2. EFFECTS SUMMARY

Since the identification in 1957 of 2,3,7,8-TCDD as a chloracnegen, more than 5000 publications have discussed its biological and toxicological properties. A large number of the effects of dioxin and related compounds have been discussed in detail throughout the chapters in Part II of this assessment. These discussions illustrate the wide range of effects produced by this class of compounds. The majority of effects have been identified in experimental animals; some have also been identified in exposed human populations. Although past EPA risk assessments have focused on cancer estimates based on extrapolation models as the major concern for dioxin and related compounds, more recent data suggest that noncancer effects may be occurring at or near human background steady-state body burden levels in animals and in humans. Evaluation of noncancer effects and their relationship to past and current body burdens and intake levels is an important feature of this reassessment. Direct comparisons between various noncancer effects and cancer in animals and humans and exposures of interest are presented in the form of *margins of exposure* (MOE).

Cross-sectional studies have been conducted to evaluate the prevalence or extent of disease in living 2,3,7,8-TCDD-exposed groups (Suskind and Hertzberg, 1984; Moses et al., 1984; Lathrop et al., 1984, 1987; Roegner et al., 1991; Grubbs et al., 1995; Sweeney et al., 1989; CDC Vietnam Experience Study, 1988; Webb et al., 1989; Ott and Zober, 1994). The limitations of the cross-sectional study design for evaluating hazard and risk are discussed in Part II, Chapter 7b, Section 7.11. Many of the earliest studies were unable to define exposure-outcome relationships owing to a variety of shortcomings, including small sample size, poor participation, short latency periods, selection of inappropriate controls, and the inability to quantify exposure to 2,3,7,8-TCDD or to identify confounding exposures.

Cohort and case-control studies have been used to investigate hypothesized increases in malignancies among the various 2,3,7,8-TCDD-exposed populations (Fingerhut et al., 1991a, b; Manz et al., 1991; Eriksson et al., 1990). In more recent analyses of occupational cohorts (Steenland et al., 1999; Ott and Zober, 1996; Flesch-Janys et al., 1998), cross-sectional studies of U.S. chemical workers (Sweeney et al., 1989), U.S. Air Force Ranch Hand personnel (Roegner et al., 1991; Grubbs et al., 1995), and Missouri residents (Webb et al., 1989), serum or adipose tissue levels of 2,3,7,8-TCDD were measured to evaluate 2,3,7,8-TCDD-associated effects in exposed populations. The ability to measure tissue or serum levels of 2,3,7,8-TCDD for all or a large sample of the subjects confirmed exposure to 2,3,7,8-TCDD and permitted the investigators to test hypothesized dose-response relationships.

A large number of effects of exposure to TCDD and related compounds have been documented in the scientific literature. Although many effects have been demonstrated in multiple species (see Table 2-1), other effects may be specific to the species in which they are measured and may have limited relevance to the human situation. Although the potential species-specific responses are an important consideration for characterizing potential hazard, all the observed effects of 2,3,7,8-TCDD illustrate the multiple sequelae that are possible when primary impacts are at the level of signal transduction and gene transcription. Even though not all observed effects may be characterized as "adverse" (i.e., some may be responses within the normal range or adaptive or compensatory and of unknown or neutral consequence), they represent a continuum of response expected from the fundamental changes in biology caused by exposure to dioxin-like compounds. As discussed in the following sections, the doses associated with this plethora of effects are best compared across species using a common measurement unit of steady-state body burden of 2,3,7,8-TCDD and other dioxin-like compounds, as opposed to the level or rate of exposure/intake.

The low end of the range of experimental lowest-observed-adverse-effect levels (LOAELs), no-observed-adverse-effect levels (NOAELs), and effective doses at the 1% response level (ED_{01}s) for critical endpoints from animal studies is compiled in Table 5-6 and Appendix A. These selected endpoints cover a spectrum from overt toxicity (e.g., fetal mortality, cancer), through developmental and reproductive toxicity endpoints, to enzyme induction as a marker of intracellular dioxin activity. Many of the studies report multiple statistically significant effects related to dioxin exposure. From these results, the values tabulated were selected on the basis of the lowest dose at which significant effects occurred—findings that were generally highlighted by the authors of the publication. In the event that multiple endpoints were elicited at the same dose, the effect considered of most consistency across studies and relevance to human risk assessment was selected (e.g., decreased sperm counts).

A variety of methods were employed to estimate body burdens corresponding to the LOAELs/NOAELs/ED_{01}s, including using measured body burden and lipid concentration data, absorption adjustments for single-dose studies, and first-order pharmacokinetic modeling estimates using absorbed dose and halflife. Additional details on study design, endpoint selection, and calculation of body burdens are included in Appendix A and can also be found in Sections 5.2 and 6.0 of this document and in other chapters of the dioxin reassessment. Human equivalent intakes for the body burden endpoints were calculated according to formulae discussed in Part II, Chapter 8 of this report and are displayed in order corresponding to the preceding three results columns in Table 5-6 and Appendix A. These comparisons result in the finding that, when animal data associated with effects at the low end of the range of experimental

observation (NOAELs/LOAELs/ED_{01}s) are compared to current average human body burdens of approximately 5 ng TEQ_{DFP}-WHO_{98}/kg—representing lifetime average intake values of approximately 3 pg TEQ_{DFP}-WHO_{98}/kg/day—or to current intake values of 1 pg TEQ_{DFP}-WHO_{98}/kg/day, relatively small MOEs are obtained. Similarly, some human noncancer effects (e.g., developmental delay, neurobehavioral outcomes, and impact on thyroid function in Dutch children) and cancer outcomes show comparatively small MOEs.

In the following sections which discuss these general effects, the focus is on developing an understanding of dioxin hazard and risk. This discussion is, by its nature, selective of findings that inform the risk assessment process. Readers are referred to the more comprehensive chapters for further discussion of the broader epidemiologic and toxicologic database.

2.1. BIOCHEMICAL RESPONSES (Cross-reference: Part II, Chapters 2, 3, and 8)

As described later in Section 3, mechanistic studies can reveal the biochemical pathways and types of biological events that contribute to adverse effects from exposure to dioxin-like compounds. For example, much evidence indicates that 2,3,7,8-TCDD acts via an intracellular protein, AhR, which is a ligand-dependent transcription factor that functions in partnership with a second protein (known as the AhR nuclear translocator, or Arnt) to alter gene expression. In addition, receptor binding may result in release of cytoplasmic proteins that, in turn, alter the expression or activity of cell-regulatory proteins (e.g., increases in Src activity). Therefore, from a mechanistic standpoint, TCDD's adverse effects appear likely to reflect alterations in gene expression or protein activity that occur at an inappropriate time and/or for an inappropriate length of time. Mechanistic studies also indicate that several other proteins (e.g. hif α, Rb, relA, src, sim, etc.) contribute to TCDD's gene-regulatory effects and that the response to 2,3,7,8-TCDD involves a relatively complex interplay between multiple genetic and environmental factors. This model is illustrated in Figure 2-1 (from Part II, Chapter 2). Comparative binding studies and other data suggest that biochemical events observed in response to TCDD exposure are also seen with other dioxin-like compounds in proportion to their TEFs.

Comparative data from animal and human cells and tissues suggest a strong qualitative similarity across species in response to dioxin-like chemicals. This further supports the applicability to humans of the generalized model of initial events in response to dioxin exposure. These biochemical and biological responses are sometimes considered adaptive or reflective of exposure to dioxin-like compounds. When they are seen within normal homeostatic limits, these biochemical changes are often not considered adverse in and of themselves. However, many of these changes are potentially on a continuum of dose-response relationships that leads to adverse responses and, considering the potential to shift population distributions in response, may be of

concern. Because of the distribution of responses and sensitivity within a population, it is possible that adaptive responses for some are frankly adverse for those at the tails of the distribution. For this reason, a balanced approach must be used when describing these events, recognizing that they may be adaptive or simply biomarkers of exposure to dioxin-like compounds, or they may represent early events in a pathway resulting in a risk of adverse effects in some humans.

If, as we can infer from the evidence, 2,3,7,8-TCDD and other dioxin-like compounds operate through these mechanisms, there are constraints on the possible models that can plausibly account for dioxin's biological effects and also on the assumptions used during the risk assessment process. For instance, the linear relationship expected between ligand concentration and receptor binding may or may not be reflective of dose-response relationships for downstream events requiring complex interactions of other regulatory proteins with the activated receptor. Puga et al. (2000a) have shown that interactions of TCDD with the AhR alters expression of over 300 genes in a single cell line at one time point and one dose. These data suggest that mechanisms of toxic action may be very complicated and that additional research will be necessary to further unravel the mechanistic relationships underpinning dioxin's toxicity.

Mechanistic knowledge of dioxin action may also be useful in other ways. For example, knowledge of genetic polymorphisms that influence 2,3,7,8-TCDD responsiveness may also allow the identification of individuals either refractory to or at particular risk from exposure to dioxin. In addition, knowledge of the biochemical pathways that are altered by dioxin-like compounds may help in the development of approaches to intervention or to drugs that can prevent dioxin's adverse effects.

As described in Part II, Chapter 2, biochemical and genetic analyses of the mechanisms by which dioxin modulates particular genes have revealed the outline of a novel regulatory system whereby a chemical signal can alter cellular regulatory processes. Future studies of dioxin action have the potential to provide additional insights into mechanisms of mammalian gene regulation that are of relatively broad interest. Additional perspectives on dioxin action can be found in several recent reviews (Birnbaum, 1994a, b; Schecter, 1994; Hankinson, 1995; Schmidt and Bradfield, 1996; Rowlands and Gustafsson, 1997; Gasiewicz, 1997; Hahn, 1998; Denison et al., 1998; Wilson and Safe, 1998; Schecter and Gasiewicz, 2003).

The ability of 2,3,7,8-TCDD and other dioxin-like compounds to modulate a number of biochemical parameters in a species-, tissue-, and temporal-specific manner is well recognized. Despite the ever-expanding list of these responses from the past 20 years and the elegant work on the molecular mechanisms mediating some of these, there still exists a considerable gap between our knowledge of individual biochemical changes and the degree to which they are related to the

more complex biological and toxicological endpoints elicited by these chemicals. A framework for considering these responses in a mode of action context is discussed later in this document.

TCDD-elicited activation of the AhR has been clearly shown to mediate altered transcription of a number of genes, including several oncogenes and those encoding growth factors, receptors, hormones, and drug-metabolizing enzymes. Table 2-2 provides an illustrative list of gene products whose regulation or activity is modulated by 2,3,7,8-TCDD. Although this list is not meant to be exhaustive, it demonstrates the range of potential dioxin impacts on pathways with potential to lead to adverse effects.

As discussed in Part II, Chapter 2, it is possible that the TCDD-elicited alteration of activity of these genes may occur through a variety of mechanisms. The transcription of some genes may be directly regulated by the activated AhR. Other alterations in gene expression may be secondary to the initial biochemical events directly regulated transcriptionally by the AhR. Some of the changes may also occur by post-transcriptional processes such as messenger ribonucleic acid (mRNA) stabilization or altered protein phosphorylation (Gaido et al., 1992; Matsumura, 1994). Nie et al. (2001) described cross-talk between Arnt-requiring pathways resulting in interactions between the AhR and the hypoxia signaling pathways. Thus, the molecular mechanisms by which many if not most of the biochemical processes discussed herein are altered by 2,3,7,8-TCDD treatment remain to be determined. Nevertheless, it is assumed, based on the cumulative evidence available, that all of these processes are mediated by the binding of 2,3,7,8-TCDD to the AhR. Although evidence has accumulated for the involvement of the AhR in many but not all of these processes, structure-activity relationships, genetic data, and reports from the use of biological models such as "knockout" mice that are lacking the AhR ($AhR^{-/-}$) are consistent with the involvement of the AhR as the initial step leading to these biochemical alterations. In fact, for every biochemical response that has been well studied, the data are consistent with the particular response being dependent on the AhR.

The dioxin-elicited induction of certain drug-metabolizing enzymes such as CYP1A1, CYP1A2, and CYP1B1 is clearly one of the most sensitive responses observed in a variety of different animal species, including humans, and it occurs at body burdens as low as 3–8 ng TCDD/kg in animals (see Part II, Chapter 8, Sections 8.3 and 8.4). These and other enzymes are responsible for the metabolism of a variety of exogenous and endogenous compounds. Several lines of experimental evidence suggest that these enzymes may be responsible for either enhancing or protecting against the toxic effects of a variety of agents, including known carcinogens as well as endogenous substrates such as hormones. These interactive effects are dependent on the compounds and the experimental system examined.

Several reports (Kadlubar et al., 1992; Esteller et al., 1997; Ambrosone et al., 1995; Kawajiri et al., 1993) provide evidence that human polymorphisms in CYPIA1 and CYPIA2 that result in higher levels of enzyme activity are associated with increased susceptibility to colorectal, endometrial, breast, and lung tumors. Also, exposure of AhR-deficient ("knockout") mice to benzo[a]pyene (BaP) results in no tumor response, suggesting a key role for the AhR—and perhaps CYPIA1 and CYPIA2—in BaP carcinogenesis (Dertinger et al., 1998; Shimizu et al., 2000). Modulation of these enzymes by dioxin may play a role in chemical carcinogenesis. However, the exact relationship between the induction of these enzymes and any toxic endpoint observed following dioxin exposure has not been clearly established.

In addition to what is known about the P450 isozymes (CYP1A1, CYP1A2, and CYP1B1), there exists some evidence from experimental animal data to indicate that the alteration of certain other biochemical events might have a more direct relationship to sensitive toxic responses observed following TCDD exposure. Some of these may be relevant to responses observed in humans, and further work in these areas is likely to lead to data that would assist in the risk characterization process. For example, changes in EGFR have been observed in tissues from dioxin-exposed animals and humans (see Part II, Chapter 3, Section 3.5, and Chapter 6, Section 6.5). EGF and its receptor possess diverse functions relevant to cell transformation and tumorigenesis, and changes in these functions may be related to a number of dioxin-induced responses, including neoplastic lesions, chloracne, and a variety of reproductive and developmental effects. Likewise, the known ability of TCDD to directly or indirectly alter the levels and/or activity of other growth factors and hormones, such as estrogen, thyroid hormone, testosterone, and gonadotropin-releasing hormone and their respective receptors as well as enzymes involved in the control of the cell cycle (Safe, 1995b), may affect growth patterns in cells/tissues, leading to adverse consequences. In fact, most of the effects that the dioxins produce at the cellular and tissue levels are due not to cell/tissue death but to altered growth patterns (Birnbaum, 1994b). Many of these alterations may occur at critical times in development and/or maturation and thus may be irreversible.

