al., 1996) and these TCDD-induced increases were not observed in ovariectomized rats. Other evidence to support this hypothesis includes the observation that mathematical modeling of the development of altered hepatocellular foci indicates that TCDD may have an effect on the initiation rate within the framework of a one-cell two-stage initiation-promotion model (Portier et al., 1996; Moolgavkar et al., 1996)(see Chapter 8). However, alternate two-cell models for tumor promotion do not suggest an effect on the initiation rate (Conolly et al., 1997).

6.4.2. Endocrine Disruption/Growth Dysregulation/Altered Signal Transduction

One of the characteristics of TCDD is that it is a potent growth dysregulator and alters the signaling of numerous hormonal systems. TCDD induces the expression of a large number of genes involved in growth regulation, hormonal signaling and signal transduction, and hormone metabolism. In addition to these effects, which are presumably mediated through the AhR-ARNT heterodimer, there are also AhR-dependent effects on signaling pathways independent of activation of gene expression by the AhR-ARNT heterodimer that may be related to the mechanism of toxicity of TCDD. These effects are described in more detail in Chapter 2. Although many of the effects of TCDD have not been directly assessed for their role in the carcinogenicity of TCDD, it is likely that sex, species, and tissue specificity of dioxin carcinogenicity is due to a combination of these effects. Consequently, it is unlikely that a single

mechanism is responsible for all the carcinogenic effects of TCDD in all tissues and species. However, it is now accepted by the scientific community that most, if not all, of TCDD's toxic and biochemical effects, including tumor promotion, are AhR dependent and that TCDD provides an example for evaluating the issues relevant to risk assessment for receptor-mediated carcinogens.

The list of biochemical effects produced by TCDD in humans, experimental animals, and cell systems is expanding. These effects include those that may alter normal cell regulatory processes, such as cell proliferation and differentiation, metabolic capacity, and hormonal pathways. Potentially the effects of TCDD on the endocrine system and tissue differentiation may play a role in susceptibility to carcinogenesis induced by other compounds, that is distinct from effects on metabolism of procarcinogens. Brown and co-workers showed that prenatal exposure of female rats to TCDD resulted in an increased susceptibility to DMBA-induced mammary adenocarcinomas. This was likely due to an increase in mammary gland terminal end buds as a result of prenatal exposure (Brown et al., 1998).

6.4.3. Cell Replication/Apoptosis and Tumor Promotion

One mechanism that has been proposed for the reduced tumor promotion capacity of TCDD in ovariectomized rats is the effect of TCDD on cell proliferation. TCDD did not stimulate cell proliferation rates in ovariectomized rats, whereas a mean increase of tenfold was apparent in intact rats receiving 100 ng TCDD/kg for 30 weeks (Table 6-5) (Lucier et al., 1991). There was considerable interindividual variation in both cell proliferation rates and enzyme-altered foci in the DEN/TCDD groups. Comparisons of the two data sets revealed a strong positive correlation between enzyme-altered foci and cell proliferation, although the importance of this finding is diminished by the fact that cell proliferation was quantified in nonlesioned hepatocytes. The mechanism whereby ovarian hormones and TCDD interact to produce cell proliferation in hepatocytes may involve growth factor pathways. Consistent with this idea, TCDD induced a loss of plasma membrane epidermal growth factor receptor (EGFR) in intact rats but not in ovariectomized rats (Sewall et al., 1993) EGF is thought to provide a mitogenic stimulus in hepatocytes and to play a key role in hepatocarcinogenesis (Vickers and Lucier, 1991; Velu, 1990; Shi and Yager, 1989; Eckl et al., 1988). A schematic representation of a plausible mechanism for the role of estrogen in TCDD-mediated liver cancer in rats is given in Figure 6-2.

These observations of the ovarian hormone-dependent increase in hepatocyte replication following chronic exposure to TCDD (Lucier et al., 1991) parallel the observed sex-dependent induction of liver tumors in rats. This observation has led to the hypothesis that the induction of cell replication by TCDD may be a critical event in the mechanism of hepatocarcinogenesis. This

hypothesis was supported by the observation that hepatocyte replication was dose-dependently increased after chronic exposure to TCDD (Maronpot et al., 1993)(Table 6-8).

Other studies, however, have failed to observe any effect of TCDD on nonfocal hepatocyte replication (Buchmann et al., 1994; Stinchcombe et al., 1995). More recently, it was shown that induction of hepatocyte replication is exposure-duration dependent and is only observed following 30 weeks of exposure to TCDD (Walker et al., 1998). Indeed, after 14 weeks of exposure, hepatocyte replication is lower in TCDD-treated animals than in controls. These data indicate that the induction of hepatocyte replication is not an early event in tumor promotion by TCDD and likely represents a secondary response to the induction of putatively preneoplastic AHF. However, data are insufficient to conclude that induction of hepatocyte replication is not involved in development of liver tumors.

Although cell replication is not seen after subchronic exposure to TCDD, it was observed that there was a suppression of hepatocyte apoptosis following TCDD treatment (Stinchcombe et al., 1995). The suppression of UV-inducible apoptosis by TCDD has also been observed in vitro (Worner et al., 1996), suggesting that this suppression may be an early event in tumor promotion. The suppression of apoptosis by TCDD in AHF may provide a growth advantage to these preneoplastic lesions, and therefore may be involved in the mechanism of hepatocarcinogenesis.

6.4.4. Thyroid Cancer–Proposed Mechanism of Action

TCDD causes a dose-related increase in thyroid follicular cell adenomas and carcinomas in rats and mice. One hypothesis for the induction of thyroid tumors involves the disruption of thyroid hormone homeostasis via induction of phase II enzymes UDP-glucuronosyltransferases (UGTs) (Hurley, 1998; Hill et al., 1998). Dioxin-like compounds induce the synthesis of UDP-glucuronosyltransferase-1 (UGT1) mRNA by an AhR-dependent transcriptional mechanism (Bock et al., 1998; Nebert et al., 1990). It is proposed that dioxin-like compounds increase the incidence of thyroid tumors through an extrathyroidal mechanism. Dioxin-like compounds induce hepatic UGT resulting in increased conjugation and elimination of thyroxine (T4), leading to reduced serum T4 concentrations. T4 production is controlled by the thyroid stimulating hormone (TSH) which is under negative and positive regulation from the hypothalamus, pituitary, and thyroid by the thyrotrophin-releasing hormone (TRH), TSH, T4, and triiodothyronine (T3). Consequently, the reduced serum T4 concentrations would lead to a decrease in the negative feedback inhibition on the pituitary gland. This would then lead to a rise in the secreted TSH and stimulation of the thyroid. The persistent induction of UGT by dioxins and subsequent prolonged stimulation of the thyroid would result in thyroid follicular cell hyperplasia and hypertrophy of the thyroid, thereby increasing the risk of progression to neoplasia. In support of this hypothesis, Kohn et al. modeled the effect of TCDD on UGTS and thyroid hormones in female rats within the

framework of a pharmacologically based-pharmacokinetic (PBPK) model (Kohn et al., 1996). This mathematical model described release and uptake of thyroid hormones, metabolism, TCDD induction of UGT1, regulation of TSH release from the pituitary by T4 and feedback on TRH and somatostatin which inhibits TSH release. The model successfully reproduced the observed effects of TCDD on serum T3, T4, and TSH, and UGT1 mRNA and enzyme activity, suggesting that this is a plausible mechanism for an indirect role of TCDD on the thyroid. This model is supported by the more recent experimental work of Schuur et al. which demonstrated the extrathyroidal effects of TCDD on thyroid hormone turnover (Schuur et al., 1997).

6.4.5. Mechanisms of Reduced Spontaneous Tumor Incidence

In the Kociba study, there was a significant reduction in the incidence of spontaneous benign tumors of the uterus, benign neoplasms of the mammary gland, mammary carcinomas, and pituitary adenomas in female rats, and pheochromocytoma of the adrenal gland and pancreatic adenomas in male rats. It is likely that the reduction in body weight gain as a result of TCDD exposure is responsible for the reduced incidence in these spontaneous lesions. Studies by the National Toxicology Program (Haseman and Johnson, 1996; Seilkop, 1995) indicate that chemical exposures resulting in a reduction of body weight by 10%-20% is correlated with a decrease in incidence of both mammary tumors and anterior pituitary tumors. This phenomenon may be related to a homeostatic growth suppression in reproductive organs during periods of reduced nutritional status. In addition, reduction in pancreatic adenomas (Kociba et al., 1978) has been observed in diet-restricted animals, and consequently, this reduction in incidence may be due to TCDD induced body weight reduction. The reduction in pheochromocytoma in male rats does not appear to be a consequence of changes in body weight, yet it exhibited a dose-dependent reduction in TCDD exposed males. The mechanism for the reduction in pheochromocytoma is unknown. The decrease in hormone-dependent cancers also may be related to the ability of TCDD to alter estrogen metabolism and/or its ability to act in some tissues/cell systems as an antiestrogen. Increased estrogen metabolism may result from increased expression of CYP1 cytochromes P450, UGT, and GST. Decreased estrogen action could result from effects on the estrogen receptor (ER) levels or ER transcriptional function. TCDD may also induce the inactivation of estrogen in target cells by metabolism by TCDD inducible cytochromes CYP1A2, CYP1B1, or conjugating enzymes such as UGT1/GST without altering circulating estradiol concentrations.

