

# Animal Models of Human Response to Dioxins

Jean A. Grassman, Scott A. Masten, Nigel J. Walker, and George W. Lucier

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

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent member of a class of chlorinated hydrocarbons that interact with the aryl hydrocarbon receptor (AhR). TCDD and dioxinlike compounds are environmentally and biologically stable and as a result, human exposure is chronic and widespread. Studies of highly exposed human populations show that dioxins produce developmental effects, chloracne, and an increase in all cancers and suggest that they may also alter immune and endocrine function. In contrast, the health effects of low-level environmental exposure have not been established. Experimental animal models can enhance the understanding of the effects of low-level dioxin exposure, particularly when there is evidence that humans respond similarly to the animal models. Although there are species differences in pharmacokinetics, experimental animal models demonstrate AhR-dependent health effects that are similar to those found in exposed human populations. Comparisons of biochemical changes show that humans and animal models have similar degrees of sensitivity to dioxin-induced effects. The information gained from animal models is important for developing mechanistic models of dioxin toxicity and critical for assessing the risks to human populations under different circumstances of exposure. — *Environ Health Perspect* 106(Suppl 2):761–775 (1998). <http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/761-775grassman/abstract.html>

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## Introduction

Dioxins are a class of highly toxic and broadly dispersed environmental contaminants that may pose a significant risk to human health. Dioxins include 75 polychlorinated dibenzodioxins (PCDDs), 135 polychlorinated dibenzofurans (PCDFs), and nine coplanar and mono-*ortho*-substituted polychlorinated biphenyls (PCBs) that are structurally similar to PCDDs and PCDFs (1,2). The most potent member of this family is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which also has the greatest affinity for the aryl hydrocarbon

receptor (AhR). For simplicity, the term dioxin will be used to refer to any of these compounds that act as AhR ligands and elicit dioxinlike effects.

Dioxins are the unintentional contaminants of many processes involving organic materials and chlorine. Combustion, incineration, synthesis of phenoxy herbicides and wood preservatives, and industrial and municipal processes such as paper manufacturing are the principal sources of dioxins (3). Most dioxins are resistant to environmental and biologic degradation

and once formed, disperse throughout the atmosphere, soil, and water (3). Although environmental concentrations of dioxins are generally low, they bioaccumulate in human foodstuffs because of their lipophilicity and stability.

Human exposures are lifelong and universal and usually consist of a mixture of dioxins. Approximately 80% of human environmental exposure to dioxins comes from the consumption of fat-containing foods such as milk, meat, and fish (4,5). Although dioxins can be detected in water, soil, and dust, these sources contribute relatively little to human exposure (6).

Total exposure to mixtures of dioxins can be assessed by calculating the total toxic equivalent (TEQ). A toxic equivalency factor (TEF) is assigned to each dioxin based on its potency compared to TCDD. The TEQ is the sum of magnitude of exposure for each constituent dioxin multiplied by its respective TEF (7,8). The use of the TEQ is appropriate for PCDDs and PCDFs where TCDD alone or a mixture with an equivalent TEQ produce similar biologic effects (9).

Humans metabolize dioxins slowly, as evidenced by the estimated TCDD half-life of 5.8 to 14.1 years (10,11). Many PCDFs have somewhat shorter half-lives of 2 to 4 years (12), whereas 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) has an estimated half-life of 19.7 years in humans (13). The limited capacity for the metabolism of dioxins results in accumulation in the body, particularly in adipose tissue. Residents of the United States have an average concentration of approximately 5 ppt TCDD in their adipose tissue (14).

Because all humans contain dioxins in their bodies, background exposures may result in disease even if the incidence is low. It is expected that humans will differ in their susceptibility to the health effects produced by dioxins. Factors such as gender, developmental stage, and the effect of Phase I and II enzyme polymorphisms are likely to contribute to the variation in susceptibility. Therefore, investigative approaches that promote a better understanding of low-level human responses and variability are needed.

## Animal Models

Animal models for dioxin response can be used to establish causation, study the underlying mechanisms of action, aid in developing biomarkers of risk in human

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Address correspondence to Dr. J.A. Grassman, National Institute of Environmental Health Sciences, P.O. Box 12233, MD C4-05, Research Triangle Park, NC 27709. Telephone: (919) 541-3466. Fax: (919) 541-1479. E-mail: grassman@niehs.nih.gov

Abbreviations used: AhR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; AUC, area under the curve; CYP1A1, cytochrome P450 1A1; CYP1A2, cytochrome P450 1A2; CYP1B1, cytochrome P450 1B1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EROD, ethoxyresorufin deethylase; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofuran, PeCDF, 2,3,4,7,8-pentachlorodibenzofuran; T<sub>3</sub>, 3,5,3'-triiodothyronine; T<sub>4</sub>, thyroxine; TEF, toxic equivalency factor; TEQ, toxic equivalent; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TSH, thyroid stimulating hormone; UDP-GT, uridine diphosphate-glucuronosyltransferase.

populations, and improve the science base for quantitative risk assessment. Integration of all available information describing the toxicokinetics, biochemistry, and health effects of dioxins in both human studies and animal models can be used to develop mechanistic models for risk assessment (15,16). The conceptual framework for considering human and animal data forms a parallelogram (Figure 1). This approach can be used to infer human responses by comparing human and animal *in vitro* data with animal *in vivo* effects. The relationship between responses in surrogate and target tissue can be similarly inferred through the use of an animal model. A useful animal model of human response to dioxins requires consideration of the characteristics of human exposure, the use of comparable dosimetry to describe exposure, establishing similarity in biologic responses, and demonstrating that the observed biologic responses are produced by similar biochemical mechanisms. Criteria for establishing such a model may be summarized as follows:

- Human exposure: Known levels of human exposures should define the relevant ranges of exposure for animal models.
- Comparable dosimetry: Because of different pharmacokinetics, the relationship between administered dose and internal dose may differ by species. Responses observed in different species should be compared using dose metrics that correspond to the biologically effective dose.
- Similar biologic responses: The animal model should display health effects or biologic responses that are similar to those observed in human populations.
- Similar mechanisms for biochemical effects: The relevance of complex phenomena observed in animal models, such as changes in cellular proliferation,

depends on a similarity in the underlying mechanisms.

## Human Exposure

The doses used in animal models should be based on realistic levels of human exposure and the internal doses produced by those exposures. Human exposure is assessed by measuring the concentrations of dioxins in the lipid fraction of the blood. Environmental sources, including dietary exposure, give rise to detectable serum levels of dioxins in virtually all individuals (4,6). Adults typically have total dioxin levels of 36 to 58 ppt TEQ (17) (Table 1). Of this total, the coplanar and mono-*ortho*-substituted dioxinlike PCBs contribute from 8 to 17 ppt TEQ (17).

There is considerable variation in normal human exposure to dioxins. Dietary practices, such as the consumption of foodstuffs that bioaccumulate dioxins, can significantly contribute to exposure in adults. Heavy consumption of crabs by Norwegian fishermen results in blood levels of PCDDs and PCDFs that exceed the levels in nonconsumers by 5-fold (18). Breast-fed infants may be exposed when lipophilic dioxins are excreted in breast milk. This is supported by very limited data that show slightly higher tissue levels in infants that are breast-fed rather than formula-fed (19). Based on analysis of breast milk, infants have a daily exposure of approximately 70 pg TEQ/kg/day, which exceeds average adult daily exposures by an order of magnitude (20).

Several human populations have been highly exposed because of accidental releases, employment in the chemical industry, and gross contamination of foodstuffs. Studies of these populations have proved invaluable for understanding the

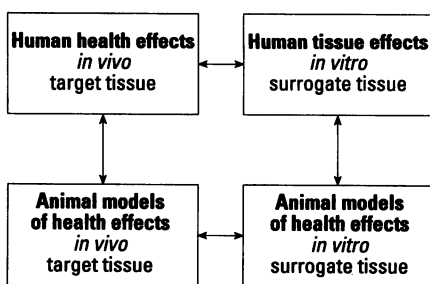
occurrence of dioxin-induced health effects in humans. Lipid-adjusted serum levels exceeding 500 ppt have been measured in some members of these populations decades after cessation of high exposure. Accounting for the passing of several half-lives, many individuals have had serum lipid dioxin levels in excess of 1000 ppt.

## Comparable Dosimetry

Qualitative differences in exposure estimates can arise because humans are exposed to a mixture of dioxins whereas most animal models use only a single compound. Accordingly, human exposure in human-animal model comparisons should be expressed as the total TEQ rather than the single compound used in the animal model. This is most important with low-level environmental exposures where TCDD usually makes up only 20 to 33% of the total TEQ (21). Under these circumstances, comparisons based on TCDD values rather than the total TEQ will overestimate the impact of TCDD. Estimating the total TEQ is less critical in situations if the population was highly exposed to a single dioxin as with the TCDD release in Seveso, Italy.

Several alternative dose metrics can be used for quantitative comparisons of dioxin response between species. Among them are intake, tissue concentration, body burden, and total cumulative dose expressed as area under the curve (AUC).

Intake of dioxins is usually expressed as average daily dose (picograms/kilograms/day). Intake is the most convenient method of measuring exposures in animal models. In humans, average daily dose can be estimated by measuring levels in foodstuffs and monitoring consumption. In contrast, estimation of daily dose stemming



**Figure 1.** A conceptual model for integrating animal and human health effects. Adapted from Clark et al. (74).

**Table 1.** Dioxin exposure of selected human populations based on contemporary lipid-adjusted serum levels.

Population	Exposure source	TEQ, ppt lipid	Reference
U.S. adults	Background	28–41 <sup>a</sup>	DeVito et al. (17)
	Environmental	8–17 <sup>b</sup>	
Norwegian males <sup>c</sup> Infants	Seafood (crab) consumption	110 <sup>a</sup>	Johansen et al. (18)
	Breast milk <sup>d</sup> Infant formula <sup>e</sup>	3.4 <sup>a</sup> (adipose) 2.8, 2.1 <sup>a</sup> (adipose)	Beck et al. (19)
Chemical industry workers, BASF cohort <sup>f</sup>	Occupational, accidental	<1–553 <sup>g</sup>	Ott et al. (183)
Chemical industry workers, Boehring cohort <sup>h</sup>	Occupational, 1952–1984	29–500 <sup>a</sup>	Flesch-Janys et al. (13)
Residents of Seveso, Italy, Seveso cohort <sup>i</sup>	Accidental, 1976	1–90 <sup>g</sup>	Landi et al. (181)

<sup>a</sup>TEQ based on PCDDs and PCDFs. <sup>b</sup>TEQ based on coplanar and mono-*ortho*-substituted PCBs. <sup>c</sup>n=9. <sup>d</sup>n=1. <sup>e</sup>n=2. <sup>f</sup>n=138. <sup>g</sup>TCDD only. <sup>h</sup>n=45. <sup>i</sup>n=103.

from episodes of high or changing dioxin exposure is not straightforward. The daily intake for highly exposed human populations is usually based on current serum dioxin levels and time elapsed since the exposure. This value has a high degree of uncertainty because of the length of time since last exposure, heterogeneity of dioxin half-life in humans, and changes in daily exposures over time.

Tissue concentrations, body burden, and AUC more closely estimate individual internal dose than expressions of exposure such as intake. As such, estimates can reflect the impact of intra- and interspecies differences in half-life for TCDD, which varies from 15 days in the mouse (9) to as long as 14.1 years in humans (10,11) (Table 2). Metrics of internal dose can also incorporate the impact of physiologic factors. For instance, aging increases the body burden of dioxins in both rodents and humans (14,22). For comparison, the concentration of dioxins residing within the lipid fraction are estimated and reported. The adjustment for lipids is based on the assumptions that dioxins reside solely within the lipid compartment and that the dioxin concentration in lipid is uniform throughout the body.

Dioxin exposures expressed as body burdens are based on measurements of lipid dioxin levels corrected for the proportion of the body mass consisting of lipid, usually 22% in humans (17). AUC is a dose metric that estimates the total cumulative internal

dose by integrating dose magnitude with its duration. Differences in life expectancies limit the use of AUC for interspecies comparisons because of uncertainties in scaling exposure duration. Also, past episodes of exposure, such as those encountered in highly exposed human populations, may be difficult to reconstruct if only contemporary dioxin values are available.