There does not yet exist a precise understanding of the relationships between the alteration of specific biochemical processes and particular toxic responses observed in either experimental animals or humans exposed to the dioxins. This is due predominantly to our incomplete understanding of the complex and coordinated molecular, biochemical, and cellular interactions that regulate tissue processes during development and under normal homeostatic conditions. A further understanding of these processes and how 2,3,7,8-TCDD may interfere

with them remains an important goal that would greatly assist in the risk characterization process. In particular, knowledge of the causal association of these responses coupled with dose-response relationships may lead to a better understanding of sensitivity to various exposure levels of the dioxin-like compounds. Nevertheless, it is important to recognize that many of the biochemical and biological changes observed are consistent with the notion that 2,3,7,8-TCDD is a powerful growth dysregulator. This hypothesis may play a considerable role in the risk characterization process by providing a focus on those processes, such as development, reproduction, immunity, and carcinogenesis, that are highly dependent on coordinated growth regulation.

2.2. ADVERSE EFFECTS IN HUMANS AND ANIMALS

2.2.1. Cancer (Cross-reference: Part II, Chapters 6, 7, and 8)

2.2.1.1. *Epidemiologic Studies*

Since the last formal EPA review in 1988 of the human database relating to the carcinogenicity of TCDD and related compounds, a number of new follow-up mortality studies have been completed. This body of information is described in Part II, Chapter 7a, Section 7.5, of this assessment, and summaries appear in an International Agency for Research on Cancer monograph (IARC, 1997), the Agency for Toxic Substances and Disease Registry (ATSDR) ToxProfile (ATSDR, 1999a), and the National Toxicology Program's report on carcinogens (NTP, 2001). Among the most important of these are the ones by Fingerhut et al. (1991a) and Steenland et al. (1999, 2001) from NIOSH of 5172 U.S. chemical manufacturing workers and the independent analyses by Aylward et al. (1996) and Salvan et al. (2001) and followup of the Dow sub-cohort by Bodner et al. (2003); a study of 2479 German workers involved in the production of phenoxy herbicides and chlorophenols by Becher et al. (1996, 1998) and by others in separate publications (Manz et al., 1991; Nagel et al., 1994; Flesch-Janys et al., 1995, 1998); a study of more than 2000 Dutch workers in two plants involved in the synthesis and formulation of phenoxy herbicides and chlorophenols (Bueno de Mesquita et al., 1993) and subsequent follow-up and expansion by Hooiveld et al., 1998); a smaller study by Zober et al. (1990) of 247 workers involved in a chemical accident cleanup and subsequent follow-up (Ott and Zober, 1996b); and an international study by Saracci et al. (1991) of more than 18,000 workers exposed to phenoxy herbicides and chlorophenols, with subsequent follow-up and expansion by Kogevinas et al. (1997). Recent reports also indicate increased cancer risks among the Seveso population (Bertazzi et al. 2001a, Warner et al. 2002).

Although uncertainty remains in interpreting these cohort results because not all potential confounders have been ruled out and coincident exposures to other carcinogens are likely (see Cole et al., 2003 for a critique), all provide support for an association between exposure to dioxin

and related compounds and increased cancer mortality. Strong inference regarding carcinogenic hazard often relies on the availability of studies with well-documented exposures. One of the strengths of these studies is that each has some exposure information that permits an assessment of dose response. Some of these data have, in fact, served as the basis for fitting the dose-response models in Part II, Chapter 8, Section 8.4.

In addition, during the development of its monograph on PCDDs/PCDFs (IARC, 1997), the IARC Working Group abstracted from the published literature data concerning the most highly exposed populations in the world. The group focused its attention on the most exposed subcohorts within cohorts with adequate latency. IARC suggests that if associations between exposure and risk are truly causal, they will become more apparent in these highly exposed subcohorts with adequate latency. Increased risk for all cancers combined and lung cancer mortality were consistent findings in the occupational cohort studies. Although the increase was generally low (20–50%), it was highest in the subcohorts with the presumed heaviest exposure. The results of the IARC Working Group's analysis regarding all cancer and lung cancer mortality in the recent studies are summarized in Table 2-3. Observed numbers of cases, standardized mortality ratios (SMR) and 95% confidence intervals (CI) are given for each of these two findings for each study.

In addition, the Working Group developed overall SMRs for the combined studies. The group state clearly that, although these total SMRs are low (1.4, 95% CI = 1.2–1.6 for all cancers and 1.4, 95% CI = 1.1–1.7 for lung cancer), these results are unlikely to be due to chance, nor can confounding by cigarette smoking likely account for the increase in lung cancer. Positive dose-response trends in the German studies and increased risk in the longer duration U.S. subcohort and the most heavily exposed Dutch workers support this view. In the opinion of these experts, increases of this magnitude in all cancers combined have rarely been found in occupational cohorts. These results are also supported by significantly increased mortality from lung and liver cancers subsequent to the Japanese rice oil poisoning accident where exposure to high levels of PCDFs and PCBs occurred (Kuratsune et al., 1988; Kuratsune, 1989).

Although smoking as a confounder cannot be totally eliminated as a potential explanation of the occupational studies results, analyses conducted to date (Fingerhut et al., 1991b; Ott and Zober, 1996b) suggest that smoking is not likely to explain the entire increase in lung cancer and may even suggest synergism between occupational exposure to dioxin and smoking. These analyses have not been deemed entirely satisfactory by some reviewers of the literature. The question of confounding exposures such as to asbestos and other chemicals in addition to smoking has not been entirely ruled out and must be considered as potentially adding to the observed increases. Although increases of cancer at other sites (e.g., non-Hodgkin's lymphoma,

soft tissue sarcoma, gastrointestinal cancer) have been reported (see Part II, Chapter 7a, Section 7.5), the data for an association with exposure to dioxin-like chemicals are less compelling due to the limited numbers of observed tumors at any specific site.

As discussed by IARC (McGregor et al., 1998) and Smith and Lopipero (2001), it is unusual for a cancer hazard characterization to focus on the "all cancers combined" category of epidemiological results, and continuing uncertainties regarding site-specific cancer increases following dioxin exposure remain a factor in concluding that the epidemiological information is limited. McGregor et al. (1998) note, however, that the predominant cancer promotion mechanism of action for dioxin will theoretically elicit pre-existing initiated cell lines. These promotional effects would be expected in multiple tissues, especially those most sensitive to the effects of dioxin. In epidemiological studies, there may not be a statistically increased tumor site(s), but rather a pattern of smaller increases that could vary across study populations because of differences in life histories, exposures, and pre-existing initiating events.

The cancer-promotion mechanism may also serve to accentuate existing tumor rate increases following other carcinogenic exposures, thereby acting in a synergistic manner. Timing of tumor induction may differ between a cancer promoter and initiator, where the effects of a promoter may not be monotonic with time, but rather may exhibit an earlier onset, harvesting effect, where the total cancer burden may not have changed but the onset has been accelerated. These timing issues are exacerbated by the pharmacokinetics of dioxin elimination, where initial peak body burdens during employment or after accidental exposures decline gradually after cessation of exposure.

Mathematically, a net carcinogenic effect in one or more organ sites must, by definition, increase the "all cancers combined" risk for the exposed population if the exposed and control groups are matched (i.e., they have the same background cancer rate absent the exposure). Thus, an increase in the all cancers category should be considered an expected result of a carcinogen exposure, not an unusual event. The statistical power of a study to detect such an effect is, however, the limiting factor in the presence of stochastic events and imperfect matching. This constraint is particularly applicable to rare tumor sites, but it also occurs for common tumor sites such as lung, colon, breast (♀), and prostate (♂) or for mechanistically linked sites (e.g., hormonally related breast, ovary, uterus), where substantial increases in site-specific relative risks are necessary to impact the all cancer category.

Ionizing radiation (a mutagenic carcinogen) provides an example where small increased relative risks at multiple sites lead to a significantly increased relative risk for "all nonleukemic cancers." In atomic bomb survivors, the relative risk for all nonleukemic cancers at 100 rads was 1.17 ($p<0.01$), comprised principally of small but statistically significant increases in stomach

(relative risk [RR] = 1.11), lung (1.33), breast (1.69), ovary (1.52), and bladder-kidney (1.55) cancers and nonstatistically significant increases in esophagus (1.23), liver (1.35), ovary (1.52), and multiple myeloma (1.51). Although the relative risk for leukemia was 3.95 ($p<0.01$), the excess cancer burden from nonleukemic sites in the exposed population was over twice that due to the leukemias (Hoel, 1987).

Some studies that are discussed in Part II, Chapter 7a, report small or no increased risk of cancer from exposure to 2,3,7,8-TCDD or its congeners. These studies generally suffer from one or more deficiencies that limit their ability to determine the carcinogenic hazard of dioxins. These deficiencies fall into the following categories: little statistical power to detect an effect of exposure because the measured exposures are lower than those seen in the studies cited above and are more similar to those of the comparison population; no measurements of internal exposure to 2,3,7,8-TCDD and potential for misclassification of exposure; and inadequate latency or follow-up.

The Ranch Hand study of U.S. Air Force personnel who sprayed the defoliant Agent Orange during the Vietnam War provides an illustrative example of statistical power constraints in the presence of low predicted relative risks. Statistical power is the ability of a study to detect a real difference between two groups at pre-defined levels of statistical significance (usually $p \leq 0.05$) and relative risk. Statistical power analysis based on the detailed dosimetry and health status data available for this cohort indicates insufficient statistical power to detect an elevated all-cancers risk at levels consistent with the occupational dose-response data. A predicted relative risk for all cancers combined can be estimated for the Ranch Hands by calculating the difference between their dose and that of the control group (mean background of 4.25 ppt TCDD in lipid) (Michalek et al., 1998) and then multiplying this dose increment by an estimated cancer risk slope factor for TCDD. The median AUC increment value for the overall Ranch Hand group is 468 ng TCDD/kg lipid * years, and for the high dioxin group the median is 2280 ng TCDD/kg lipid * years. Using the Becher et al. (1998) linear formula (RR = 1 + 0.000016 x AUC ng-TCDD/kg lipid * years, which equals ~ 3×10^{-3} risk/pg/kg/day) described in Section 5.3 and Table 5-2 of this document, the estimated all-cancers relative risk for the overall Ranch Hand cohort is approximately 1.01, and for the high-exposure group it is 1.04 as compared to the control population. Using formulae in Fleiss (1981) and Cohen (1977) and assuming two-sided testing at a significance level of 5%, the study has no power to detect 1 to 4% increases in relative risk. Data on the overall prevalence of cancer in the comparison group (18.9%) and sample sizes (all Ranch Hand 845 vs. 1224 controls; high category 241 vs. 1200 controls) used in the above analysis were obtained from the 1997 Ranch Hand morbidity report (http://www.brooks.af.mil/AFRL/HED/hedb/afhs/.html).

Recent suggestive cancer findings from the Ranch Hand database are consistent with these calculations, both in the magnitude of the risk ratios under review and in the constraints on statistical methods to detect such levels of incremental risk. Akhtar et al. (2003) provide results that suggest exposure to dioxin-contaminated herbicides may be associated with cancer, based on a statistically significant positive trend in "any site" cancer relative risk with exposure group, accompanied by a non-significant increase in the any site cancer standardized incidence ratio of 1.09 (Obs. 134, Exp 123.34, p=0.34).

In addition, one of the earliest reported associations between exposure to dioxin-like compounds in dioxin-contaminated phenoxy herbicides and increased cancer risk involved an increase in soft tissue sarcomas (Hardell and Sandstrom, 1979; Eriksson et al., 1981; Hardell and Eriksson, 1988; Eriksson et al., 1990). In this and in other recent evaluations of the epidemiologic database, many of the earlier epidemiological studies that suggested an association between dioxin exposure and soft tissue sarcoma have been criticized for a variety of reasons. Arguments regarding selection bias, lack of exposure or differential exposure misclassification, confounding, and chance in each individual study, which increases uncertainty around this association, have been presented in the scientific literature. Nonetheless, the incidence of soft tissue sarcoma is elevated, although not statistically so, in several of the most recent studies (Bertazzi et al., 1993, 1997, 1999; Fingerhut et al., 1991a; Hertzman et al., 1997; Kogevinas et al., 1997; Lampi et al., 1992; Lynge, 1998; Pesatori et al., 1999; Saracci et al., 1999; Vineis et al., 1986). It is probable that soft tissue sarcomas are not unlike other site-specific cancers whose risks from exposure to TCDD are difficult to define because of small numbers and lack of measures of internal exposure.

The accidental exposure of the population at Seveso, Italy, serves as an example of a more highly exposed group where, in previous assessments, latency was considered to be inadequate. Although Bertazzi and coworkers published results of cancer mortality after 10 and 15 years of latency, results are suggestive but not definitive regarding an association between exposure to TCDD and cancer deaths. Results of the analysis of 20 years of follow-up have recently been published (Bertazzi et al., 2001). This more recent follow-up of the same group of residents in zones A and B was completed after 20.5 years to December 31, 1996. The authors stated that their results support the evaluation of TCDD as a human carcinogen, especially with the increased estimates of relative risk for all cancer mortality and for several specific sites of cancer in the >15 year latency period. No soft tissue sarcomas were observed in zones A and B. However, less than one case would have been expected to occur by the end of the follow-up. In Zone A, where exposure was highest, the expectation of a soft tissue sarcoma was only 0.1. There was little power to detect a significant risk in that region.

In a commentary by Smith and Lopipero (2001) on this study, two "key" problems were identified. The "likely" exposure levels back-calculated to the time when the exposures occurred indicate that the weighted average for the two highest exposure zones in Seveso is only 136 ng/kg TCDD (lipid adjusted) versus a mean of 3600 ng/kg TCDD (lipid adjusted) in the combined U.S. industrial cohorts. This interpretation is consistent with the data in Figure 5-1 of this document. On this basis, one would not expect to find significant increases in all cancers combined based on extra risk estimates from the occupational cohorts. This situation is not unlike the one described above for the Ranch Hand cohort. However, in this case, associations with exposure to TCDD and cancer risk are being reported.

The other issue raised by these authors is the potential for smoking-related causes of disease to be confounders in this study. The relatively low dioxin exposure and the increase in major smoking-related causes of death raise questions regarding the attribution of these cancer effects to TCDD exposure. Other data are consistent with potential dioxin hazard in this exposed population, for example, the finding of increased diabetes mortality among women. Bertazzi (2001b) takes exception to these interpretations and argues against the perception of "low" exposure and smoking as a confounder. It is clear that the question of whether the Bertazzi (2001a) study contributes to the weight of evidence for carcinogenicity awaits further follow-up and improved exposure assessment.

