9.4.1. Levels in the Environment and in Food

CDDs, CDFs, and PCBs have been found throughout the world in practically all media, including air, soil, water, sediment, fish and shellfish, and agricultural food products such as meat and dairy products. The highest levels of these compounds are found in soils, sediments, and biota; very low levels are found in water and air. The widespread occurrence observed, particularly in industrialized countries, is not unexpected, considering the numerous sources that emit these compounds into the atmosphere and the overall resistance of these compounds to biotic and abiotic transformation.

The average levels of these compounds found in the various media in North America have been compiled in the Exposure Document. The levels shown for environmental media and for food in North America are based on few samples and must be considered uncertain. However, they seem reasonably consistent with levels measured in a number of studies in Western Europe and Canada. The consistency of these levels across industrialized countries adds some confidence to the limited data from the United States and provides some reassurance that the U.S. estimates are reasonable. A major concern raised regarding all of these data is that few if any of these studies had a statistical design that was satisfactory for generalization to national food supplies. This adds to the uncertainty of extrapolations using these findings and argues for additional data collection to evaluate national and regional differences of levels of dioxin-like compounds in the environment and in food.

This assessment proposes the hypothesis that the primary mechanism by which dioxin-like compounds enter the terrestrial food chain is via atmospheric deposition. Dioxin and related compounds enter the atmosphere directly through air emissions or indirectly, for example, through volatilization from land or water or from resuspension of particles. Deposition can occur directly onto soil or onto plant surfaces. Soil deposits can enter the food chain via direct ingestion (e.g., grazing animals, earth worms, fur preening by burrowing animals). Dioxin-like compounds in soil can become available to plants by volatilization and vapor absorption or particle resuspension and adherence to plant surfaces. In addition, dioxin-like compounds in soil can adsorb directly to underground portions of plants. Uptake from soil via the roots into above-ground portions of plants is thought to be insignificant.

Support for this air-to-food hypothesis is provided by Hites (1991) who concluded that "background environmental levels of dioxin-like compounds are caused by dioxin-like compounds entering the environment through the atmospheric pathway." His conclusion was based on demonstrations that the congener profiles in lake sediments could be linked to congener profiles of combustion sources. Further arguments supporting this hypothesis include: (1) numerous measurements show that emissions occur from multiple sources and deposition occurs in most areas, including remote locations; (2) atmospheric transport and deposition are the only mechanisms that could explain the widespread distribution of these compounds in soil; and (3) other mechanisms of uptake into food, for instance, from direct contamination or through packaging, are much less plausible. Direct uptake into food from soil or sediments is possible and could be important for "local" exposures. These routes are less likely to explain the general background level of dioxin and related compounds found in the diet of the general population.

At present, it is unclear whether atmospheric deposition represents primarily "new" contributions of dioxin and related compounds from all media reaching the atmosphere or whether it is "old" dioxin and related compounds that persist and recycle in the environment. Understanding the relationship between these two scenarios will be particularly important in understanding the relative contributions of individual point sources of these compounds to the food chain and assessing the effectiveness of control strategies focused on either "new" or "old" dioxins in attempting to reduce the levels in food.

9.4.2. Background Exposure Levels

The term "background" exposure has been used throughout this reassessment to describe exposure of the general population, who are not exposed to readily identifiable point sources of dioxin-like compounds, that results in widespread, low-level circulation of dioxin-like compounds in the environment. The primary route of this exposure is thought to be the food supply, and most of the dioxin-like compounds are thought to come from non-natural sources. For the purposes of estimating background exposures to dioxin-like compounds via dietary intake the upper-range background toxicity equivalent values (i.e., those calculated using one-half the detection limit for the nondetects) were used in the Exposure Document.

Uncertainties associated with the use of TEQs have been described throughout this chapter. The estimates are based on intake of dioxin-like CDDs and CDFs and do not include estimates for dioxin-like PCBs or other dioxin-like compounds. Inclusion of dioxin-like PCBs could raise these estimates by 35-50%. The net effect of these calculations is that we may be overestimating background levels based on the use of one-half of the detection limit and underestimating background levels by not including the dioxin-like PCBs or other dioxin-like compounds.

A background exposure level of 120 pg TEQ/day for the United States was estimated. These estimates are comparable to analogous estimates for European countries. These include estimates for Germany, which range from 79 pg TEQ/day based on Furst et al. (1990) to 158 pg TEQ/day based on Furst et al. (1991), 118-126 pg TEQ/day exposure via numerous routes in The Netherlands (Theelen, 1991), and 140-290 pg TEQ/day for the typical Canadian exposed mainly through food ingestion (Gilman and Newhook, 1991). It is generally concluded by these researchers that dietary intake is the primary pathway of human exposure to CDDs and CDFs. These investigators among others suggest that greater than 90 percent of human exposure occurs through the diet, with foods from animal origins being the predominant pathway.

This conclusion, that food is the predominant pathway of exposure, remains to be validated in the United States. Although data are derived from multiple studies from around the world, the data represent limited numbers of samples. Use of one-half of the detection level for nondetects is a reasonable but conservative approach to estimating low levels in samples. For some data sets, use of zero values for nondetects would result in significantly lower estimates. Setting nondetects equal to zero, however, does not significantly change the average TEQ levels estimated for most categories of U.S. food. In the current assessment, similar estimates of TEQs derived from different data sets, developed by different investigators in several countries, strengthen the probability that this inference represents the exposure of the general population in industrialized countries to dioxin and related compounds.

Data on human tissue levels suggest that body-burden levels among industrialized nations are reasonably similar (Schecter, 1991). These data can also be used to estimate

background exposure through the use of pharmacokinetic models. Using this approach, exposure levels to 2,3,7,8-TCDD in industrialized nations are estimated to be about 20 to 40 pg TCDD/day (0.3-0.6 pg TCDD/kg/day). This is generally consistent with the estimates derived using diet-based approaches to estimate total TCDD intake. Pharmacokinetic approaches have not been applied to estimate exposures to CDDs or CDFs other than TCDD, which contribute substantially to the body burden of dioxin-like compounds. Estimates of exposure to dioxin-like CDDs and CDFs based on dietary intake are in the range of 1-3 pg TEQ/kg/day. Estimates based on the contribution of dioxin-like PCBs to toxicity equivalents raise the total to 3-6 pg TEQ/kg/day. This range is used throughout this characterization as an estimate of average background exposure to dioxin-like CDDs, CDFs, and PCBs.

The U.S. study of CDD/F body burdens contained in the National Human Adipose Tissue Survey (NHATS) (U.S. EPA, 1991) analyzed for CDD/Fs in 48 human tissue samples which were composited from 865 samples. These samples were collected during 1987 from autopsied cadavers and surgical patients. While this was an important study of chemical residues occurring in human fat, numerous technical shortcomings of this study have been described. For instance, the sample compositing prevents use of these data to examine the distribution of CDD/F levels in tissue among individuals. However, it did allow conclusions in the following areas:

- **National Averages:** The national averages for all TEQ congeners (but excluding dioxin-like PCBs) were estimated and totaled to 28 pg TEQ/g lipid adjusted value (28 ppt).
- **Age Effects:** Tissue concentrations of CDD/Fs were found to increase with age.
- **Geographic Effects:** In general, the average CDD/F tissue concentrations appeared fairly uniform geographically.
- **Race Effects:** No significant differences in CDD/F tissue concentrations were found on the basis of race.
- **Sex Effects:** No significant differences in CDD/F tissue concentrations were found between males and females.

- **Temporal Trends:** The 1987 survey showed decreases in tissue concentrations relative to the 1982 survey for all congeners. However, it is not known whether these declines were due to improvements in the analytical methods or actual reductions in body-burden levels. The fact that areas surveyed in this study changed over time (due to drop-out of areas) also makes interpretation of time trends difficult. The percent reductions among individual congeners varied from 9% to 96%. The relationship of these data to an apparent declining trend of dioxin-like compounds in environmental samples, especially sediments, is currently unclear.

More recent data (Patterson et al., 1994) show similar decreasing trends with regard to levels of dioxin-like PCBs in blood and fat. In addition, these data showed a wide variability of PCB congeners in human adipose tissue samples as compared to concentrations of CDDs and CDFs, which were less variable.

Inclusion of dioxin-like PCBs in TEQ calculations raises the average body burden to 40-60 pg TEQ/g (40-60 ppt). Because available data from the two studies discussed above do not provide a representative population sample, these conclusions must be regarded as uncertain. Additional measurements will be necessary to confirm this hypothesis. Use of a protocol for sampling that allows an evaluation of age-adjusted population averages will be critical for understanding the current body-burden situation and evaluating impacts of future efforts to further reduce exposures to this class of compounds.

Levels of dioxin-like compounds found in human tissue/blood appear similar in Europe and North America. Schecter (1991) compared levels of dioxin-like compounds found in blood among people from U.S. pooled samples (100 subjects) and Germany (85 subjects). Although mean levels of individual congeners differed by as much as a factor of two between the two populations, the total TEQ averaged 42 pg TEQ/g (42 ppt) in the German subjects and was 41 pg TEQ/g (41 ppt) in the pooled U.S. samples. These values do not include TEQs for PCBs.