6.5. BIOCHEMICAL RESPONSES

This section will summarize some of the changes produced by TCDD, including discussion of (1) possible relevance of the response to TCDD-mediated cancer, (2) whether the response is AhR mediated, (3) whether information is available on the role of transcriptional activation, (4) dose-response relationships, and (5) whether animal models are consistent with human responses. This chapter will not attempt to evaluate all of the biochemical and molecular responses to TCDD, but will focus on the ones that are either the most relevant to carcinogenic responses or have received the most study. The responses include induction of P4501A1 (CYP1A1), cytochrome P4501A2 (CYP1A2), EGFR, estrogen receptor (ER), and UDP-glucuronosyltransferase (UDPGT). Table 6-9 lists many of the biochemical changes affected by TCDD in in vivo and/or in vitro systems and some information on mechanisms of action.

6.5.1. Cytochrome P450

The most studied response to TCDD has been induction of cytochrome P450 isozymes (Whitlock, 1990; Silbergeld and Gasiewicz, 1989; Poland and Knutson, 1982). The first reports of P450 induction in vivo and in vitro appeared in 1973 (Lucier et al., 1973; Greig and DeMatteis, 1973; Poland and Glover, 1973), and hundreds of papers have been published on the subject since that time. These papers have dealt with various aspects of TCDD-mediated induction of P450, such as isozyme specificity, time course, structure-activity relationships, molecular mechanisms of transcriptional activation of the CYP1A1 gene, identification of transcriptional activating factors, tissue and cell specificity, and dose-response relationships. The molecular mechanisms responsible for enzyme induction are described elsewhere in this volume.

The mechanistic relationship of CYP1A1 and 1A2 induction to cancer or any other toxic endpoint following dioxin exposure has not yet been demonstrated, yet considerable controversy exists on this subject (Roberts, 1991). Because CYP1A1 catalyzes the metabolic activation of many chemicals, such as the PAHs, to DNA-reactive metabolites, it has been postulated that induction of CYP1A1 might enhance the carcinogenic actions from a given exposure level to many PAHs. Recently it has been shown that benzo(a)pyrene is not carcinogenic in transgenic mice that are AhR deficient (Shimizu et al., 2000). The lack of carcinogenicity is presumably due to the lack of induction of CYP1A1 by B(a)P in these animals, supporting a proposed role for CYP1A1 in the carcinogenicity of B(a)P. Usually, however, preinduction of CYP1A1 diminishes the carcinogenic potency of PAHs such as 3-methylcholanthrene, benzo(a)pyrene, and 7,2-dimethylbenzanthracene if exposure to an inducing agent (such as TCDD) is short term (Parkinson and Hurwitz, 1991; Wattenberg, 1985; Cohen et al., 1979; Wattenberg, 1978; Miller et al., 1958). Induction also protects against the carcinogenic actions of aflatoxin, diethylnitrosamine, arylamines, and urethane. Protection occurs at numerous cancer sites,

including liver and lung. Several lines of evidence support the idea that enzyme induction is the mechanism responsible for the protective effect. First, treatment of mice deficient in AhR with inducers does not protect against PAH-mediated cancer (Kouri et al., 1978). Second, the ability of inducing agents to protect against cancer is positively correlated with their potency as inducing agents (Wattenberg and Leong, 1970; Arcos et al., 1961). Third, the inducing agent must be administered at least 1 day prior to treatment, which allows sufficient time for the inducer to produce elevated levels of CYP1A1 (Parkinson et al., 1983; Wheatley, 1968).

The most probable mechanism for the protective effect of enzyme induction is that it leads to decreased concentrations of promutagenic DNA adducts in target tissues. These findings appear to contradict the knowledge that CYP1A1 is required for the metabolism of PAHs, aflatoxin, and several other carcinogens to DNA-reactive arene oxides (Guengerich, 1988; Levin et al., 1982; Conney, 1982). For example, the promutagenic DNA adduct of benzo(a)pyrene appears to be a 7,8-diol-9,10 epoxide metabolite adducted to deoxyguanosine, and formation of this metabolite requires two separate actions of CYP1A1. The contradiction can be resolved by analysis of all the metabolic pathways for chemical carcinogens whose potencies are decreased by pretreatment with inducing agents. In addition to CYP1A1-mediated increases in metabolic activation, CYP1A1 also converts PAHs to inactive metabolites (Thakker et al., 1985; Pelkonnen and Nebert, 1982). Moreover, induction of uridine diphosphoglucuronyltransferase also occurs concurrently with CYP1A1 induction (Lucier et al., 1986). This enzyme also detoxifies metabolites of PAHs and other carcinogens and facilitates their excretion from the body (Thakker et al., 1985; Nemoto and Gelboin, 1976). Therefore, it appears that TCDD-mediated enzyme induction increases the rate of detoxification of some carcinogens to a greater extent than it increases the rate of formation of DNA-damaging metabolites.

Increased frequency of sister chromatid exchanges was observed in lymphocytes of people exposed to pentachlorinated dibenzo-*p*-dioxins (PCDFs) in Taiwan when those lymphocytes were challenged with beta-naphthoflavone (Lundgren et al., 1986, 1988). This may be because the PCDFs cause increased rates of metabolic activation of beta-naphthoflavone to DNA-reactive metabolites (Lundgren et al., 1987). These findings are consistent with the idea that TCDD's ability to induce drug-metabolizing enzymes (CYP1A1 and 1A2) may lead to an increased rate of formation of DNA-reactive metabolites of some carcinogens, most notably the PAHs and aromatic amines. However, there is evidence that the opposite effect occurs in some cases, because in vivo exposure to CYP1A1 inducers actually leads to a decrease in DNA adducts in target tissue following in vivo exposure to PAHs such as benzo(a)pyrene (Cohen et al., 1979; Parkinson and Hurwitz, 1991). It can reasonably be concluded that TCDD exposure may increase the rate of DNA adduct formation for some carcinogens but decreases the rate for others, and that

predictions should not be made without experimental data on DNA adduct concentrations in control and TCDD-treated animals.

Although there is no clear mechanistic link between CYP1A1 induction and cancer, it is important to note that many CYP1A1 inducers are themselves carcinogens when encountered in chronic dosing regimens; therefore, the protective effect of inducing agents appears to be limited to short-term exposure. For example, benzo(a)pyrene, 3-methylcholanthrene, and TCDD are CYP1A1 inducers and multisite carcinogens (Vanden Heuvel and Lucier, 1993; Levin et al., 1982; Slaga et al., 1979; Sims and Glover, 1974).

The relationship of CYP1A2 induction to the carcinogenic actions of other compounds is less clear than it is for CYP1A1. For example, CYP1A2 catalyzes the formation of catechol estrogens from 17-beta-estradiol (Graham et al., 1988). The catechol estrogens are considered to be possible toxic metabolites because they could lead to increased free radical damage to cellular macromolecules such as DNA (Li and Li, 1990; Metzler, 1984; Yager and Liehr, 1996). This mechanism could be responsible, in part, for the findings that TCDD is a hepatocarcinogen in female rats but not male rats, and that ovariectomy protects against the hepatocarcinogenic actions of TCDD. Also consistent with the hepatocarcinogenicity data is the observation that CYP1A2 is induced in liver but not in extrahepatic organs, with the possible exception of the nasal mucosa (Goldstein and Linko, 1984). In contrast, CYP1A1 induction occurs in virtually every tissue of the body, which is consistent with the observation that the AhR is found in a wide variety of cell types.

In addition to the well-characterized induction of CYP1A1 and CYP1A2, TCDD also induces another cytochrome P450, CYP1B1, that has been identified in humans and rodents (Bhattacharyya et al., 1995; Savas et al., 1994; Sutter et al., 1994; Walker et al., 1995). CYP1B1 is expressed in a variety of human tissues and is inducible by TCDD in numerous human cell and rodent tissues including liver, lung, and kidney (Hayes et al., 1996, Sutter et al., 1994; Walker et al., 1995). CYP1B1 is active in the metabolism of numerous polycyclic aromatic hydrocarbons and arylamines (Otto et al., 1992; Shimada et al., 1996; Crofts et al., 1998) and can catalyze the 4-hydroxylation of 17-beta-estradiol in humans cells (Hayes et al., 1996). The potent carcinogenicity of 4-hydroxyestradiol in Syrian Golden hamsters (Liehr et al., 1986) and the observed elevation of 4-hydroxylase activity in human tumors (Liehr et al., 1996) suggest that the estradiol hydroxylase activity of CYP1B1 may play a critical role in tumorigenesis. This implication has been further extended to suggest that the induction of CYP1B1 in rat liver may play a role in the ovarian hormone-dependent hepatocarcinogenicity of TCDD (Yager et al., 1996). However, there are no reports in the literature that CYP1B1 in rodents has any significant estradiol hydroxylase activity, and therefore it is not clear if CYP1B1 is involved in the mechanism of hepatocarcinogenesis in rats.