Carcinogenic doses can be used to compare alternative dose metrics in rats, mice, and humans, as shown in Table 2. The average daily dose associated with cancer is greater in mice than in rats, suggesting a possible difference in sensitivity. A meaningful average daily intake is difficult to estimate for the human population because human high-level exposures took place between 1952 and 1984 (13) and were followed by several years of low-level environmental exposures.

There are limitations in the use of tissue-specific comparisons between rodents and humans due to the dose-dependent sequestration of dioxins in the rodent liver. Typically, the TCDD concentration in rodent liver tissue ranges from 1 to 10.5 times the concentration in adipose tissue, depending on the dosing regimen (23,24). Because of the sequestration, hepatic tissue concentrations in the exposed rat may not be representative of tissue levels in serum and potential target organs such as the thyroid (Table 2). In humans the sequestration of dioxins in hepatic tissue is not well understood. With low-level environmental

exposures, similar concentrations of TCDD are found in the liver and adipose tissues of humans (25,26). Cross-species comparisons of tissue concentrations should be approached with caution.

Characterization of the carcinogenic dose on the basis of body burdens, however, suggests that despite the large difference in intake and tissue concentrations, similar body burdens are associated with cancer in rats, mice, and humans (Table 2). The comparability of the carcinogenic doses across species suggests that rats and mice could serve as experimental animal models for further studies of the mechanism of response.

As in Table 2, the average dioxin exposure from environmental sources results in tissue lipid levels (36–58 ppt TEQ) and body burdens (8–13 ppt TEQ) that are more than 2 orders of magnitude lower than the carcinogenic doses in either highly exposed humans or rats. The human cancer risk posed by this low-dose exposure is unknown. The use of experimental animal models could assist in defining the low-dose region by comparing the dosimetry of dioxin-dependent biochemical changes associated with cancer in both humans and animals.

## Biologic Response to Dioxins in Humans and Corresponding Animal Models

The biologic responses attributed to dioxins in humans and the corresponding responses found in animal models are discussed below. Because of the volume of literature that examines the effects of dioxins in humans, a comprehensive review is not possible. Representative studies examining comparable outcomes in human and animal models were selected. Whenever possible, studies that demonstrated dose dependency and adequately controlled covariates were chosen. The principal dioxin-induced biologic responses occurring in both humans and experimental animal models include the following:

- Chloracne
- Altered sex hormone levels
- Altered developmental outcomes
- Altered thyroid function
- Altered immune function
- Cancer
- Presence and functionality of the AhR
- Phase I and Phase II enzyme induction
- Altered epidermal growth factor receptor signaling
- Altered cellular growth and differentiation

**Table 2.** Comparison of TCDD half-life and alternative dose metrics in rats, mice, and humans.

	Sprague-Dawley rat	B6C3F <sub>1</sub> mouse	Humans	
			Adult high exposure	Adult low exposure
TCDD half-life	12–31 days (2)	15 days (9)	5.8–14.1 years (10,11)	
Health effect	Thyroid cancer (72)	Liver cancer (72)	All cancers <sup>a</sup> (185)	Not known
Average daily intake, pg/kg/day	1400	71,000	Not known	1–10 TEQ <sup>b,c</sup> (186)
Tissue, ppt lipid	6500 (liver) <sup>d</sup> 1250 (serum) <sup>d</sup>	ND	545–4362 <sup>e,f</sup> (serum)	36–58 TEQ <sup>b</sup> (serum) (17)
Body burden, ppt	73 (liver) <sup>g</sup> 250 (serum) <sup>h</sup>	944 (17)	120–960 <sup>e,i</sup>	8–13 TEQ <sup>b</sup> (17)

ND, no data. <sup>a</sup>Relative risk ratio 2.7 (confidence index 1.7–4.4); significant increase ( $p < 0.01$ ) based on linear trend for exposure quintiles plus 9th and 10th deciles; exposure data shown for the most highly exposed decile. <sup>b</sup>TEQ based on PCDDs, PCDFs, and coplanar and mono-*ortho*-substituted PCBs. <sup>c</sup>Intake estimated from food consumption and dioxin concentrations. <sup>d</sup>Liver and serum concentrations were determined by linear regression of averaged daily dose and the corresponding tissue levels of TCDD in 30-week-old animals treated biweekly with TCDD for 30 weeks; assumes lipid content of rat liver and serum of 4 and 0.4%, respectively (GW Lucier, unpublished observations). <sup>e</sup>TEQ based on PCDDs and PCDFs. <sup>f</sup>Extrapolated to last known exposure based on serum TCDD concentration, first order elimination kinetics, and elimination half-lives measured in Flesch-Janys et al. (13). <sup>g</sup>Calculated using the liver concentration of TCDD and assuming a liver:adipose ratio of 1 and that liver and adipose contribute 85% of the total body burden of TCDD. <sup>h</sup>Calculated using the lipid-adjusted serum level and assuming a total body lipid content of 20%. <sup>i</sup>Body burden calculated based on contemporary serum concentrations extrapolated to last known exposure and an average of 22% body fat.

### Acute and High-Dose Effects

Dioxins produce a syndrome of toxicity, characterized by progressive weight loss and delayed lethality, in rats, mice, rabbits, guinea pigs, hamsters, and nonhuman primates (27). Acute toxicity has not been observed among highly exposed humans despite the occurrence of high accidental exposures that exceed the doses known to produce acute toxicity in guinea pigs (28). The elicitation of the acute toxicity syndrome in rhesus macaques by PeCDF (29) suggests that whereas humans are tolerant, similar effects would be possible with sufficient exposure.

Chloracne is a reversible but often disfiguring skin condition characterized by acneiform eruptions due to epidermal hyperkeratosis and hyperplasia (30). High exposures, where plasma levels exceed 100 ppt lipid, may produce chloracne in many, but not all, individuals (31,32). Individuals who consumed up to 1 g of PCDFs and PCBs in contaminated rice oil developed chloracne and dermatologic and metabolic disorders that are collectively referred to as Yu-cheng (33). Animal species known to develop chloracnelike lesions include rhesus macaques, rabbits, and rodents (17,27,29). In experimental animal models such as the rhesus macaque, the chloracnelike lesions may be accompanied by epidermal changes affecting the toenails and sebaceous glands (29). Similar changes have been observed in human children exposed prenatally to high concentrations of PCDFs and PCBs (34).

### Reproductive, Developmental, and Immunologic Effects

Exposure to dioxins has been linked to alterations in endocrine and possibly reproductive capabilities in humans. In a population of highly exposed chemical plant workers, plasma levels of luteinizing hormone and follicle stimulating hormone were positively correlated to blood lipid dioxins that ranged from below 20 to 3400 ppt. There was also a trend for reduced testosterone in these workers (35).

Few studies have examined reproductive outcome as a function of dioxin exposure in human populations. Indirect evidence that alterations in endocrine status may affect reproduction comes from a study of the gender ratio of children born to parents exposed by the accidental release of TCDD in Seveso. During the 7 years following the release, the proportion of female children was significantly elevated (48 females compared to 26 males) (36).

In male rats, exposure to TCDD produces testicular abnormalities and reduces plasma testosterone levels (37). Developmental exposure to TCDD in the male rat results in subsequent impairment of reproductive capabilities (38).

Early exposure to toxicants can produce developmental effects in the form of physical malformations and altered growth and maturation. Some developmental effects, such as physical malformations, may be detectable at birth whereas others, such as changes in growth, can be manifested at any time until the onset of sexual maturity (39).

The children of Taiwanese women with Yu-cheng were exposed to high concentrations of PCBs and PCDFs transplacentally and in some instances through breast milk. At ages 6 to 7 they exhibited disordered behavior, increased activity (40), and impaired cognitive development (41).

A Dutch study examined the effect of the dioxins in normal breast milk by comparing breast-fed and formula-fed infants (42). Standardized tests of mental and psychomotor development suggested a transitory reduction in psychomotor skills in the breast-fed infants that was related to their intake of contaminants. Even so, as a group, breast-fed infants performed better than formula-fed infants (42). The children were also exposed to nondioxinlike PCBs, which other investigators have found to be related to reductions in psychomotor function (43). The effect of the nondioxinlike PCBs was correlated with transplacental rather than perinatal exposure (43).

Dioxins in breast milk may also alter the level of thyroid hormones in human infants. Highly exposed infants had elevated thyroid stimulating hormone (TSH) levels and lower free thyroxine ( $T_4$ ) levels than less-exposed infants. In all cases hormone levels were within normal clinical ranges (44).

Experimental animal models support the putative developmental effects seen in humans. Exposure to dioxins adversely affected learning in young rhesus macaques (45), whereas toxic levels of the dioxin PeCDF reduced the level of circulating  $T_4$  and increased TSH levels in adult rhesus macaques (29). A similar pattern of altered plasma thyroid hormone was produced by TCDD in rats (46). Exposure of rodents to dioxins either *in utero* or neonatally via maternal milk reduced body weight, delayed development, and caused immunologic disturbances (47,48). Dioxins are potent murine teratogens that produce hydronephrosis and cleft palates at doses

below those producing maternal toxicity (23,49). In the Long-Evans rat, maternal exposure to low levels of TCDD results in urogenital tract abnormalities in both males and females and altered reproductive behavior in the adult male (50).

Endometriosis, a disorder with both endocrine and immunologic features, is found only in female primates and is characterized as the nonmalignant proliferation of the endometrial tissue outside the uterus. An estimated 20 to 40% of women have endometriosis, which can produce chronic abdominal pain and reduce fertility (51). The development of endometriosis in female rhesus macaques treated with TCDD led to the hypothesis that dioxins may cause endometriosis in humans (52). Furthermore, individuals exposed by the Seveso accident were estimated to have total cumulative TCDD exposures exceeding the exposure of the affected female rhesus macaques (53). The effect of dioxins on endometriotic tissue has been investigated in a murine model in which endometriosis can be surgically implanted (54). Dioxins, defined by their cytochrome P450 1A1 (CYP1A1) enzyme-inducing activity, enhanced the growth of endometriotic lesions, whereas compounds lacking such activity failed to stimulate the growth of lesions (55). The occurrence of endometriosis in dioxin-exposed human populations is currently under investigation.

Immunotoxicologic investigations in humans often evaluate the peripheral immune system rather than host immunity or immunodeficiency. Most approaches either enumerate the constituents of the circulating immune system or assess the function of cellular components *in vitro*.

Dioxin exposure has been linked with changes in the number and function of lymphocytes. A dose-dependent increase in the number of peripheral helper T lymphocytes was found in industrial workers with 25 to 522 ppt TEQ in their blood lipids (56). In a group of 11 industrially exposed individuals, dioxin exposure was correlated with altered lymphocyte function (57). The proliferative response to interleukin-2 and allogeneic lymphocytes from human lymphocyte antigen-unrelated individuals and the ability to stimulate the proliferation of pooled allogeneic lymphocytes was reduced (57). The functional changes suggest that dioxin exposure may have either impaired helper T lymphocyte function or enhanced suppressor function without altering the proportion of T lymphocyte subpopulations (57).

Studies of early human development suggest that low-level environmental exposure may also produce changes in the immune system. Prenatal exposure in human infants was estimated from maternal and infant plasma concentrations of planar, mono-, and di-*ortho*-substituted PCBs and PCDDs/PCDFs. Higher levels of exposure corresponded with fewer circulating T lymphocyte receptor subpopulations, monocytes, and granulocytes at birth and at 3 months. Prenatal exposure was also positively correlated with increased numbers of cytotoxic T cells at 18 months of age (58). Immune function in children aged 6 to 10 years exposed during the Seveso accident showed no clinical evidence of immune abnormalities based on serum immunoglobulin concentrations and responses to T- and B-cell mitogens (59). Subsequent to the TCDD release, 20 of the 44 children developed chloracne, which demonstrates that as a group their exposure to TCDD was considerable (59).

In murine models, dioxins suppress antibody responses and to a lesser extent T lymphocyte responses. Low-dose exposure to dioxins decreased the frequency of memory T helper lymphocytes and suppressed both T-dependent and T-independent antibody responses (60,61). Dioxins may also affect nonspecific immunity by augmenting inflammatory responses (62). Rodent studies show that dioxins impair immune function and, as a consequence, resistance to viral and parasitic infections is reduced (63,64). Dioxins produce involution of the thymus at doses that exceed those required to alter immune function (62).