New information on levels of dioxin-like compounds in human adipose tissue and blood has recently been published (Patterson et al., 1994). This study reports measurements of dioxin-like PCB congeners as well as CDD and CDF levels in samples from 28 Atlanta

residents. These measurements show that concentrations of dioxin-like PCBs can be more than an order of magnitude higher than concentrations of TCDD. Comparison with other published information suggests much higher levels of nondioxin-like congeners of PCBs and the possibility that concentrations of both types of congeners will depend heavily on previous human activities such as fish consumption. These data are consistent with the previous statement that dioxin-like PCBs may account for approximately one-third of the total TEQ in the general population. Values in Patterson's study calculated TEQs for PCBs using the data of Safe (1990), which were acknowledged by the author as being conservative and, based on more recent data, overestimate the contribution of dioxin-like PCBs.

While, as described above, evaluation of the range of background population blood levels is difficult given existing data, the NHATS tissue data show that the maximum measured concentrations were about two times higher than the average for most congeners (U.S. EPA, 1991). These results are based on composite samples that each included approximately 20 individual samples. This high level of compositing will greatly reduce the individual variability of samples. Consequently, the range in body burdens in the entire population is expected to be larger than that found among the samples in this study. The Patterson et al. (1994) data show that the maximum 2,3,7,8-TCDD concentration was about three to four times higher than the average. Similar results were seen for PCB 126. These results are based on samples of 28 individuals. Again, the range of body burdens in the entire population will be greater than that found among these 28 individuals. Accordingly, it can be concluded that body burdens of dioxin-like compounds are likely to be at least three to four times higher than the average for some members of the population and, perhaps, even higher. While it is difficult to know the full extent of the range of body burdens, the Patterson data were found to fit reasonably well as a log-normal distribution. This observation has also been made for other data sets (Sielken, 1987). With such distributions in large populations, it is not unusual to see values that extend three standard deviations beyond the mean. The body burdens corresponding to three standard deviations beyond the mean (99th percentile) have been estimated (using a log-scale calculation) to be approximately seven times higher than the arithmetic mean. Whether individuals with background levels of dioxin-like compounds of this magnitude exist in the general population

is unknown, but these calculations provide support for the inference that the general population may have a wide range of body burdens and, therefore, both average and high end values should be considered when evaluating potential for adverse impacts of background exposures.

9.4.3. Highly Exposed Populations

In addition to general population exposure, some individuals or groups of individuals may also be exposed to dioxin-like compounds from discrete sources or pathways locally within their environment. Examples of these "special" population exposures include occupational exposures, direct or indirect exposure to local populations from discrete local sources, exposure to nursing infants from mother's milk, or exposures to subsistence or recreational fishers. These exposures have been discussed previously in terms of increased exposure due to dietary habits (see Exposure Document) or due to occupational conditions or industrial accidents (see Chapter 7). Although exposures to these populations may be significantly higher than to the general population, they usually represent relatively small numbers of individuals. Inclusion of their levels of exposure in the general population estimates would have little impact on average estimates and would obscure the potential significance of elevated exposures for these subpopulations.

For example, consumption of breast milk by nursing infants may lead to higher levels of exposure during the early postnatal period as compared to intake in the diet later in life. Schecter et al. (1992) report that a study of 42 U.S. women found an average of 16 pg TEQ/g (16 ppt), 3.3 ppt of which was 2,3,7,8-TCDD, in the lipid portion of breast milk. A much larger survey in Germany (n=728) found an average of 31 pg TEQ/g (31 ppt) with a range of 6 to 87 pg TEQ/g in the lipid portion of breast milk (Beck et al., 1991). These estimates do not include a contribution to total TEQ from dioxin-like PCBs. The level in human breast milk can be predicted on the basis of the estimated dioxin intake by the mother. Such procedures are presented in Volume II of the Exposure Document.

Elimination of 2,3,7,8-TCDD through mother's milk can result in higher exposure levels to the infant than for the general population. Assuming that an infant breast feeds for one year (a conservative assumption since, in the United States, 6 months of breast feeding is

more typical), has an average weight during this period of 10 kg (which is on the high end [90-98th percentile] of the average weight distribution for the first year of life), ingests 0.8 kg/d of breast milk and that the dioxin concentration in milk fat is 20 pg/g (20 ppt) of TEQ, the average daily dose to the infant over this period is predicted to be about 60 pg TEQ/kg/d, not including dioxin-like PCBs. This value is 10 to 20 times higher than the estimated range for background exposure to adults (i.e., 3-6 pg TEQ including dioxin-like PCBs/kg/d) and would have been even higher if dioxin-like PCBs had been included in this sample analysis. A range of alternative assumptions could be made regarding the nursing time period, infant's body weight, and milk ingestion rate. None of these factors is likely to vary individually by more than a factor of two and, when combined, will likely result in less than multiplicative variability in estimates of daily intake. WHO (1988) suggested that a reasonable average nursing scenario would be 6 months duration, 0.7 L/day ingestion rate, and a milk fat content of 3.5%. Using a milk ingestion rate of 120 mL/kg/day (compared to 80 mL/kg/day used above) and a milk concentration of 16.9 pg TEQ/g, WHO estimated a daily intake of 70 pg TEQ/kg/day.

If a 70-year averaging time is used to obtain an added increment of lifetime daily dose, then the increment of lifetime average daily dose attributable to the EPA nursing scenario is estimated to be 0.8 pg of TEQ/kg/d. On a mass basis, the cumulative dose to the infant under this scenario is about 210 ng compared to a lifetime background intake of about 1,700 to 5,100 ng (suggesting that 4% to 12% of the lifetime intake may occur as a result of breast feeding for the first year of life). WHO (1988) estimated that 4% of the lifetime intake would occur during the 6 months of nursing in their scenario. This percentage, as well as the daily intake rate, is nearly identical to the estimates presented in the Exposure Document although based on somewhat different assumptions. Traditionally, EPA has used the lifetime average daily dose as the basis for evaluating incremental cancer risk and the average daily dose (i.e., the daily exposure per unit body weight occurring during an exposure event) as the more appropriate indicator of risk for certain noncancer end points. The use of a lifetime average daily dose for high-level, early exposures may underestimate cancer risk if dose rate or perinatal sensitivity is important in the ultimate carcinogenic outcome. The average daily dose approach may be particularly important for the evaluation

of noncancer end points if exposure is occurring during windows of sensitivity during prenatal and postnatal development. However, data are currently insufficient to verify this hypothesis.

In addition, consumption of unusually high levels of fish, meat, or dairy products containing elevated levels of dioxin and related compounds can lead to elevated blood levels in comparison to the general population. Most people eat fish from multiple sources, both fresh and salt water, where levels of dioxin-like compounds are likely to be low. Even if large quantities of fish are consumed, most people are not likely to have unusually high exposures to dioxin-like compounds. However, individuals who fish regularly for purposes of basic subsistence are likely to obtain their fish from a few sources and may have the potential for elevated exposures. Such individuals may also consume large quantities of fish. Although average consumers may eat a few fish meals a month (an average intake of approximately 6.5 grams of fish a day), many recreational anglers near large water bodies may consume, on average, four to five times as much (approximately 30 grams per day). Of course, these averages include some individuals who eat no fish at all. Some individuals at the high end of the consumption range may eat, on average, as much as 140 grams per day. Certain members of ethnic groups who are subsistence fishers may consume two to three times this high-end amount as an upper estimate (up to 400 grams or approximately 1 pound per day). If high-end consumers obtain their fish from areas where content of dioxin-like chemicals in the fish is high, they may constitute a highly exposed subpopulation. Svensson et al. (1991) found elevated blood levels of CDDs and CDFs in high fish consumers living near the Baltic Sea in Sweden. The highest consumers, fishermen or workers in the fish industry, had blood level TEQs that were approximately three times that of non-fish consumers (60 pg TEQ/g lipid versus 20 pg TEQ/g lipid). The difference in levels of dioxin-like compounds was particularly apparent for the CDFs. Dioxin-like PCBs were not accounted for in this study. Studies are currently under way to examine fish consumption patterns in several Native American groups. Recent results (Columbia River Intertribal Fish Commission, 1994) suggest that Native Americans living along the Columbia River may consume an average of 30 grams of fish a day; some individuals consume much higher levels. Studies are currently under way to determine levels of dioxin-like compounds in fish

from this region. No measurements of dioxin-like chemicals in the blood of these Native American populations are currently available.

Dewailly et al. (1994) observed elevated levels of coplanar PCBs in the blood of fishermen on the north shore of the Gulf of the St. Lawrence River who consume large amounts of seafood. Coplanar PCB levels were 20 times higher among the 10 highly exposed fishermen than among controls. This study also reported elevated levels of coplanar PCBs in the breast milk of Inuit women of Arctic Quebec. The principal source of protein for the Inuit people is fish and sea mammal consumption.