In general, both past and more recent human studies have focused on males. Although males comprise all the case-control studies and the bulk of the cohort study analyses, animal and mechanism studies suggest that males and females might respond differently to TCDD. There are now, however, some limited data suggesting carcinogenic responses associated with dioxin exposure in females. The only report of a female cohort that had good TCDD exposure surrogate information was that of Manz et al. (1991), which found a borderline statistically significant increase in breast cancer. Although Saracci et al. (1991) did report reduced female breast and genital organ cancer mortality, the finding was based on few observed deaths and on chlorophenoxy herbicide rather than TCDD exposures. In the later update and expansion of this cohort, Kogevinas et al. (1997) provided evidence of a reversal of this deficit and reported a borderline significant excess risk of breast cancer in females.

Bertazzi et al. (1993, 1997, 1998) reported nonsignificant decreases in breast cancer and endometrial cancer in women living in geographical areas around Seveso that were contaminated by dioxin. Breast cancer rates in women who had been exposed as infants at the time of the Seveso explosion were increased. On the basis of 15 (1.5%) confirmed breast cancer cases in the Seveso Women's Health Study, a Cox proportional hazard ratio for breast cancer of 2.1 fold (95% CI 1.0 - 4.6) was reported for a ten-fold increase in serum TCDD levels (Warner et al.,

2002). Although Kogevinas et al. (1993) saw an increase in cancer incidence among female workers most likely exposed to TCDD, no increase in breast cancer was observed in their small cohort. In short, TCDD cancer experience for women may differ from that of men, but currently there are few data to adequately address this question.

Both laboratory animal data and mechanistic inferences suggest that males and females may respond differently to the carcinogenic effects of dioxin-like chemicals. Further data will be needed to address this question of differential response between sexes, especially to hormonally mediated tumors. In addition, studies by Brown et al. (1998) demonstrated that prenatal exposure of rats to 2,3,7,8-TCDD enhances their sensitivity as adults to chemical carcinogenesis. A mechanistic understanding of the impact of gestational dioxin exposure on mammary tissue development has been provided by the work of Fenton and coworkers (Fenton et al., 2002; Vorderstrasse et al., 2004). The experimental data in laboratory animals suggest that exposure to women or perinatal exposures may result in carcinogenic responses. The epidemiological data examining the association between exposure of adult women to dioxin and cancer is limited. No epidemiological data are available to address the question of the potential impact of exposure to dioxin-like compounds on childhood cancers or the effects of perinatal exposures on the development of cancers later in life. The epidemiological data to date have not adequately addressed these issues.

In summary, 2,3,7,8-TCDD and, by inference from more limited data, other dioxin-like compounds are described as potentially multisite carcinogens in the more highly exposed human populations—consisting primarily of adult males that have been studied. Although the epidemiologic data by themselves are not sufficient to infer a causal association between exposure to TCDD and other dioxin-like chemicals and increased cancer in humans (IARC, 1997; ATSDR, 1999a; DHHS, 2001), this "limited" epidemiologic database has been strengthened by emerging data that reflect further follow-up and better exposure metrics. Although uncertainty remains, the cancer findings in the epidemiologic literature are generally consistent with results from studies of multiple laboratory animal species, where dioxin-like compounds have clearly been identified as multisite carcinogens and tumor promoters.

2,3,7,8-TCDD has also been demonstrated to promote dose-dependent clonal expansion and neoplastic transformation in human epidermal keratinocytes immortalized by simian adenovirus SV40 exposure, leading to fixed alterations in regulatory gene expression (Yang et al., 1999) and squamous cell carcinoma when inoculated into athymic nude mice (Yang et al., 1992). These phenomena did not occur in the absence of SV40 virus induction or in control cell lines, including the immortalized cell culture.

Thus, the findings of increased risk at multiple sites in occupationally exposed humans appear to be plausible, given what is known about mechanisms of dioxin action and the fundamental level at which this class of compounds appears to act on gene expression and cellular regulation in target tissues. Although several studies found a positive trend in dose-response and have been the subject of empirical risk modeling (see Part II, Chapter 8, and Becher et al., 1998, and Steenland et al., 2001), the epidemiologic data alone provide little insight into the shape of the dose-response curve below the range of observation in these occupationally exposed populations. However, Mackie et al. (2003) suggest that there is no evidence of a dioxin cancer threshold from the epidemiology data. Steenland and Deddens (2003) also reported that the results of quantitative exposure-response analyses for low environmental levels based on the NIOSH cohort are consistent with the results from the Becher cohort and demonstrate that a doubling of background levels of exposure will increase lifetime risk of cancer death between 0.1 and 1%. The issue of the shape of the dose-response curve in occupational cohorts is further discussed in Section 5.2.1 of this document.

2.2.1.2. *Animal Carcinogenicity (Cross-reference, Part II: Chapters 6 and 8)*

An extensive database on the carcinogenicity of dioxin and related compounds in laboratory studies exists and is described in detail in Part II, Chapter 6. There is adequate evidence that 2,3,7,8-TCDD is a carcinogen in laboratory animals, based on long-term bioassays conducted in both sexes of several strains of rats and mice, hamsters, and fish (U.S. EPA, 1985; Huff et al., 1991; Zeise et al., 1990; IARC, 1997; DHHS, 2001). All the studies produced positive results, leading to conclusions that TCDD is a multi-site carcinogen that increases the incidence of tumors at sites distant from the site of treatment and at doses well below the maximum tolerated dose. Since this issue was last reviewed by the Agency, in 1988, TCDD has been shown to be a carcinogen in hamsters (Rao et al., 1988), which are relatively resistant to the lethal effects of TCDD. Other preliminary data have also shown TCDD to be a liver carcinogen in the small fish *Medaka* (Johnson et al., 1992).

In the past, limited attempts had been made to demonstrate the carcinogenicity of other dioxin-like compounds. A mixture of two isomers of hexachlorodibenzo-*p*-dioxin (HCDDs) produced liver tumors in both sexes of rats and mice when given by the gavage route (NTP, 1980), but not by the dermal route in Swiss mice (NTP, 1982a,b). Reports from Rozman (1999, 2000) and Rozman et al. (2000) demonstrated lung cancer in female rats given gavage exposures of 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin(HpCDD).

Recently, the National Toxicology Program (NTP, 2003 a-d) has conducted chronic bioassays to test the relative carcinogenic potency of four dioxin-like congeners (TCDD,

2,3,4,7,8-PeCDD, PCB 118, and PCB 126), both alone and in combination. In these studies, TCDD, PCB 126 and 2,3,4,7,8-PeCDF, were tested individually or in an equally potent mixture of all three chemicals in a 2-year bioassay in female Sprague-Dawley rats. The NTP study also included PCB 118, but the results and interpretation of this bioassay remain under review due to substantial contamination by PCB 126. Initial reports from the NTP study indicate that there is clear evidence of carcinogenicity for both TCDD and PCB 126. In these studies, both TCDD and PCB 126 exposures increases the incidence of cholangiocarcinoma of the liver, cystic keratinizing epithelioma of the lung, and gingival squamous cell carcinoma of the oral mucosa. Under the conditions of the 2-year study, there was some evidence of carcinogenic activity for the 2,3,4,7,8-PeCDF based on increased incidences of cholangiocarcinoma of the liver, cystic keratinizing epithelioma of the lung and gingival squamous cell carcinoma of the oral mucosa. The results from the mixture study also indicate clear evidence of carcinogenicity as evidenced by dose dependent increases in cholangiocarcinomas in the liver and cystic keratinizing epitheliomas of the lung. The data on the three individual chemicals and mixtures demonstrate consistent increases in the incidence of three tumor types. This evidence provides support that the carcinogenicity of dioxin-like chemicals is mediated through their interactions with the Ah receptor and that the TEF methodology may provide a useful tool in estimating the potential carcinogenic risks of dioxin-like chemicals.

TCDD is characterized as a nongenotoxic carcinogen because it is negative in most assays for DNA-damaging potential and is a potent "promoter" and a weak initiator or noninitiator in two-stage initiation-promotion (I-P) models for liver, skin, and lung. The liver response is characterized by increases in altered hepatocellular foci (AHF), which are considered to be preneoplastic lesions because increases in AHFs are associated with liver cancer in rodents. The results of the multiple I-P studies enumerated in Table 6-5 and in Part II, Chapter 6, Section 6.3, have been interpreted as showing that induction of AHFs by TCDD is dose-dependent (Maronpot et al., 1993; Teeguarden et al., 1999), exposure-duration dependent (Dragan et al., 1992; Teeguarden et al., 1999; Walker et al., 2000), and partially reversible after cessation of treatment (Dragan et al., 1992; Tritscher et al., 1995; Walker et al., 2000).

Other studies indicate that other dioxin-like compounds have the ability to induce AHFs. These studies showed that the compounds demonstrate a rank-order of potency for AHF induction that is similar to that for CYP1A1 (Flodstrom and Ahlborg, 1992; Waern et al., 1991; Schrenk et al., 1994). Non-ortho-substituted, dioxin-like PCBs have also induced the development of AHFs according to their potency to induce CYP1A1 (Hemming et al., 1995; van der Plas et al., 1999). It is interesting to note that liver I-P studies carried out in ovariectomized rats demonstrated the influence that the intact hormonal system has on AHF development. AHF

were significantly reduced in the livers of ovariectomized female rats (Graham et al., 1988; Lucier et al., 1991).

I-P studies on skin have demonstrated that TCDD is a potent tumor promoter in mouse skin as well as rat liver. Early studies demonstrated that TCDD is at least two orders of magnitude more potent than the "classic" promoter tetradecanoyl phorbol acetate (Poland et al., 1982), that TCDD skin tumor promotion is AhR dependent (Poland and Knutsen, 1982), that TCDD had weak or no initiating activity in the skin system (DiGiovanni et al., 1977), and that TCDD's induction of drug-metabolizing enzymes is associated with both metabolic activation and deactivation of initiating agents, as described by Lucier et al. (1979). More recent studies show that the skin tumor-promoting potencies of several dioxin-like compounds reflect relative AhR binding and pharmacokinetic parameters (Hebert et al., 1990).

Although few I-P studies have demonstrated lung tumors in rats or mice, the study by Clark et al. (1991) is particularly significant because of its use of ovariectomized animals. In contrast to liver tumor promotion, lung tumors were seen only in initiated (diethylnitrosamine [DEN]), TCDD-treated rats. No tumors were seen in DEN-only, TCDD-only, control, or DEN/TCDD intact rats. Liver tumors are ovary dependent, but ovaries appear to protect against TCDD-mediated tumor promotion in female rat lung. Perhaps the use of transgenic animal models will allow further understanding of the complex interaction of factors associated with carcinogenesis in rodents and, by extension, in humans. Several such systems are being evaluated (Eastin et al., 1998; van Birgelen et al., 1999; Dunson et al., 2000).

The tumor-promoting ability of a number of dioxin-like chemicals has been examined. As discussed in Part II, Chapter 6, Section 6, 1,2,3,7,8-PCDD; 1,2,3,4,6,7,8-HpCDD; 2,3,4,7,8-PCDF; 1,2,3,4,7,8-HCDF; PCB126; and PCB105 all promote the development of AHF within rodent liver, suggesting that they, like TCDD, are tumor promoters. (For a summary of positive tumor-promotion studies for PCDDs and PCDFs in rats, see Part II, Chapter 6, Table 6-5). In addition, complex mixtures of dioxins and furans and commercial PCB mixtures act as promoters of liver AHF. For the five principle dioxins, furans, and coplanar PCBs that comprise approximately 80% of the current, total dioxin/furan/PCB TEQ in human blood, all are positive in either rodent bioassays or rodent liver tumor-promotion studies or mouse skin tumor-promotion studies. Although the majority of dioxin-like congeners have not been tested for carcinogenicity in chronic rodent bioassays, these data suggest that it is likely that those individual congeners and mixtures of dioxin-like compounds that comprise the majority of the dioxin-like activity in human tissues are likely to be carcinogenic to rodents.

van den Berg et al. (2000) present a summary of the data (their Table 1) relied on by WHO's European Centre for Environment and Health (WHO-ECEH) and IPCS in their joint

consensus re-evaluation of the TEFs for PCDDs, PCDFs, and dioxin-like PCBs for mammals. These TEFs were derived using a tiered approach in which in vivo toxicity data were given more weight than in vitro data, toxicity more than biochemical endpoints, and chronic more than acute data. Table 2-4 summarizes the tumor incidence and promotion data that were cited in the development of these $TEFs_{DFP}$-WHO_{98}. The data presented are for those congeners that are principal contributors to the background body burden of dioxin TEQs in the United States (see Part I, Chapter 3). For 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF, the TEF was used to adjust the dose from the studies by Waern et al. (1991), and for PCB 126 similar dose adjustments are included from Hemming et al. (1995; their Fig. 4). For the comparison of TCDD to the HxCDDs, the primary TCDD data points from the Kociba et al. (1978) bioassay were graphed for both the original tumor count data and for the revised tumor counts from Goodman and Sauer (1992). This presentation of both the original and the revised tumor counts for TCDD reflects the contemporaneous performance and analysis of the HxCDD and TCDD bioassays and pathology and the recognition that the HxCDD pathology has not been re-analyzed.

Table 2-3 illustrates the comparability of the TCDD and other congener data sets based on TEFs. This analysis also demonstrates that the development of the TEFs for all of the congeners that contribute substantially to the background dioxin TEQ appropriately reflect either cancer bioassay or tumor promotion data. Furthermore, when one considers the impact of current TEF values on compounds that made up the majority of the TEQ prior to 1990, it is clear that more than 80% of the TEQ for either dioxins/furans or PCBs was made up of compounds for which the current TEF is supported by data on relative potencies which included tumor promotion or carcinogenic endpoints. This point is illustrated in Part II, Chapter 6, Table 6-10.

2.2.1.3. *Plausible Mode(s) of Carcinogenic Action*

Several potential mechanisms for TCDD carcinogenicity are discussed above and in Part II, Chapter 6, Section 6.4. These include oxidative stress, indirect DNA damage, endocrine disruption/growth dysregulation/altered signal transduction, and cell replication/apoptosis leading to tumor promotion. All of these mechanisms are biologically plausible as contributors to the carcinogenic process in humans, and none are mutually exclusive. Several biologically based models that encompass many of these activities are described in Part II, Chapter 8, Section 8.4. Further work is needed to elucidate a detailed mechanistic model for any particular carcinogenic response in animals or in humans; however, plausible modes of action with probable relevance to human carcinogenicity are discussed below.