### Cancer

Below is a summary of studies performed in highly exposed human populations that have the greatest risk of developing dioxin-induced cancer. The 1997 International Agency for Research on Cancer assessment of the carcinogenicity of TCDD (6) contains a comprehensive review of the evidence for the carcinogenicity of PCDDs and PCDFs in human populations.

In 1976, an accidental release of TCDD from a trichlorophenol manufacturing facility contaminated the countryside surrounding the town of Seveso, exposing approximately 38,000 people. Rather than estimating individual exposures, zones of exposure were defined according to TCDD levels measured in neighboring soils. These estimates, which roughly corresponded to serum lipid TCDD levels (28), were used for a series of epidemiologic studies that

examined cancer mortality and morbidity in the years following the release (65–67). The outcomes observed in the Seveso population are particularly informative because in contrast to the occupational cohorts, substantial numbers of females and children were exposed.

Up to 15 years after the release, neither the incidence of cancer mortality nor morbidity in the zone with the highest level of contamination was elevated (65–67). This seemingly contradictory finding is not surprising because the expected numbers of many rare cancers are less than one in the subpopulation of approximately 800 individuals (67). In the zone with intermediate contamination and a population numbering approximately 6000, an excess of hematopoietic cancers, including leukemia and Hodgkin's lymphoma was found among males living 15 years after the incident (67). Females from this zone also showed an increased risk of Hodgkin's lymphoma and multiple myeloma (67). A statistically significant increase in the incidence of hepatobiliary and extrahepatic cancers was detected among females 10 years after the accident (66), although mortality from hepatobiliary cancers in females was not elevated after 15 years (67). At 10 years, an increased incidence of brain cancer was observed among women living in the zone with the lowest contamination (66).

The highest human exposures to dioxins are found within industrial populations involved in the manufacture of phenoxy herbicides and chlorophenols. Many individuals involved in manufacturing have blood dioxin levels that exceed background levels by more than an order of magnitude years after termination of exposure. In most studies exposure was based on work history rather than quantitative measurement of serum levels, making it difficult to attribute the outcomes to dioxins. Employment, hence exposure, often lasted for decades. However, assessment of the risk to these cohorts is complicated by the presence of numerous other chemical exposures. In addition most of the industrially exposed populations are predominantly, if not entirely, male.

A meta-analysis of selected cohorts showed excess mortality from cancers for all sites combined, lung cancer, and non-Hodgkin's lymphoma, and weak evidence for an elevation in soft-tissue sarcoma and gastrointestinal cancers. (6). The association of TCDD with an increase in all cancers was further strengthened by the dose dependence of the risk (13,68). In addition

a nested case-control study showed that the odds ratio for non-Hodgkin's lymphoma and soft-tissue sarcoma was dependent on the magnitude of the estimated exposure (69).

Animal models support the carcinogenic activity of TCDD based on studies of Syrian golden hamsters, rats, and mice (70). Cancers observed in human populations, such as non-Hodgkin's lymphoma, gastrointestinal cancers, and soft-tissue sarcomas, are not replicated in the animal models. However, comparison of the experimental animal models shows that specific cancers may be species, strain, or gender specific. In both Sprague-Dawley and Osborne-Mendel rats, female but not male rats develop liver cancer (71,72), whereas thyroid cancers are found in excess only among male Osborne-Mendel rats (72). TCDD-treated female Osborne-Mendel rats and Swiss Webster mice develop subcutaneous fibrosarcomas (72). TCDD induces tongue, nasal turbinate, hard palate, lung, and adrenal cancers in both male and female rats. Both male and female B6C3F<sub>1</sub> mice develop lymphoma and liver cancers, whereas thyroid cancers are only found in females (70). Only Syrian golden hamsters develop squamous carcinomas of the facial skin (70).

### Biochemical Effects of Dioxins in Humans and Animal Models

Estimates of the risk posed by dioxins would be enhanced by a better understanding of the mechanisms of effect, characterization of low dose response, and a more thorough understanding of the range of susceptibility in human populations. The previous section demonstrates that a variety of human responses have been linked with dioxin exposure although the noncancer effects are usually small with uncertain health significance. Both the cancer and noncancer effects may be difficult to attribute to dioxins because of the presence of other chemicals such as nondioxinlike PCBs. The dioxin-related effects seen in experimental animal models are consistent with those observed in humans and further indicate the possibility of adverse developmental, reproductive, and other health outcomes.

The health effects induced by dioxins are the ultimate outcome of a receptor-mediated process characterized by diverse responses that include the induction of enzymes, changes in growth factors and hormones, and alterations in cellular proliferation and differentiation. The contribution

of experimental animal models lies in the ability to examine responses to dioxin in both the intact animal and within the affected tissues to better understand the mechanism. Furthermore, animal models permit the examination of the effects of precisely measured concentrations of specific dioxins in both the whole animal and the target organ. This can be accomplished without the many competing exposures found in human populations. Most important, responses can also be examined in tissues such as peripheral blood lymphocytes, which permits direct comparisons with human response.

We are currently studying the effect of dioxins on human health by integrating data obtained from experimental animal models with results obtained from human tissues with either environmental or *in vitro* exposure. A Sprague-Dawley rat tumor-promotion model has been used to study the dose-response characteristics of dioxin-related responses (73). Initiation is accomplished by the administration of a single dose of diethylnitrosamine followed by multiple doses of TCDD, usually at biweekly intervals for 30 to 60 weeks. Murine models include the sensitive C57BL/6J mice with the high affinity form of the AhR (74) and strains of mice congeneric for the AhR (75).

In conjunction with the animal studies, parallel investigations of the biochemical effects of dioxin exposure are being examined in several human populations with different patterns and levels of exposure. The populations include women who consumed PCDF- and PCB-contaminated rice oil (Yu-cheng) (76), people who were living near Seveso at the time of the accidental release of TCDD in 1976 (28,59,77), workers employed in the manufacture of phenoxy herbicides (31) at a Boehringer-Ingelheim chemical plant in Germany, and volunteers with the low but detectable exposures typical of all residents of the United States (78,79). The range of serum concentrations found in the highly exposed cohorts and their controls are listed in Table 1.

Human biochemical response, by necessity, must be characterized in an easily obtainable and renewable tissue. Peripheral blood, especially lymphocytes and to a lesser extent placental tissue, have proved invaluable for this work. Human blood samples can be readily obtained and the lymphocytes assayed for gene expression, enzyme activity, and protein concentration of dioxin-responsive end points. Growth factors, hormones, and dioxin congener

concentrations can be measured in the plasma. Lymphocytes are also amenable to *in vitro* manipulations. This enables the treatment of lymphocytes with known concentrations of dioxins and permits *in vitro* culture where the capacity to respond to dioxin can be assessed. The degree to which lymphocytes reflect the dose-response characteristics of other tissues poses a potential limitation in their use. Also, biochemical responses, such as the expression of cytochrome P450 1A2 (CYP1A2) may not be detectable in lymphocytes.

#### Aryl Hydrocarbon Receptor and Aryl Hydrocarbon Receptor Nuclear Translocator

Several lines of evidence indicate that most if not all of the biologic responses elicited by dioxins are mediated by a cellular protein termed AhR to which TCDD binds specifically and with high affinity. The AhR normally exists as a multiprotein cytosolic complex consisting of the AhR ligand-binding subunit, heat shock proteins, and possibly other proteins [reviewed by Okey et al. (80)]. Upon ligand binding the AhR complex dissociates, moves to the nucleus, and interacts with the AhR nuclear translocator (ARNT). The AhR:ARNT heterodimer binds to specific response elements on DNA and functions as a ligand-activated transcription factor (81,82). The more limited studies with the human AhR indicate a similar conservation of ligand and DNA-binding function in animals and humans (80,83). Studies in animal models and human and animal cell systems that evaluate the structure-activity relationships for the various dioxins have shown a positive correlation between AhR binding affinity and the induction of aryl hydrocarbon hydroxylase (CYP1A1) activity, lethality, thymic atrophy, weight loss, and immunotoxicity (27,80).

The AhR is expressed in all tissues and species examined thus far (80,84,87). Although an endogenous ligand for the AhR has not been identified, the high degree of conservation of functional domains across taxonomic lines suggests that the AhR is necessary for important cellular processes, particularly during development (83). Evidence from transgenic mice lacking a functional AhR also supports this hypothesis (88,89).

The use of the resistant DBA/2J and the sensitive C57BL/6J mouse strains have shown that sensitivity of response to dioxins segregates with the AhR locus and is due to receptor structure rather than quantity (90).

In AhR genetic knockout mice, TCDD treatment fails to induce expression of hepatic Cyp1a1 and Cyp1a2 (89), and UDP-glucuronosyltransferase (UDP-GT), or to produce other signs of acute toxicity such as thymic atrophy and hepatomegaly (88). The constitutive expression of hepatic Cyp1a2 and UDP-GT is reduced by 85 to 90%, indicating that the AhR also plays a role in basal gene expression of these genes (88).

Thus, current evidence suggests that different AhR alleles, at least in animal models, influence sensitivity of response without affecting the spectrum of biochemical changes produced by dioxins (90). The reduced affinity of the human AhR for TCDD (91) suggests that humans may be somewhat less sensitive than mice and raises the possibility that as yet unidentified variant AhR alleles in humans may influence susceptibility to dioxin-mediated adverse effects. Although human AhR structural variants have been identified (91,92), a relationship between a specific AhR genotype and responsiveness to dioxins has not been demonstrated in human populations. The AhR and ARNT are present and functional in mitogen-activated human lymphocytes and human lymphocyte cell lines (74,85,93-95). Furthermore, AhR and ARNT mRNA are detectable in resting human lymphocytes (79,85) and after mitogen stimulation AhR and ARNT mRNA and protein expression increases in a time-dependent manner (79). We are currently examining the relationship between interindividual differences in AhR and ARNT expression levels and dioxin responsiveness in dioxin-exposed human populations.

The expression of genes under the control of the AhR such as CYP1A1 and CYP1A2 are ideal candidates for a quantitative comparison of dioxin responsiveness across species. Safe (96) has published a more complete review of the dioxin-inducible AhR-dependent genes. Although a direct causal role for these genes in the pathway of dioxin toxicity remains to be demonstrated, the mechanistic link between dioxin exposure and AhR-dependent gene expression makes them potentially sensitive indicators of dioxin exposure, response, or susceptibility to adverse effects.

#### Cytochrome P450 1A1

The sequence of events leading from the occupancy of the AhR to the induction of gene expression is best characterized for the CYP1A1 gene (81,82,97). Only limited

evidence currently exists for this mechanism in the dioxin-regulated expression of other genes, but is assumed to occur by a similar mechanism.

Treatment of human and murine lymphoid tissue *in vitro* with TCDD allows for the comparison of possible species-specific dose-response characteristics. Recent evidence from this laboratory showed that the dioxin responsiveness of human and murine lymphocytes were qualitatively and quantitatively similar. Human lymphocytes cultured with mitogen and TCDD showed increased CYP1A1 (EROD) activity at concentrations as low as 0.5 nM in cell culture medium (74) (SA Masten, in preparation). Spleen lymphocytes from C57BL/6J mice cultured similarly to the human lymphocytes showed comparable median effective concentration values of approximately 1 to 2 nM (74). Human lymphocytes, however, demonstrated a greater capacity for CYP1A1 induction with maximal induction values approximately 20-fold greater than the maximal levels of murine lymphocytes. The relative level of CYP1A1 induction in murine spleen was also less than human lymphocytes; 3- to 6-fold compared to 10- to 20-fold (74).

Although dioxins clearly induce CYP1A1 in cultured human cells, the effect of environmental or occupational dioxin exposure on CYP1A1 expression in human tissues remains relatively unexamined. Other CYP1A1 inducers such as cigarette smoke are associated with elevated CYP1A1 levels in tissues that are relevant for assessing dioxin toxicity including placenta (98), lymphocytes (99), and lung (100).

Women suffering from Yu-cheng had elevated levels of placental CYP1A1 activity 4 years after consumption of PCDF and PCB-contaminated rice oils (101). Placental TEQ levels in these women averaged approximately 9700 ppt lipid based primarily on the presence of PeCDF and 1,2,3,4,7,8-HexaCDF (102), which accounted for >95% of the TEQ. CYP1A1 activity ranged from nondetectable to 64 pmol/min/mg protein in placentas from the exposed women while the placentas from unexposed women had no detectable CYP1A1 activity (103). CYP1A1 activity did not correlate with placental PCDF or PCB concentration, possibly because of individual differences in response and unknown agonist/antagonist activity of PCBs.