CYP1B1 in both humans and rodents is active in the metabolism of PAHs and arylamines, and therefore, like CYP1A1, CYP1B1 may play a role in modulating the carcinogenicity of procarcinogens in both humans and experimental models. A recent report indicates that CYP1B1-dependent DMBA metabolism is required for the induction of DMBA-induced lymphomas in mice (Buters et al., 1999).

There are a number of studies on dose-response relationships for TCDD's effects on CYP1A1 and 1A2 (DeVito et al., 1991; Lin et al., 1991a; Kedderis et al., 1991; Harris et al., 1990a; Goldstein and Safe, 1989; Abraham et al., 1988; Lucier et al., 1986; Vecchi et al., 1983; Kitchin and Woods, 1979; Poland and Glover, 1973). These studies (Tritscher et al., 1992; Graham et al., 1988; Sloop and Lucier, 1987) include single and chronic dosing, time-course evaluations, and species comparisons. Dose-response relationships have been evaluated by quantitation of CYP1-dependent enzyme activities, quantitation of mRNA levels by Northern blot analysis, and quantitation of CYP1 protein by radioimmunoassay and immunolocalization in tissue sections. Dose-response modeling of these studies is described in detail in Chapter 8 of this document. Evaluations of various data sets for TCDD-mediated dose-response relationships have revealed some interesting information. One way of analyzing data for linearity or nonlinearity of dose-response for receptor-mediated events is the Hill equation (Hayashi and Sakamoto, 1986). A Hill coefficient of 1 suggests a linear relationship between exposure and dose throughout the experimental dose range, and would predict a proportional relationship between target tissue concentration of TCDD and biological response at all dose levels. This would imply that the response had no practical threshold or “no effect level.” Hill coefficients greater than 1 would indicate sublinearity in dose-response, whereas a Hill coefficient of less than 1 would indicate supralinearity for response in the low-dose region. Analyses of single-exposure and chronic exposure data for CYP1A1 and CYP1A2 induction in rat or mouse liver indicate a Hill coefficient of slightly greater than 1 for CYP1A1 and slightly less than 1 for CYP1A2 (Portier et al., 1992; Kohn et al., 1993). Although these analyses involve an extrapolation beyond the range of experimental data, they are consistent with the hypothesis that there is no threshold for TCDD-mediated induction of CYP1A1 and 1A2. Time-course and dose-response analyses indicate that CYP1B1 is expressed at significantly lower levels than either CYP1A isozyme and is induced only at higher doses than those required for CYP1A1 or CYP1A2 (Santostefano et al., 1997; Walker et al., 1999). Furthermore, the Hill coefficient for CYP1B1 induction is greater than that for CYP1A1 (Walker et al., 1999). A more detailed analysis of dose-response relationships for cytochrome P450 induction and other dioxin-inducible responses can be found in Chapter 8 of this volume.

Immunological detection of induced CYP1A1 and 1A2 in liver sections obtained from rats exposed chronically to TCDD indicates hepatocyte heterogeneity in response to TCDD (Tritscher

et al., 1992; Bars and Elcombe, 1991). For example, relatively low doses of TCDD (1 ng/kg/day) appear to maximally induce some cells around the centrilobular region. Increasing doses of TCDD increase the number of cells responding, rather than the amount of induction in responding cells. Like CYP1A1 and CYP1A2, CYP1B1 is also induced by TCDD in the rat liver in a centrilobular pattern of expression (Walker et al., 1997). It has been suggested that the heterogeneous pattern of expression may be due to differences in expression of the AhR across the acinus (Lindros et al., 1997) or to differences in binding affinity (Andersen et al., 1997). Alternatively, the observation that CYP1A2 is responsible for hepatic sequestration of TCDD (Diliberto et al., 1999) suggests that the heterogeneity in expression of the CYPs may be in part due to a heterogeneity in distribution of TCDD across the liver acinus. In support of this theory, the concentration of TCDD in periportal hepatocytes is higher than that seen in centrilobular hepatocytes (Santostefano et al., 1999). These data, which document cell differences in sensitivity to induction, complicate evaluation of dose-response relationships. For example, some hepatocytes appear to be maximally induced by low doses of TCDD, whereas other hepatocytes exhibit no detectable P450 induction response at the same doses. As discussed earlier, a mechanistic link between P450 induction and cancer has not been established. Evaluations of P450 induction and TCDD-mediated cell proliferation by immunocytochemical methods in rat liver reveal that cells expressing CYP1A1 and 1A2 are different from those exhibiting TCDD-mediated increases in DNA replication (Lucier et al., 1992).

Placentas from Taiwanese women exposed to rice oil contaminated with polychlorinated dibenzofurans have markedly elevated levels of CYP1A1 (Lucier et al., 1987; Wong et al., 1986). Comparison of these data with induction data in rat liver suggests that humans are at least as sensitive as rats to the enzyme-inductive actions of TCDD and its structural analogues (Lucier, 1991). Consistent with this contention, the in vitro EC_{50} for TCDD-mediated induction of CYP1A1-dependent enzyme activities is approximately 1.5 nM when using either rodent or human lymphocytes (Clark et al., 1992). Also, binding of TCDD to the AhR occurs with a higher affinity in rat cellular preparations compared with humans (Lorenzen and Okey, 1991; Okey et al., 1989). This difference may be related to the greater lability of the human receptor during tissue preparation and cell fractionation procedures, or to an inherent property of the human AhR (Manchester et al., 1987). In any event, it does appear that humans contain a fully functional AhR (Cook and Greenlee, 1989), as evidenced by significant CYP1A1 induction in tissues from exposed humans, and this response occurs with sensitivity similar to that observed in experimental animals.

6.5.2. Epidermal Growth Factor Receptor

EGF is a potent mitogen and stimulates the generation of mitotic signals in both normal and neoplastic cells (Stoscheck and King, 1986; Carpenter and Cohen, 1979). Several lines of evidence suggest that the EGF receptor and its ligands, including transforming growth factor-alpha, possess diverse functions relevant to cell transformation and tumorigenesis (Velu, 1990; Marti et al., 1989; Mukku and Stancel, 1985). In fact, the mechanism of action for several tumor promoters, such as phenobarbital and the phorbol esters, is thought to involve the EGF receptor pathway (Stoscheck and King, 1986). A schematic representation of the proposed mechanism for EGF-stimulated mitogenesis is given in Figure 6-3.

Several studies have shown that TCDD decreases the binding capacity of the plasma membrane EGF receptor for its ligand without a change in K_d (Clark et al., 1991a; Lin et al., 1991a; Abbott and Birnbaum, 1990; Astroff et al., 1990; Sunahara et al., 1989; Stoscheck and King, 1986; Hudson et al., 1985; Madhukar et al., 1984). One study used a range of TCDD doses (3.5 to 125 ng/kg/day) for 30 weeks to evaluate the effects of TCDD exposure on EGF receptor in rat liver plasma membranes. There was a clear dose-response relationship for TCDD's effects on the total binding capacity of the EGF receptor, although TCDD did not produce a change in binding affinity of the receptor. The maximal effect was a threefold decrease in the concentration of plasma membrane EGF receptor; the ED_{50} was ~10 ng/kg/day based on administered dose and ~2 ppb TCDD based on liver TCDD concentration. These values are similar to the ED_{50} for induction of CYP1A1 and CYP1A2 for 30-week exposures. The dose-response data, like the data for CYP1A1 and CYP1A2 induction, were subjected to curve-fitting analyses using the Hill equation (Portier et al., 1992). This analysis indicated that a Hill coefficient of 1 provided the best fit, suggesting that there is a linear relationship between target tissue dose and response for effects on the EGF receptor. Although Hill analyses of dose-response data for TCDD's effects on the EGF receptor, CYP1A1 induction, and CYP1A2 induction are inconsistent with the idea of a threshold, the lowest dose used in these experiments was 100 pg/kg/day, so the possibility exists that dose-response relationships are different in the very low-dose region (1 to 10 pg/kg/day) encountered as background human exposures.

Dose-response data on EGFR were compared with dose-response relationships for TCDD-mediated increases in cell proliferation and growth of preneoplastic lesions within the framework of a two-stage model for hepatocarcinogenesis in rats (Lucier et al., 1992, Sewall et al., 1993, 1995a). Results indicate that cell proliferation and the growth of preneoplastic lesions are less sensitive responses to TCDD than is loss of plasma membrane EGF receptor. Therefore, the EGF receptor may be involved in the hepatocarcinogenic actions of TCDD, but dose-response relationships for this effect may be different from dose-response relationships for liver cancer in

rats. These data reflect the knowledge that several steps and/or several genes are involved in the modulation of coordinated biological responses.