The possibility of high exposures to dioxin-like chemicals as a result of consuming meat and dairy products is most likely to occur in situations where individuals consume large quantities of these foods from a locality where the level of these compounds is elevated. Most people eat meat and dairy products from multiple sources and, even if large quantities are consumed, are not likely to have unusually high exposures. However, individuals who raise their own livestock for basic subsistence have the potential for higher exposures if local levels of dioxin-like compounds are high. Volume III of the Exposure Document presents methods for evaluating this type of exposure scenario and concludes that indirect exposures via consumption of locally produced foods represent a major pathway for human exposure for a limited number of individuals in the population. In an example analysis contained in Volume III of the Exposure Document based on proximity to combustor emissions, the high end exposure estimates from food consumption were found to be about two orders of magnitude higher than inhalation exposures at the same location. However, it should be noted that no studies were found in the literature to demonstrate this potential increased exposure based on measurements of dioxin-like chemicals from source to livestock to humans.

Although the subpopulations discussed above have the potential for high exposure to dioxin-like compounds, a careful evaluation of dietary habits and proximity to sources of dioxin and related compounds is needed. It would generally be inappropriate to compute the total intake of dioxin-like compounds in a subpopulation by simply adding the dioxin intake from highly consumed food to the general population intake level. The general population background estimate assumes a typical pattern of food ingestion, whereas a subpopulation

that has a high consumption rate of one particular food type is likely to eat less of other food types. Ideally, the evaluation should be based on the entire diet of the subpopulation and use case-specific values for food ingestion rates and concentrations of dioxin-like compounds.

High blood levels of dioxin and related compounds based on high levels of exposure have been documented for industrial exposures in segments of the chemical industry and for industrial accidents. Health effects studies in human populations have focused on these groups of highly exposed individuals. Results of these studies are described in detail in Chapter 7.

9.5. DISPOSITION AND PHARMACOKINETICS

The disposition and pharmacokinetics of 2,3,7,8-TCDD and related compounds have been investigated in several species and under various exposure conditions. These data and models derived from them are critical in understanding the sequelae of human exposure. Data related to disposition and pharmacokinetics of dioxin and related compounds and efforts to develop models to further understand tissue dosimetry are described in detail in Chapter 1 of the Health Assessment Document.

The gastrointestinal, dermal, and transpulmonary absorptions of these compounds represent potential routes for human uptake. Findings of studies in experimental animals indicate that oral exposure to 2,3,7,8-TCDD in the diet or in an oil vehicle results in the absorption of >50%, and often closer to 90%, of the administered dose. Gastrointestinal absorption of related compounds is variable, incomplete, and congener specific. More soluble congeners, such as 2,3,7,8-TCDF, are almost completely absorbed, while the extremely insoluble OCDD is very poorly absorbed. In some cases, absorption has been found to be dose dependent, with increased absorption occurring at lower doses (2,3,7,8-TBDD, OCDD). The limited data base also suggests that there are no major interspecies differences in the gastrointestinal absorption of these compounds among mammals. Limited data (Poiger and Schlatter, 1986) from a single human volunteer suggest a high level (>87%) of absorption of 2,3,7,8-TCDD in corn oil from the gastrointestinal tract. Following absorption, a half-life for elimination was estimated to be 2,120 days (5.8 years). It should be noted that this estimate of half-life is for a single individual and that longer

median half-lives for 2,3,7,8-TCDD have been estimated (7.1 and 11.3 years) in other studies described in this chapter and in Chapter 1.

Additional data also indicate the importance of the formulation or vehicle containing the toxicant(s) on the relative bioavailability of 2,3,7,8-TCDD and related compounds after exposure. For instance, rodent feeding studies indicate that the bioavailability of 2,3,7,8-TCDD from soil varies between sites and 2,3,7,8-TCDD content alone may not be indicative of potential human hazard from contaminated environmental materials. Although data indicate that substantial absorption may occur from contaminated soil, soil type and duration of contact may substantially affect the absorption of 2,3,7,8-TCDD from soils obtained from different contaminated sites. This uncertainty should be kept in mind as intake values and the assumption of 50-100% absorption are often used to estimate potential risk from environmental samples.

In experiments measuring dermal absorption for 2,3,7,8-TCDD and several CDFs, the percentage of administered dose absorbed decreased with increasing dose while the amount absorbed increased with dose. Results also suggest that the majority of the compound remaining at the skin exposure site was associated with the outer skin layer (the stratum corneum) and did not penetrate through to the dermis. Together, these results on dermal absorption indicate that at ≤ 0.1 μmol/kg, a greater percent of this administered dose of 2,3,7,8-TCDD and three CDFs was absorbed. Nonetheless, even following a low-dose dermal application of 200 pmol (1 nmol/kg), the rate of absorption of 2,3,7,8-TCDD is still very slow (rate constant of 0.005 hour^{-1}). Dermal exposure of humans to 2,3,7,8-TCDD and related compounds usually occurs as a complex mixture of these contaminants in soil, oils, or other mixtures that would be expected to alter absorption. Available data suggest that the dermal absorption of 2,3,7,8-TCDD depends on the formulation (vehicle or adsorbent) containing the toxicant. Although no data are available to directly evaluate human dermal absorption, the data available from in vitro and animal studies suggest slow dermal absorption of these compounds, which is likely to be dependent on the vehicle or adsorbent containing the compounds and the duration of the contact.

The use of incineration as a means of solid and hazardous waste management results in the emission of vapors and contaminated particles that may contain TCDD and related

compounds into the environment. Thus, exposure to TCDD and related compounds may result from inhalation of contaminated fly ash, dust, and soil or from ingestion if air-transported particles are deposited on fruits and vegetables. Direct exposure by the inhalation route is usually relatively low as a percentage of overall intake. Systemic effects occur in animals after pulmonary exposure to TCDD, suggesting that transpulmonary absorption of TCDD does occur. Further results suggest that the transpulmonary absorption of 2,3,7,8-TCDD and 2,3,7,8-TBDD was similar to that observed following oral exposure. These limited data provide evidence of efficient transpulmonary absorption after intratracheal instillation in laboratory animals. No data from humans or primates are available to address this issue. However, these data provide support for the inference that efficient absorption will occur when vapors and particles containing dioxin and related compounds are inhaled by humans.

Once absorbed into blood, 2,3,7,8-TCDD and related compounds readily distribute to all organs. Tissue distribution within the first hour after exposure reflects physiological parameters such as blood flow to a given tissue and relative tissue size. There do not appear to be major species or strain differences in the tissue distribution of 2,3,7,8-TCDD and 2,3,7,8-TCDF in mammals, with the liver and adipose tissue being the primary disposition sites although human data to address this issue are quite limited. The tissue distribution of the coplanar PCBs and PBBs also appears to be similar to that of 2,3,7,8-TCDD and 2,3,7,8-TCDF based on evaluation in experimental animals.

Multiple studies suggest that distribution of this class of compounds to internal organs is dose dependent. At low doses in animal studies, adipose tissue serves as the major depot; at high doses, a major fraction is sequestered in the liver. The biochemical basis for this observation is under investigation. Induction of a hepatic binding protein has been hypothesized to play a major role.

As discussed above, levels of 2,3,7,8-TCDD averaging 5-10 pg/g lipid (ppt) have been reported for background populations. Sielken (1987) evaluated these data and concluded that the levels of 2,3,7,8-TCDD in human adipose are log-normally distributed and positively correlated with age. Among the observed U.S. background levels of 2,3,7,8-TCDD in human adipose tissue, more than 10% were >12 pg/g (ppt).

Human body-burden measurements on dioxins were initially conducted using adipose tissue, which required surgical samples, or, occasionally for women, using breast milk. Patterson et al. (1988) showed that human serum was an accurate and more practical surrogate for human adipose tissue. They found that the partitioning ratio of 2,3,7,8-TCDD between adipose tissue and serum was approximately 1.09 when the concentrations were adjusted for lipid content. This relationship appears to hold for at least a thousandfold concentration range in excess of background levels. This correlation indicates that serum 2,3,7,8-TCDD, coupled with measurement of serum lipid content, provides a valid estimate of the 2,3,7,8-TCDD concentration in adipose tissue under steady-state, low-dose conditions.