CYP1A1 enzyme activity cannot be detected in resting human lymphocytes but is detectable in lymphocytes that have been

incubated with mitogens and TCDD (104). CYP1A1 inducibility in human lymphocytes varies considerably between individuals and this variation has been shown to have a genetic component with approximately 10% of the population having the high inducibility phenotype (105). Following an interesting observation by Kellermann et al. (106) showing higher prevalence of the highly inducible phenotype in lung cancer cases compared to controls, many subsequent studies have evaluated CYP1A1 inducibility in human lymphocytes as a possible marker for susceptibility to smoking-related lung cancer, with equivocal results (107,108).

Two genetic polymorphisms have been identified in the *CYP1A1* gene; a *Msp* I restriction fragment length polymorphism in the 3' noncoding region and a single nucleotide substitution resulting in an amino acid change (Ile/Val) in the domain responsible for catalytic activity. Population-based studies have yielded conflicting evidence for an association between these polymorphisms and either *in vitro* inducibility of CYP1A1 or risk for specific types of cancer (107). This may be accounted for by an absence of functional significance for the genetic polymorphisms. Indeed, the Ile/Val polymorphism within the CYP1A1 catalytic domain was recently shown to have no effect on catalytic activity (109,110). Ethnic differences in allele frequencies may also account for the conflicting results. The CYP1A1 *Msp* I variant allele associated in some studies with high enzyme inducibility has the greatest prevalence in Asian populations (111). In a recent meta-analysis of several published studies, there was a significant link between the two CYP1A1 genetic polymorphisms and cancer risk in Japanese populations but not Caucasians or other ethnic groups (111).

Although an association between known CYP1A1 genetic polymorphisms and inducibility has been noted in some studies (107), it is unlikely these polymorphisms account for the observed range of CYP1A1 inducibility phenotypes in the human population. Indeed, recent genetic evidence has shown the CYP1A1 high inducibility phenotype segregates with the chromosomal region containing the AhR structural gene (112). In the context of our current studies in dioxin-exposed populations, we are evaluating CYP1A1 levels as possible biomarkers of exposure and susceptibility and attempting to identify factors that may explain and/or modify the interindividual variation in dioxin responsiveness. Preliminary

evidence suggests that the distribution of CYP1A1 inducibility in human populations may be associated with the magnitude and/or pattern of exposure. A 7-fold range of inducibility was obtained in lymphocytes from 15 individuals with presumed background dioxin exposures. Lymphocytes from 85 occupationally exposed individuals showed a 27-fold variation in inducibility (104), whereas those exposed in the Seveso accident showed an 11-fold variation (113). Further analyses that correlate individual CYP1A1 activity with exposure status will determine the degree to which these differences correspond to either dioxin exposure or responsiveness.

Although it is possible to measure CYP1A1 induction after *in vivo* dioxin exposure in human tissues such as lymphocytes, the analysis of CYP1A1 expression in animal models has been critical in characterizing the dose response for effects of TCDD exposure on gene expression *in vivo*. CYP1A1 is inducible by TCDD and other AhR agonists in multiple animal models and in tissues, including liver, lung, kidney, and small intestine (114-116). In the rat liver, immunohistochemical analysis of the dose-dependent expression of CYP1A1 protein in chronically exposed animals shows that the number of cells expressing CYP1A1 increases with increasing dose in a centrilobular-specific pattern (73). Even at high doses some cells are not induced, which suggests that individual cells may differ in their TCDD dose-response characteristics (73). CYP1A1 expression has been observed in human liver and also exhibits a similar pattern of expression (117), although it is not known whether this pattern is related to dioxin exposures. Induction of CYP1A1 expression in the rat liver is the most sensitive response observed *in vivo* following exposure to TCDD (118). Significant increases in CYP1A1 expression have been observed at liver TCDD concentrations as low as 64 ppt lipid (assuming a lipid concentration of 4%), a level that is elevated but within the range of dioxin concentrations observed in human livers (6).

While CYP1A1 induction *in vivo* is highly correlated with dioxin exposure in animal models and indicative of a functional AhR signal transduction pathway, the role of CYP1A1 in the development of adverse health effects associated with dioxin exposure is unknown.

### Cytochrome P450 1A2

CYP1A2 is the second member of the TCDD-inducible CYP1A subfamily and,

like CYP1A1, is induced by TCDD via an AhR-dependent pathway and a mechanism similar to that of CYP1A1. In contrast to CYP1A1, CYP1A2 expression is observed in normal livers and inducibility by AhR agonists is primarily localized to the liver (114), although expression has been detected in other tissues.

CYP1A2 expression has been observed in the liver of humans with presumed background environmental exposure to dioxins (119). The levels of CYP1A2 mRNA in the liver of these individuals varies about 20-fold and is from 2 to 30 times higher than the expression of CYP1A1 molecules (119). Whether the variability in CYP1A1 and CYP1A2 levels is related to dioxin exposure is not known.

CYP1A2 activity can be detected in humans indirectly by monitoring metabolites in urine (120) or exhaled air (121) after the administration of caffeine. CYP1A2 expression is induced in humans by ingestion of omeprazole, smoking blonde tobacco (120), and eating pan-fried meats (122). Elevated CYP1A2 activities have also been observed in chemical plant workers who had serum levels as high as 147 ppt of TCDD (123), although these findings were not statistically significant.

In rats chronically exposed to TCDD, CYP1A2 protein is induced in the liver approximately 10-fold, compared to 200-fold for CYP1A1, yet the maximum level of expression of the two CYP1A isozymes is similar (73,124). Like CYP1A1, the induction of CYP1A2 with increasing liver TCDD burden exhibits a centrilobular-specific pattern of expression (73). CYP1A2 expression is an important factor in comparative pharmacokinetics of TCDD between species because it functions as a TCDD binding protein (125), and as a result is responsible for hepatic sequestration of TCDD in rodents (126).

In contrast to CYP1A1, the expression of CYP1A2 is implicated in one mechanism of hepatocarcinogenesis of TCDD in rats. CYP1A2 is involved in the metabolism of estrogen to catechol estrogens (127), which may serve as a source for reactive oxygen species and may potentially result in oxidative stress and indirect genotoxicity (128). CYP1A2 also catalyzes the *N*-oxidation of arylamines (129) and has been linked to an increase in risk for colorectal cancer (130).

### Cytochrome P450 1B1

Cytochrome P450 1B1 (CYP1B1) is the first member of a TCDD-inducible CYP1B subfamily of cytochromes P450 that has

been cloned from humans (131,132), mice (133,134), and rats (135,136). Human and rodent CYP1B1 proteins catalyze the bioactivation of a broad spectrum of compounds including polycyclic aromatic hydrocarbons and aromatic amines (137–139). Truncating mutations in human CYP1B1 are the primary cause of congenital glaucoma in humans (140), which suggests that CYP1B1 is involved in the metabolism of endogenous substrates involved in normal cell growth and differentiation. Human CYP1B1 is active in the formation of the catechol estrogen 4-hydroxyestradiol (141), which is carcinogenic in male hamsters (142). The formation of 4-hydroxyestradiol has been implicated in the development of human mammary tumors and uterine leiomyoma (143), and CYP1B1 protein expression is frequently observed in wide variety of malignant tumors (144). CYP1B1 therefore represents a TCDD-inducible gene that is plausibly linked in multiple mechanisms of carcinogenesis through the formation of potentially genotoxic metabolites of estrogens, the activation of procarcinogens, and altered cell growth and differentiation.

CYP1B1 is normally expressed in human fetal heart, brain, and kidney (138) and in rat adrenal glands and testes (135,136). Expression of CYP1B1 is inducible by TCDD in multiple human cell lines (131,132,145) and in multiple rodent tissues, including liver, lung, and kidney (135). CYP1B1 also exhibits a centrilobular pattern of expression in TCDD-treated rat liver (146) but is expressed at lower levels and exhibits a TCDD dose response different from that of CYP1A1 (147,148).

CYP1B1 expression is detectable in human lymphocytes and inducible by TCDD in mitogen-stimulated lymphocytes treated with TCDD *in vitro* (104). Expression of CYP1B1 is higher in bronchial epithelial cells obtained from smokers than from nonsmokers (149), suggesting that CYP1B1 may be inducible *in vivo* by Ah receptor agonists present in cigarette smoke. Current studies in our laboratory are evaluating the relationship between prior TCDD exposure and CYP1B1 expression in lymphocytes from several different human populations.

The following biochemical responses include changes in differentiation and proliferation ascertained either through direct effects on target tissue such as the liver or by alterations in growth factors or their receptors. Unlike the cytochrome P450s, these dioxin-induced changes are not the

result of direct transcriptional control by the AhR, but integrate many biochemical changes subsequent to AhR binding and effects on gene expression.

### Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein with tyrosine kinase activity that mediates the actions of the polypeptide hormones epidermal growth factor (EGF) and transforming growth factor alpha. Ligand binding results in receptor autophosphorylation and internalization accompanied by phosphorylation and activation of downstream effector proteins (150). The EGFR is widely expressed and functions in cellular growth and differentiation in multiple tissues including the liver and gastrointestinal tract (151). The EGFR also regulates the implantation, growth, and differentiation of the placenta (152), and alterations in EGFR signaling pathways may be involved in carcinogenesis (153).

EGF-stimulated autophosphorylation of the EGFR in placentas from women with Yu-cheng was reduced by approximately 60% compared to unexposed controls (76). The reduction in autophosphorylation of the EGFR was highly correlated with the reduction in birth weight seen in the offspring of the exposed mothers, and with placental PCB, but not PCDF, concentration. TCDD treatment also reduces EGFR binding capacity in human keratinocytes (154).

TCDD-mediated decreases in EGFR binding capacity were observed in both mice (155) and rats (156). In the rat tumor promotion model, TCDD treatment led to a dose-dependent reduction in EGFR-stimulated autophosphorylation, EGFR protein, and EGFR maximal binding capacity while the ligand-binding affinity of the receptor was unaffected (156,157). The magnitude of the decrease (60%) was similar to that seen in placentas from women with Yu-cheng. In the rat model the reduction in EGFR binding was correlated with increased hepatocyte labeling index (156), suggesting a possible relationship to tumor promotion. Both the decrease in EGFR levels and the increased proliferation depend on the presence of ovarian hormones because the TCDD-mediated changes were greater in intact than ovariectomized female rats (156).

Despite a similar maximal magnitude of reduction in EGFR autophosphorylation in the rat model and women with Yu-cheng, EGFR maximal binding capacity



was not reduced in the women with Yu-cheng (103). This difference may be due to a difference in the level of exposure because the average dioxin concentrations in the placentas from the exposed women was approximately 100 ppt TEQ wet weight while the lowest liver concentration producing changes in maximal binding capacity in the rat model was approximately 1800 ppt TCDD wet weight (156). On a body burden basis, however, this effect occurred at a similar dose in rats and humans (2158 vs 2582 ng/kg).

### Thyroid Function

TCDD causes dose-related significant increases in thyroid follicular cell adenomas in female B6C3F<sub>1</sub> mice and male Osborne-Mendel rats (70). One putative mechanism for thyroid carcinogenesis is that decreased concentrations of serum thyroid hormones leads to a negative feedback regulation on the hypothalamic and pituitary glands, which results in an increase in secretion of TSH. Prolonged stimulation of the thyroid gland by TSH may lead to follicular cell hyperplasia and subsequently to tumors (158). UDP-GT is a Phase II conjugating enzyme that glucuronidates thyroid hormones and is active in the excretion of a variety of other endogenous and exogenous molecules (159). UDP-GT is inducible by TCDD in human and rat tissues (160,161), and several animal studies have shown that TCDD as well as other compounds that increase hepatic levels of UDP-GT lower circulating levels of thyroid hormones, presumably through a UDP-GT-mediated increase in clearance from the body (162,163). Evidence also exists that dioxin exposure is associated with altered thyroid hormone status in human populations (44,164,165).