The mechanism by which TCDD alters EGF receptor-binding capacity is not fully understood, although TCDD does not appear to decrease EGF receptor mRNA (Lin et al., 1991a; Osborne et al., 1988). By using congenic mice deficient in the high-affinity AhR, TCDD's effects on the EGF receptor were shown to require the AhR (Lin et al., 1991a). In control animals, the EGF receptor is distributed on the surface of the plasma membrane and is composed of an external ligand-binding domain, a transmembrane domain, and an intercellular domain (Velu, 1990; Carpenter, 1987). Ligands for the EGF receptor (EGF or TGF-alpha) in the intracellular space bind the EGF receptor, producing a conformational change that stimulates the intercellular region to catalyze phosphorylation of the receptor itself as well as other proteins involved in cell regulation. The process results in internalization of the receptor, characterized by an increase in cytosolic EGFR coupled with a decrease in membrane-bound receptor. The effects of TCDD and CDFs on the number of binding sites for the plasma membrane EGF receptor are correlated with a concomitant decrease in EGF-stimulated autophosphorylation of the EGF receptor, indicating that TCDD produces a true functional change in the EGF receptor (Clark et al., 1991a; Sunahara et al., 1989; Nelson et al., 1988; Sunahara et al., 1988). Importantly, the addition of EGF to hepatocytes or several cell lines in culture produces a loss of plasma membrane EGF receptor coupled with a loss of EGF-stimulated autophosphorylation (Velu, 1990; Carpenter, 1987). Therefore, TCDD produces an EGF receptor-like response consistent with the idea that TCDD enhances the generation of cellular mitotic signals.

Although TCDD exposure mimics EGF actions in hepatocytes, TCDD itself does not appear to bind to the EGF receptor. The most plausible mechanism for effects on the EGF receptor involves the finding that TCDD induces production of TGF-alpha in hepatocytes as well as human keratinocytes (Choi et al., 1991). This response could alter control of normal growth patterns because TGF-alpha binds the EGF receptor with high affinity, leading to enhanced production of mitogenic signals. Alternatively, TCDD may affect EGF receptor transcription. In fact, TCDD has been shown to decrease uterine EGF receptor mRNA levels (Astroff et al., 1990). Receptor concentrations may also be altered by other events including posttranslational glycosylation, increased lysosomal degradation, or alterations in signal transduction pathways such as protein kinases (Madhukar et al., 1988). It is also possible that TCDD alters phosphorylation of the EGF receptor by activation of protein kinase C, resulting in decreased binding capacity of the plasma membrane EGF receptor. This effect occurs following exposure to the tumor promoter TPA and is associated with decreased autophosphorylation rates and EGF receptor internalization (Beguinot et al., 1985; Cochet et al., 1984). In any event,

TCDD-mediated alterations in EGF receptor pathways may, in part, be responsible for the tumor-promoting actions of TCDD by enhancement of mitotic signals.

The effects on the EGF receptor system may be mediated by estrogen action, and it has been postulated that the estrogen and EGF receptor pathways are integrated by "cross talk" mechanisms (Ignar-Trowbridge et al., 1992; Astroff et al., 1990). In vivo and in vitro studies have demonstrated that TCDD alters the ER (DeVito et al., 1992; Lin et al., 1991a; Clark et al., 1991a; Umbreit and Gallo, 1988; Romkes et al., 1987) and estrogens can, in turn, alter EGF receptor binding and cellular distribution (Vickers and Lucier, 1991; Vickers et al., 1989; Mukku and Stancel, 1985). Moreover, studies conducted within the framework of a two-stage model for hepatocarcinogenesis have demonstrated that TCDD-mediated decreases in plasma membrane EGF receptor are ovarian hormone dependent (Sewall et al., 1993). These studies concluded that ovarian hormones are essential to the tumor-promoting actions of TCDD because TCDD does not induce hepatocyte proliferation or stimulate the growth of preneoplastic lesions in ovariectomized rats (Section 6.3, Initiation/Promotion Studies).

Evidence indicates that TCDD and its structural analogues produce the same effects on the EGF receptor in human cells and tissues as observed in experimental animals. First, incubation of human keratinocytes with TCDD decreases plasma membrane EGF receptor, and this effect is associated with increased synthesis of TGF-alpha (Choi et al., 1991; Hudson et al., 1985). Second, placentas from humans exposed to rice oil contaminated with polychlorinated dibenzofurans exhibit markedly reduced EGF-stimulated autophosphorylation of the EGF receptor, and this effect occurred with similar sensitivity to that observed in rats (Lucier, 1991; Sunahara et al., 1989). The magnitude of the effect on autophosphorylation was positively correlated with decreased birth weight of the offspring.

6.5.3. UDP-Glucuronosyltransferases

Several studies have shown that TCDD induces synthesis of at least one isozyme of UDPGT (Lucier et al., 1973, 1974, 1986) by a mechanism that requires the AhR (Bock, 1991). The gene UGT-1 regulates synthesis of the UDPGT isozyme, which conjugates numerous substrates including 1-naphthol, p-nitrophenol, and thyroxine (Burchell et al., 1991). This gene contains a TCDD-responsive element that permits transcriptional activation following binding of the TCDD-AhR complex. Other chemicals that bind the AhR, such as 3-methylcholanthrene and benzo(a)pyrene, also induce UGT-1 (Bock, 1991). UDPGTs are considered a deactivation pathway for numerous environmental chemicals and endogenous compounds, such as steroid hormones, by rendering them water soluble and excretable as a consequence of the catalytic addition of a glucuronide moiety (Tephly and Burchell, 1990). Therefore, induction of UDPGT

may be responsible, in part, for the finding that pretreatment with TCDD leads to diminished DNA adducts for PAHs and decreased concentrations of some steroid hormones.

Conjugation of thyroxine by UGT-1 leads to deactivation and elimination of this thyroid hormone (Henry and Gasiewicz, 1987; Bastomsky, 1977). The decreased levels of thyroxine associated with UDPGT induction produce decreased feedback inhibition of the pituitary gland, which responds by secreting increased amounts of TSH (Sanders et al., 1988; Barter and Klaassen, 1992). Several studies have provided evidence that prolonged stimulation by TSH produces an oncogenic effect on the thyroid (Hill et al., 1989). Interestingly, rat liver EGF receptor may, in part, be regulated by thyroid hormones (Mukku, 1984). Increased incidence of thyroid tumors is the most sensitive endpoint in cancer bioassays, as evidenced by a statistically significant increase at a dose of 1.4 ng/kg/day. Consistent with this hypothesis, rodent studies have shown that TCDD and other inducers of hepatic UDPGT decrease thyroxine concentration in blood, which is associated with increased levels of thyroid-stimulating hormone (Barter and Klaassen, 1992; Henry and Gasiewicz, 1987).

Dose-response studies for TCDD's inductive effects on hepatic UDPGT in rats have demonstrated that the single dose ED50 is approximately 0.7 μg/kg, which is similar to the ED50 for CYP1A1 induction (Lucier et al., 1986). Furthermore, the shape of the dose-response curve for both responses is similar. Analysis of the expression of UGT1A1 in rodent liver showed that induction of UGT1A1 RNA was dose dependently increased following a single dose of TCDD (Vanden Heuvel et al., 1994). Further studies showed that chronic exposure of female rats to 0-125 ng TCDD/kg/day for 30 weeks led to a significant increase in UGT and subsequent alterations in thyroid function (Sewall et al., 1995b). A mathematical pharmacokinetic-pharmacodynamic model for TCDD (Kohn et al., 1993) was modified to include effects of TCDD on UGT and thyroid hormone levels (Kohn et al., 1996) (see Chapter 8). Model outcomes accurately predicted changes in thyroid hormone levels in TCDD-treated female rats and lend support to the hypothesis that induction of UGT, and subsequent persistent stimulation of the thyroid by TSH, may be involved in the promotion of thyroid tumorigenesis. It is noteworthy, however, that these data were obtained from female Sprague-Dawley rats and that the thyroid carcinogenicity of TCDD was observed in male but not female Osborne-Mendel rats. Furthermore, in the Kociba study chronic exposure to TCDD did not induce thyroid tumors in female Sprague-Dawley rats. Although gender-specific difference in carcinogenicity may be due to higher circulating levels of TSH in male rats, the model predictions increase confidence in the hypothesis that the induction of UDPGT by TCDD is directly involved in the mechanism.

Because humans have the dioxin-responsive UDPGT (UGT-1) (Burchell et al., 1991) and TCDD induces UDPGT in human hepatocyte cell cultures, it is reasonable to assume that TCDD

and its structural analogues would induce UDPGT in humans, although laboratory data are needed to validate this assumption.