TCDD is a potent tumor promoter in rat and mouse liver and in initiated human skin cells. In general terms, it is believed that cancer is likely due to the clonal expansion of damaged

cells that have a heritable genetic defect. Increased growth and accumulation of damage in critical genes ultimately aid in the progression into tumors. Consequently, promotion of carcinogenesis by TCDD may occur at several steps: (1) increased formation of initiated/susceptible cells through DNA mutation and/or increase rate of fixation of damaged DNA into the genome, (2) reduced loss of initiated cells through a suppression of apoptosis, (3) increase in growth rate and clonal expansion of initiated cells, and (4) accumulation of DNA damage in critical genes resulting in the progression of clonally expanded cell populations into tumors. Within this framework, it is hypothesized that TCDD may be acting as a tumor promoter through multiple mechanisms. Primarily, the activation of the AhR leads to alteration in genes that are involved in normal cell growth and differentiation pathways.

TCDD may contribute to the formation and accumulation of DNA damage via an indirect mechanism involving the production of reactive oxygen species. These reactive oxygen species may be formed as a result of autooxidation during futile metabolism of TCDD by the induction of CYP1 enzymes or via the CYP1-dependent production of estrogen metabolites capable of redox cycling. The clonal expansion of these damaged cells by TCDD and related chemicals is likely to occur through the altered expression and activity of a number of genes that regulate the cell-cycle. Activation of the AhR by TCDD results in altered expression or activity of the EGF receptor, retinoblastoma protein, TGF-beta, and many others. These proteins all regulate the cell cycle, and alterations of these proteins would alter cell growth properties.

The contribution of these two pathways in the carcinogenic actions of TCDD remains uncertain. However, Portier et al. (1996) have proposed a model in which the contribution of TCDD to the number of DNA damaged or initiated cells plays a significant role in its carcinogenic response. In contrast, Conolly and Andersen (1997) have proposed a tumor promotion model based on a negative selection mechanism in which the actions of TCDD are focused on its ability to alter cell growth properties. Descriptions of these models are provided in Part II, Chapter 8. Interestingly, the use of the model by Portier and colleagues leads to a result that is consistent with low-dose linearity, whereas the Andersen and Conolly model predicts highly nonlinear dose response relationships in the low-dose region. Presently, the available data do not allow for adequate discrimination between these two models.

TCDD causes a dose-related increase in thyroid follicular cell adenomas and carcinomas in rats and mice. One hypothesis for the induction of thyroid tumors involves the disruption of thyroid hormone homeostasis via the induction of the phase II enzymes UDP-glucuronosyltransferases (UGTs) (Hurley, 1998; Hill et al., 1998). Dioxin-like compounds induce the synthesis of UDP-glucuronosyltransferase-1 (UGT1) mRNA by an AhR-dependent transcriptional mechanism (Bock et al., 1998; Nebert et al., 1990). It is proposed that dioxin-like

chemicals increase the incidence of thyroid tumors through an extrathyroidal mechanism. Dioxin-like chemicals induce hepatic UGT, resulting in increased conjugation and elimination of thyroxine (T4) and leading to reduced serum T4 concentrations. T4 production is controlled by thyroid stimulating hormone (TSH), which is under negative and positive regulation from the hypothalamus, pituitary, and thyroid by thyrotrophin releasing hormone (TRH), TSH itself, thyroxine (T4), and triiodothyronine (T3). Consequently, the reduced serum T4 concentrations would lead to a decrease in the negative feedback inhibition on the pituitary gland. This would then lead to a rise in secreted TSH and stimulation of the thyroid. The persistent induction of UGT by dioxins and subsequent prolonged stimulation of the thyroid would result in thyroid follicular cell hyperplasia and hypertrophy of the thyroid, thereby increasing the risk of progression to neoplasia.

In support of this hypothesis, Kohn et al. (1996) modeled the effect of 2,3,7,8-TCDD on UGTs and thyroid hormones in female rats within the framework of a PBPK model. This mathematical model described release and uptake of thyroid hormones, metabolism, 2,3,7,8-TCDD induction of UGT1, regulation of TSH release from the pituitary by T4, and feedback on TRH and somatostatin, which inhibits TSH release. The model successfully reproduced the observed effects of 2,3,7,8-TCDD on serum T3, T4, and TSH and UGT1 mRNA and enzyme activity, suggesting that this is a plausible mechanism for an indirect role of 2,3,7,8-TCDD on the thyroid. This model is supported by the more recent experimental work of Schuur et al. (1997), which demonstrated the extrathyroidal effects of 2,3,7,8-TCDD on thyroid hormone turnover.

Although this discussion illustrates that there is no defined molecular mechanism leading to cancer in either liver or thyroid, it does demonstrate the concept of "mode of action" as defined in the Agency's proposed cancer guidelines (U.S. EPA, 1996, 1999, 2003). In each case, critical "key events" that correlate with carcinogenicity can be identified and measured, and these same events occur in both animals and humans. Although these relationships and linkages remain to be detailed, they form plausible, testable hypotheses whose acceptance by the scientific community is growing.

Despite this lack of a defined mechanism at the molecular level, there is a consensus that 2,3,7,8-TCDD and related compounds are receptor-mediated carcinogens in that (1) interaction with the AhR is a necessary early event; (2) 2,3,7,8-TCDD modifies a number of receptor and hormone systems involved in cell growth and differentiation, such as the EGFR and estrogen receptor; and (3) sex hormones exert a profound influence on the carcinogenic action of 2,3,7,8-TCDD.

2.2.1.4. *Other Data Related to Carcinogenesis*

Despite the relatively large number of bioassays on 2,3,7,8-TCDD, those by Kociba et al. (1978) and NTP (1982a), because of their multiple dose groups and wide dose range, continue to be the focus of dose-response modeling efforts and of additional review. Goodman and Sauer (1992) reported a re-evaluation of the female rat liver tumors in the Kociba study using the latest pathology criteria for such lesions. The review confirmed only approximately one-third of the tumors of the previous review (Squire, 1980). Although this finding did not change the determination of carcinogenic hazard—as 2,3,7,8-TCDD induced tumors in multiple sites in this study—it did have an effect on evaluation of dose-response and on estimates of risk at low doses. These issues are discussed in a later section of this document.

One of the more intriguing findings in the Kociba bioassay was reduced tumor incidences of the pituitary, uterus, mammary gland, pancreas, and adrenals in exposed female rats as compared to controls. Although this finding, coupled with evaluation of epidemiologic data, has led some authors to conclude that dioxin possesses "anticarcinogenic" activity (Kayajanian, 1997, 1999), it should be noted that in the Kociba study, the decreased incidence of tumors, with the exception of mammary gland tumors, is associated with significant weight loss in these rats. Examination of the data from NTP also demonstrates a significant decrease in these tumor types when there is a concomitant weight loss in the rodents, regardless of the chemical administered (Haseman and Johnson, 1996). It is also worth noting that the decrease in mammary tumors was only observed in one of seventeen rodent carcinogenesis studies, and was not observed in the recent NTP studies on TCDD, PCB 126, and 2,3,4,7,8-PeCDF (NTP, 2003 a-d).

As discussed in Section 3.2.3, under certain circumstances exposure to 2,3,7,8-TCDD may elicit beneficial effects. For example, 2,3,7,8-TCDD protects against the subsequent carcinogenic effects of polycyclic aromatic hydrocarbons (PAHs) in mouse skin, possibly reflecting induction of detoxifying enzymes (Cohen et al., 1979; DiGiovanni et al., 1980). In other situations, 2,3,7,8-TCDD-induced changes in estrogen metabolism may alter the growth of hormone-dependent tumor cells, producing a potential anticarcinogenic effect (Spink et al., 1990; Gierthy et al., 1993). While TCDD has been shown to inhibit the growth of certain breast cancer cell lines, Warner et al. (2002) have demonstrated an increase in breast cancer in highly exposed women from Seveso. Because the mechanism of the decreases in the tumor cells is unknown, extrapolation of these effects to humans is premature.

In considering overall risk, one must take into account factors such as the range of doses to target organs and hormonal state to obtain a complete picture of hazard and risk. Although exposure to dioxins may influence cancer response directly or indirectly and positively or negatively, it is unlikely that such data will be available to argue that dioxin exposure provides a

net benefit to human health. It is also important to note that the doses at which the incidence of certain tumors may decrease is in the same range at which adverse noncancer effects occur (see Appendix A).

2.2.1.5. *Cancer Hazard Characterization*

TCDD, CDDs, CDFs, and dioxin-like PCBs are a class of well-studied compounds whose human cancer potential is supported by a large database, including "limited" epidemiological support, unequivocal animal carcinogenesis, and biologic plausibility based on mode of action data. In 1985, EPA classified 2,3,7,8-TCDD and related compounds as "probable" human carcinogens, based on the available data. During the intervening years, the database relating to the carcinogenicity of dioxin and related compounds has grown and strengthened considerably. In addition, EPA guidance for carcinogen risk assessment has evolved (U.S. EPA, 1996, 1999, 2003). Under EPA's current approach, complex mixtures of dioxin and related compounds are considered "likely to be carcinogenic to humans," as are individual dioxin-like congeners other than TCDD. This descriptor is based primarily on the concept of toxic equivalency but also on the data available to support this characterization for individual congeners. Positive lifetime bioassays are available for a number of the principal congeners contributing to human TEQ body burden, specifically TCDD, 2,3,4,7,8-PeCDF, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, and PCB 126 (Kociba et al., 1978; NTP, 1980; NTP, 2003 a-d).

2,3,7,8-TCDD is best characterized as "carcinogenic to humans." This means that, based on the weight of all of the evidence (human, animal, mode of action), 2,3,7,8-TCDD meets the stringent criteria that allows EPA and the scientific community to accept a causal relationship between exposure and cancer hazard. The guidance (see EPA, 2003, section 2.6) suggests that "carcinogenic to humans" is an appropriate descriptor of carcinogenic potential when there is an absence of conclusive epidemiologic evidence to clearly establish a cause-and-effect relationship between human exposure and cancer but there is compelling carcinogenicity data in animals and mechanistic information in animals and humans demonstrating similar modes of carcinogenic action.

The "carcinogenic to humans" descriptor is suggested for 2,3,7,8-TCDD because *all* of the following conditions are met:

- Occupational epidemiologic studies all show an association between 2,3,7,8-TCDD exposure and increases in the all-cancers-combined category, in lung cancer, and perhaps in cancers at other sites, but the data are insufficient on their own to demonstrate a causal association.

- There is extensive carcinogenicity in both sexes of multiple species of animals at multiple sites.

- There is general agreement that the mode of 2,3,7,8-TCDD's carcinogenicity is AhR dependent and proceeds through modification of the action of a number of receptor and hormone systems involved in cell growth and differentiation, such as the EGFR and estrogen receptors.

- The human AhR and the rodent AhR are similar in structure and function and, once transformed, both bind to the same DNA response elements, designated DRE's.

- Human and rodent tissue and organ cultures respond to TCDD and related chemicals in a similar manner and at similar concentrations.

Other dioxin-like compounds are characterized as "likely to be carcinogenic to humans," primarily because of the lack of epidemiological evidence associated with their carcinogenicity, although there is a strong inference based on toxic equivalency that they would behave in humans as 2,3,7,8-TCDD does. Each of the congeners that contributes substantially to human body burden has been evaluated in vivo in cancer bioassays or tumor promotion assays. Each has a large database demonstrating AhR-mediated dioxin-like activities. Each has physico-chemical properties that contribute to their persistence. For each congener, the degree of certainty of carcinogenic hazard is dependent on the available congener-specific data and its consistency with the generalized mode of action that underpins toxic equivalency for 2,3,7,8-TCDD and related compounds. For the congeners most frequently encountered in human blood, milk, and adipose tissue, the database in support of 2,3,7,8-TCDD-like carcinogenic hazard is strong; those with weaker data supporting 2,3,7,8-TCDD-like carcinogenicity contribute relatively little to total TEQ.

On the basis of this logic, all complex environmental mixtures of 2,3,7,8-TCDD and dioxin-like compounds would be characterized as "likely" carcinogens, but the degree of certainty of the cancer hazard would be dependent on the major constituents of the mixture. For instance, the hazard potential, although still considered "likely," would be characterized differently for a mixture whose TEQ was dominated by octachlorodibenzo-*p*-dioxin as compared to one dominated by other PCDDs.

2.2.2. Reproductive and Developmental Effects

Several sections of this reassessment (Part II, Chapter 5 and Chapter 7b) have focused on the variety of effects that dioxin and dioxin-like agents can have on human reproductive health and development. The emphasis in each of these chapters has been on the discussion of the more recent reports of the impact of dioxin-like compounds on reproduction and development. These reports have been put into context with previous reviews of the literature applicable in risk assessment (Hatch, 1984; Sweeney, 1994; Kimmel, 1988) to develop a profile of the potential for dioxin and dioxin-like agents to cause reproductive or developmental toxicity, based on the available literature. An earlier version of the literature review and discussion contained in Part II, Chapter 5, has been previously published (Peterson et al., 1993).

The origin of concerns regarding a potential link between exposure to chlorinated dioxins and adverse developmental events can be traced to early animal studies reporting increased incidence of developmental abnormalities in rats and mice exposed early in gestation to 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Courtney and Moore, 1971). 2,4,5-T is a herbicide that contains dioxin and related compounds as impurities. Its use was banned in the late 1970s, but exposure to human populations continued as a result of past production, use, and disposal.

2.2.2.1. *Human Effects*

The literature base with regard to potential human effects is detailed in Part II, Chapter 7b, Section 7.13. In general, there is limited epidemiological evidence to make a direct association between exposure to TCDD or other dioxin-like compounds and effects on human reproduction or development. One effect that may illustrate this relationship is the altered sex ratio (increased females) seen in the 6 years after the Seveso, Italy, accident (Mocarelli et al., 1996, 2000), and in a heavily exposed occupational cohort in Russia (Ryan et al., 2002). Particularly intriguing in these evaluations is the observation that exposure before and during puberty is linked to this sex ratio effect, and predominantly through the paternal side. Other sites have been examined for the effect of TCDD exposure on sex ratio with mixed results but with smaller numbers of offspring. Data on these sites are still preliminary, but effects similar to the Seveso findings are being reported. Continued evaluation of the Seveso population may provide other indications of impacts on reproduction and development but, for now, such data are limited and further research is needed.