Dioxin-related changes in the serum levels of T<sub>4</sub>, 3,5,3'-triiodothyronine (T<sub>3</sub>), TSH, and UDP-GT mRNA were measured in a rat tumor promotion model (166). TCDD treatment led to a dose-dependent decrease in serum T<sub>4</sub>, which correlated with liver TCDD burden and increased UDP-GT mRNA. These changes were accompanied by increased serum TSH and thyroid follicular cell hyperplasia. Significant changes in these end points occurred at liver burdens of 450 to 1800 ppt TCDD wet weight, equivalent to a daily dose of 3.5 to 10.7 ng/kg/day for 30 weeks.

The mechanisms and dosimetry underlying these observed biologic phenomena were further examined by physiological

modeling of the alterations in thyroid hormones and induction of UDP-GT observed in the rat (167). The thyroid hormones T<sub>3</sub> and T<sub>4</sub> were added to an existing mechanistic model of TCDD (168) by adding compartments and terms for secretion, uptake, transport, glucuronidation, and deiodination. The model incorporates the induction of UDP-GT by TCDD via the AhR complex and the subsequent increase in glucuronidation of T<sub>4</sub>. T<sub>4</sub> secretion was modeled as regulated by hypothalamic and pituitary factors, which in turn were based on a feedback mechanism controlled by blood levels of T<sub>4</sub>. The resultant model successfully reproduced blood T<sub>3</sub>, T<sub>4</sub>, and TSH levels in rats receiving biweekly oral dosing. The ability of this model to accurately represent biologic processes is demonstrated by the correct prediction of liver TCDD concentrations and UDP-GT expression. Data measuring these end points were not used to construct the model, but were confirmed experimentally (167).

### Cellular Proliferation and Differentiation

Although a direct causal relationship between changes in cellular growth and differentiation and adverse health effects is not known, disruption of cellular homeostasis could certainly be a risk factor for carcinogenicity or other toxic effects.

Changes in cellular proliferation and differentiation represent critical steps in dioxin carcinogenicity that cannot readily be studied in exposed human populations. Chronic exposure to TCDD in animal models results in a broad spectrum of changes in cell proliferation and differentiation (169,170). This likely results from the effect of dioxins on multiple hormone and growth factor signal transduction pathways that are involved in cell growth and homeostasis. The development of altered hepatic foci, characterized by the expression of the enzymes  $\gamma$ -glutamyl-transpeptidase and placental glutathione S-transferase (124,171), is the result of a complex interaction between cell proliferation, apoptosis, and hepatocyte differentiation. In a rat two-stage tumor promotion model, chronic exposure to increasing doses of TCDD up to 125 ng/kg/day for 30 weeks results in increased cell proliferation and altered hepatic foci formation (172,173). Both the number and size of the foci are increased by dioxin exposure (172), although there is considerable

interindividual variability in response that does not correlate with either liver TCDD concentration or with the induction of cytochrome P450s (172). TCDD-induced increases in cell proliferation and foci development are dependent on the presence of ovarian hormones (124), and this correlates with the female-specific induction of tumors in rats (71). One of the characteristics of tumor promotion is reversibility on cessation of treatment. Withdrawal of TCDD treatment leads to a reduction in number of altered hepatic foci; however, the focal size and percent of liver occupied by foci increases (173-175). These data suggest that TCDD treatment may promote the development of a population of foci that may be able to continue to develop in the absence of TCDD.

Although the response to dioxins has been best characterized in liver tissue, animal models show that dioxin alters proliferation and differentiation in other tissues as well. In mice, prenatal TCDD exposure results in cleft palate due to a disruption in proliferation and differentiation of epithelial cells required for fusion of the palatal shelves (176). The expression of AhR and ARNT in human embryonic craniofacial tissues indicates the machinery for responding to TCDD is present (177). A single dose of TCDD (either 2.5 or 25  $\mu$ g/kg) in pubertal female Sprague-Dawley rats reduced ovarian weight and inhibited mammary cell development and differentiation by reducing the number of terminal ductal structures and gland size (178).

*In vitro* systems allow for the study of the effects of TCDD on growth and proliferation of human cells including primary and immortalized keratinocytes, embryonic craniofacial tissues, and MCF-7 breast cancer cell line. Normal human epidermal keratinocytes treated with 10 nM TCDD showed an increase in markers of terminal differentiation while the total number of cells remained constant (179). The estrogen-dependent growth of MCF-7 cells was inhibited by 0.3 nM TCDD (180). In addition, several studies have shown that TCDD can induce transformation of both rodent and human cells *in vitro* (170).

### Gender Differences in Response

Evidence from the analysis of serum TCDD levels in the Seveso population suggests that dioxin pharmacokinetics may differ in respect to gender. Twenty years after the initial exposure, the average serum level in females was approximately three times that found in males (181). This observation

could not be accounted for by differences in the initial exposure, percentage of body fat, or several other covariates.

Rodent cancer bioassays indicate that TCDD is a hepatic carcinogen in female but not male rats (71), implying that the presence of estrogens influences the carcinogenic response. Indications that a similar process occurs in humans comes from the 10-year follow-up of those exposed in the Seveso incident. Women but not men showed an excess of liver cancer (66). This excess was present but not statistically significant 15 years after the accident (67).

Rodent models suggest that the presence of ovarian hormones, presumably estrogens, play a role in modifying many effects of TCDD that are correlated either with the development of hepatic preneoplastic changes or tumors. Compared to ovariectomized rats, the livers of intact animals treated with TCDD for 30 weeks showed a decrease in EGF receptor (156), a greater hepatic proliferative response (73,124), a greater volume occupied by putative preneoplastic lesions (124), and increased oxidative DNA damage (182), while the quantity of CYP1A2 was not affected (124).

## Summary

The adverse effects of dioxins are well established based on studies of experimental animal models and highly exposed human populations. From these investigations, the current view of dioxins as potent

toxicants capable of producing a multitude of diverse biologic effects has emerged.

Much less is known about the risk posed to humans by lifelong low-level environmental exposure. Better characterization of dose-response relationships and the factors associated with individual variation in susceptibility are needed to evaluate the risk to humans from background exposures. These questions can best be addressed with experimental animal models, provided the response of the animal species accurately replicates the human response. The relevance of a particular animal model for evaluating or predicting human response is determined by a number of properties—factors that can be manipulated, such as dose regimens, as well as factors that are intrinsic to the animal model, such as biologic response. They can be summarized as follows:

- The reproductive, developmental, immunologic, and carcinogenic responses to dioxins seen in humans also occur in animal models.
- The preponderance of biochemical effects induced by dioxins in both animals and humans are mediated by the AhR.
- Animal dosing regimens can be varied to examine the range of exposures encountered in human populations.
- Dose metrics based on internal dose (tissue dose and body burden) can be used to compare responses across species as these parameters take into account species differences in clearance rates.

- The biochemical responses to dioxins in animal models show qualitative and quantitative similarity to those observed in humans.

Based on reviews of exposed humans, the corresponding experimental animal models, and the biochemical responses in human and animals, biologic responses to dioxins are qualitatively and often quantitatively similar. Exposure to dioxins has been implicated in a wide range of human health effects related to reproduction, immune function, growth and development, and cancer. The role of dioxins in producing these effects is supported by corresponding outcomes in animal models. The similarity in biochemical responses makes it possible to study the underlying mechanisms of dioxin toxicity relevant for humans. Most important, animal models make it possible to study the relationship between simple biochemical responses and more complex responses, such as altered growth and differentiation, which lead to adverse health effects. This information is integrated with human studies to infer responses that would not otherwise be possible, such as target tissue effects. Current efforts are underway to characterize the effect of susceptibility factors such as genetics, gender, age, and the dosimetry of dioxin-dependent biochemical changes in human populations. Parallel studies in animal models can be used to understand how these biochemical changes can lead to adverse health effects.

## REFERENCES AND NOTES

1. Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit Rev Toxicol* 24:87-149 (1994).
2. Van den Berg M, De Jongh J, Poiger H, Olson JR. The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *Crit Rev Toxicol* 24:1-74 (1994).
3. Zook DR, Rappe C. Environmental sources, distribution, and fate of polychlorinated dibenzodioxins, dibenzofurans, and related organochlorines. In: *Dioxins and Health* (Schecter A, ed). New York:Plenum Press, 1994;710.
4. Schecter A. Exposure assessment: measurement of dioxins and related chemicals in human tissues. In: *Dioxins and Health* (Schecter A, ed). New York:Plenum Press, 1994;449-485.
5. Wesp HF, Rippen G, Fiedler H, Lau C, Hutzinger O, Sievers S, Friesel P, Gras B, Reich T, Schacht U et al. Dioxin mass balance for the City of Hamburg, Germany. Part 3: Update of food consumption data and human exposure. *Organohalogen Compounds* 30:37-42 (1996).
6. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 69: Polychlorinated Dibenzopara-Dioxins and Polychlorinated Dibenzofurans. Lyon:International Agency for Research on Cancer, 1997.
7. Safe S. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit Rev Toxicol* 21:51-88 (1990).
8. NATO. International Toxicology Equivalency Factor Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds Report 176. Brussels:North Atlantic Treaty Organization, Committee on the Challenges of Modern Society, 1988.
9. DeVito MJ, Birnbaum LS. The importance of pharmacokinetics in determining the relative potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran. *Fundam Appl Toxicol* 24:145-148 (1995).
10. Michalek JE, Pirkle JL, Caudill SP, Tripathi RC, Patterson DG, Jr., Needham LL. Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year follow-up. *J Toxicol Environ Health* 47:209-220 (1996).
11. Wolfe WH, Michalek JE, Miner JC, Pirkle JL, Caudill SP, Patterson DG Jr, Needham LL. Determinants of TCDD half-life