6.5.4. Estrogen Receptor

Several lines of evidence have demonstrated that interactions of TCDD and estrogens are critical to some of the carcinogenic responses to TCDD. Although the precise mechanisms of those interactions have not been established, recent data indicate that TCDD effects on the ER and on estrogen metabolism are involved. The mechanisms for TCDD/estrogen interactions appear to be tissue specific. Of particular interest is the finding that TCDD increases liver tumor incidence in rats, and at the same time decreases tumor incidence in organs such as the mammary gland, uterus, and pituitary (Kociba et al., 1978). Therefore, TCDD/estrogen interactions will be examined separately for liver and other endocrine organs.

The liver contains a fully functional ER that possesses characteristics similar to those identified for ER in the mammary gland and uterus (Mastri and Lucier, 1983; Powell-Jones et al., 1981; Eisenfeld et al., 1976). For example, the liver exhibits high-affinity binding for 17-beta-estradiol and other potent estrogens, liver ER binding is specific for estrogens, the ligand receptor complex interacts reversibly with DNA, and this interaction leads to transcriptional activation of estrogen-responsive genes. Synthesis of hepatic ER, unlike ER in other target tissues, is under pituitary control (Lucier et al., 1981). Treatment of rats with a single dose of TCDD decreases binding capacity of the hepatic ER, and this effect is correlated with a decrease in ER protein (Zacharewski et al., 1991, 1992; Harris et al., 1990b; Romkes and Safe, 1988; Romkes et al., 1987). TCDD also decreases rat hepatic ER in chronic exposure experiments, with a threefold decrease evident following a dose of 100 ng/kg/day for 30 weeks (Clark et al., 1991b). TCDD also decreases hepatic ER binding in C57Bl6 mice, but a much higher dose is needed to produce this effect in congenic mice deficient in the high-affinity AhR, indicating that TCDD-mediated decreases in ER are dependent on the AhR (Lin et al., 1991b). Dose-response studies in mice demonstrate that the single-dose ED50 is ~0.7 μg TCDD/kg, similar to the ED50 for other biochemical end points such as CYP1A1 induction, loss of plasma membrane EGF receptor, and induction of UDPGT. The observation that TCDD decreases hepatic ER is in apparent contradiction to the finding that TCDD increases hepatocyte proliferation, because the ER is thought to produce mitogenic signals. However, quantitation of ER in control and TCDD-treated rats was done using preparations from liver homogenates. Immunolocalization studies are needed so that the relationship of ER concentrations to cell proliferation in normal and preneoplastic cells can be more carefully evaluated.

In addition to effects on hepatic ER, TCDD may influence estrogen action in another way. CYP1A2 efficiently catalyzes the conversion of estrogens to catechol estrogens in liver (Graham

et al., 1988; Dannan et al., 1986). CYP1A2 is not found in extrahepatic tissues, with the possible exception of the nasal cavity, so catechol estrogen formation would be expected to occur only in liver. Catechol estrogens have been postulated to possess macromolecule- damaging properties as a consequence of free radical generation (Li and Li, 1990; Metzler, 1984). Therefore, TCDD may increase the DNA-damaging capacity of estrogens in liver as a function of CYP1A2 induction. This effect may, in part, explain the carcinogenic actions of TCDD in female rat liver, and is consistent with the knowledge that ovariectomy protects against the hepatocarcinogenic actions of TCDD and that male rats do not appear to be susceptible to TCDD-induced liver tumors (Lucier et al., 1991; Kociba et al., 1978). It is important to note that cancer is more than a two-stage process, and the stage-specific actions of TCDD in multistage cancer models are not known, although TCDD-mediated cell proliferation and possible indirect genotoxic effects may be critical at more than one stage. A hypothetical mechanistic scheme for TCDD-mediated liver cancer is shown in Figure 6-2.

The finding that chronic TCDD exposure decreases tumor incidences in the pituitary, mammary gland, and uterus may also reflect TCDD's effects on ER and estrogen metabolism. As discussed above, TCDD decreases uterine ER concentrations in cytosolic and nuclear fractions of rats and mice, and these changes are associated with diminished estrogen action in both in vivo and in vitro studies. TCDD also increases estrogen metabolism, presumably as a consequence of CYP1A2 in liver and UDPGT induction in liver and extrahepatic tissues (Shiverick and Muther, 1982). Likewise, the addition of TCDD to a breast cancer cell line (MCF-7) results in increased estrogen degradation (Gierthy et al., 1988). However, there are only small effects on serum 17-beta estradiol levels following administration of TCDD to either rats or mice (Shiverick and Muther, 1983). Therefore, the effect on serum estradiol is considerably less sensitive than the effects on the uterine receptor. This comparison has led investigators to conclude that the antiestrogenic actions of dioxins are primarily caused by effects on ER levels in reproductive tract tissues. Consistent with this hypothesis, Fernandez and Safe (1992) have shown that TCDD is antimitogenic in human breast cancer cells. Final evaluation of the role of estrogen metabolism awaits data on concentrations of estrogens in responsive cells of control and TCDD-treated rats, which may be different from serum estradiol levels. In any event, it appears clear that TCDD does possess antiestrogenic properties that are likely to be important to decreased tumor incidences in some reproductive tract and endocrine organs. Numerous studies have documented that the ER is found in virtually every tissue of the body, although the effects of TCDD on human ER in vivo have not been studied.

6.5.5. Other Biochemical Endpoints

TCDD alters a number of other pathways involved in the regulation of cell differentiation and proliferation (see Chapter 3). The specific relationships of these effects to multistage carcinogenesis are not known, but the broad array of effects on hormone systems, growth factor pathways, cytokines, and signal transduction components is consistent with the notion that TCDD is a powerful growth dysregulator (Table 6-9). It is also consistent with the findings that TCDD alters cancer risks at a large number of sites, possibly reflecting multiple mechanisms of carcinogenicity. Biochemical/molecular/endocrine changes produced by TCDD include the glucocorticoid receptor (Sunahara et al., 1989), tyrosine kinase (Madhukar et al., 1988), gastrin (Mably et al., 1990), interleukin-1beta (Sutter et al., 1991), plasminogen activator inhibitor (Sutter et al., 1991), tumor necrosis factor-alpha (Clark et al., 1991b), gonadotropin-releasing hormone (Moore et al., 1989), testosterone (Moore et al., 1985), and luteinizing hormone (Mably et al., 1992). The importance of these responses to the carcinogenic process should not be diminished by the lack of detail presented here. In every case studied, these responses have been shown to be dependent on the AhR.

6.6. SUMMARY AND WEIGHT OF EVIDENCE FROM ANIMAL STUDIES

There have been several long-term studies designed to determine if TCDD is a carcinogen in experimental animals. All of these studies have been positive and demonstrate that TCDD is a multisite carcinogen, is a carcinogen in both sexes and in several species including the Syrian hamster, is a carcinogen in sites remote from the site of treatment, and increases cancer incidence at doses well below the MTD. In two-stage models for liver and skin cancer, it is clear that TCDD is a potent promoting agent with weak or no initiating activity. Of those compounds that make up the bulk of the human body burdens of TEQ, only three of them, namely TCDD and a mixture of two congeners of hexachlorodibenzo-*p*-dioxin (HCDD) (1,2,3,6,7,8 and 1,2,3,7,8,9), have been tested in chronic rodent bioassays. In both cases, there was clear evidence of carcinogenicity. Commercial mixtures of PCBs that have a high TEQ also have been shown to be carcinogenic in rat liver. While many of the other dioxin-like compounds have not been tested in chronic carcinogenicity studies, 1,2,3,7,8-PCDD; 1,2,3,4,6,7,8-HpCDD; 2,3,4,7,8-PCDF; 1,2,3,4,7,8-HCDF; PCB126; and PCB105 all promote the development of putatively preneoplastic altered hepatocellular foci (AHF) within rodent liver suggesting that they also act as tumor promoters like TCDD (see Table 6-5). Together, dioxins and furans comprising 94% of the total dioxin/furan TEQ, and PCBs comprising 85% of the total coplanar PCB TEQ, have all shown to be positive in either rodent bioassays, rodent liver tumor promotion studies, or mouse skin tumor promotion studies. In addition, complex mixtures of dioxins and furans and commercial PCB mixtures act as promoters of liver AHF. These data suggest that, while the

majority of dioxin-like congeners have not been tested for carcinogenicity in chronic rodent bioassays, it is likely that those individual congeners and mixtures of dioxin-like compounds, which comprise the majority of the dioxin-like activity in human tissues, are carcinogenic to rodents. Furthermore, when one considers the impact of current TEF values on compounds that make up the majority of the current TEQ, it is clear that more than 90% of the current TEQ for either dioxins/furans or PCBs is made up of compounds for which the current TEF is supported by data on relative potencies based on tumor promotion or carcinogenic endpoints. More information on this point is provided in Table 6-10.