Positive human data on developmental effects of dioxin-like compounds are limited to a few studies of populations exposed to a complex mixture of potentially toxic compounds (e.g., developmental studies from the Netherlands and effects of ingestion of contaminated rice oil in Japan [Yusho] and Taiwan [Yu-Cheng]). In the latter studies, however, all four manifestations

of developmental toxicity (reduced viability, structural alterations, growth retardation, and functional alterations) were observed to some degree following exposure to dioxin-like compounds as well as other agents. Data from the Dutch cohort of children exposed to PCBs and dioxin-like compounds (Huisman et al., 1995a, b; Koopman-Esseboom et al., 1994a-c; 1995a, b, 1996; Pluim et al., 1992, 1993, 1994; Weisglas-Kuperus et al., 1995; Patandin et al., 1998, 1999; ten Tusscher et al., 2003; Vreugdenhil et al., 2002) suggest impacts of background levels of dioxin and related compounds on neurobehavioral outcomes, thyroid function, immune function, and liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Although these effects cannot be attributed solely to dioxin and related compounds, several associations suggest that these are, in fact, likely to be AhR-mediated effects. Similarly, it is highly likely that the developmental effects in human infants exposed to a complex mixture of PCBs, PCDFs, and polychlorinated quaterphenyls (PCQs) in the Yusho and Yu-Cheng poisoning episodes may have been caused by the combined exposure to those PCB and PCDF congeners that are AhR agonists (Lü and Wong, 1984; Kuratsune, 1989; Rogan, 1989). However, it is not possible to determine the relative contributions of individual chemicals to the observed effects.

The incidents at Yusho and Yu-Cheng resulted in increased perinatal mortality and low birth weight in infants born to women who had been exposed. Rocker bottom heal was observed in Yusho infants, and functional abnormalities have been reported in Yu-Cheng children. Not all the effects that were seen are attributable only to dioxin-like compounds. The similarity of effects observed in human infants prenatally exposed to this complex mixture with those reported in adult monkeys exposed only to TCDD suggests that at least some of the effects in the Yusho and Yu-Cheng children are due to the TCDD-like congeners in the contaminated rice oil ingested by the mothers of these children. The similar responses include a clustering of effects in organs derived from the ectodermal germ layer, referred to as ectodermal dysplasia, including effects on the skin, nails, and Meibomian glands and developmental and psychomotor delay during developmental and cognitive tests (Chen et al., 1992). Some investigators believe that because some of the effects in the Yusho and Yu-Cheng cohorts do not correlate with TEQ, such effects could be exclusively due to nondioxin-like PCBs or to an interaction between the dioxins and the nondioxin-like congeners.

Of particular interest is the common developmental origin (ectodermal layer) of many of the organs and tissues that are affected in humans. An ectodermal dysplasia syndrome involving hyperpigmentation, deformation of the fingernails and toenails, conjunctivitis, gingival hyperplasia, and abnormalities of the teeth has been clearly associated with the Yusho and Yu-Cheng episodes, and in the non-human primate studies. Alaluusua et al. (1996, 1999)

investigated dioxin exposure and tooth development in Finnish children as a result of studies of dental effects in dioxin-exposed rats, mice, and nonhuman primates (Part II, Chapter 5, Section 5.2) and in PCB-exposed children (Rogan et al., 1988). The Finnish investigators examined enamel hypomineralization of permanent first molars in 6–7-year-old children. The length of time that infants breast-fed was not significantly associated with either mineralization changes or with TEQ levels in the breast milk. However, when the levels and length of breast-feeding were combined in an overall score, a statistically significant association was observed (r= 0.3, p=0.003, regression analysis). These data are discussed further in Part II, Chapter 7b, Section 7.13. Follow-up mechanistic studies on tooth development in TCDD sensitive and resistant rats revealed a relatively high dose impact on epithelial-mesenchymal interactions, particularly the mesenchymal odontocytes. This effect that was not associated with differential resistance to acute TCDD toxicity (Kiukkonen et al., 2002).

Other investigations into noncancer effects of human exposure to dioxin have provided human data on TCDD-induced changes in circulating reproductive hormones. This was one of the effects judged as having a positive relationship with exposure to TCDD in Part II, Chapter 7b, Section 7.13. Levels of reproductive hormones have been measured with respect to exposure to 2,3,7,8-TCDD in three cross-sectional medical studies. Testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured in trichlorophenol (TCP) and 2,4,5-T production workers from the NIOSH cohort (Egeland et al., 1994), in Army Vietnam veterans (CDC Vietnam Experience Study, 1988), and in Air Force Ranch Hands, who handled and/or sprayed Agent Orange during the Vietnam War (Roegner et al., 1991; Grubbs et al., 1995). A recent study also demonstrated an inverse correlation between TCDD levels and prolactin in 2,4,5,-T herbicide sprayers (Johnson et al., 2001). Alterations in breast development have been reported in young women, where a doubling of the serum dioxin concentration (CALUX assay) increased the odds of not having reached the adult stage of breast development by 2.3 fold (P<0.02) in the women (~17 yo) studied (Den Hond et al., 2002). Alterations in menstrual duration and flow have been reported in women exposed as premenarcheal girls 20 years previously as a result of the Seveso incident (Eskenazi et al., 2002a).

The risk of abnormally low testosterone was two to four times higher in exposed workers who had serum 2,3,7,8-TCDD levels above 20 ng/g than in unexposed referents (Egeland et al., 1994). In both the 1987 and 1992 examinations, mean testosterone concentrations were slightly but not significantly higher in Ranch Hands (Thomas et al., 1990; Grubbs et al., 1995). FSH and LH concentrations were no different between the exposed and comparison groups. No significant associations were found between Vietnam experience and altered reproductive

hormone levels (CDC Vietnam Experience Study, 1988). Only the NIOSH study (Egeland et al., 1994) found an association between serum 2,3,7,8-TCDD level and increases in serum LH.

The findings of the NIOSH and Ranch Hand studies are plausible, given the pharmacological and toxicological properties of 2,3,7,8-TCDD in animal models, which are discussed in Part II, Chapters 5 and 7. One plausible mechanism responsible for the effects of dioxins may involve their ability to influence hormone receptors. The AhR, to which 2,3,7,8-TCDD binds, and the hormone receptors are signaling pathways that regulate homoeostatic processes. These signaling pathways are integrated at the cellular level, and there is considerable "cross-talk" between these pathways. For example, studies suggest that 2,3,7,8-TCDD modulates the concentrations of numerous hormones and/or their receptors, including estrogen (Romkes and Safe, 1988; Romkes et al., 1987), progesterone (Romkes et al., 1987), glucocorticoid (Ryan et al., 1989), and thyroid hormones (Gorski and Rozman, 1987; Pavuk et al., 2003).

In summary, the results from both the NIOSH and the Ranch Hand studies are limited by the cross-sectional nature of the data and the type of clinical assessments conducted. However, the available data provide evidence that small alterations in human male reproductive hormone levels are associated with serum 2,3,7,8-TCDD.

2.2.2.2. *Experimental Animal Effects*

The extensive experimental animal database with respect to reproductive and developmental toxicity of dioxin and dioxin-related agents is discussed in Part II, Chapter 5. Dioxin exposure has been observed to result in both male and female reproductive effects as well as developmental effects. These latter effects are among the most responsive health endpoints to dioxin exposure (see Part II, Chapter 8, Section 8.3). In general, the prenatal and developing postnatal animal is more sensitive to the effects of dioxin than is the adult. In several instances (e.g., fetotoxicity in hamsters, rats, mice, and guinea pigs), the large species differences seen in acute toxicity are greatly reduced when developing animals are evaluated. Most of the data reviewed are from studies of six genera of laboratory animals. Although much of the data come from animals exposed only to TCDD, more recent studies of animals exposed to mixtures of PCDD/PCDF/ PCB congeners provide results that are consistent with the studies of TCDD alone.

2.2.2.2.1. ***Developmental toxicity.*** Dioxin exposure results in a wide variety of developmental effects; these are observed in three different vertebrate classes and in several species within each class. All four of the manifestations of developmental toxicity have been observed following

exposure to dioxin: reduced viability, structural alterations, growth retardation, and functional alterations. As summarized previously (Peterson et al., 1993), increased prenatal mortality (rat and monkey), functional alterations in learning (rat, mouse, and monkey) and sexual behavior (rat), and changes in the development of the reproductive system (rat, hamster, and mouse) occur at the lowest exposure levels tested (see also Part II, Chapter 8, Section 8.3).

Dioxin exposure has resulted in reduced prenatal or postnatal viability in virtually every species in which it has been tested. Previously, increased prenatal mortality appeared to be observed only at exposures that also resulted in maternal toxicity. However, the studies of Olson and McGarrigle (1990) in the hamster and Schantz and Bowman (1989) in the monkey suggested that this was not the case in all species. Although the data from these two studies were limited, prenatal death was observed in cases where no maternal toxicity was evident. In the rat, Peterson's laboratory (Bjerke et al., 1994a, b; Roman et al., 1995) reported increased prenatal death following a single exposure to TCDD during gestation that did not cause maternal toxicity, and Gray et al. (1995a) observed a decrease in postnatal survival under a similar exposure regimen. Although identifying the presence or absence of maternal toxicity may be instructive as to the specific origin of the reduced prenatal viability, it does not alter the fact that pre- and postnatal deaths were observed. In either case, the Agency considers these effects as being indicators of developmental toxicity in response to the exposure (U.S. EPA, 1991b).

Some of the most striking findings regarding dioxin exposure relate to the effects on the developing reproductive system in laboratory animals. Only a single, low-level exposure to TCDD during gestation is required to initiate these developmental alterations. Mably et al. (1992a-c) originally reported that a single exposure of the Holtzman maternal rat to as little as 0.064 μg/kg could alter normal sexual development in the male offspring. A dose of 0.064 μg/kg in these studies resulted in a maximal body burden in the maternal animal of 64 ng/kg during critical windows in development. More recently, these findings of altered normal sexual development have been further defined (Bjerke et al., 1994a, b; Gray et al., 1995a; Roman et al., 1995) and extended to female offspring and other strains (Faqi et al., 1998; Ohsako et al., 2001) and species (hamsters and mice) (Gray et al., 1995b; Theobald et al., 1997). In general, the findings of these later studies have produced qualitatively similar results that define a significant effect of dioxin on the developing reproductive system.

In the developing male rat, TCDD exposure during the prenatal and lactational periods results in delay of the onset of puberty, as measured by age at preputial separation. There is a reduction in testis weight, sperm parameters, and sex accessory gland weights. In the mature male exposed during the prenatal and lactational periods, there is an alteration of normal sexual behavior and reproductive function. Males exposed to TCDD during gestation are

demasculinized. Feminization of male sexual behavior and a reduction in the number of implants in females mated with exposed males have also been reported, although these effects have not been consistently found. These effects do not appear to be related to reductions in circulating androgens, which were shown in the most recent studies to be unaffected by TCDD. Most of these effects have occurred in a dose-related fashion, some at doses of 0.05 μg/kg and 0.064 μg/kg, the lowest doses tested (Mably et al., 1992c; Gray et al., 1997a).

In Part II, Chapter 8, ED_{01} values were estimated from the Mably et al. (1992a-c) and Gray et al. (1997a) reports. In these two studies more than 44 data sets were modeled, and 17 of these data sets had body burden ED_{01}s lower than 50 ng/kg. For the 12 endpoints in the Mably et al. studies that were modeled in Part II, Chapter 8, the median body burden ED_{01} estimate is 5.2 ng TCDD/kg. Although not modeled in Part II, Chapter 8, the data from Faqi et al. (1998) and Ohsaka et al. (2001) have LOAELs and NOAELs for developmental reproductive effects of TCDD in male rats ranging from body burdens of 12.5–200 ng TCDD/kg, which is consistent with the Mably et al. and Gray et al. studies.

In the developing female rat, Gray and Ostby (1995) demonstrated altered sexual differentiation in both the Long Evans and Holtzman strains. The effects observed depended on the timing of exposure. Exposure during early organogenesis altered the cyclicity, reduced ovarian weight, and shortened the reproductive lifespan. Exposure later in organogenesis resulted in slightly lowered ovarian weight, structural alterations of the genitalia, and a slight delay in puberty. However, cyclicity and fertility were not affected with the later exposure. The most sensitive dose-dependent effects of TCDD in the female rat were the structural alterations of the genitalia that occurred at 0.20 μg TCDD/kg administered to the dam (Gray et al., 1997b).

As described above, studies demonstrating adverse health effects from prenatal exposures often involved a single dose administered at a discrete time during pregnancy. The production of prenatal effects at a given dose appears to require exposure during critical times in fetal development. This concept is well supported by a recent report (Hurst et al., 2000) that demonstrated the same incidence of adverse effects in rat pups born to dams with a single exposure of 0.2 μg TCDD/kg body weight on gestation day 15 versus 1.0 μg TCDD/kg body weight on gestation day 8. Both of these experimental exposure paradigms resulted in the same fetal tissue concentrations and body burdens during the critical window of sensitivity. For example, exposure to 0.2 μg TCDD/kg on day 15 resulted in 13.2 pg TCDD/g fetal tissue on day16; exposure to 1.0 μg TCDD/kg on day 8 resulted in 15.3 pg TCDD/g fetus on day 16. This study demonstrates the appropriateness of the use of body burden to describe the effects of TCDD when comparing different exposure regimens. The uncertainties introduced when trying to compare studies with steady-state body burdens with single-dose studies may make it difficult

to determine a lowest effective dose. Application of pharmacokinetic models (described in Parts I and II) to estimate body burdens at the critical time of development is expected to be a sound method for relating chronic background exposures to the results obtained from single-dose studies.

Structural malformations, particularly cleft palate and hydronephrosis, occur in mice administered TCDD. The findings, although not representative of the most sensitive developmental endpoints, indicate that exposure during the critical period of organogenesis can affect the processes involved in normal tissue formation. The TCDD-sensitive events appear to require the AhR. Mouse strains that produce AhRs with relatively high affinity for TCDD respond to lower doses than do strains with relatively low-affinity receptors. Moreover, congeners that have a greater affinity for the AhR are more developmentally toxic than those that have a lower affinity. This is consistent with the rank ordering of toxic potency based on affinity for the receptor, as discussed in Part II, Chapter 9, Section 9.3. In addition, mice in which the Ah receptor has been knocked out do not develop cleft palate.

Recent work, not elaborated upon here, has demonstrated that developmental exposure of rodents to dioxin also permanently alters the development of the prostate in wild type but not AhR null mutant mice (Lin et al., 2003), and mammary development in rats and mice (Fenton et al, 2002; Vorderstrasse et al., 2003). The key role of the Ah receptor has also been demonstrated in the developing heart of AhR null mice (Lund et al., 2003).

2.2.2.2.2. ***Adult female reproductive toxicity.*** The primary effects of TCDD on female reproduction in animals appear to be decreased fertility, inability to maintain pregnancy for the full gestational period, and, in the rat, decreased litter size. In some studies of rats and of primates, signs of ovarian dysfunction such as anovulation and suppression of the estrous cycle have been reported (Kociba et al., 1976; Barsotti et al., 1979; Allen et al., 1979; Li et al., 1995a, b). Although the majority of reproductive effects are associated with high-dose exposures in experimental animals, the induction of endometriosis in primates occurs at body burdens near background human exposures. This effect is discussed further below.