- in veterans of Operation Ranch Hand. *J Toxicol Environ Health* 41:481-488 (1994).
12. Ryan JJ, Gasiewicz TA, Brown JF Jr. Human body burden of polychlorinated dibenzofurans associated with toxicity based on the Yusho and Yucheng incidents. *Fundam Appl Toxicol* 15:722-731 (1990).
  13. Flesch-Janys D, Becher H, Gurn P, Jung D, Konietzko J, Manz A, Papke O. Elimination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in occupationally exposed persons. *J Toxicol Environ Health* 47:363-378 (1996).
  14. Orban JE, Stanley JS, Schwemberger JG, Remmers JC. Dioxins and dibenzofurans in adipose tissue of the general US population and selected subpopulations. *Am J Public Health* 84:439-445 (1994).
  15. Lucier GW, Portier CJ, Gallo MA. Receptor mechanisms and dose-response models for the effects of dioxins. *Environ Health Perspect* 101(1):36-44 (1993).
  16. Portier CJ. Mechanistic modelling and risk assessment. *Pharmacol Toxicol* 72(Suppl 1):28-32 (1993).
  17. DeVito MJ, Birnbaum LS, Farland WH, Gasiewicz TA. Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. *Environ Health Perspect* 103(9):820-831 (1995).
  18. Johansen HR, Alexander J, Rosslund OJ, Planting S, Lovik M, Gaarder PI, Gdynia W, Bjerve KS, Becher G. PCDDs, PCDFs, and PCBs in human blood in relation to consumption of crabs from a contaminated Fjord area in Norway. *Environ Health Perspect* 104(7):756-764 (1996).
  19. Beck H, Dross A, Kleeman WJ, Mathar W. PCDD and PCDF concentrations in different organs from infants. *Chemosphere* 20:903-910 (1990).
  20. Pohl HR, Hibbs BF. Breast-feeding exposure of infants to environmental contaminants-A public health risk assessment viewpoint: chlorinated dibenzodioxins and chlorinated dibenzofurans. *Toxicol Environ Health* 12:593-611 (1996).
  21. Patterson DG Jr, Todd GD, Turner WE, Maggio V, Alexander LR, Needham LL. Levels of non-*ortho*-substituted (coplanar), mono- and di-*ortho*-substituted polychlorinated biphenyls, dibenzo-*p*-dioxins, and dibenzofurans in human serum and adipose tissue. *Environ Health Perspect* 102(Suppl 1):195-204 (1994).
  22. Vanden Heuvel JP, Clark GC, Tritscher A, Lucier GW. Accumulation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in liver of control laboratory rats. *Fundam Appl Toxicol* 23:465-469 (1994).
  23. Birnbaum LS. Distribution and excretion of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in congenic strains of mice which differ at the Ah locus. *Drug Metab Dispos* 14:34-40 (1986).
  24. Diliberto JJ, Jackson JA, Birnbaum LS. Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) disposition following pulmonary, oral, dermal, and parenteral exposures to rats. *Toxicol Appl Pharmacol* 138:158-168 (1996).
  25. Thoma H, Mucke W, Kauert G. Comparison of the polychlorinated dibenzo-*p*-dioxin and dibenzofuran in human tissue and human liver. *Chemosphere* 20:433 (1990).
  26. Leung HW, Wendling JM, Orth R, Hileman F, Paustenbach DJ. Relative distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human hepatic and adipose tissues. *Toxicol Lett* 50:275-282 (1990).
  27. Poland A, Knutson JC. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu Rev Pharmacol Toxicol* 22:517-554 (1982).
  28. Mocarelli P, Needham LL, Marocchi A, Patterson DG Jr, Brambilla P, Gerthoux PM, Meazza L, Carreri V. Serum concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and test results from selected residents of Seveso, Italy. *J Toxicol Environ Health* 32:357-366 (1991).
  29. Brewster DW, Elwell MR, Birnbaum LS. Toxicity and disposition of 2,3,4,7,8-pentachlorodibenzofuran (4PeCDF) in the rhesus monkey (*Macaca mulatta*). *Toxicol Appl Pharmacol* 93:231-246 (1988).
  30. Coenraads PJ, Brouwer A, Olie K, Tang N. Chloracne. Some recent issues. *Dermatol Clin* 12:569-576 (1994).
  31. Manz A, Berger J, Dwyer JH, Flesch-Janys D, Nagel S, Waltsgott H. Cancer mortality among workers in chemical plant contaminated with dioxin. *Lancet* 338:959-964 (1991).
  32. Neuberger M, Landvoigt W, Derntl F. Blood levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in chemical workers after chloracne and in comparison groups. *Int Arch Occup Environ Health* 63:325-327 (1991).
  33. Lu YC, Wong PN. Dermatological, medical, and laboratory findings of patients in Taiwan and their treatments. *Am J Ind Med* 5:81-115 (1984).
  34. Gladen BC, Taylor JS, Wu YC, Ragan NB, Rogan WJ, Hsu CC. Dermatological findings in children exposed transplacentally to heat-degraded polychlorinated biphenyls in Taiwan. *Br J Dermatol* 122:799-808 (1990).
  35. Egeland GM, Sweeney MH, Fingerhut MA, Wille KK, Schnorr TM, Halperin WE. Total serum testosterone and gonadotropins in workers exposed to dioxin. *Am J Epidemiol* 139:272-281 (1994).
  36. Mocarelli P, Brambilla P, Gerthoux PM, Patterson DG Jr, Needham LL. Change in sex ratio with exposure to dioxin [Letter]. *Lancet* 348:409 (1996).
  37. Moore RW, Potter CL, Theobald HM, Robinson JA, Peterson RE. Androgenic deficiency in male rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Appl Pharmacol* 79:99-111 (1985).
  38. Gray LE Jr, Ostby JS. *In utero* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. *Toxicol Appl Pharmacol* 133:285-294 (1995).
  39. Kimmel CA, Kimmel GL. Risk assessment for developmental toxicity. In: *Developmental Toxicology* (Kimmel CA, Buleke-Sam J, eds). New York:Raven Press, 1994:429-453.
  40. Chen YC, Yu ML, Rogan WJ, Gladen BC, Hsu CC. A 6-year follow-up of behavior and activity disorders in the Taiwan Yucheng children. *Am J Public Health* 84:415-421 (1994).
  41. Chen YC, Guo YL, Hsu CC, Rogan WJ. Cognitive development of Yu-Cheng ("oil disease") children prenatally exposed to heat-degraded PCBs. *JAMA* 268:3213-3218 (1992).
  42. Koopman Esseboom C, Weisglas Kuperus N, de Ridder MA, Van der Pauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. *Pediatrics* 97:700-706 (1996).
  43. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. *Ann Epidemiol* 1:407-413 (1991).
  44. Koopman Esseboom C, Morse DC, Weisglas Kuperus N, Lutkeschipholt IJ, Van der Pauw CG, Tuinstra LG, Brouwer A, Sauer PJ. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. *Pediatr Res* 36:468-473 (1994).
  45. Schantz SL, Bowman RE. Learning in monkeys exposed perinatally to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Neurotoxicol Teratol* 11:13-19 (1979).
  46. Bastomsky CH. Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Endocrinology* 101:292-296 (1977).
  47. Faith RE, Moore JA. Impairment of thymus-dependent immune functions by exposure of the developing immune system to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *J Toxicol Environ Health* 3:451-464 (1977).
  48. Thiel R, Koch E, Ulbrich B, Chahoud I. Peri- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: effects on physiological development, reflexes, locomotor activity and learning behaviour in Wistar rats. *Arch Toxicol* 69:79-86 (1994).
  49. Birnbaum LS, Harris MW, Barnhart ER, Morrissey RE. Teratogenicity of three polychlorinated dibenzofurans in C57BL/6N mice. *Toxicol Appl Pharmacol* 90:206-216 (1987).
  50. Birnbaum LS. Developmental effects of dioxins. *Environ Health Perspect* 103(Suppl 7):89-94 (1995).

51. Mahmood TA, Templeton A. Prevalence and genesis of endometriosis. *Hum Reprod* 6:544–549 (1991).
52. Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Fundam Appl Toxicol* 21:433–441 (1993).
53. Bois FY, Eskenazi B. Possible risk of endometriosis for Seveso, Italy, residents: an assessment of exposure to dioxin. *Environ Health Perspect* 102(5):476–477 (1994).
54. Cummings AM, Metcalf JL. Induction of endometriosis in mice: a new model sensitive to estrogen. *Reprod Toxicol* 9:233–238 (1995).
55. Johnson KL, Cummings AM, Birnbaum LS. Promotion of endometriosis in mice by polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls. *Environ Health Perspect* 105(7):750–755 (1997).
56. Neubert R, Maskow L, Webb J, Jacob Muller U, Nogueira AC, Delgado I, Helge H, Neubert D. Chlorinated dibenzo-*p*-dioxins and dibenzofurans and the human immune system. 1: Blood cell receptors in volunteers with moderately increased body burdens. *Life Sci* 53:1995–2006 (1993).
57. Tonn T, Esser C, Schneider EM, Steinmann Steiner Haldenstatt W, Gleichmann E. Persistence of decreased T-helper cell function in industrial workers 20 years after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ Health Perspect* 104(4):422–426 (1996).
58. Weisglas-Kuperus N, Sas TCJ, Koopman-Esseboom C, van der Swan C, de Ridder MAJ, Beishuizen A, Hooijkaas H, Sauer PJ. Immunological effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in infants. *Organohalogen Compounds* 30:205–208 (1996).
59. Mocarelli P, Marocchi A, Brambilla P, Gerthoux P, Young DS, Mantel N. Clinical laboratory manifestations of exposure to dioxin in children. A six-year study of the effects of an environmental disaster near Seveso, Italy. *JAMA* 256:2687–2695 (1986).
60. Vecchi A, Mantovani A, Sironi M, Luini W, Spreafico F, Garattini S. The effect of acute administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on humoral antibody production and cell-mediated activities in mice. *Arch Toxicol Suppl* 4:163–165 (1980).
61. Dooley RK, Holsapple MP. Elucidation of cellular targets responsible for tetrachlorodibenzo-*p*-dioxin (TCDD)-induced suppression of antibody responses. I: The role of the B lymphocyte. *Immunopharmacology* 16:167–180 (1988).
62. Kerkvliet NI. Immunological effects of chlorinated dibenzo-*p*-dioxins. *Environ Health Perspect* 103(Suppl 9):47–53 (1995).
63. Bursleson GR, Lebrec H, Yang YG, Ibanes JD, Pennington KN, Birnbaum LS. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on influenza virus host resistance in mice. *Fundam Appl Toxicol* 29:40–47 (1996).
64. Luebke RW, Copeland CB, Diliberto JJ, Akubue PI, Andrews DL, Riddle MM, Williams WC, Birnbaum LS. Assessment of host resistance to *Trichinella spiralis* in mice following preinfection exposure to 2,3,7,8-TCDD. *Toxicol Appl Pharmacol* 125:7–16 (1994).
65. Bertazzi PA, Zocchetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L. Ten-year mortality study of the population involved in the Seveso incident in 1976. *Am J Epidemiol* 129:1187–1200 (1989).
66. Bertazzi PA, Pesatori AC, Consonni D, Tironi A, Landi MT, Zocchetti C. Cancer incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin. *Epidemiology* 4:398–406 (1993).
67. Bertazzi PA, Zocchetti C, Guercilena S, Consonni D, Tironi A, Landi MT, Pesatori AC. Dioxin exposure and cancer risk: a 15-year mortality study after the “Seveso accident.” *Epidemiology* 8:646–652 (1997).
68. Ott MG, Zober A. Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. *Occup Environ Med* 53:606–612 (1996).
69. Kogevinas M, Kauppinen T, Winkelmann R, Becher H, Bertazzi PA, Bueno de Mesquita HB, Coggon D, Green L, Johnson E, Littorin M et al. Soft tissue sarcoma and non-Hodgkin’s lymphoma in workers exposed to phenoxy herbicides, chlorophenols, and dioxins: two nested case-control studies. *Epidemiology* 6:396–402 (1995).
70. Huff JE, Salmon AG, Hooper NK, Zeise L. Long-term carcinogenesis studies on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and hexachlorodibenzo-*p*-dioxins. *Cell Biol Toxicol* 7:67–94 (1991).
71. Kociba RJ, Keyes DG, Beyer JE, Carreon RM, Wade CE, Dittenber DA, Kalnins RP, Frauson LE, Park CN, Barnard SD et al. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Toxicol Appl Pharmacol* 46:279–303 (1978).
72. U.S. NTP. Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (CAS No 1746-01-6) in Osborne-Mendel Rats and B6C3F<sub>1</sub> Mice (Gavage Study). TR-209. Research Triangle Park, NC:U.S. National Toxicology Program, 1982.
73. Tritscher AM, Goldstein JA, Portier CJ, McCoy Z, Clark GC, Lucier GW. Dose-response relationships for chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a rat tumor promotion model: quantification and immunolocalization of CYP1A1 and CYP1A2 in the liver. *Cancer Res* 52:3436–3442 (1992).
74. Clark G, Tritscher A, Bell D, Lucier G. Integrated approach for evaluating species and interindividual differences in responsiveness to dioxins and structural analogs. *Environ Health Perspect* 98:125–132 (1992).
75. Lin FH, Stohs SJ, Birnbaum LS, Clark G, Lucier GW, Goldstein JA. The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the hepatic estrogen and glucocorticoid receptors in congenic strains of Ah responsive and Ah nonresponsive C57BL/6J mice. *Toxicol Appl Pharmacol* 108:129–139 (1991).
76. Sunahara GI, Nelson KG, Wong TK, Lucier GW. Decreased human birth weights after *in utero* exposure to PCBs and PCDFs are associated with decreased placental EGF-stimulated receptor autophosphorylation capacity. *Mol Pharmacol* 32:572–578 (1987).
77. Bertazzi PA. Long-term effects of chemical disasters. Lessons and results from Seveso. *Sci Total Environ* 106:5–20 (1991).
78. Grassman J, Clark G, Yang X-P, Masten S, Spencer D, Landi MT, Miller C, Walker N, Lucier G. Estimating variability in response to dioxin after exposure in Seveso, Italy. *Organohalogen Compounds* 30:302–307 (1996).
79. Masten SA, Yang X, Grassman JA, Walker NJ, Miller CR, Spencer DL, Lanier KM, Clark GC, Sutter TR, Lucier GW. Ah receptor (AhR) and Ah receptor nuclear translocator (ARNT) levels as predictors of dioxin responsiveness in human lymphocytes [Abstract]. *Fundam Appl Toxicol* 36:127 (1997).
80. Okey AB, Riddick DS, Harper PA. The Ah receptor: mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. *Toxicol Lett* 70:1–22 (1994).
81. Schmidt JV, Bradfield CA. Ah receptor signaling pathways. *Ann Rev Cell Dev Biol* 12:55–89 (1996).
82. Rowlands JC, Gustafsson JA. Aryl hydrocarbon receptor-mediated signal transduction. *Crit Rev Toxicol* 27:109–134 (1997).
83. Dolwick KM, Schmidt JV, Carver LA, Swanson HI, Bradfield CA. Cloning and expression of a human Ah receptor cDNA. *Mol Pharmacol* 44:911–917 (1993).
84. Denison MS, Wilkinson CF. Identification of the Ah receptor in selected mammalian species and induction of aryl hydrocarbon hydroxylase. *Eur J Biochem* 147:429–435 (1985).
85. Hayashi S, Watanabe J, Nakachi K, Eguchi H, Gotoh O, Kawajiri K. Interindividual difference in expression of human Ah receptor and related P450 genes. *Carcinogenesis* 15:801–806 (1994).
86. Li W, Donat S, Dohr O, Unfried K, Abel J. Ah receptor in different tissues of C57BL/6J and DBA/2J mice: use of competitive polymerase chain reaction to measure Ah-receptor mRNA expression. *Arch Biochem Biophys* 315:279–284 (1994).