The finding of weak or no initiating activity is not surprising because TCDD does not form DNA adducts and is negative in short-term tests for genetic toxicity. The general consensus is that TCDD is an example of receptor-mediated carcinogenesis in that (1) interaction with the AhR appears to be a necessary early step, (2) TCDD modifies a number of receptor and hormone systems involved in cell growth and differentiation, such as the epidermal growth factor receptor and the ER, and (3) sex hormones exert a profound influence on the carcinogenic actions of TCDD. Although tumor promotion data for the polychlorinated dibenzofurans and coplanar polychlorinated biphenyls are limited, it appears that these compounds are liver tumor promoters with potencies dependent on their binding affinity to the AhR.

Some of the central issues in the risk assessment of TCDD and its structural analogues are (1) characterization of the shape of the dose-response curve for receptor-mediated events, (2) evaluation of the relevance of animal data in estimating human risks, and (3) the health consequences of background exposures (1 to 10 pg TEQ/kg/day) of dioxin and its structural analogues. With regard to the shape of the dose-response curve, it is clear from animal studies that there are different dose-response curves for different TCDD effects, which is consistent with the generally accepted dogma for steroid receptor-mediated responses. In general, the biochemical/molecular responses such as cytochrome P450 induction do not show evidence for a threshold, although unequivocal conclusions cannot be made about the mechanistic link, if any, between biochemical responses and toxic effects. In fact, coordinated biological responses such as TCDD-mediated cell proliferation and growth of preneoplastic lesions (foci of cellular alterations in liver) appear to be less sensitive endpoints, although evaluation of these responses is complicated by a high degree of interindividual variation: some animals do not exhibit any increase in cell proliferation in response to TCDD exposure.

The mechanistic basis for interindividual variation is unclear, and this lack of knowledge complicates approaches to estimate human risks from experimental animal data. However, several studies indicate that, for the most part, humans appear to respond like experimental animals for biochemical and carcinogenic effects. However, data from epidemiology studies are difficult to evaluate because the carcinogenic effects, if any, resulting from background TCDD

exposures are not known, although biochemical effects such as cytochrome P450 induction may be produced by background exposures.

Table 6-1. Sites for increased cancer in animal bioassays

Species/Strain	Sex	Site	Reference
Rats/Sprague-Dawley	Male	Tongue Nasal turbinates/hard palate	Kociba et al., 1978
	Female	Lung Nasal turbinates/hard palate Liver	
Rats/Osborne-Mendel	Male	Thyroid Adrenal cortex	NTP, 1982a
	Female	Liver Adrenal cortex Subcutaneous fibrosarcoma	
Mice/B6C3F1	Male	Liver	NTP, 1982a
	Female	Liver Thyroid Subcutaneous fibrosarcoma	
Mice/B6C3 and B6C	Male	Thymic lymphomas	Della Porta et al., 1987
	Female	Liver	
Hamsters/Syrian Golden	Male	Facial skin carcinoma	Rao et al., 1988

Table 6-2. Different evaluations of Kociba study liver tumor data in female rats

			TCDD(ng/kg/day)		
Evaluation	**Tumor classification**	**Control**	**1**	**10**	**100**
Kociba et al., 1978	Hyperplastic nodule	8/86 $p<0.0001$[a]	3/50	18/50 $p<0.001$[b]	23/49 $p<0.001$
	Hepatocellular carcinoma	1/86 $p<0.0001$	0/50	2/50	11/49 $p<0.001$
	Hyperplastic nodule; hepatocellular carcinoma[c]	9/86 $p<0.001$	3/50	18/50 $p<0.001$	34/48 $p<0.001$
Squire, 1980	Neoplastic nodule[d]: hepatocellular carcinoma	16/86 $p<0.0001$	8/50	27/50 $p<0.001$	33/47 $p<0.001$
Goodman and Sauer, 1992	Hepatocellular adenoma	2/86 $p<0.0001$	1/50	9/50 $p<0.01$	14/45 $p<0.001$
	Hepatocellular carcinoma	0/86 $p<0.01$	0/50	0/50	4/45 $p<0.05$
	Hepatocellular adenoma; hepatocellular carcinoma	2/86 $p<0.0001$	1/50	9/50 $p<0.01$	18/45 $p<0.001$

[a]*p*-values for Mantel-Haenszel trend tests are given below the control group incidences (Huff et al., 1991).
[b]*p*-values for Fisher exact tests are given below the incidence data for TCDD-treated animals.
[c]Combined incidence data for hyperplastic nodule and hepatocellular carcinoma in the Kociba study is as described by Huff et al., 1991.
[d]Hyperplastic nodule, neoplastic nodule, and hepatocellular adenoma are interchangeable lesions.

Table 6-3. Tumor incidences[a] in Osborne-Mendel rats

			TCDD (ng/kg/day)		
Sex	**Target organ**	**Control**	**1.4**	**7.1**	**71**
Male	Thyroid: follicular cell adenoma	1/69 p=0.006[b]	5/48 p=0.042 [c]	6/50 p=0.021	10/50 p=0.001
	Liver: neoplastic nodule	0/74 p=0.005	0/50 —	0/50 —	3/50 p=0.6
	Adrenal cortex: adenoma	6/72 p=0.26	9/50 p=0.09	12/49 p=0.015	9/49 p=0.09
Female	Liver: neoplastic nodule	5/75 p<0.001	1/49 —	3/50 —	12/49 p=0.006
	Adrenal cortex: adenoma or carcinoma	11/73 p=0.014	9/49 p=0.4	5/49 —	14/46 p=0.039
	Subcutaneous fibrosarcoma	0/75 —	2/50 p =0.16	3/50 p=0.06	4/49 p=0.023

[a]NTP, 1982a; Huff et al., 1991.
[b]p value obtained from Cochran-Armitage test for dose-related trend.
[c]p value obtained from Fisher exact test compared with control group.

Table 6-4. Tumor incidences[a] in B6C3F$_1$ mice

		TCDD (ng/kg/day)			
Sex	**Target organ**	**Control**	**1.4**	**7.1**	**71**
Male	Liver: carcinoma	8/73 *p*=0.002[b]	9/49 *p*=0.19[c]	8/49 *p*=0.28	17/50 *p*=0.002
	Liver: adenoma	7/73 *p*=0.024	3/49 —	5/49 *p*=0.6	10/50 *p*=0.09
	Lung: adenoma or carcinoma	10/71 *p*=0.004	2/48 —	4/48 —	13/50 *p*=0.08

		TCDD (ng/kg/day)			
Sex	**Target organ**	**Control**	**5.7**	**28.6**	**286**
Female	Subcutaneous fibrosarcoma	1/74 *p*=0.007	1/50 *p*=0.6	1/48 *p*=0.6	5/47 *p*=0.032
	Liver: carcinoma	1/73 *p*=0.008	2/50 *p*=0.4	2/48 *p*=0.4	6/47 *p*=0.014
	Liver: adenoma	2/73 *p*=0.11	4/50 *p*=0.2	4/48 *p*=0.2	5/47 *p*=0.8
	Thyroid: adenoma	0/69 *p*=0.016	3/50 *p*=0.07	1/47 *p*=0.4	5/46 *p*=0.009
	Hematopoietic: all lymphomas	18/74 *p*=0.011	11/50 —	13/48 *p*=0.4	20/47 *p*=0.029

[a]NTP, 1982a; Huff et al., 1991.
[b]*p* value obtained from Cochran-Armitage test for dose-related trend.
[c]*p* value obtained from Fisher exact test compared with control group.

Table 6-5. Summary of positive tumor promotion studies for PCDDs and PCDFs in rats

Strain/sex	Initiator	Promoter	Site	Reference
SD/F	PH/DEN	TCDD	Liver	Pitot et al., 1980
F344/F	PH/DEN	TCDD	Liver	Pitot et al., 1987
F344/F	PH/DEN	TCDD	Liver	Hendrich et al., 1986
SD/F	DEN	TCDD	Liver	Graham et al., 1988
SD/F	DEN	TCDD	Liver	Flodstrom and Ahlborg, 1991
SD/F	DEN	TCDD	Liver	Lucier et al., 1991
SD/F	DEN	TCDD	Liver	Clark, 1991
SD/F (ovx)	DEN	TCDD	Lung	Clark, 1991
SD/F	PH/DEN	TCDD	Liver	Flodstrom et al., 1991
F344/F	PH/DEN	TCDD	Liver	Dragan et al., 1991
SD/F	PH/DEN	TCDD	Liver	Waern et al., 1991
SD/F	DEN	TCDD	Liver	Flodstrom and Ahlborg, 1992
SD/F	DEN	PCDFs	Liver	Flodstrom and Ahlborg, 1992
CR/F	PH/DEN	TCDD	Liver	Dragan et al., 1992
SD/F	DEN	TCDD	Liver	Maronpot et al., 1993
SD/F	DEN	PCB 126, PCB 105	Liver	Hemming et al., 1993
Wistar/F	DEN	TCDD, HCDD	Liver	Buchmann et al., 1994
Wistar/F	NNM	TCDD, HCDD, PCDD	Liver	Schrenk et al., 1994
SD/F	DEN	TCDD	Liver	Sills et al., 1994
SD/F	DEN	TCDD, PCB126	Liver	Hemming et al., 1995
Wistar/F	DEN	TCDD	Liver	Stinchcombe et al., 1995
SD/F	DEN	TCDD	Liver	Tritscher et al., 1995
SD/F	DEN	PCB 118	Liver	Haag-Gronlund et al., 1997
SD/F	DEN	TCDD	Liver	Walker et al., 1997
SD/F	DEN	TCDD	Liver	Mann, 1997
SD/F	DEN	TCDD	Liver	Wyde et al., 1999
SD/F	PH/DEN	TCDD	Liver	Teeguarden et al., 1999
SD/F	PH/DEN	PCDD, PCDF, PCB	Liver	van der Plas, 1999
SD/F	DEN	TCDD	Liver	Walker et al., 2000

Abbreviations: DEN, diethylnitrosamine; PH, 2/3 hepatectomy; SD, Sprague-Dawley; F, female; M, male; NNM, N-nitrosomorpholine; ovx, ovariectomized.