In a study of potentially heavily exposed Vietnam veterans, the Centers for Disease Control and Prevention (MMWR, 1988) reported an Air Force study of Ranch Hand veterans who were either herbicide loaders or herbicide specialists in Vietnam. The herbicide 2,4,5-T (Agent Orange) that was used in Vietnam was contaminated with a low percentage of 2,3,7,8-TCDD. The mean serum 2,3,7,8-TCDD level of 147 Ranch Hand personnel was 49 pg/g (ppt) in 1987, based on total lipid-weight, while the mean serum level of the 49 control was 5 pg/g (ppt). In addition, 79% of the Ranch Hand personnel and 2% of the controls had 2,3,7,8-TCDD levels ≥ 10 pg/g (ppt). The distribution of 2,3,7,8-TCDD levels in this phase of the Air Force health study indicates that Ranch Hand veterans have had higher lifetime exposures than controls and that a small number of Ranch Hand personnel had unusually heavy 2,3,7,8-TCDD exposure. Pirkle et al. (1989) estimated the median half-life of 2,3,7,8-TCDD in humans to be approximately 7 years on the basis of 2,3,7,8-TCDD levels in serum samples taken in 1982 and 1987 from 36 of the Ranch Hand personnel who had 2,3,7,8-TCDD levels > 10 pg/g (ppt) in 1987. Similar tissue concentrations were obtained by Kahn et al. (1988) in a report comparing 2,3,7,8-TCDD levels in blood and adipose tissue of moderately exposed Vietnam veterans who handled herbicides regularly while in Vietnam and matched controls. Although this study can distinguish moderately exposed men from others, the data do not address the question of the difficulty of characterizing the exposures of persons whose exposures are relatively low and who constitute the bulk of the population, both military and civilian, who may have been exposed to greater than background levels of 2,3,7,8-TCDD. Despite the fact that their exposures

may result in slightly elevated levels of 2,3,7,8-TCDD, these individuals are indistinguishable from the general population with similar blood levels spanning a range of nondetect to >10 ppt. Recently, a follow-up analysis to the Ranch Hand study described above has been published. This study (Wolfe et al., 1994) describes half-life measurements based on 337 Ranch Hand veterans. The estimate of the median half-life of TCDD is predicted to be 11.3 years. The implications of this longer half-life on our understanding of TCDD kinetics and on the back-calculations of historic intake values and body burdens will need to be fully described in future versions of this report.

The metabolism of 2,3,7,8-TCDD and related compounds is required for urinary and biliary elimination and therefore plays a major role in regulating the rate of excretion of these compounds and determining their half-life. Although early in vivo and in vitro investigations were unable to detect the metabolism of 2,3,7,8-TCDD, there is now evidence that a wide range of mammalian and aquatic species are capable of slowly biotransforming 2,3,7,8-TCDD to polar metabolites. Although metabolites of 2,3,7,8-TCDD have not been directly identified in humans, recent analytic data from feces samples from an individual in a self-dosing experiment suggest that humans can slowly metabolize 2,3,7,8-TCDD (Wendling and Orth, 1990). Direct intestinal excretion of the parent compound is another route for excretion of 2,3,7,8-TCDD and related compounds that is not regulated by metabolism.

Some investigators have questioned whether the parent compound or metabolites are responsible for dioxin toxicity. Structure-activity studies of 2,3,7,8-TCDD and related compounds support the widely accepted principle that the parent compound is the active species, and the relative lack of biological activity of readily excreted monohydroxylated metabolites of 2,3,7,8-TCDD and 3,3′,4,4′-TCB suggests that metabolism is a detoxification process necessary for the biliary and urinary excretion of these compounds. This concept has also been generally applied to 2,3,7,8-TCDD-related compounds, although data are lacking on the structure and toxicity of metabolites of other CDDs, BDDs, CDFs, BDFs, PCBs, and PBBs. It is still possible, however quite unlikely, that low levels of unextractable and/or unidentified metabolites may contribute to one or more of the toxic responses of 2,3,7,8-TCDD and related compounds.

Physiologically based pharmacokinetic (PB-PK) models have been developed for 2,3,7,8-TCDD in mice, rats, and humans. PB-PK models incorporate known or estimated anatomical, physiological, and physicochemical parameters to describe quantitatively the disposition of a chemical in a given species. PB-PK models can assist in the extrapolation of high-to-low dose kinetics within a species, estimating exposures by different routes of administration, calculating effective doses, and extrapolating these values across species. These models are particularly important given the limited empirical data on individual dioxin-like congeners.

Chapter 8 contains a review of biologically based models of dioxin pharmacokinetics. The early studies in rodents have recently been extended to describe protein induction and tissue distribution data in the mouse (Leung et al., 1990b) and rat (Leung et al., 1990a). Andersen et al. (1993) refined the model to include induction of CYP1A1 and diffusion-limited tissue distribution. CYP1A1 is one of a family of proteins involved in the activation and detoxification of both endogenous and exogenous chemicals. The model described by Kedderis et al. (1993) for 2,3,7,8-tetrabromodibenzo-*p*-dioxin extended the use of PBPK models to the brominated congener of TCDD. Portier et al. (1993) modeled the steady-state induction of CYP1A1 and CYP1A2 using Hill equations. Their analysis stressed the importance of the mechanism of endogenous protein expression on the shape of the dose-response curve in the low-dose region. Kohn and Portier (1993) extended this result to a general class of models and discussed implications of these models for risk assessment. Kohn et al. (1993) used approaches to describe tissue dosimetry of TCDD and additionally incorporated dioxin-mediated effects on growth factors, induction of the Ah-receptor, and several models for endogenous induction of CYP1A1, CYP1A2, and the EGF receptor. Other models have been proposed recently to describe effects of TCDD on lipid metabolism (Roth et al., 1993).

An empirical dose-dependent model by Carrier (1991) relates the varying fraction of the body burden of TCDD associated with the liver in humans to the total body burden of TCDD. This model is consistent with the animal results described by the PB-PK models of Andersen et al. (1993) and Kohn et al. (1993).

Our uncertainty in the validity of predictions from PB-PK models is primarily driven by the limited availability of congener and species-specific data that accurately describe the dose- and time-dependent disposition of 2,3,7,8-TCDD and related compounds. As additional data become available, particularly on the dose-dependent disposition of these compounds, more accurate models can be developed. In developing a suitable model in the human, it is also important to consider that the half-life estimate of 7.1 years for 2,3,7,8-TCDD was based on two serum values taken 5 years apart, with the assumption of a single compartment, and assuming a first-order elimination process (Pirkle et al., 1989). It is likely that the excretion of 2,3,7,8-TCDD in humans is more complex, involving several compartments, tissue-specific binding proteins, and a continuous daily background exposure. Furthermore, changes in body weight and body composition should also be considered in developing PB-PK models for 2,3,7,8-TCDD and related compounds in humans. Data contained in the recently reported, expanded study of half-life of 2,3,7,8-TCDD in Ranch Hand Veterans (Wolfe et al., 1994) and additional follow-up studies using blood level information from the 1992-1993 physical examination should allow for better estimates of TCDD half-life, provide important additional data to evaluate whether TCDD follows single-compartment, first-order kinetics, and provide additional information with which to study the influence of percent body fat on TCDD elimination in these veterans.

It is known that exposure occurs to the developing fetus through placental transfer of dioxin-like compounds in maternal blood via the placenta. In addition, exposure is likely to increase in the early postnatal period through intake of mother's milk containing dioxin-like compounds. Redistribution of body burdens is likely to occur with growth and development, depending on relative intakes and changes in body fat content. Fasting, aging, and disease are all thought to alter steady-state levels of dioxin during life. These changes complicate standard pharmacokinetic models and present the possibility for temporary but potentially important increases in blood or tissue levels of dioxin-like compounds during critical periods of development, growth, and aging. Additional data on both distribution and dose to target organs and response to the tissue-specific dose in relation to development and growth will be required to refine our perspectives on the importance of these issues in evaluating dioxin hazards and risks.

An understanding of the relationship between exposure and dose is an important aspect of an adequate characterization of risk. The data base relating to this issue is extensive for 2,3,7,8-TCDD but is lacking for many of the related compounds. Nonetheless, evaluation of available data and the development of physiologically based models has led to a better understanding of the disposition and pharmacokinetics of dioxin and related compounds than for most other environmental chemicals. This is particularly important because this characterization relies extensively on estimates of body burden, which is a function of the uptake, distribution, metabolism, and excretion of this complex mixture of structurally related compounds. Estimates of half-life in the body facilitate the understanding of bioaccumulation as a function of intake over a lifetime and of the impact of incremental exposures on blood or tissue levels both over the short and long term. In addition, these estimates allow some estimation of historical body burdens to complement effects analysis in human populations presumed to have high exposures in earlier decades.

9.6. MECHANISMS OF DIOXIN ACTION

Knowledge of the mechanisms of dioxin action may facilitate the risk assessment process by imposing bounds on the assumptions and models used to describe possible responses to exposure to dioxin. In this document, the relatively extensive data base on dioxin action has been reviewed, with emphasis on the contribution of the specific cellular receptor for dioxin and related compounds, the Ah receptor, to the mechanism(s) of action. Other reviews referenced in Chapter 2 provide additional background on the subject. Discussion in this chapter will focus on aspects of our understanding of mechanism(s) of dioxin action that are particularly important in understanding and characterizing dioxin risk, including:

- Similarities at the biochemical level between humans and other animals with regard to receptor structure and function;
- Relationship of receptor binding to toxic effects; and
- Role that the purported mechanism(s) of action might contribute to the diversity of biological response seen in animals and, to some extent, in humans.

The remarkable potency of TCDD in eliciting its toxic effects in animals suggested the possible existence of a receptor for dioxin. Biochemical and genetic evidence implicates the TCDD-receptor in the biological responses to dioxin-like compounds. Electrophoretic studies to evaluate the properties of specific proteins from inbred mouse strains reveal the existence of several forms of the TCDD-binding protein. These observations imply the existence of multiple alleles at the Ah locus in mice. The biochemical properties of the different forms of the Ah receptor remain to be described. In particular, the extent to which the different receptor forms affect the sensitivity to TCDD is not known.