2.2.2.2.3. ***Adult male reproductive toxicity.*** TCDD and related compounds decrease testis and accessory sex organ weights, cause abnormal testicular morphology, decrease spermatogenesis, and reduce fertility when given to adult animals in doses sufficient to reduce feed intake and/or body weight. In the testes of these different species, TCDD effects on spermatogenesis are characterized by loss of germ cells, the appearance of degenerating spermatocytes and mature spermatozoa within the lumens of seminiferous tubules, and a reduction in the number of tubules

containing mature spermatozoa (Allen and Lalich, 1962; Allen and Carstens, 1967; McConnell et al., 1978; Chahoud et al., 1989). This suppression of spermatogenesis is not a highly sensitive effect when TCDD is administered to postweanling animals, as an exposure of 1 μg/kg/day over a period of weeks appears to be required to produce these effects.

2.2.2.3. ***Other Data Related to Developmental and Reproductive Effects***

2.2.2.3.1. ***Endometriosis.*** The association of dioxin with endometriosis was first reported in a study of rhesus monkeys that had been exposed for 4 years to dioxin in their feed and then held for an additional 10 years (Rier et al., 1993). There was a dose-related increase in both the incidence and severity of endometriosis in the exposed monkeys as compared to controls. Follow-up on this group of monkeys revealed a clear association with total TEQ. A study in which rhesus monkeys were exposed to PCBs for up to 6 years failed to show any enhanced incidence of endometriosis (Arnold et al., 1996). However, many of these monkeys were no longer cycling, and the time may not have been adequate to develop the response. In the TCDD monkey study, it took 7 years before the first case of endometriosis was noted (Rier et al., 1993).

A recent study in Cynomolgus monkeys showed promotion of surgically induced endometriosis by TCDD within 1 year after surgery (Yang et al., 2000). Studies using rodent models for surgically induced endometriosis have also shown the ability of TCDD to promote lesions in a dose-related manner (Cummings et al., 1996, 1999; Johnson et al., 1997; Bruner-Tran et al., 1999). This response takes at least 2 months to be detected (Cummings et al., 1996, 1999; Johnson et al., 1997). Another study in mice that failed to detect dioxin promotion of surgically induced endometriosis held the mice for only 1 month, not long enough to detect a response (Yang et al., 1997). Prenatal exposure of mice also enhanced the sensitivity of the offspring to the promotion of surgically induced endometriosis by TCDD (Cummings et al., 1999).

The effects of TCDD in the murine model of endometriosis appear to be AhR-mediated, as demonstrated in a study in which AhR ligands were able to promote the lesions, whereas non-AhR ligands, including a nondioxin-like PCB, had no effect on surgically induced endometriosis (Johnson et al., 1997). Dioxin has also been shown to result in endometriosis with human endometrial tissue implanted in nude mice (Bruner-Tran et al., 1999).

Data on the relationship of dioxins to endometriosis in humans is intriguing, but preliminary. Studies in the early 1990s suggested that women who had higher levels of persistent organochlorines were at increased risk for endometriosis (Gerhard and Runnebaum, 1992). This was followed by the observation that Belgian women, who have the highest levels of dioxins in their background population, had higher incidences of endometriosis than those reported from

other populations (Koninckx et al., 1994). A study from Israel then demonstrated that there was a correlation between detectable TCDD in women who had surgically confirmed endometriosis in comparison to those who had no endometriosis (Mayani et al., 1997).

Recent studies from Belgium indicate that women with higher body burdens, based on serum TEQ determinations, are at greater risk for endometriosis (Pauwels et al., 1999). No association was seen with total PCBs in this study. A small study in the United States that did not involve surgically confirmed endometriosis saw no association between TCDD and endometriosis (Boyd et al., 1995). Likewise, a study in Canada saw no association between total PCBs and endometriosis (Lebel et al., 1998). The lack of an association with total PCBs is not surprising, because the rodent studies have indicated that this response is AhR-mediated (Johnson et al., 1997). The Seveso Women's Health Study reported "...a doubled, non-significant risk for endometriosis among women with serum TCDD levels of 100 ppt or higher, but no clear dose-response. Unavoidable disease misclassification in a population-based study may have led to an underestimate of the true risk of endometriosis"(Eskenazi et al., 2002b).

The animal results lend biological plausibility to the epidemiology findings (Birnbaum and Cummings, 2002). Endometriosis is not only an endocrine disorder, it is also associated with immune system alterations (Rier et al., 1995; Rier and Foster, 2002). Dioxins are known to be potent modulators of the animal immune system and to affect estrogen homeostasis. Further studies are clearly needed to provide additional support to this association of endometriosis and dioxins, as well as to demonstrate causality.

2.2.2.3.2. ***Androgenic deficiency.*** The effects of TCDD on the male reproductive system when exposure occurs in adulthood are believed to be due in part to an androgenic deficiency. This deficiency is characterized in adult rats by decreased plasma testosterone and 5α-dihydrotestosterone concentrations, unaltered plasma LH concentrations, and unchanged plasma clearance of androgens and LH (Moore et al., 1985, 1989; Mebus et al., 1987; Moore and Peterson, 1988; Bookstaff et al., 1990a). The cause of the androgenic deficiency was believed to be due to decreased testicular responsiveness to LH and increased pituitary responsiveness to feedback inhibition by androgens and estrogens (Moore et al., 1989, 1991; Bookstaff et al., 1990a, b; Kleeman et al., 1990). The single dose used in some of those earlier studies (15 µg TCDD/kg body weight) is now known to affect Leydig cells (Johnson et al., 1994).

2.2.2.4. *Developmental and Reproductive Effects Hazard Characterization*

There is limited direct evidence addressing the issues of how or at what levels humans will begin to respond to dioxin-like compounds with adverse impacts on development or reproductive function. The series of published Dutch studies suggest that pre- and early postnatal exposures to PCBs and other dioxin-like compounds may impact developmental milestones at levels at or near current average human background exposures. Although it is unclear whether these measured responses indicate a clearly adverse impact, if humans respond to TCDD similarly to animals in laboratory studies, there are indications that exposures at relatively low levels might cause developmental effects and at higher levels might cause reproductive effects. There is especially good evidence for effects on the fetus from prenatal exposure. The Yusho and Yu-Cheng poisoning incidents are clear demonstrations that dioxin-like compounds can produce a variety of mild to severe developmental effects in humans that resemble the effects of exposure to dioxins and dioxin-like compounds in animals.

Humans do not appear to be particularly sensitive or insensitive to effects of dioxin exposure in comparison to other animals. Therefore, it is reasonable to assume that human responsiveness would lie across the middle ranges of observed responses. This assumption still does not address the issues surrounding the potentially different responses that humans (or animals) might have to the more complex and variable environmental mixtures of dioxin-like compounds. One additional key point is that most of the epidemiology studies have focused on TCDD, and not the total TEQ. Eskenazi et al. (2004) have shown that background exposure to dioxins, furans and PCBs in the referent population (zone non-ABR) cohort at Seveso was substantial,with non-ABR residents having average serum 2,3,7,8-TCDD and TEQ levels of 20.2 ppt and 100.4 ppt, respectively. The exposure zone A median serum TCDD level was 272 ppt and zone B was 47 ppt. The authors suggest that previous Seveso studies "that considered only TCDD exposure, may have underestimated health effects due to total TEQ concentrations."

TCDD and related compounds have reproductive and developmental toxicity potential in a broad range of wildlife and domestic and laboratory animals. Many of the effects have been shown to be TCDD dose-related. The effects on perinatal viability and male reproductive development are among the most sensitive effects reported, occurring at a single prenatal exposure range of as little as 0.05–0.075 μg/kg, resulting in calculated fetal tissue concentrations of 3–4 ng/kg in the rat (Hurst et al., 2000). In these studies, effects were often observed at the lowest exposure level tested, thus a NOAEL has not been established for several of these endpoints. In general, the structure-activity results are consistent with an AhR-mediated mechanism for the developmental effects that are observed in the low-dose range. The structure-activity relationship in laboratory mammals appears to be similar to that for AhR binding. This

is especially the case with cleft palate in the mouse, but has also been seen with hydronephrosis in the mouse, and developmental reproductive effects in rats.

It is assumed that the responses observed in animal studies are indicative of the potential for reproductive and developmental toxicity in humans. This is an established assumption in the risk assessment process for developmental toxicity (U.S. EPA, 1991b). It is supported by the number of animal species and strains in which effects have been observed. The limited human data are consistent with an effect following exposure to TCDD or TCDD-like agents. In addition, the phylogenetic conservation of the structure and function of the AhR also increases our confidence that these effects may occur in humans.

There is extensive evidence in experimental animals (mice, rats, monkeys) that exposure to dioxin-like chemicals during development produces neurobehavioral effects. In fact, recent studies in rodents demonstrate effects on brain development (Zareba et al., 2002), attention (Markowski et al., 2002), and behavior (Hojo et al., 2002) at doses close to current human body burdens. The situation in humans is more complex. Studies in humans demonstrate associations between dioxin exposure and alterations in neurological development. These same studies often show similar associations between exposure to nondioxin-like PCBs and these same effects. On the basis of the human studies, it is possible that the alterations in neurological development are due to an interaction between the dioxins and the nondioxin-like PCBs. At present there are limited data that define the roles of the dioxins versus the nondioxin-like PCBs in these effects on neurological development.

In general, the structure-activity results on dioxin-like compounds are consistent with an AhR-mediated mechanism for many of the developmental effects that are observed. The structure-activity relationship in laboratory mammals appears to be similar to that for AhR binding. This is especially the case with teratogenesis in the mouse. However, a direct relationship with AhR binding has not yet been proven for those involving the developing nervous system.

2.2.3. Immunotoxicity

2.2.3.1. *Epidemiologic Findings*

The available epidemiologic studies on immunologic function in humans relative to exposure to 2,3,7,8-TCDD do not describe a consistent pattern of effects among the examined populations. Two studies of German workers in which one cohort was exposed to 2,3,7,8-TCDD (Ott et al., 1994), and the other to 2,3,7,8-tetrabrominated dioxin and furan (Zober et al., 1992), found dose-related increases of complements C3 or C4, whereas the Ranch Hands have continued to exhibit elevations in immunoglobulin A (IgA) (Roegner et al., 1991; Grubbs et al.,

1995). Other studies of groups with documented exposure to 2,3,7,8-TCDD have not examined complement components to any great extent or observed significant changes in IgA. Suggestions of immunological disturbances have been observed in a small group of exposed workers (Tonn et al., 1996) and in perinatally exposed children (ten Tusscher et al., 2003), providing support for a testable hypothesis to be evaluated in other exposed populations.

Comprehensive evaluation of immunologic status and function of the NIOSH (Halperin et al., 1998), Ranch Hand (Michalek et al., 1999b), and Hamburg chemical workers (Jung et al., 1998; Ernst et al., 1998) cohorts found no consistent differences between exposed and unexposed groups for lymphocyte subpopulations, response to mitogen stimulation, or rates of infection. However, recent data from the Seveso experience demonstrate subtle effects on immune function (Baccarelli et al., 2002).

More comprehensive evaluations of immunologic function with respect to exposure to 2,3,7,8-TCDD and related compounds are necessary to assess more definitively the relationships observed in nonhuman species. Longitudinal studies of the maturing human immune system may provide the greatest insight, particularly because animal studies have found significant results in immature animals, and human breast milk is a source of 2,3,7,8-TCDD and other related compounds. The studies of Dutch infants (ten Tusscher et al., 2003) described earlier provide an example of such a study design. Additional studies of highly exposed adults may also shed light on the effects of long-term chronic exposures through elevated body burdens. Therefore, there appears to be too little information to suggest definitively that 2,3,7,8-TCDD, at the levels observed, causes long-term adverse effects on the immune system in adult humans.

2.2.3.2. *Animal Findings*

Cumulative evidence from a number of studies indicates that the immune system of various animal species is a target for toxicity of TCDD and structurally related compounds, including other PCDDs, PCDFs, and PCBs. Both cell-mediated and humoral immune responses are suppressed following TCDD exposure, suggesting that there are multiple cellular targets within the immune system that are altered by TCDD. Evidence also suggests that the immune system is indirectly targeted by TCDD-induced changes in nonlymphoid tissues. TCDD exposure of experimental animals results in decreased host resistance following challenge with certain infectious agents, which likely result from TCDD-induced suppression of immunological functions.

The primary antibody response to the T cell-dependent antigen, sheep red blood cells (SRBCs), is the most sensitive immunological response that is consistently suppressed in mice exposed to TCDD and related compounds. The degree of immunosuppression is related to the

potency of the dioxin-like congeners. There is remarkable agreement among several different laboratories for the potency of a single acute dose of TCDD (i.e., suppression at a dose as low as 0.1 μg TCDD/kg with an average 50% immunosupressive dose [ID_{50}] value of approximately 0.7 μg TCDD/kg) to suppress this response in Ah-responsive mice. Results of studies that have compared the effects of acute exposure to individual PCDDs, PCDFs, and PCB congeners (which differ in their binding affinity for the AhR) on this response have provided critical evidence that certain dioxin-like congeners are also immunosuppressive. The degree of immunosuppression has been found to be related to potency of the dioxin-like congeners. Antibody responses to T cell-independent antigens such as trinitrophenyl-lipopolysaccharide and the cytotoxic T lymphocyte (CTL) response are also suppressed by a single acute exposure to TCDD, albeit at higher doses than those that suppress the SRBC response. Although a thorough and systematic evaluation of the immunotoxicity of TCDD-like congeners in different species and for different immunological endpoints has not been performed, it can be inferred from the available data that dioxin-like congeners are immunosuppressive.

Perinatal exposure of experimental animals to TCDD results in suppression of primarily T cell immune functions, with suppression persisting into adulthood. In mice, the effects on T cell functions appear to be related to the fact that perinatal TCDD exposure alters thymic precursor stem cells in the fetal liver and bone marrow and thymocyte differentiation in the thymus. These studies suggest that perinatal development is a critical and sensitive period for TCDD-induced immunotoxicity. Further efforts should be made to determine the consequences of perinatal exposure to TCDD and related compounds and mixtures on immune system integrity.

2.2.3.3. *Other Data Related to Immunologic Effects*

In addition to the TCDD-like congener results, studies using strains of mice that differ in the expression of the AhR have provided critical evidence to support a role for Ah-mediated immune suppression following exposure to dioxin-like compounds. Recent in vitro work also supports a role for Ah-mediated immune suppression. Other in vivo and in vitro data, however, suggest that non-Ah-mediated mechanisms may also play some role in immunotoxicity induced by dioxin-like compounds. However, more definitive evidence remains to be developed to support this latter view.