87. Carver LA, Hogenesch JB, Bradfield CA. Tissue specific expression of the rat Ah-receptor and ARNT mRNAs. *Nucleic Acids Res* 22:3038-3044 (1994).
88. Fernandez Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, Nebert DW, Rudikoff S, Ward JM, Gonzalez FJ. Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science* 268:722-726 (1995).
89. Schmidt JV, Su GH, Reddy JK, Simon MC, Bradfield CA. Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. *Proc Natl Acad Sci USA* 93:6731-6736 (1996).
90. Birnbaum LS. Evidence for the role of the Ah receptor in response to dioxin. In: *Receptor-Mediated Biological Processes: Implications for Evaluating Carcinogenesis*. New York:Wiley-Liss, 1994;139-154.
91. Ema M, Ohe N, Suzuki M, Mimura J, Sogawa K, Ikawa S, Fujii Kuriyama Y. Dioxin binding activities of polymorphic forms of mouse and human arylhydrocarbon receptors. *J Biol Chem* 269:27337-27343 (1994).
92. Kawajiri K, Watanabe J, Eguchi H, Nakachi K, Kiyohara C, Hayashi S. Polymorphisms of human Ah receptor gene are not involved in lung cancer. *Pharmacogenetics* 5:151-158 (1995).
93. Lorenzen A, Okey AB. Detection and characterization of Ah receptor in tissue and cells from human tonsils. *Toxicol Appl Pharmacol* 107:203-214 (1991).
94. Masten SA, Shiverick KT. Characterization of the aryl hydrocarbon receptor complex in human B lymphocytes: evidence for a distinct nuclear DNA-binding form. *Arch Biochem Biophys* 336:297-308 (1996).
95. Waithe WI, Michaud M, Harper PA, Okey AB, Anderson A. The Ah receptor, cytochrome P450A1 mRNA induction, and aryl hydrocarbon hydroxylase in a human lymphoblastoid cell line. *Biochem Pharmacol* 41:85-92 (1991).
96. Safe SH. Modulation of gene expression and endocrine response pathways by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds. *Pharmacol Ther* 67:247-281 (1995).
97. Whitlock JP Jr. Mechanistic aspects of dioxin action. *Chem Res Toxicol* 6:754-763 (1993).
98. Whyatt RM, Garte SJ, Cosma G, Bell DA, Jedrychowski W, Wahrendorf J, Randall MC, Cooper TB, Ottman R, Tang D et al. CYP1A1 messenger RNA levels in placental tissue as a biomarker of environmental exposure. *Cancer Epidemiol Biomarkers Prev* 4:147-153 (1995).
99. Vanden Heuvel JP, Clark GC, Thompson CL, McCoy Z, Miller CR, Lucier GW, Bell DA. CYP1A1 mRNA levels as a human exposure biomarker: use of quantitative polymerase chain reaction to measure CYP1A1 expression in human peripheral blood lymphocytes. *Carcinogenesis* 14:2003-2006 (1993).
100. Yoshikawa M, Arashidani K, Kawamoto T, Kodama Y. Aryl hydrocarbon hydroxylase activity in human lung tissue: in relation to cigarette smoking and lung cancer. *Environ Res* 65:1-11 (1994).
101. Lucier GW, Nelson KG, Everson RB, Wong TK, Philpot RM, Tiernan T, Taylor M, Sunahara GI. Placental markers of human exposure to polychlorinated biphenyls and polychlorinated dibenzofurans. *Environ Health Perspect* 76:79-87(1987).
102. Lucier GW. Humans are a sensitive species to some of the biochemical effects of structural analogs of dioxin. *Environ Toxicol Chem* 10:727-735 (1991).
103. Wong TK, Domin BA, Bent PE, Blanton TE, Anderson MW, Philpot RM. Correlation of placental microsomal activities with protein detected by antibodies to rabbit cytochrome P-450 isozyme 6 in preparations from humans exposed to polychlorinated biphenyls, quaterphenyls, and dibenzofurans. *Cancer Res* 46:999-1004 (1986).
104. Masten SA, Grassman JA, Yang X, Miller CR, Spencer DL, Lanier KM, Walker NJ, Jung D, Konietzko J, Edler L et al. Mechanistically based markers of exposure and response to dioxin in occupationally exposed individuals. *Organohalogen Compounds* 34:80-85 (1997).
105. Kouri RE, McKinney CE, Slomiany DJ, Snodgrass DR, Wray NP, McLemore TL. Positive correlation between high aryl hydrocarbon hydroxylase activity and primary lung cancer as analyzed in cryopreserved lymphocytes. *Cancer Res* 42:5030-5037 (1982).
106. Kellermann G, Shaw CR, Luyten-Kellerman M. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. *N Engl J Med* 289:934-937 (1973).
107. Rannug A, Alexandrie AK, Persson I, Ingelman Sundberg M. Genetic polymorphism of cytochromes P450 1A1, 2D6 and 2E1: regulation and toxicological significance. *J Occup Environ Med* 37:25-36 (1995).
108. Nebert DW, Petersen DD, Puga A. Human AH locus polymorphism and cancer: inducibility of *CYP1A1* and other genes by combustion products and dioxin. *Pharmacogenetics* 1:68-78 (1991).
109. Persson I, Johansson I, Ingelman-Sundberg M. *In vitro* kinetics of two human *CYP1A1* variant enzymes suggested to be associated with interindividual differences in cancer susceptibility. *Biochem Biophys Res Commun* 231:227-230 (1997).
110. Zhang ZY, Fasco MJ, Huang L, Guengerich FP, Kaminsky LS. Characterization of purified human recombinant cytochrome P450A1-Ile462 and -Val462: assessment of a role for the rare allele in carcinogenesis. *Cancer Res* 56:3926-3933 (1996).
111. d'Errico A, Taiolo E, Chen X, Vineis P. Genetic polymorphisms and the risk of cancer: a review of the literature. *Biomarkers* 1:149-173 (1996).
112. Micka J, Milatovich A, Menon A, Grabowski GA, Puga A, Nebert DW. Human Ah receptor (AHR) gene: localization to 7p15 and suggestive correlation of polymorphism with *CYP1A1* inducibility. *Pharmacogenetics* 7:95-101 (1997).
113. Grassman JA, Masten SA, Spencer DL, Yang X, Miller CR, Clark GC, Lanier KM, Needham LL, Landi MT, Walker NJ et al. Unpublished data.
114. Goldstein JA, Linko P. Differential induction of two 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible forms of cytochrome P-450 in extrahepatic versus hepatic tissues. *Mol Pharmacol* 25:185-191 (1984).
115. Diliberto JJ, Akubue PI, Luebke RW, Birnbaum LS. Dose-response relationships of tissue distribution and induction of CYP1A1 and CYP1A2 enzymatic activities following acute exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in mice. *Toxicol Appl Pharmacol* 130:197-208 (1995).
116. Okey AB. Enzyme induction in the cytochrome P-450 system. *Pharmacol Ther* 45:241-298 (1990).
117. McKinnon RA, Hall PD, Quattrochi LC, Tukey RH, McManus ME. Localization of CYP1A1 and CYP1A2 messenger RNA in normal human liver and in hepatocellular carcinoma by *in situ* hybridization. *Hepatology* 14:848-856 (1991).
118. Vanden Heuvel JP, Clark GC, Kohn MC, Tritscher AM, Greenlee WF, Lucier GW, Bell DA. Dioxin-responsive genes: examination of dose-response relationships using quantitative reverse transcriptase-polymerase chain reaction. *Cancer Res* 54:62-68 (1994).
119. Schweikl H, Taylor JA, Kitareewan S, Linko P, Nagorney D, Goldstein JA. Expression of *CYP1A1* and *CYP1A2* genes in human liver. *Pharmacogenetics* 3:239-249 (1993).
120. Butler MA, Lang NP, Young JF, Caporaso NE, Vineis P, Hayes RB, Teitel CH, Massengill JP, Lawsen MF, Kadlubar FF. Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. *Pharmacogenetics* 2:116-127 (1992).
121. Lambert GH, Hsu CC, Guo L, Ryan JJ, Schoeller DA. The dose response relationship between the serum levels of polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) and cytochrome P450 1A2 activity as determined by the caffeine breath test. *Organohalogen Compounds* 21:485-488 (1994).
122. Sinha R, Rothman N, Brown ED, Mark SD, Hoover RN, Caporaso NE, Levander OA, Knize MG, Lang NP, Kadlubar FF. Pan-fried meat containing high levels of heterocyclic aromatic amines but low levels of polycyclic aromatic