Human cells contain an intracellular protein whose properties resemble those of the Ah receptor in animals. Binding studies and hydrodynamic analyses have identified an Ah receptor-like protein(s) in a variety of human tissues. Functional Ah receptors have been found in many human tissues, including lymphocytes, liver, lung, and placenta. By analogy with the existence of multiple receptor forms in mice, it is reasonable to anticipate that the human population will also be polymorphic with respect to Ah receptor structure and function. Therefore, it is also reasonable to expect that humans may differ from one another in their susceptibilities to TCDD. The binding and hydrodynamic properties of the Ah receptor differ relatively little across species and tissues yet responses vary widely; it is impossible, therefore, to account for the diversity of TCDD's biological effects by characteristics of the receptor alone.

TCDD acts via an intracellular protein (the Ah receptor), which is a ligand-dependent transcription factor that functions in partnership with a second protein (known as Arnt); therefore, from a mechanistic standpoint, TCDD's adverse effects appear likely to reflect sustained alterations in gene expression. Mechanistic studies also indicate that several proteins contribute to TCDD's gene regulatory effects and that the response to TCDD probably involves a relatively complex interplay between multiple genetic and environmental factors. Such mechanistic information imposes constraints on the possible models that can plausibly account for TCDD's biological effects and, therefore, on the assumptions used during the risk assessment process. Mechanistic knowledge of dioxin action may also be useful in other ways. For example, knowledge of genetic polymorphisms that influence TCDD responsiveness may allow the identification of individuals at particular risk from

exposure to dioxin. In addition, mechanistic knowledge of the biochemical pathways that are altered by TCDD may identify novel targets for the development of drugs that can antagonize dioxin's adverse effects.

As described below, biochemical and genetic analyses of the mechanism by which dioxin induces CYP1A1 gene transcription have revealed the outline of a novel regulatory system whereby a chemical signal can alter the expression of specific mammalian genes. The evidence to date implies that the Ah receptor participates in every biological response to TCDD. For example, studies of structure-activity relationships among congeners of TCDD reveal a correlation between a compound's specific binding affinity and its potency in eliciting biochemical responses, such as enzyme induction. Furthermore, inbred mouse strains in which TCDD binds with lower affinity to the receptor exhibit decreased sensitivity to dioxin's biological effects, such as thymic involution, cleft palate formation, and hepatic porphyria. While there are a few investigators who believe that dioxin may act directly on specific cellular and biological processes without Ah-receptor mediation, the majority of investigators believe that most, if not all, biological responses to dioxin and related compounds are Ah-receptor mediated. A simplified diagram of this hypothesis is presented in Figure 9-2. This hypothesis predicts that TCDD will be found to activate the transcription of other genes via a receptor- and enhancer-dependent mechanism analogous to that described for the cytochrome P4501A1 (CYP1A1) gene.

Compensatory changes, which occur in response to TCDD's primary effects, can complicate the analysis of dioxin action in intact animals. For example, TCDD can produce changes in the levels of steroid hormones, peptide growth factors, and/or their cognate cellular receptors. In turn, such alterations have the potential to produce a series of subsequent biological effects, which are not directly mediated by the Ah receptor. Furthermore, the hormonal status of an animal appears to influence its susceptibility to the hepatocarcinogenic effects of TCDD (Lucier et al., 1991). Likewise, exposure to other chemicals can alter the developmental toxicity of TCDD (Couture et al., 1990). Therefore, in some cases, TCDD may act in combination with other chemicals to produce its biological effects. Such phenomena increase the difficulty of analyzing dioxin action in intact animals

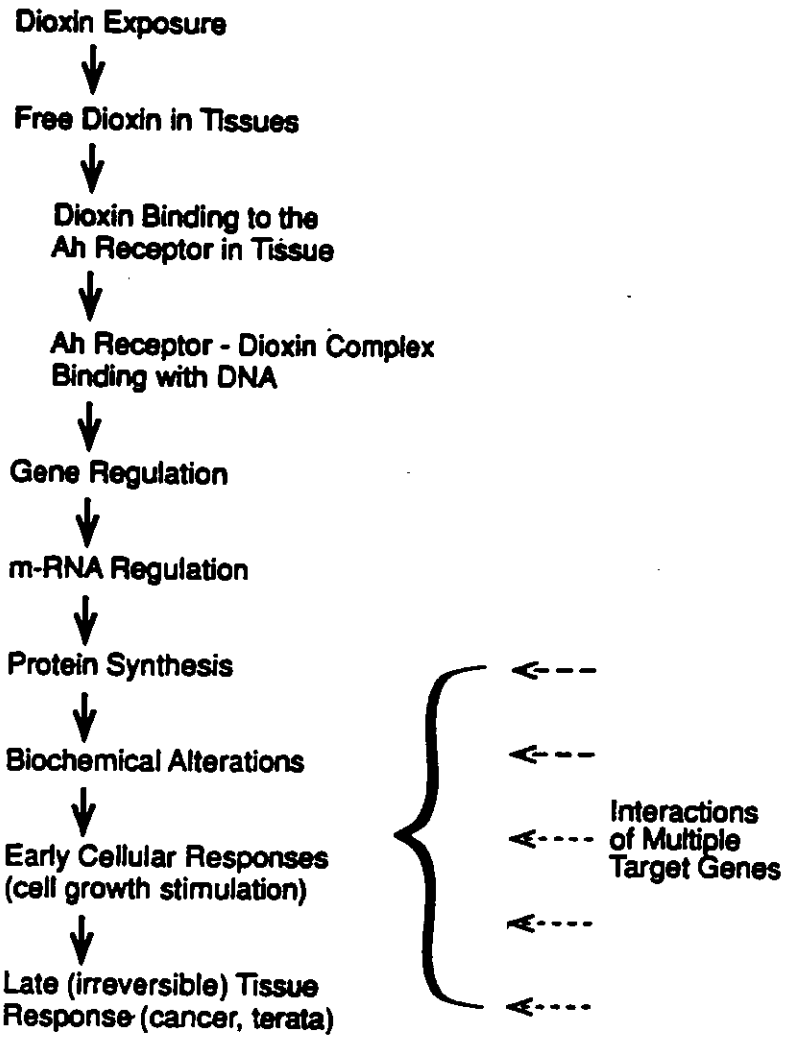

Figure 9-2. Schematic representation of the complex sequence of molecular and biological events involved in dioxin-mediated toxicants.

and increase the complexity of risk assessment, given that humans are routinely exposed to a wide variety of chemicals.

The fact that TCDD may induce a cascade of biochemical changes in the intact animal raises the possibility that dioxin might produce a response such as cancer by mechanisms that differ among tissues. These mechanisms are discussed in detail in Chapter 8, along with the supporting biological data and dose-response models. One possible mechanism discussed in Chapter 8 is that TCDD might activate a gene(s) that is directly involved in tissue proliferation. A second mechanism involves TCDD-induced changes in hormone metabolism, which may lead to tissue proliferation secondary to increased secretion of a trophic hormone, and/or to changes in metabolism, which might lead to indirect mutagenic effects. Thus, while this reassessment has identified a number of hypothetical mechanisms for cancer induction by TCDD, there remains considerable uncertainty about which mechanisms occur, with what levels of sensitivity, and in which species. Advances in knowledge regarding the role of such activities in dioxin toxicity will facilitate the development of more definitive biologically based models of dioxin action.

Under some circumstances, TCDD can protect against the carcinogenic effects of polycyclic aromatic hydrocarbons in mouse skin; this may reflect the induction of detoxifying enzymes by dioxin (Cohen et al., 1979; DiGiovanni et al., 1980). In other situations, TCDD-induced changes in hormone metabolism may alter the growth of hormone-dependent tumor cells, producing a potential anticarcinogenic effect (Spink et al., 1990). There is considerable uncertainty about the magnitude and importance of these effects in relation to both dose and response characteristics of dioxins in various species. Nonetheless, these (and perhaps other) effects of TCDD complicate the risk assessment process for dioxin.

A substantial body of biochemical and genetic evidence indicates that the Ah receptor mediates the biological effects of TCDD. This evidence implies that a response to dioxin requires the formation of ligand-receptor complexes. TCDD-receptor binding appears to obey the law of mass action and, therefore, depends on (1) the concentration of ligand in the target cell; (2) the concentration of receptor in the target cell; and (3) the binding affinity of the ligand for the receptor. In principle, some TCDD-receptor complexes will form even at very low levels of dioxin exposure. However, in practice, at some finite concentration of

TCDD, the formation of TCDD-receptor complexes may be insufficient to elicit detectable effects. Furthermore, biological events subsequent to TCDD-receptor binding may or may not exhibit a linear response to dioxin. However, recent studies in several laboratories have indicated no evidence of a threshold for relatively simple responses to dioxin-like compounds such as CYP1A1 induction and others. Further information will be required to determine if other responses to dioxin-like compounds requiring gene transcription will also demonstrate low-dose linear behavior.