The immunosuppressive potency of individual dioxin-like compounds in mice is related to their structural similarity to TCDD. However, the immunotoxicity of TCDD and related congeners can be modified by co-exposure to nondioxin-like PCBs in simple binary or more complex mixtures, resulting in additive or antagonistic interactions. There is a need for the

generation of dose-response data of acute, subchronic, and chronic exposure to the individual congeners in a mixture and for the mixture itself in order to fully evaluate potential synergistic, additive, or antagonistic effects of environmentally relevant mixtures. A preliminary report demonstrating that the immunotoxicity of a food-like mixture of dioxins was well-predicted by the TEQ has been presented (Smialowicz et al., 1997).

Animal host resistance models that mimic human disease have been used to assess the effects of TCDD on altered host susceptibility. TCDD exposure increases susceptibility to challenge with bacteria, viruses, parasites, and tumors. Mortality is increased in TCDD-exposed mice challenged with certain bacteria. Increased parasitemia occurs in TCDD-exposed mice and rats challenged with parasitic infections. Low doses of TCDD also alter resistance to virus infections in rodents. Increased susceptibility to infectious agents is an important benchmark of immunosuppression; however, the role that TCDD plays in altering immune-mediated mechanisms important in murine resistance to infectious agents remains to be elucidated. Also, because little is known about the effects that dioxin-like congeners have on host resistance, more research is recommended in this area.

Studies in nonhuman primates exposed acutely, subchronically, or chronically to halogenated aromatic hydrocarbons (HAH) have revealed variable alterations in lymphocyte subpopulations, primarily T lymphocyte subsets. In three separate studies in which monkeys were exposed subchronically or chronically to PCBs, the antibody response to SRBC was consistently found to be suppressed. These results in nonhuman primates are important because they corroborate the extensive database of HAH-induced suppression of the antibody response to SRBC in mice and thereby provide credible evidence for immunosuppression by HAHs across species. In addition, these data indicate that the primary antibody response to this T cell-dependent antigen is the most consistent and sensitive indicator of HAH-induced immunosuppression.

The available database derived from well-controlled animal studies on TCDD immunotoxicity can be used for the establishment of NOELs. As the antibody response to SRBCs has been shown to be dose-dependently suppressed by TCDD and related dioxin-like compounds, this database is best suited for the development of dose-response modeling.

2.2.3.4. *Immunologic Effects Hazard Characterization*

Accidental or occupational exposure of humans to TCDD and/or related compounds variably affects a number of immunological parameters. Unfortunately, the evaluation of immune system integrity in humans exposed to dioxin-like compounds has provided data that are inconsistent across studies. The broad range of "normal" responses in humans due to the large

amount of variability inherent in such a heterogenous population, the limited number and sensitivity of tests performed, and poor exposure characterization of the cohorts in these studies compromise any conclusions about the ability of a given study to detect immune alterations. Consequently, there are insufficient clinical data from these studies to fully assess human sensitivity to TCDD exposure. Nevertheless, based on the results of the extensive animal work, the database is sufficient to indicate that immune effects could occur in the human population from exposure to TCDD and related compounds at some dose level. At present, it is EPA's scientific judgment that TCDD and related compounds should be regarded as nonspecific immunosuppressants and immunotoxicants until better data to inform this judgment are available.

It is interesting that a common thread in several human studies is the observed reduction in $CD4^+$ T helper cells, albeit generally within the "normal" range, in cohorts exposed to dioxin-like compounds. Even though these reductions may not translate into clinical effects, it is important to note that these cells play an important role in regulating immune responses and that their reduction in clinical diseases is associated with immunosuppression. It is also important to realize that those at the extremes of the population distribution may be at special risk of such alterations. Another important consideration is that a primary antibody response following immunization was not evaluated in any of the human studies. Because this immune parameter has been revealed to be the most sensitive in animal studies, it is recommended that TCDD and related compounds be judged immunosupressive and that this parameter be included in future studies of human populations exposed to TCDD and related compounds. It is also recommended that research focused on delineating the mechanism(s) underlying dioxin-induced immunotoxicity and immunosuppression continue.

2.2.4. Chloracne

Chloracne and associated dermatologic changes are widely recognized responses to TCDD and other dioxin-like compounds in humans. Along with the reproductive hormones discussed above and gamma glutamyl transferase (GGT) levels, which are discussed below, chloracne is one of the noncancer effects that has a strong positive association with exposure to TCDD in humans (see Part II, Chapter 7b, Section 7.13). Chloracne is a severe acne-like condition that develops within months of first exposure to high levels of dioxin and related compounds. For many individuals, the condition disappears after discontinuation of exposure, despite initial serum levels of dioxin in the thousands of parts per trillion; for others, it may remain for many years. The duration of persistent chloracne is on the order of 25 years, although

cases of chloracne persisting for more than 40 years have been noted (see Part II, Chapter 7b, Section 7.13).

In general, chloracne has been observed in most incidents where substantial dioxin exposure has occurred, particularly among TCP production workers and Seveso residents (see Part II, Chapter 7b). The amount of exposure necessary for development of chloracne has not been resolved, but studies suggest that high exposure (both high acute and long-term exposure) to 2,3,7,8-TCDD increases the likelihood of chloracne, as evidenced by chloracne in TCP production workers and Seveso residents who had documented high serum 2,3,7,8-TCDD levels (Beck et al., 1989; Fingerhut et al., 1991a; Mocarelli et al., 1991; Neuberger et al., 1991) or in individuals who had a work history with long duration of exposure to 2,3,7,8-TCDD-contaminated chemicals (Bond et al., 1989).

In earlier studies, chloracne was considered to be a "hallmark of dioxin intoxication" (Suskind, 1985). However, in only two studies were risk estimates calculated for chloracne. Both were studies of different cohorts of TCP production workers, one of which was employed in a West Virginia plant (Suskind and Hertzberg, 1984), the other in a plant in Michigan (Bond et al., 1989). Of the 203 West Virginia workers, 52.7% ($p<0.001$) were found to have clinical evidence of chloracne, and 86.3% reported a history of chloracne ($p<0.001$). None of the unexposed workers had clinical evidence or reported a history of chloracne. Among the Michigan workers, the relative risk for cases of chloracne was highest for individuals with the longest duration of exposure ($\geq$ 60 months; RR = 3.5, 95% CI = 2.3–5.1), those with the highest cumulative dose of TCDD (based on duration of assignment across and within 2,3,7,8-TCDD-contaminated areas in the plant) (RR = 8.0, 95% CI = 4.2–15.3), and those with the highest intensity of 2,3,7,8-TCDD exposure (RR = 71.5, 95% CI = 32.1–159.2).

Studies in multiple animal species have been effective in describing the relationship between 2,3,7,8-TCDD and chloracne, particularly in rhesus monkeys (McNulty, 1977; Allen et al., 1977; McConnell et al., 1978). Subsequent to exposure to 2,3,7,8-TCDD, monkeys developed chloracne and swelling of the meibomian glands, the modified sebaceous glands in the eyelid. The histologic changes in the meibomian glands are physiologically similar to those observed in human chloracne (Dunagin, 1984).

In summary, the evidence provided by the various studies convincingly supports what is already presumed—that chloracne is a common sequel of high levels of exposure to 2,3,7,8-TCDD and related compounds. More information is needed to determine the level and frequency of exposure to dioxin-like compounds needed to cause chloracne and whether personal susceptibility plays a role in the etiology. Finally, it is important to recall that the absence of chloracne does not imply lack of exposure (Mocarelli et al., 1991).

2.2.5. Diabetes

Diabetes mellitus is a heterogeneous disorder that is a consequence of alterations in the number or function of pancreatic beta cells responsible for insulin secretion and carbohydrate metabolism. Diabetes and fasting serum glucose levels were evaluated in more recent cross-sectional medical studies because of the apparently high prevalence of diabetes and abnormal glucose tolerance tests in one case report of 55 TCP workers (Pazderova-Vejlupkova et al., 1981). Recent epidemiology studies, as well as early case reports, have indicated a weak association between serum concentrations of dioxin and diabetes. This association was first noted in the early 1990s when a decrease in glucose tolerance was seen in the NIOSH cohort. This was followed by a report of an increase in diabetes in the Ranch Hand cohort (Michalek et al., 1999a; Longnecker and Michalek, 2000). An increase in diabetes in other occupational cohorts (Steenland et al., 1999; Vena et al., 1998) as well as in the Seveso population (Pesatori et al., 1998) has also been reported. There was not a significant increase in diabetes in the NIOSH mortality study, although 6 of the 10 most highly exposed workers did have diabetes (Calvert et al., 1999). However, mortality studies are limited in their ability to assess risk from diabetes mellitus because the prevalence of disease may not be available from death certificates.

A paper by Longnecker and Michalek (2000) found a pattern suggesting that low levels of dioxin may influence the prevalence of diabetes. However, these results did not show an exposure-response relationship. Because it is the only study of its type to have been published, additional population-based studies are warranted to validate its findings. A recent update of the Ranch Hand study shows a 47% excess of diabetes in the most heavily exposed group of veterans (Michalek et al., 1999a).

Most of the data suggest that the diabetes observed in the studies is Type II, or adult-onset diabetes, rather than insulin dependent, or Type I. Aging and obesity are the key risk factors for Type II diabetes. However, dioxins may shift the distribution of sensitivity, putting people at risk at younger ages or when they have less weight. Dioxin alters lipid metabolism in multiple species, including humans (Sweeney et al., 1997; Pohjanvirta and Tuomisto, 1994), and it also alters glucose uptake into both human and animal cells in culture (Enan and Matsumura, 1994; Olsen et al., 1994). Mechanistic studies have demonstrated that dioxin affects glucose transport (Enan and Matsumura, 1994), a property under the control of the hypoxia response pathway (Ouiddir et al., 1999). A key regulatory protein in this pathway is the partner of the AhR, Arnt (also known as HIF1-beta) (Gu et al., 2000; Taylor and Zhulin, 1999). Activation of the AhR by dioxin may compete with other pathways for Arnt, such as the hypoxia-inducible factor (HIF) pathway (Gradin et al., 1992). Dioxin has also been shown to downregulate the insulin growth factor receptor (Liu et al., 1992). These three issues—altered lipid metabolism, altered glucose

transport, and alterations in the insulin signaling pathway—all provide biological plausibility to the association of dioxins with diabetes.

A causal relationship between diabetes and dioxin has not been established, although both the toxicologic and epidemiological data are suggestive of a plausible association (Remillard and Bunce, 2002). Many questions have yet to be answered. For example, does diabetes alter the pharmacokinetics of dioxin? Diabetes is known to alter the metabolism of several drugs in humans (Matzke et al., 2000) and may also alter dioxin metabolism and kinetics. Because adult-onset diabetes is also associated with being overweight, and body composition has been shown to modify the apparent half-life of dioxin, could the rate of elimination of dioxins be lowered in people who have diabetes, causing them to have higher body burdens? This may be relevant to the background population, but it is hardly likely to be an explanation in highly exposed populations.

Key research needs are twofold. The first is to develop an animal model with which to study the association between dioxins and diabetes and glucose perturbation. Several rodent models for Type II diabetes exist and may be used. The second is to conduct population-based incidence studies that take into account dioxin levels as well as the many known factors associated with diabetes. Although diabetes may cause the underlying pathology leading to death, it is often not attributed as the cause of death and thus limits the utility of mortality studies.

2.2.6. Other Effects

2.2.6.1. *Elevated GGT*

As mentioned above, there appears to be a consistent pattern of increased GGT levels among individuals exposed to 2,3,7,8-TCDD-contaminated chemicals. Elevated levels of serum GGT were observed within a year after exposure in Seveso children (Caramaschi et al., 1981; Mocarelli et al., 1986) and 10 or more years after cessation of exposure among TCP and 2,4,5-T production workers (May, 1982; Martin, 1984; Moses et al., 1984; Calvert et al., 1992) and among Ranch Hands (Roegner et al., 1991; Grubbs et al., 1995). All of these groups had a high likelihood of substantial exposure to 2,3,7,8-TCDD. In addition, for those studies that evaluated dose-response relationships with 2,3,7,8-TCDD levels, the effect was observed only at the highest levels or categories of 2,3,7,8-TCDD and, in the NIOSH study, only in workers who reported drinking high levels of alcohol.

In contrast, although background levels of serum 2,3,7,8-TCDD suggested minimal exposure in Army Vietnam veterans, GGT was increased at borderline significance among Vietnam veterans as compared to non-Vietnam veterans (CDC Vietnam Experience Study,

1988). In addition, despite the increases observed in some studies of occupational cohorts, other studies of TCP production workers from West Virginia or Missouri residents measured but did not report elevations in GGT levels (Suskind and Hertzberg, 1984; Webb et al., 1989).

In clinical practice, GGT is often measured because it is elevated in almost all hepatobiliary diseases and is used as a marker for alcoholic intake (Guzelian, 1985). In individuals with hepatobiliary disease, elevations in GGT are usually accompanied by increases in other hepatic enzymes, for example, AST and ALT, and metabolites, for example, uro- and coproporphyrins. Significant increases in hepatic enzymes other than GGT and metabolic products were not observed in individuals whose GGT levels were elevated 10 or more years after exposure ended, suggesting that the effect may be GGT-specific. These data suggest that in the absence of increases in other hepatic enzymes, elevations in GGT are associated with exposure to 2,3,7,8-TCDD, particularly among individuals who were exposed to high levels.

The animal data with respect to 2,3,7,8-TCDD-related effects on GGT are sparse. Statistically significant changes in hepatic enzyme levels, particularly AST, ALT, and alkaline phosphatase, have been observed after exposure in rats and hamsters (Gasiewicz et al., 1980; Kociba et al., 1978; Olson et al., 1980). Only one study evaluated GGT levels (Kociba et al., 1978); moderate but statistically nonsignificant increases were noted in rats fed 0.10 μg/kg 2,3,7,8-TCDD daily for 2 years, and no increases were observed in control animals.

In summary, GGT is the only hepatic enzyme examined that was found in a number of studies to be chronically elevated in adults exposed to high levels of 2,3,7,8-TCDD. The consistency of the findings in a number of studies suggests that the elevation may reflect a true effect of exposure, but its clinical significance is unclear. Long-term pathological consequences of elevated GGT have not been illustrated by excess mortality from liver disorders or cancer or in excess morbidity in the available cross-sectional studies.

It must be recognized that the absence of an effect—for example, liver enzymes—in a cross-sectional study does not obviate the possibility that the enzyme levels may have increased concurrently with the exposure but declined after cessation. The apparently transient elevations in ALT levels among the Seveso children suggest that hepatic enzyme levels other than GGT may react in this manner to 2,3,7,8-TCDD exposure.