- hydrocarbons induces cytochrome P4501A2 activity in humans. *Cancer Res* 54:6154–6159 (1994).
123. Halperin W, Kalow W, Sweeney MH, Tang BK, Fingerhut M, Timpkins B, Wille K. Induction of P-450 in workers exposed to dioxin. *Occup Environ Med* 52:86–91 (1995).
  124. Lucier GW, Tritscher A, Goldsworthy T, Foley J, Clark G, Goldstein J, Maronpot R. Ovarian hormones enhance 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated increases in cell proliferation and preneoplastic foci in a two-stage model for rat hepatocarcinogenesis. *Cancer Res* 51:1391–1397 (1991).
  125. Voorman R, Aust SD. TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) is a tight binding inhibitor of cytochrome P-450d. *J Biochem Toxicol* 4:105–109 (1989).
  126. Diliberto JJ, Burgin D, Birnbaum LS. Role of CYP1A2 in hepatic sequestration of dioxin: studies using CYP1A2 knock-out mice. *Biochem Biophys Res Commun* 236:431–433 (1997).
  127. Aoyama T, Korzekwa K, Nagata K, Gillette J, Gelboin HV, Gonzalez FJ. Estradiol metabolism by complementary deoxyribonucleic acid-expressed human cytochrome P450s. *Endocrinology* 126:3101–3106 (1990).
  128. Yager JD, Liehr JG. Molecular mechanisms of estrogen carcinogenesis. *Annu Rev Pharmacol Toxicol* 36:203–232 (1996).
  129. Shimada T, Guengerich FP. Activation of amino-carboline, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine, and a copper phthalocyanine cellulose extract of cigarette smoke condensate by cytochrome P-450 enzymes in rat and human liver microsomes. *Cancer Res* 51:5284–5291 (1991).
  130. Lang NP, Butler MA, Massengill J, Lawson M, Stotts RC, Hauer Jensen M, Kadlubar FF. Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. *Cancer Epidemiol Biomarkers Prev* 3:675–682 (1994).
  131. Sutter TR, Guzman K, Dold KM, Greenlee WF. Targets for dioxin: genes for plasminogen activator inhibitor-2 and interleukin-1 beta. *Science* 254:415–418 (1991).
  132. Sutter TR, Tang YM, Hayes CL, Wo YY, Jabs EW, Li X, Yin H, Cody CW, Greenlee WF. Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. *J Biol Chem* 269:13092–13099 (1994).
  133. Savas U, Bhattacharyya KK, Christou M, Alexander DL, Jefcoate CR. Mouse cytochrome P-450EF, representative of a new 1B subfamily of cytochrome P-450s. Cloning, sequence determination, and tissue expression. *J Biol Chem* 269:14905–14911 (1994).
  134. Shen Z, Liu J, Wells RL, Elkind MM. cDNA cloning, sequence analysis, and induction by aryl hydrocarbons of a murine cytochrome P450 gene, *Cyp1b1*. *DNA Cell Biol* 13:763–769 (1994).
  135. Walker NJ, Gastel JA, Costa LT, Clark GC, Lucier GW, Sutter TR. Rat CYP1B1: an adrenal cytochrome P450 that exhibits sex-dependent expression in livers and kidneys of TCDD-treated animals. *Carcinogenesis* 16:1319–1327 (1995).
  136. Bhattacharyya KK, Brake PB, Eltom SE, Otto SA, Jefcoate CR. Identification of a rat adrenal cytochrome P450 active in polycyclic hydrocarbon metabolism as rat CYP1B1. Demonstration of a unique tissue-specific pattern of hormonal and aryl hydrocarbon receptor-linked regulation. *J Biol Chem* 270:11595–11602 (1995).
  137. Otto S, Bhattacharyya KK, Jefcoate CR. Polycyclic aromatic hydrocarbon metabolism in rat adrenal, ovary, and testis microsomes is catalyzed by the same novel cytochrome P450 (P450RAP). *Endocrinology* 131:3067–3076 (1992).
  138. Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich FP, Sutter TR. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res* 56:2979–2984 (1996).
  139. Crofts FG, Strickland PT, Hayes CL, Sutter TR. Metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP) by human cytochrome P4501B1. *Carcinogenesis* 18:1793–1798 (1997).
  140. Stoilov I, Akarsu AN, Sarfarazi M. Identification of three different truncating mutations in cytochrome P4501B1 (CYP1B1) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. *Hum Mol Genet* 6:641–647 (1997).
  141. Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR. 17 $\beta$ -estradiol hydroxylation catalyzed by human cytochrome P450 1B1. *Proc Natl Acad Sci USA* 93:9776–9781 (1996).
  142. Liehr JG, Fang WF, Sirbasku DA, Ari-Ulubelen A. Carcinogenicity of catechol estrogens in Syrian hamsters. *J Steroid Biochem* 24:353–356 (1986).
  143. Liehr JG, Ricci MJ, Jefcoate CR, Hannigan EV, Hokanson JA, Zhu BT. 4-Hydroxylation of estradiol by human uterine myometrium and myoma microsomes: implications for the mechanism of uterine tumorigenesis. *Proc Natl Acad Sci USA* 92:9220–9224 (1995).
  144. Murray GI, Taylor MC, McFadyen MC, McKay JA, Greenlee WF, Burke MD, Melvin WT. Tumor-specific expression of cytochrome P450 CYP1B1. *Cancer Res* 57:3026–3031 (1997).
  145. Spink DC, Hayes CL, Young NR, Christou M, Sutter TR, Jefcoate CR, Gierthy JF. The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on estrogen metabolism in MCF-7 breast cancer cells: evidence for induction of a novel 17 beta-estradiol 4-hydroxylase. *J Steroid Biochem Mol Biol* 51:251–258 (1994).
  146. Walker NJ, Crofts FG, Li Y, Lax SF, Hayes CL, Strickland PT, Lucier GW, Sutter TR. Induction and localization of cytochrome P450 1B1 (CYP1B1) protein in the livers of TCDD-treated rats: detection using polyclonal antibodies raised to histidine-tagged fusion proteins produced and purified from bacteria. *Carcinogenesis* 19:101–108 (1998).
  147. Walker NJ, Tritscher A, Sutter TR, Lucier GW. CYP1B1 and CYP1A1 exhibit differential dose-response relationships in the TCDD-treated female Sprague-Dawley rat liver. *Organohalogen Compounds* 34:10–14 (1997).
  148. Santostefano MJ, Ross DG, Savas U, Jefcoate CR, Birnbaum LS. Differential time-course and dose-response relationships of TCDD-induced CYP1B1, CYP1A1, and CYP1A2 proteins in rats. *Biochem Biophys Res Commun* 233:20–24 (1997).
  149. Willey JC, Coy EL, Frampton MW, Torres A, Apostolakis MJ, Hoehn G, Schuermann WH, Thilly WG, Olson DE, Hammersley JR et al. Quantitative RT-PCR measurement of cytochromes P450 1A1, 1B1, and 2B7, microsomal epoxide hydrolase, and NADPH oxidoreductase expression in lung cells of smokers and nonsmokers. *Am J Respir Cell Mol Biol* 17:114–124 (1997).
  150. Carpenter G. Receptors for epidermal growth factor and other polypeptide mitogens. *Annu Rev Biochem* 56:881–914 (1987).
  151. Marti U, Burwen SJ, Jones AL. Biological effects of epidermal growth factor, with emphasis on the gastrointestinal tract and liver: an update. *Hepatology* 9:126–138 (1989).
  152. Maruo T, Matsuo H, Otani T, Mochizuki M. Role of epidermal growth factor (EGF) and its receptor in the development of the human placenta. *Reprod Fertil Dev* 7:1465–1470 (1995).
  153. Stoscheck CM, King LE Jr. Role of epidermal growth factor in carcinogenesis. *Cancer Res* 46:1030–1037 (1986).
  154. Hudson LG, Toscano WA Jr, Greenlee WF. Regulation of epidermal growth factor binding in a human keratinocyte cell line by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Appl Pharmacol* 77:251–259 (1985).
  155. Lin FH, Clark G, Birnbaum LS, Lucier GW, Goldstein JA. Influence of the Ah locus on the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the hepatic epidermal growth factor receptor. *Mol Pharmacol* 39:307–313 (1991).
  156. Sewall CH, Lucier GW, Tritscher AM, Clark GC. TCDD-mediated changes in hepatic epidermal growth factor receptor may be a critical event in the hepatocarcinogenic action of TCDD. *Carcinogenesis* 14:1885–1893 (1993).
  157. Sewall CH, Clark GC, Lucier GW. TCDD reduces rat hepatic epidermal growth factor receptor: comparison of binding,

- immunodetection, and autophosphorylation. *Toxicol Appl Pharmacol* 132:263–272 (1995).
158. Hill RN, Erdreich LS, Paynter OE, Roberts PA, Rosenthal SL, Wilkinson CF. Thyroid follicular cell carcinogenesis. *Fundam Appl Toxicol* 12:629–697 (1989).
  159. Bock KW. Roles of UDP-glucuronosyltransferases in chemical carcinogenesis. *Crit Rev Biochem Mol Biol* 26:129–150 (1991).
  160. Munzel PA, Bruck M, Bock KW. Tissue-specific constitutive and inducible expression of rat phenol UDP-glucuronosyltransferase. *Biochem Pharmacol* 47:1445–1448 (1994).
  161. Munzel PA, Bookjans G, Mehner G, Lehmkoetter T, Bock KW. Tissue-specific 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible expression of human UDP-glucuronosyltransferase UGT1A6. *Arch Biochem Biophys* 335:205–210 (1996).
  162. Liu J, Liu Y, Barter RA, Klaassen CD. Alteration of thyroid homeostasis by UDP-glucuronosyltransferase inducers in rats: a dose-response study. *J Pharmacol Exp Ther* 273:977–985 (1995).
  163. Schuur AG, Boekhorst FM, Brouwer A, Visser TJ. Extrathyroidal effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on thyroid hormone turnover in male Sprague-Dawley rats. *Endocrinology* 138:3727–3734 (1997).
  164. Ott MG, Zober A, Germann C. Laboratory results for selected target organs in 138 individuals occupationally exposed to TCDD. *Chemosphere* 29:2423–2437 (1994).
  165. Pluim HJ, de Vijlder JJ, Olie K, Kok JH, Vulsma T, van Tijn DA, van der Slikke JW, Koppe JG. Effects of pre- and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. *Environ Health Perspect* 101(6):504–508 (1993).
  166. Sewall CH, Flagler N, Vanden Heuvel JP, Clark GC, Tritscher AM, Maronpot RR, Lucier GW. Alterations in thyroid function in female Sprague-Dawley rats following chronic treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Appl Pharmacol* 132:237–244 (1995).
  167. Kohn MC, Sewall CH, Lucier GW, Portier CJ. A mechanistic model of effects of dioxin on thyroid hormones in the rat. *Toxicol Appl Pharmacol* 136:29–48 (1996).
  168. Kohn MC, Lucier GW, Clark GC, Sewall C, Tritscher AM, Portier CJ. A mechanistic model of effects of dioxin on gene expression in the rat liver. *Toxicol Appl Pharmacol* 120:138–154 (1993).
  169. Whitlock JP Jr. The aromatic hydrocarbon receptor, dioxin action, and endocrine homeostasis. *Trends Endocrinol Metab* 5:183–188 (1994).
  170. Yang JH, Rhim JS. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: molecular mechanism of carcinogenesis and its implication in human *in vitro* model. *Crit Rev Oncol Hematol* 18:111–127 (1995).
  171. Pitot HC, Goldsworthy T, Campbell HA, Poland A. Quantitative evaluation of the promotion by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of hepatocarcinogenesis from diethylnitrosamine. *Cancer Res* 40:3616–3620 (1980).
  172. Maronpot RR, Foley JF, Takahashi K, Goldsworthy T, Clark G, Tritscher A, Portier C, Lucier G. Dose response for TCDD promotion of hepatocarcinogenesis in rats initiated with DEN: histologic, biochemical, and cell proliferation endpoints. *Environ Health Perspect* 101(7):634–642 (1993).
  173. Tritscher AM, Clark GC, Sewall C, Sills RC, Maronpot R, Lucier GW. Persistence of TCDD-induced hepatic cell proliferation and growth of enzyme altered foci after chronic exposure followed by cessation of treatment in DEN initiated female rats. *Carcinogenesis* 16:2807–2811 (1995).
  174. Dragan YP, Xu XH, Goldsworthy TL, Campbell HA, Maronpot RR, Pitot HC. Characterization of the promotion of altered hepatic foci by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the female rat. *Carcinogenesis* 13:1389–1395 (1992).
  175. Walker NJ, Tritscher AM, Sills RC, Lucier GW. Hepatocarcinogenesis in a Sprague-Dawley rat initiation/promotion model following discontinuous exposure to TCDD. *Organohalogen Compounds* 34:150–153 (1997).
  176. Abbott BD, Perdew GH, Buckalew AR, Birnbaum LS. Interactive regulation of Ah and glucocorticoid receptors in the synergistic induction of cleft palate by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and hydrocortisone. *Toxicol Appl Pharmacol* 128:138–150 (1994).
  177. Abbott BD, Probst MR, Perdew GH. Immunohistochemical double-staining for Ah receptor and ARNT in human embryonic palatal shelves. *Teratology* 50:361–366 (1994).
  178. Brown NM, Lamartiniere CA. Xenoestrogens alter mammary gland differentiation and cell proliferation in the rat. *Environ Health Perspect* 103(7–8):708–713 (1995).
  179. Osborne R, Greenlee WF. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) enhances terminal differentiation of cultured human epidermal cells. *Toxicol Appl Pharmacol* 77:434–443 (1985).
  180. Gierthy JF, Bennett JA, Bradley LM, Cutler DS. Correlation of *in vitro* and *in vivo* growth suppression of MCF-7 human breast cancer by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Cancer Res* 53:3149–3153 (1993).
  181. Landi MT, Needham LL, Lucier G, Mocarelli P, Bertazzi PA, Caporaso N. Concentrations of dioxin 20 years after Seveso [Letter]. *Lancet* 349:1811 (1997).
  182. Tritscher AM, Seacat AM, Yager JD, Groopman JD, Miller BD, Bell D, Sutter TR, Lucier GW. Increased oxidative DNA damage in livers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treated intact but not ovariectomized rats. *Cancer Lett* 98:219–225 (1996).
  183. Ott MG, Messerer P, Zober A. Assessment of past occupational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using blood lipid analyses. *Int Arch Occup Environ Health* 65:1–8 (1993).
  184. Flesch-Janys D, Berger J, Gurn P, Manz A, Nagel S, Waltsgott H, Dwyer JH. Exposure to polychlorinated dioxins and furans (PCDD/F) and mortality in a cohort of workers from a herbicide-producing plant in Hamburg, Federal Republic of Germany. *Am J Epidemiol* 142:1165–1175 (1995).
  185. Birnbaum LS. The mechanism of dioxin toxicity: relationship to risk assessment. *Environ Health Perspect* 102(Suppl 9):157–167 (1994).