While much of our understanding of TCDD impacts on genetic activity is derived from studies on liver, studies of other tissues (e.g., skin, thymus) are likely to reveal additional TCDD-responsive genes, which exhibit tissue-specific expression (Sutter et al., 1991). Analyses of the mechanism of dioxin action in such systems appear likely to reveal additional factors that influence the susceptibility of a particular tissue to TCDD. In addition, studies of other TCDD-inducible genes, such as glutathione-S-transferase, quinone reductase, and aldehyde dehydrogenase, may reveal whether differences in enhancer structure, receptor-enhancer interactions, or promoter structure affect the responsiveness of the target gene to TCDD (Whitlock, 1990).

Based on our understanding of dioxin mechanism(s) to date, it is accurate to say that interaction with the Ah receptor is necessary, that humans are likely to be sensitive to the effects of dioxin, and that there is likely to be a variation between and within species and between tissue in individual species based on differential responses to receptor binding. Although threshold mechanisms may exist for some of these responses, thresholds have yet to be demonstrated. Further analyses of dioxin action may provide more insight into the mechanisms by that TCDD and related compounds produce immunological effects, reproductive and/or developmental effects or cancer, effects which are of particular public health concern. A major challenge for the future will be the establishment of experimental systems in which such complex biological phenomena are amenable to study at the molecular level.

9.7. TOXIC EFFECTS OF DIOXIN

9.7.1. General Comments

It is clear from the evaluation of the toxicologic literature that dioxin and related compounds have the ability to produce a wide spectrum of responses in animals and, presumably, in humans, if the dose is high enough (Table 9-2). Relatively few chronic effects related to exposure to dioxin-like compounds have been observed in humans. The epidemiologic data are limited due to a number of possible factors: the absence of many, specific individual measurements of dioxin exposure for the general population; a limited number of cross-sectional and prospective studies of more highly exposed populations; the limited ability of epidemiologic studies to detect significant differences between exposed and relatively unexposed populations when the outcomes are relatively rare, the exposures are low, and the population under study is small; and the difficulty in quantifying the impact of all potentially confounding exposures. Evaluation of hazard and risk for dioxin and related compounds must rely on a weight-of-the-evidence approach in which all available data (animal and human) are examined together. This process often requires extrapolation of effects across various animal species as well as to humans.

The reliability of using animal data to estimate human hazard and risk has often been questioned for this class of compounds. Although human data are limited, evidence suggests that animal models are appropriate for estimating human risk if all available data are considered. As discussed in detail in Chapters 2 and 8, humans have a fully functional Ah receptor and both in vivo and in vitro studies demonstrate comparability of biochemical responses in humans and animals (see also Table 8-5). When comparing species and strains for their responses to these compounds, a wide range of sensitivity to TCDD-induced toxicities has been noted. Qualitatively speaking, however, almost every response can be produced in every species if the appropriate dose is administered. Although outliers, i.e., species that are either very sensitive or refractory, can be identified for a particular response, no species is consistently sensitive or refractory for all effects. In addition, sensitivity for a given effect among the majority of species clusters within approximately one order of magnitude (factor of 10). Therefore, despite a range of sensitivities across species, it is reasonable to assume that humans will not be refractory to all effects nor that they will be as

Table 9-2. Effects of TCDD and Related Compounds in Different Animal Species

Effect	Human	Monkey	Guinea Pig	Rat	Mouse	Hamster	Cow	Rabbit	Chicken	Fish
Presence of AhR	+	+	+	+	+	+	+	+	+	+
Binding of TCDD: AhR Complex to the DRE (enhancer)	+		+	+	+	+	+	+	+	+
Enzyme induction	+		+	+	+	+		+	+	+
Acute lethality	0	+	+	+	+	+		+	+	+
Wasting syndrome		+	+	+	+	+		+		
Teratogenesis/fetal toxicity, mortality	+/-	+	+	+	+	+		+	+	+
Endocrine effects	+/-	+		+	+					
Immunotoxicity	+/-	+	+	+	+	+	+		+	
Carcinogenicity	+/-			+	+	+				+
Chloracnegenic effects	+	+			+			+		
Porphyria		0	0	+	+	0			+	
Hepatotoxicity		+	+/-	+	+	+/-	+	+	+	
Edema		+	0	0	+	+			+	+
Testicular atrophy		+	+	+	+					
Bone marrow hypoplasia		+	+		+/-				+	

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

sensitive as the most sensitive responder for each effect. Humans are likely, because of interindividual variability in response to a variety of toxic chemicals, which is generally greater than that found in individual species of laboratory animals, to show a wide range of sensitivities for various dioxin-induced toxicities. For purposes of the current assessment, therefore, unless there are data to identify a particular species as being representative of humans for a particular effect, average humans can be reasonably assumed to be of average sensitivity for various effects, recognizing that individuals in the population might vary widely in their sensitivity to individual effects. The uncertainty introduced by this assumption, i.e., that, on average, humans will respond as do average animal models for individual effects of exposure to dioxin-like compounds and that an unknown range of variability exists in the human population for individual effects, should be carefully considered as results of this characterization are applied to individuals or specific subpopulations.

9.7.2. Chloracne

Chloracne and associated dermatologic changes are widely recognized responses to TCDD and other dioxin-like compounds in humans. Chloracne is a severe acne-like condition that develops within months of first exposure to high levels of dioxin. For many individuals, the condition disappears after discontinuation of exposure, despite 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 over 40 years have been noted. There are very little human data from which to determine definitively the doses at which chloracne is likely to occur. Data from occupational studies suggest that persistent chloracne is more often associated with exposures of high intensity, for long duration, and commencing at an early age. Acute exposures or chronic lower level exposures, if resulting in chloracne, have generally resulted in a condition that resolves itself in a matter of months to a few years. Details of chloracnegenic response in occupationally exposed humans are described in detail in Chapter 7 of the Health Assessment Document.

Induction of chloracne in humans after exposure to dioxin and related compounds is supported by studies in laboratory animals. Rabbits, monkeys, and hairless mice have all proved useful in investigating this response. In addition, cellular systems provide a research tool in elucidating the chloracne response at the cellular level. Keratinocytes, the principal cell type in the epidermis, have been used as an in vitro model for studies of TCDD-induced hyperkeratosis, a feature of chloracne, in human- and animal-derived cell cultures. The response in these systems is analogous to the hyperkeratinization observed in vivo as a part of chloracne.

There is little doubt that chloracne is a human condition often attributable to exposure to dioxin and related compounds. The specific risk factors associated with this response are still obscure. Recognition of chloracne has been associated with high-level exposure to these compounds, and as such, may represent a biomarker of exposure. Because of the wide variability of the chloracnegenic response in humans and its varied persistence, however, the absence of chloracne is not a reliable indicator of low exposure to dioxin and related compounds.

9.7.3. Carcinogenicity

Since the last EPA review of the human data base relating to the carcinogenicity of TCDD and related compounds in 1988, several new follow-up mortality studies have been completed. Among the most important of these are a study of 5,172 workers by Fingerhut et al. (1991), a study with 1,583 workers by Manz et al. (1991), a smaller study of 247 workers by Zober et al. (1990), and a study of over 18,000 workers by Saracci et al. (1991). Although uncertainty remains in interpreting these studies because not all potential confounders have been ruled out and coincident exposures to other carcinogens is likely, all provide support for an association between exposure to dioxin and related compounds and increased cancer mortality. With the exception of the study by Saracci et al. (1991), these studies have some exposure information that permits an assessment of dose response. These data have in fact served as the basis for fitting the additive and multiplicative risk models in Chapter 8. In addition, more limited results have been presented recently on the Seveso cohort (Bertazzi et al., 1993) and on women exposed to chlorophenoxy herbicides,

chlorophenols, and dioxins (Kogevinas et al., 1993). While these two studies have methodologic shortcomings that are described in Chapter 7, they provide findings, particularly for exposure to women, that warrant additional follow-up.

While the data base from epidemiologic studies remains controversial, it is the view of this reassessment that this body of evidence supports the laboratory data indicating that TCDD probably increases cancer mortality of several types. Although not all confounders were ruled out in any one study, positive associations between surrogates of dioxin exposure, either length of occupational exposure or proximity to a known source combined with some information on body burden, and cancer have been reported. These data alone suggest a role for dioxin exposure to contribute to a carcinogenic response but do not confirm a causal relationship between exposure to dioxin and increased cancer incidence. Available human studies alone cannot demonstrate whether a cause and effect relationship between dioxin exposure and increased incidence of cancer exists. Therefore, evaluation of cancer hazard in humans must include an evaluation of all of the available animal and in vitro data as well as the data from exposed human populations.

The Peer Panel that met in September 1993 to review an earlier draft of the cancer epidemiology chapter suggested that the epidemiology data alone were still not adequate to implicate dioxin and related compounds as "known" human carcinogens but that the results from the human studies were largely consistent with observations from laboratory studies of dioxin-induced cancer and, therefore, should not be dismissed or ignored. Other scientists, including those who attended the Peer Panel meeting, felt either more or less strongly about the weight of the evidence from epidemiology studies, representing the range of opinion that still exists on the interpretation of the cancer epidemiology studies.