2.2.6.2. *Thyroid Function*

Many effects of 2,3,7,8-TCDD exposure in animals resemble signs of thyroid dysfunction or significant alterations of thyroid-related hormones. In the few human studies that have examined the relationship between 2,3,7,8-TCDD exposure and hormone concentrations in adults (CDC Vietnam Experience Study, 1988; Roegner et al., 1991; Grubbs et al., 1995;

Suskind and Hertzberg, 1984), the results are mostly equivocal. Cross-sectional analysis of the Ranch Hand cohort (Pavuk et al., 2003) found signs of elevated TSH means among the high TCDD exposure group in the 1985 and 1987 follow-ups, with an increasing trend across the decade 1982 - 1992, but no association with the occurrence of thyroid disease. Concentrations of thyroid binding globulin also appeared to be positively correlated with current levels of 2,3,7,8-TCDD in the BASF accident cohort (Ott et al., 1994). Little additional information on thyroid hormone levels has been reported for production workers and none for Seveso residents, two groups with documented high serum 2,3,7,8-TCDD levels.

Thyroid hormones play important roles in the developing nervous system in all vertebrate species, including humans—to the extent that all infants in the United States are tested for hypothyroidism shortly after birth. Several studies of nursing infants suggest that ingestion of breast milk with a higher dioxin TEQ may alter thyroid function (Pluim et al., 1993; Koopman-Esseboom et al., 1994c; Nagayama et al., 1997). These findings suggest a possible shift in the distribution of thyroid hormones, particularly T4, and point out the need for collection of longitudinal data to assess the potential for long-term effects associated with developmental exposures.

The exact processes that account for these observations in humans are unknown, but when put in perspective of animal responses, the following might apply: dioxin increases the metabolism and excretion of thyroid hormone, mainly T4, in the liver, and reduced T4 levels stimulate the pituitary to secrete more TSH, which enhances thyroid hormone production. Early in the disruption process, the body can overcompensate for the loss of T4, which may result in a small excess of circulating T4 to the increased TSH. In animals given higher doses of dioxin, the body is unable to maintain homeostasis, TSH levels remain elevated, and T4 levels decrease.

A plausible mode of action for thyroid effects is described in Section 2.2.1.3.

2.2.6.3. *Cardiovascular Disease*

Elevated cardiovascular disease has been noted in several occupational cohort studies (Steenland et al., 1999; Sweeney et al., 1997; Flesch-Janys et al., 1995) and in the Seveso (Pesatori et al., 1998) and the rice oil poisoning studies. This appears to be associated with ischemic heart disease and in some cases with hypertension. Recent data from the Ranch Hand study indicate that dioxin may be a possible risk factor for the development of essential hypertension (Grubbs et al., 1995). Elevated blood lipids have also been seen in several cohorts. The association of dioxins with heart disease in humans has biological plausibility, given the data in animals. First is the key role of hypoxia in heart disease and the potential for involvement of the activated AhR in blocking an hypoxic response (Gradin et al., 1996; Gu et al., 2000). Dioxin

has been shown to perturb lipid metabolism in multiple laboratory species (Pohjanvirta and Tuomisto, 1994). The heart—in fact the entire vascular system—is a clear target for the adverse effects of dioxin in fish and birds (Hornung et al., 1999; Cheung et al., 1981). Recent studies have demonstrated that the heart is also a target in mammals (Lund et al., 2003; NTP 2003a). In mammals, dioxin has been shown to disturb heart rhythms at high doses in guinea pigs (Gupta et al., 1973; Pohjanvirta and Tuomisto, 1994).

2.2.6.4. *Oxidative Stress*

Several investigators have hypothesized that some of the adverse effects of dioxin and related compounds may be associated with oxidative stress. Induction of CYP1A isoforms has been shown to be associated with oxidative DNA damage (Park et al., 1996). Altered metabolism of endogenous molecules such as estradiol can lead to the formation of quinones and redox cycling. This has been hypothesized to play a role in the enhanced sensitivity of female rats to dioxin-induced liver tumors (Tritscher et al., 1996). Lipid peroxidation, enhanced DNA single-strand breaks, and decreased membrane fluidity have been observed in liver as well as in extrahepatic tissues following exposure to high doses of TCDD (Stohs, 1990). A dose- and time-dependent increase in superoxide anion in peritoneal macrophages following exposure to TCDD (Alsharif et al., 1994). A recent report that low-dose (0.15 ng TCDD/kg/day) subchronic exposure can lead to oxidative changes in several tissues in mice (Slezak et al., 2000) suggests that this mechanism or mode of toxicity deserves further attention.

Table 2-1. Effects of TCDD and related compounds in different animal species

Effect	Humans	Monkey	Guinea pig	Rat	Mouse	Hamster	Cow	Rabbit	Chicken	Fish	Avian wildlife	Marine mammals	Mink
Presence of AhR	+	+	0	+	+	+	+	+	+	+	+	+	+
Binding of TCDD: AhR complex to the DRE (enhancer)	+		+	+	+	+	+	+	+	+			
Enzyme induction	+	+	+	+	+	+		+	+	+	+	+	+
Acute lethality	0	+	+	+	+	+	+	+	+	+	+	+	+
Wasting syndrome	+	+	+	+	+	+	+	+		+	+	+	+
Teratogenesis/fetal toxicity, mortality	+/-	+	+	+	+	+		+	+	+	+	+	+
Endocrine effects	+/-	+		+	+					+	+	+	+
Immunotoxicity	+/-	+	+	+	+	+	+		+	+		+	
Carcinogenicity	+/-			+	+	+				+			
Neurotoxicity	+	+		+	+				+				
Chloracnegenic effects	+	+			+		+	+		+			
Porphyria	+	0	0	+	+	0			+				
Hepatotoxicity	+	+	+/-	+	+	+/-	+	+	+	+	+	+	+
Edema		+	0	0	+	+			+	+			
Testicular atrophy		+	+	+	+								
Bone marrow hypoplasia		+	+		+/-				+				
Teeth	+	+		+									

+ = observed.
+/- = observed to limited extent, or +/- results.
0 = not observed.
Blank cells = no data.

Table 2-2. Some biochemical responses to TCDD

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

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

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

Table 2-3. Summary of the combined cohort and selected industrial cohort studies with high exposure levels, as described by IARC (1997)[a]

Reference	All cancers			Lung cancer		
	Observed	SMR	95% CI	Observed	SMR	95% CI
International cohort						
Kogevinas et al. (1997)[b]	394	1.2	1.1–1.3	127	1.2	1.0–1.4
Industrial populations (high-exposure subcohorts)						
Fingerhut et al. (1991a)[c] (USA)	114	1.5	1.2–1.8	40	1.4	1.0–1.9
Becher et al. (1996)[d] (Germany)	105	[1.3]	[1.0–1.5]	33	[1.4]	[1.0–2.0]
Hooiveld et al. (1996)[e] (Netherlands)	51	1.5	1.1–1.9	14	1	0.5–1.7
Ott and Zober (1996b)[f] (BASF accident)	18	1.9	1.1–3.0	7	2.4	1.0–5.0
TOTAL	[288]	[1.4]	[1.2–1.6]	[94]	[1.4]	[1.1–1.7]
p value	<0.001			<0.01		

[a] Adapted from IARC; Table 38 (1997); non-Hodgkin's lymphoma, soft-tissue sarcoma, and gastrointestinal results not shown. TOTALs were calculated by the IARC Working Group.

[b] Men and woman > 20 years since first exposure. These data include the cohorts of Fingerhut et al. (1991a,b), Becher et al. (1996), Hooiveld et al. (1996a), the original IARC cohort (Saracci et al., 1991), and other cohorts.

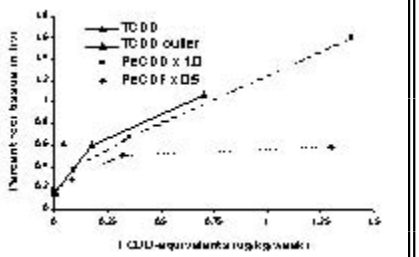
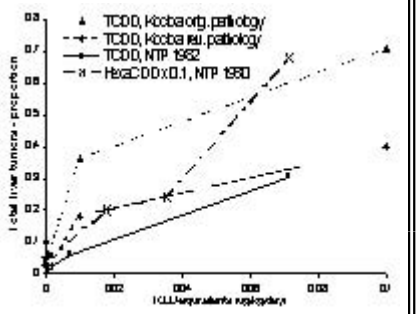
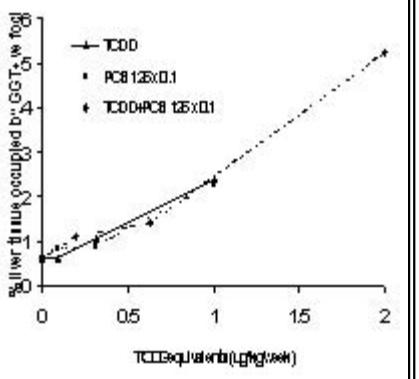
[c] Men ≥ 20 years latency and ≥ 1 year exposure.

[d] Men, cohorts I and II, summed (Boehringer-Ingelheim, Bayer-Uerdingen cohorts).

[e] Men and women, Factory A.

[f] Men, chloracne subgroup, ≥ 20 years latency. Data presented for lung cancer are all respiratory tract cancers combined.

Table 2-4. Tumor incidence and promotion data cited for the TEF-WHO$_{98}$ for principal congeners

Congener	TEF-WHO$_{98}$ tumor incidence/promotion citation[a]	TEF-WHO$_{98}$	% of adipose TEQ$_{DFP}$-WHO$_{98}$ tissue conc.[b]	Dose-response graphs: dose adjusted to reflect TEF multiplier
2,3,7,8-TCDD	TEF Standard	1	8	
1,2,3,7,8-PeCDD	Waern et al. (1991)	1	15	
2,3,4,7,8-PeCDF	Waern et al. (1991)	0.5	7	
1,2,3,6,7,8-HxCDD	NTP (1980); 1,2,3,6,7,8-HxCDD/ 1,2,3,7,8,9-HxCDD; 1:2 mixture; long-term bioassays, Osborne-Mendel rats in NTP studies, Sprague-Dawley rats in Kociba et al. (1978)	0.1	10	
1,2,3,7,8,9-HxCDD		0.1	2	
PCB 126	Hemming et al. (1995)	0.1	33	

[a] van den Berg et al., 2000. Hexa-CDD referenced to previous TEF reviews.
[b] See Part II, Chapter 4, Tables 4-46, 4-47

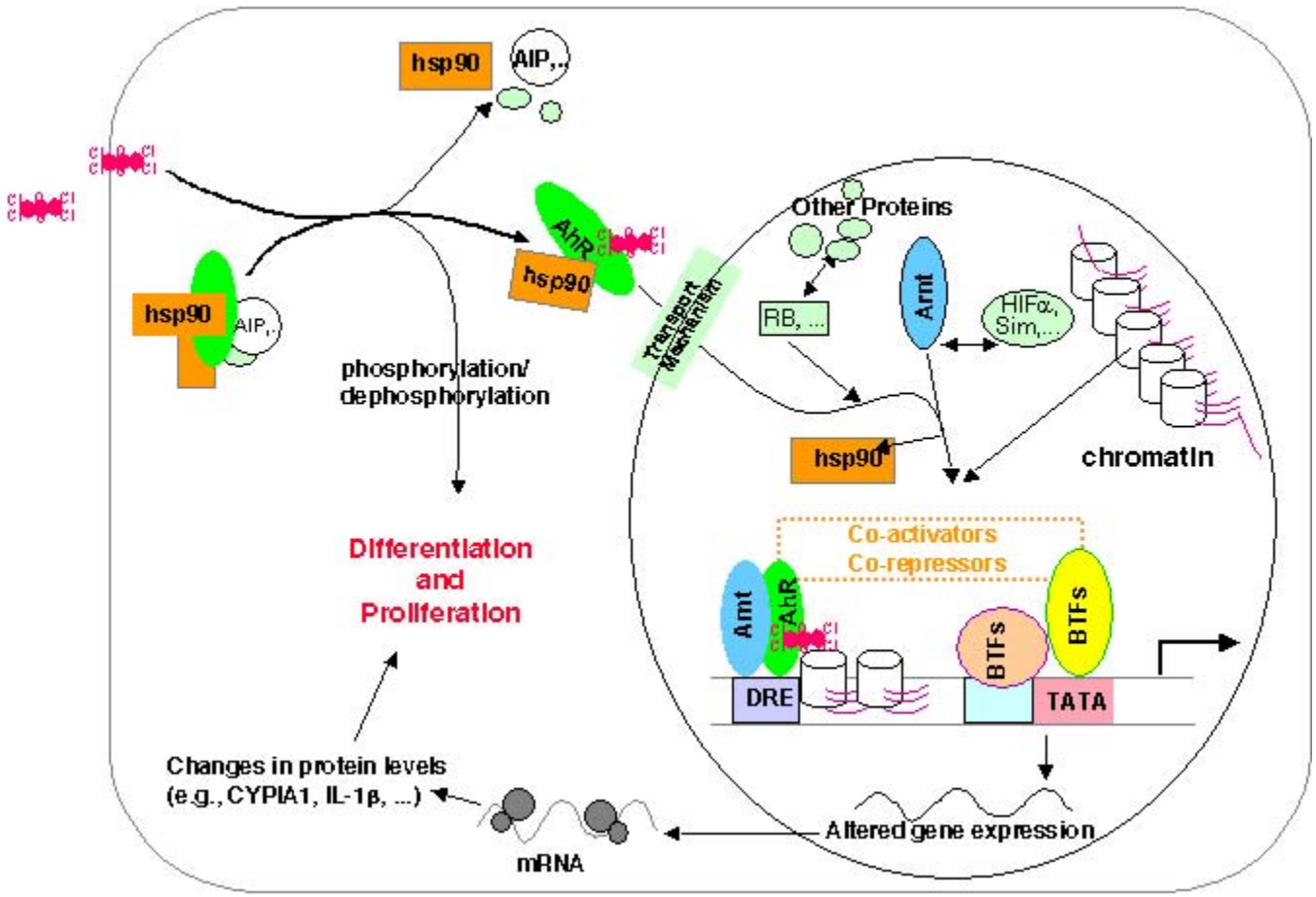


Figure 2-1. Cellular mechanism for AhR action. TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; AhR, aryl hydrocarbon receptor; AIP, associated immunophilin-like protein; hsp90, 90 kilodalton heat shock protein; p, sites of phosphorylization; Arnt, AhR nuclear translocator protein; RB, retinoblastoma protein; NF-kB, nuclear transcription factor; HIF, hypoxia inducible factor; DRE, dioxin-responsive element; BTFs, basal transcription factors; TATA, DNA recognition sequence.