Table 6-6. Summary of positive tumor promotion studies for PCDDs and PCDFs in mice

Strain/sex	Initiator	Promoter	Site	Reference
HRS/J hairless	MNNG	TCDD	Skin	Poland et al., 1982
HRS/J hairless	MNNG	TCDD PCDF HCDF	Skin	Hebert et al., 1990
C57/BL6 (M) DBA/2 (M) B6D2F1 (M) Swiss	DEN DEN DEN NDMA	TCDD, Aroclor 1254 TCDD, Aroclor 1254 TCDD, Aroclor 1254 TCDD	Liver Liver Liver Lung	Beebe et al., 1995
Tg.AC transgenic	v-Ha-ras transgene	TCDD	Skin	van Birgelen et al., 1999; Dunson et al., 2000
Tg.AC transgenic	v-Ha-ras transgene	TCDD	Skin	Eastin et al., 1998

Table 6-7. Putatively preneoplastic GGT-positive altered hepatocellular foci (AHF) after 30 weeks of treatment with TCDD as promoter[a]

Endpoint	Ovarian status	Saline		DEN-initiated[b]	
		Control	TCDD[c]	Control	TCDD
AHF/cm^3	Intact	6	5	44	387[d]
	Ovariectomized	0	0	30	80
Volume fraction	Intact	0.01	0.01	0.03	0.37[d]
	Ovariectomized	0	0	0.03	0.08
BrdU LI[e]	Intact	0.3	6.0	0.8	7.3[d]
	Ovariectomized	1.1	1.0	1.1	0.7

[a]Lucier et al., 1991.
[b]Animals were initiated with 175 mg diethylnitrosamine/kg.
[c]Biweekly treatment with 1,400 ng TCDD/kg.
[d]Significantly different from ovariectomized animals.
[e]Bromodeoxyuridine labeling indices; percentage of non-AHF hepatocyte nuclei undergoing replicative DNA synthesis in a 7-day period.

Table 6-8. Preneoplastic altered hepatocellular foci and bromodeoxyuridine labeling indices after 30 weeks of promotion with TCDD

TCDD ng/kg/day [a]	AHF [b] /cm^3	Volume fraction	Mean AHF volume	BrdU LI [c]
0[d]	442.2	0.57	13	5.3
3.5	759.2	0.85	15	3.3 [e]
10.7	791.7	1.00	11	3.3
35.7	530.4	0.93	18	6.4
125	751.7	2.23 [e]	30 [e]	14.4 [e]

[a] Daily averaged dose of a biweekly treatment of TCDD in corn oil.
[b] Placental glutathione-s-transferase positive altered hepatocellular foci (AHF)
[c] Labeling indices (LI) are the percentage of hepatocytes undergoing replicative DNA synthesis in 7 days following 30 weeks of exposure to TCDD.
[d] All animals were initiated with 175 mg diethylnitrosamine/kg, 2 weeks prior to start of treatment with TCDD.
[e] Significantly different from control values.

Source: Maronpot et al., 1993.

Table 6-9. Some biochemical responses to TCDD

CYP1A1	Human chorionic gonadotrophin
CYP1A2	Interleukin-1beta
CYP1B1	Gastrin
GST Ya	TNF alpha
GST Yb	TGF-beta
GST Yc	EGF
UDP glucuronyl transferase	Fibrinogen
QR quinone reductase/ Nmo	Plastin
Aldehyde dehydrogenase	EGFR
Ornithine decarboxylase	c-erbA related hormone receptor
Malic enzyme	Estrogen receptor
Phospholipase A2	25Dx-putative progesterone receptor
60kDa microsomal esterase	MDR-1 multidrug resistance
Aminolevulinic acid synthetase	Aryl hydrocarbon binding protein
Choline kinase	c-fos
EctoATPase	c-jun
Prostaglandin synthetase -2 (COX-2)	Cystatin-like protein
Plasminogen activator inhibitor-2	MHC-Q1
Urokinase plasminogen activator	Protein kinase C
Nedd-4-like ubiquitin protein ligase	pp60 c-src protein kinase
PEPC kinase	p21 ras
Terminal transferase	p27/Kip1
Testosterone 7alpha hydroxylase	bcl-2

Note: This list is not a comprehensive list of all responses known to be affected by TCDD.

Source: Sutter et al., 1992; Lai et al., 1996.

Table 6-10. Relative potency factors based on tumor promotion/cancer endpoints for "high TEQ contribution" dioxin-like compounds

Compound	REP[a]	TEF -WHO_{98}	% TEQ	Reference
2378-TCDD		1.0	17	
12378-PeCDD	0.8-1.04	1.0	32	Waern et al., 1991
1234678-HpCDD	0.02	0.01	3.3	Schrenk et al., 1994
123678/123789-HCDD (binary mixture)[b]	0.05	0.1/0.1	21 + 3.9	NTP, 1980
23478-PeCDF	0.1-0.21	0.5	15	Waern et al., 1991
			Total[c] =92.2 %	
33'44'5 PCB (PCB126)	0.071-0.11	0.1	64	Hemming et al., 1993, 1995
233'44' PCB (PCB105)	<0.0002	0.0001	17	Hemming et al., 1993
233'44'5 HCB (PCB156)	0.001-0.0001	0.0005	11	Haag-Grönlund et al., 1997
23'44'5 HCB (PCB118)	<0.0002	0.0001	4.3	Haag-Grönlund et al., 1997
			Total[d]=96.3 %	

[a]Range of individual relative potency factors based on cancer/tumor promotion (WHO-TEF database).
[b]Relative potency from this document (Chapter 6) based on relative dose levels.
[c]% contribution to total TEQ_{DF}-WHO_{98} in adipose tissue of humans with ambient exposures (Table 4-46 in Part I. Volume 3 Chapter 4).
[d]% contribution to total TEQ_{P}-WHO_{98} in adipose tissue of humans with ambient exposures (Table 4-47 in Part I. Volume 3 Chapter 4).