Many of the earlier epidemiological studies that suggested an association with soft tissue sarcoma were criticized for a variety of reasons. Nonetheless, the incidence of soft tissue sarcoma is elevated in several of the recent studies, supporting the findings from previous studies. The fact that similar results were obtained in independent studies of differing design and evaluating populations exposed to dioxin-like compounds under varying conditions, along with the rarity of this tumor type, weighs in favor of a consistent and real association. On the other hand, arguments regarding selection bias, differential exposure

misclassification, confounding, and chance in each individual study have been presented in the scientific literature which increase uncertainty around this association. In addition, excess respiratory cancer was noted by Fingerhut, Zober, and Manz. 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 PCDFs and PCBs occurred. Again, while smoking as a confounder cannot be totally eliminated as a potential explanation of these results, analyses conducted to date suggest that smoking is not likely to explain the entire increase in lung cancer. The question of confounding exposures, such as 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, stomach cancer) have been reported, the data for an association with exposure to dioxin-like chemicals are less compelling.

The comparison of the results of different investigations that examine the outcome of similar exposures must always be evaluated in light of factors that may influence the outcome of the study. A few of these factors include study design, potential confounding factors and exposures (extraneous factors or exposures that relate to both outcome and exposure such as age), biases that affect the selection and participation of the study population, differential exposure misclassification, variation in age of the study population, different conditions of exposure (mode, intensity, duration, and route), and differences in methods used to assess outcomes of interest. Such differences may result in some variation in the results of the compared studies. Given that the studies are well conducted and the variations noted, what is important is the within-study consistency of the results.

What emerges from an analysis of the epidemiology data is a view of dioxin-like compounds as potentially multisite carcinogens in more highly exposed human populations that have been studied, consisting primarily of adult males. There are currently very few data for women and children exposed to dioxin-like compounds. Although uncertainty in this view remains, the cancer findings are generally consistent with results from studies of laboratory animals and appear to be plausible given what is known about mechanisms of dioxin action.

While both past and more recent human studies have focused on males, there are some limited data suggesting carcinogenic responses associated with dioxin exposure in females. Because both laboratory animal data and mechanistic inferences suggest that males and females may respond differently to dioxin-like activity, further data will be needed to address this question of differential response.

An extensive data base on the carcinogenicity of dioxin and related compounds in laboratory studies exists and is described in detail in 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 rats and mice. All studies have produced positive results, leading to the conclusions that TCDD is a multistage carcinogen increasing 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, which are relatively resistant to the lethal effects of TCDD. Recent data have also shown TCDD to be a liver carcinogen in the small fish, *Medaka* (Johnson et al., 1992). Few attempts have been made to demonstrate the carcinogenicity of other dioxin-like compounds. Other than a mixture of two isomers of hexachlorodibenzodioxin (HCDDs), which produced liver tumors in both sexes of rats and mice (NTP, 1980), the more highly chlorinated CDDs and CDFs have not been studied in long-term animal cancer bioassays. However, it is generally recognized that these compounds bioaccumulate and exhibit toxicities similar to TCDD and are, therefore, also likely to be carcinogens (U.S. EPA Science Advisory Board, 1989).

In addition to the demonstration of TCDD as a complete carcinogen in long-term cancer bioassays, a number of dioxin-like PCDDs and PCDFs, as well as several PCBs, have also been demonstrated to be tumor promoters in two-stage (initiation-promotion) protocols in rodent liver and skin. In addition, a recent study has demonstrated the ability of TCDD to neoplastically transform immortalized human cells in culture at very low concentrations of TCDD. While dioxin and related compounds are not generally considered to be "genotoxic" in traditional terms, both empirical data and the results of modeling efforts suggest that they may be functioning indirectly to produce irreversible genetic changes in exposed cells. All

of these data add substantially to the weight of the evidence that dioxin and related compounds are likely to be carcinogenic, at least under some circumstances, in humans.

Despite the relatively large number of bioassays on TCDD, the study of Kociba et al. (1978) and those of the NTP (1982), because of their multiple dose groups and wide dose range, continue to be the focus of additional review. Sauer (1990) re-evaluated 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). While this finding did not change the determination of carcinogenic hazard since TCDD induced tumors in multiple sites in this study, it does have an effect on evaluation of dose-response and on estimates of risk at low doses. These issues will be discussed in a later section of this chapter.

One of the more interesting findings in the Kociba bioassay was reduced tumor incidences of the pituitary, uterus, mammary gland, pancreas, and adrenals. These findings, coupled with the sex specificity of the TCDD-induced liver tumors in rats, emphasize that the carcinogenic actions of TCDD involve a complex interaction of hormonal factors. Moreover, it is hypothesized that cell-specific factors modulate TCDD/hormone actions relevant to cancer. The findings of reduced tumor incidence in certain tissues suggest that dioxin exposure may be exerting an anticarcinogenic effect under certain circumstances or in certain tissues. The complex interplay between dioxin and hormones in terms of both carcinogenic and anticarcinogenic responses will continue to be a matter of hypothesis until specific data to address these issues are obtained.

In summary, publication of additional studies of human populations exposed to dioxin and related compounds since the last EPA assessment (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990; Saracci et al., 1991; Bertazzi et al., 1993; Kogevinas et al., 1993) has strengthened the inference, based on all the evidence from mechanistic, animal, and epidemiologic studies, that these compounds are appropriately characterized as probable human carcinogens. While the data for 2,3,7,8-TCDD are particularly comprehensive, the data on other congeners remain limited. This puts added emphasis on the assumptions and inferences regarding toxicity equivalence in evaluating complex exposures to dioxin and related compounds with regard to carcinogenicity. The evolving understanding of the

complex interplay between dioxin-like compounds and hormones and other modulators of cell growth and differentiation continues to complicate more precise determinations of cancer hazard and risk.

9.7.4. Reproductive and Developmental Effects

The potential for dioxins and related compounds to cause reproductive and developmental toxicity in animals has been recognized for many years, and the data base regarding these effects is analyzed in Chapter 5. Recent laboratory studies have suggested that altered development may be among the most sensitive TCDD end points in laboratory animal systems although the likelihood and level of response in humans are much less clear. Although the discussion of these effects in Chapter 5 is divided into developmental toxicity and male and female reproductive toxicity, it is important to recognize the interrelatedness of developmental and reproductive events at all levels of biological complexity. This point is critical for understanding and fully characterizing the hazards and risks of dioxin and related compounds. For example, effects of TCDD on circulating levels of sex hormones and/or on responsiveness to sex hormones in laboratory animals or humans may be translated into reproductive dysfunction if exposure occurs in adulthood as well as abnormal development and/or reproductive dysfunction if exposure occurs prenatally. Therefore, a similar effect of dioxin-like compounds may be manifest as a reproductive end point if exposure occurs to adults or as a developmental and/or a reproductive end point if exposure occurs to the fetus. Likewise, even though effects on organ structure and on growth are considered separate developmental end points that are associated with pre- and postnatal exposure to TCDD in laboratory animals, they are interrelated because effects on prenatal growth can significantly disrupt the structural integrity of an organ system. It is important to note that adverse developmental effects are a complex set of end points, many of which are caused by multiple factors, requiring coincidence of a number of events.

In the current data base, developmental toxicity end points have been observed at lower TCDD exposure levels than male and female reproductive toxicity end points in a number of animal systems. The lowest effective TCDD egg burden for causing developmental toxicity in fish and birds and the lowest effective maternal TCDD body

burden for producing a wide range of developmental responses in mammals are summarized in Chapter 5. Of particular interest to the risk assessment process is the fact that a wide variety of developmental events, crossing three vertebrate classes and several species within each class, can be perturbed, suggesting that dioxin has the potential to disrupt a large number of critical developmental events at specific developmental stages. Not only can these changes lead to increases in embryo/fetal mortality, but they can disrupt organ system structure and irreversibly impair organ function.

The laboratory studies demonstrating adverse health effects from prenatal exposures often involved a single dose administered at a discrete time during pregnancy. The doses that produced adverse effects, such as reproductive and developmental toxicity, can be related to longer term body burdens produced by the single dose or to background body burdens. Because the production of prenatal effects often requires exposures to occur during certain critical times during fetal development, the uncertainties in the relationship with steady-state body burdens must be carefully assessed. A single dose may cause a spike in both maternal and fetal blood concentration related to the magnitude of the dose, and the concentrations will fall rapidly as the dioxin-like compounds are redistributed to adipose and other tissues. Application of pharmacokinetic models described earlier in this chapter to estimate blood concentrations 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.

Because developmental toxicity following exposure to TCDD-like congeners occurs in fish, birds, and mammals, it is likely to occur at some level in humans. It is not currently possible to state exactly how or at what levels humans in the population will respond with adverse impacts on development or reproductive function. Data analyzed in Chapter 5 and Chapter 7 suggest, however, that adverse effects may be occurring at levels lower than originally thought to represent a no observed adverse effect level (NOAEL) in animals. Traditional toxicology studies had led to the conclusion that the NOAEL was in the range of intake values of 1 ng TEQ/kg/day. Current data suggest that the NOAEL in animals should be lower. This issue will be discussed further in the dose-response section of this chapter.