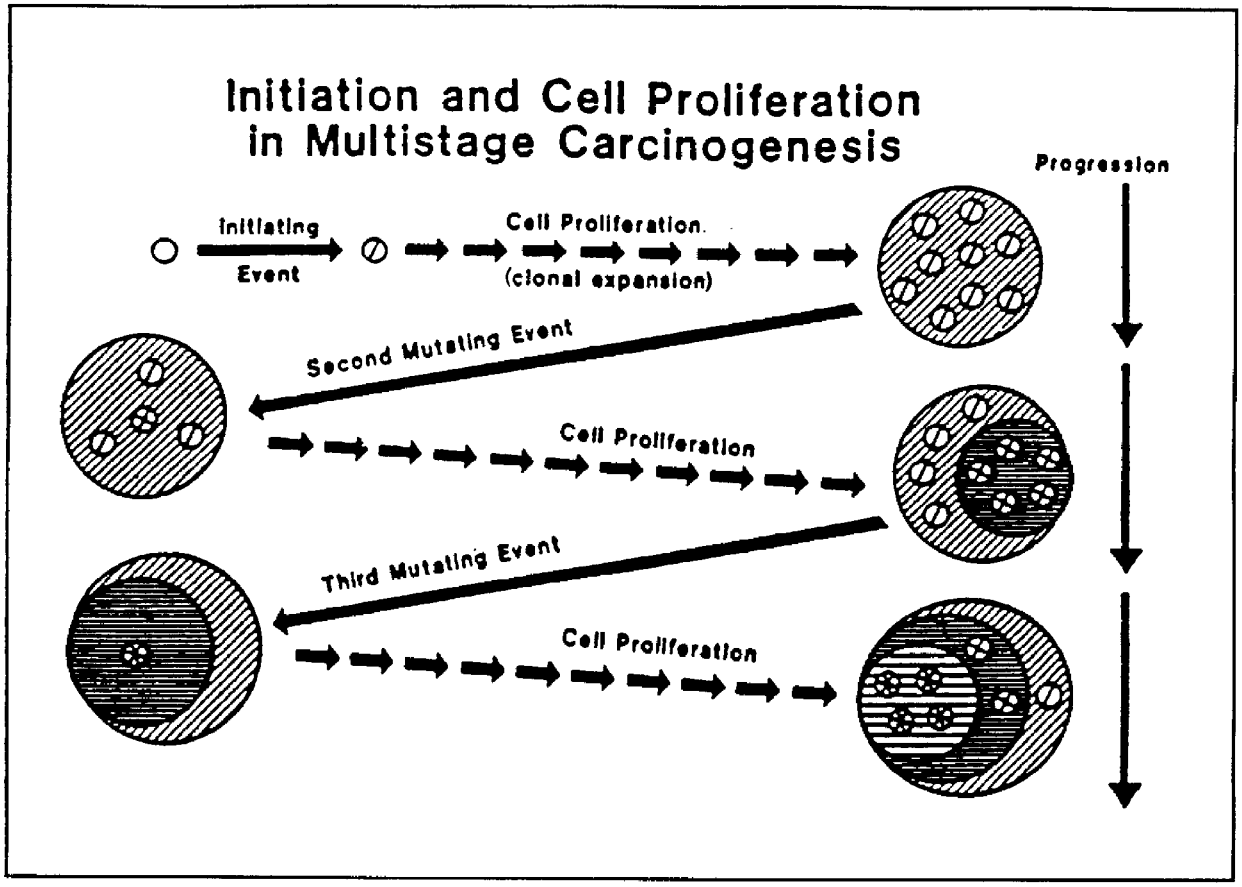

Figure 6-1. Schematic representation of multistep carcinogenesis including the roles of genetic damage and cell proliferation. It is important to note that several DNA-damaging steps and several cell proliferation steps are likely to be involved during the complete process of chemical carcinogenesis.

Source: Swenberg et al., 1987.

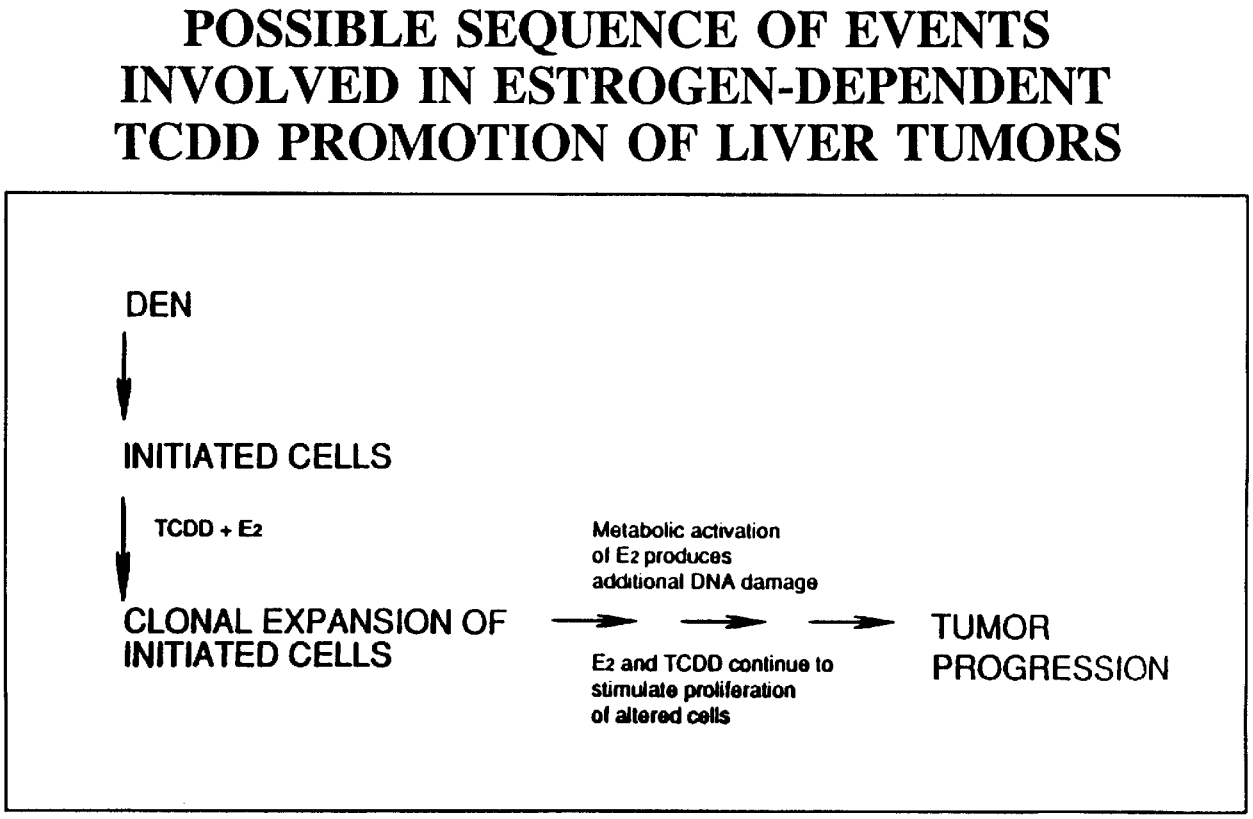

Figure 6-2. Operational model of TCDD/estrogen interactions relative to tumor promotion in a two-stage model of hepatocarcinogenesis. Clonal expansion of initiated cells may reflect stimulation of mitogenesis through receptor-mediated events involving epidermal growth factor receptor, estrogen receptor, and the AhR.

Source: Vickers and Lucier, 1991.

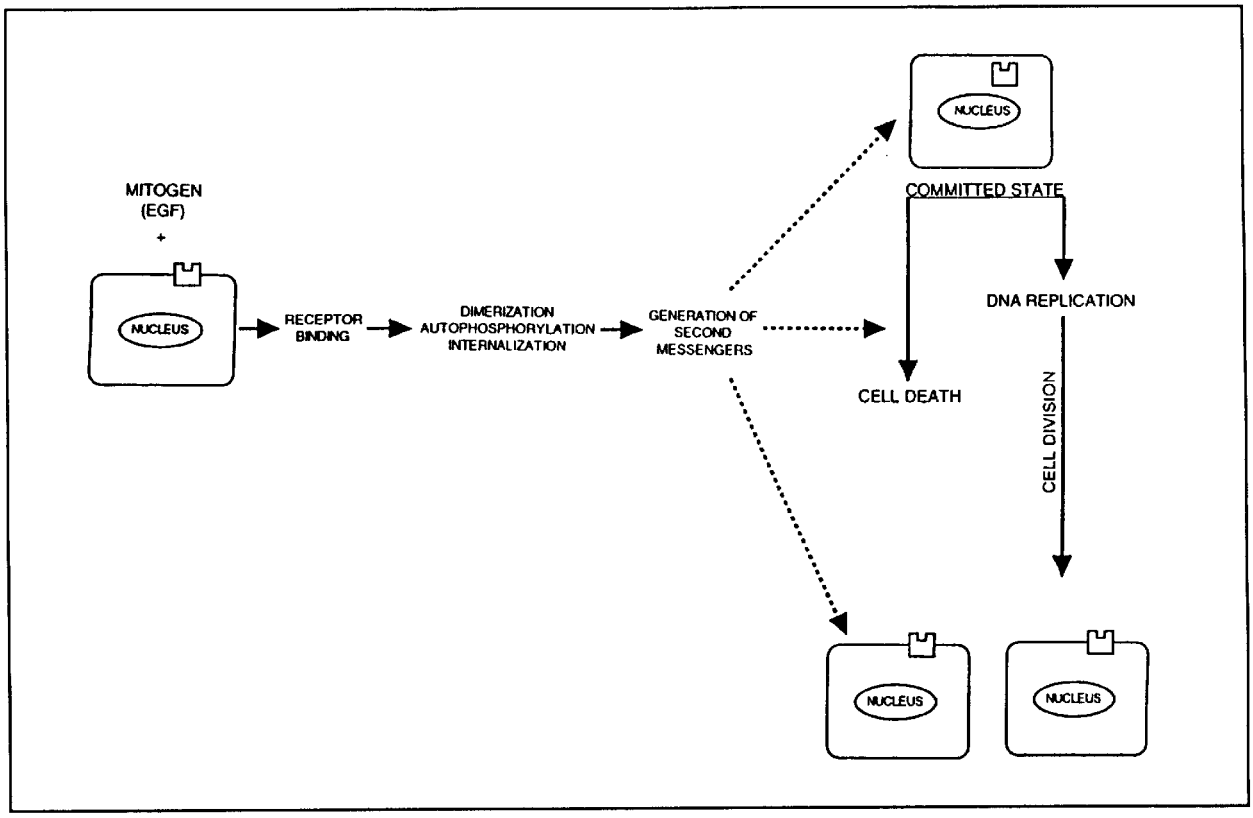

Figure 6-3. Plausible mechanism for the role of EGF-mediated stimulation of mitotic activity.

Source: Stoscheck and King, 1986.

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