While human data on potential developmental effects of dioxin-like compounds are limited, developmental effects in human infants exposed to a complex mixture of PCBs, CDFs, and PCQs in the Yusho and Yu-Cheng poisoning episodes were probably caused by the combined exposure to those PCB and CDF congeners that are Ah-receptor agonists. However, it should be noted that not all effects that are seen are attributable only to dioxin-like compounds. Similarity of the effects observed in human infants prenatally exposed to this complex mixture with those reported in adult monkeys exposed only to TCDD increases the probability 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. Most significant is a clustering of effects in organs derived from the ectodermal germ layer, a syndrome referred to as ectodermal dysplasia. Included in this syndrome are effects on the skin, nails, and meibomian glands that occur in both adult monkeys exposed to TCDD and in Yusho and Yu-Cheng infants exposed transplacentally to PCB, CDF, and PCQ contaminated rice oils. In addition, accelerated tooth eruption has been reported both in human infants affected by the Yusho and Yu-Cheng exposures and in neonatal mice exposed to TCDD. Yu-Cheng children exposed transplacentally to PCB, CDF, and PCQ contaminated rice oil have also exhibited developmental and psychomotor delay during developmental and cognitive tests (Chen et al., 1992). Some investigators believe that, because these effects do not correlate with TEQ, the effects are exclusively due to nondioxin-like PCBs or a combination of all congeners. However, monkeys pre- and postnatally exposed to TCDD are also affected by a deficit in cognitive function. Recent studies presented at Dioxin '93 (Hsu et al., 1993; Lai et al., 1993) have demonstrated that these effects persist throughout childhood, as does the growth retardation (Guo et al., 1994). The concept that the ectodermal dysplasia syndrome in Yusho and Yu-Cheng infants may be caused by the combination of PCB and CDF congeners in the rice oil that are Ah receptor agonists but are less potent than TCDD is consistent with structure-activity results for various developmental end points in different species of fish, birds, and mammals.

In mammals, postnatal functional alterations involving learning behavior and the developing reproductive system appear to be the developmental events most sensitive to prenatal dioxin exposure. The developing immune system may also be highly sensitive.

Alterations in structures and diminished prenatal viability and growth begin to predominate at maternal TCDD body burdens and/or daily TCDD doses during gestation that are above 100 ng/kg in virtually every species tested. These doses of TCDD are not maternally toxic. Higher dose levels can be demonstrated to result in prenatal mortality. A general finding in fish, bird, and mammalian species is that the embryo or fetus is more sensitive to TCDD-induced mortality than the adult. Thus, the timing of TCDD exposure during the life history of an animal can greatly influence its susceptibility to overt dioxin toxicity.

With respect to male and female reproductive end points, there are clear effects following dioxin exposure of the adult animal. Such reproductive effects generally occur at TCDD body burdens that are higher than those required to cause the more sensitive developmental end points. For example, TCDD exposure of the adult male rodent causes reduced testis and accessory sex organ weights, abnormal testis structure, decreased spermatogenesis, reduced fertility, decreased testicular testosterone synthesis, reduced plasma androgen concentrations, and altered regulation of pituitary LH secretion. However, in laboratory animal studies, these effects are detectable only at TCDD exposure levels that are overtly toxic to the animal. In the more limited studies focusing on female reproduction, the primary effects include decreased fertility, inability to maintain pregnancy, and in the rat, decreased litter size. Signs of ovarian dysfunction and alterations in hormone levels have also been reported.

Exposure of female mice and rats to TCDD has an antiestrogenic effect on the uterus. The dose of TCDD required to produce this response is generally higher than that needed to cause the most sensitive signs of developmental toxicity in these species. More specifically, hydronephrosis and cleft palate in mice and reductions in spermatogenesis in rats occur at maternal doses of TCDD that are far less than those needed to exert a demonstrable antiestrogenic effect when adult female mice and rats are exposed to dioxin. The precise mechanism of TCDD's antiestrogenic effect is not fully understood. It may be caused by both a decrease in available estrogen receptor number and/or by an increase in cytochrome P-4501A-mediated estrogen metabolism within the target cell.

These studies indicate that while there is variability between species in the profile of developmental responses elicited by TCDD, essentially all dioxin-like PCB, CDD, and CDF

congeners that have Ah receptor affinity and intrinsic activity produce the same pattern of developmental effects within a given vertebrate species if a sufficiently high dose of the congener is given. Data to support these conclusions regarding reproductive and developmental hazards of dioxin and related compounds continue to accumulate, but the weight of the evidence is still a subject of much scientific debate.

9.7.5. Immunotoxicity

Concern over the potential toxic effects of chemicals on the immune system arises from the critical role that the immune system plays in maintaining health. It is well recognized that suppressed immunological function can result in increased incidence and severity of infectious diseases as well as some types of cancer. Conversely, the inappropriate enhancement of immune function or the generation of misdirected immune responses may precipitate or exacerbate the development of allergic and autoimmune diseases. Thus, suppression as well as enhancement of immune function are considered to represent potential immunotoxic effects of chemicals.

Extensive evidence has accumulated over the past 20 years to demonstrate that the immune system is a target for toxicity of TCDD and structurally related compounds, including PCDDs, PCDFs, PCBs, and PBBs. This evidence is described in detail in Chapter 4. The evidence has derived from numerous studies in various animal species, primarily rodents, but also guinea pigs, rabbits, monkeys, marmosets, and cattle. Epidemiological studies also provide some evidence for the immunotoxicity of dioxin and related compounds in humans. In animal studies, relatively high doses of HAH produce lymphoid tissue depletion, except in the thymus where cellular depletion occurs at lower doses. Alterations in specific immune effector functions and increased susceptibility to infectious disease have been identified at doses of TCDD well below those that cause lymphoid tissue depletion. 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. In addition, in parallel with increased understanding of the cellular and molecular mechanisms involved in immunity, studies on

TCDD are beginning to establish biochemical and molecular mechanisms of TCDD immunotoxicity.

The ability of an animal to resist and/or control viral, bacterial, parasitic, and neoplastic diseases is determined by both nonspecific and specific immunological functions. Decreased functional activity in any immunological compartment may result in increased susceptibility to infectious and neoplastic diseases. In terms of risk assessment, host resistance is often accorded the "bottom line" in terms of relevant immunotoxic end points. Animal host resistance models that mimic human disease are available and have been used to assess the effect of TCDD on altered host resistance. Results from host resistance studies provide evidence that exposure to TCDD results in increased susceptibility to bacterial, viral, parasitic, and neoplastic diseases. These effects are observed at relatively low doses and likely result from TCDD-induced suppression of immunological function. The specific immunological functions targeted by TCDD in each of the host resistance models remain to be fully defined.

Despite considerable investigation, the cells that are altered by TCDD exposure, leading to suppressed immune function, have not been unequivocally identified. Direct in vitro effects of TCDD on purified B cell activity have been reported, while direct effects on macrophages and T cells in vitro have not been described. The in vitro effects of TCDD on lymphocytes, however, appear to be influenced by cell culture conditions, which may explain the discrepancies in effects observed in different laboratories. Although the direct effects of TCDD on T cells in vitro have not been demonstrated, it is clear that functional T cell responses generated in vivo are compromised following in vivo exposure. TCDD may alter immune function by indirect mechanisms. One potentially important indirect mechanism is via effects on the endocrine system. Several endocrine hormones have been shown to regulate immune responses, including glucocorticoids, sex steroids, thyroxine, growth hormone, and prolactin. Importantly, TCDD and other related compounds have been shown to alter the activity of these hormones.

It is important to consider that if an acute exposure to TCDD even temporarily raises the TCDD body burden at the time when an immune response is initiated, there may be a risk of adverse impacts even though the total body burden may indicate a relatively low

average TCDD level. Furthermore, because TCDD alters the normal differentiation of immune system cells, the human embryo may be very susceptible to long-term impairment of immune function from in utero effects of TCDD on developing immune tissue. There are currently no data to directly support this hypothesis. Concern arises as a consequence of inferences derived from an understanding of dioxin action and observations in humans and laboratory animals.

In summary, evidence has accumulated to demonstrate that the immune system is a target for toxicity of TCDD and structurally related compounds. The evidence has derived from numerous studies in various animal species. Animal studies suggest that some immunotoxic responses may be evoked at very low levels of dioxin exposure. Epidemiological studies also provide conflicting evidence for the immunotoxicity of these compounds in humans. Few changes in the immune system in humans associated with dioxin body burdens have been detected when exposed humans have been studied. Both direct and indirect (e.g., hormonally mediated) impacts on the immune system have been hypothesized to be the basis of dioxin immunotoxicity. While there is speculation that the developing immune system may be particularly sensitive to the effects of exposure to dioxin and related compounds, additional research will be needed to support this hypothesis.

9.7.6. Other Effects

A number of other effects of dioxin and related compounds have been discussed in some detail throughout the chapters in this assessment. While they illustrate the wide range of effects produced by this class of compounds, some may be specific to the species in which they are measured and may have limited relevance to the human situation. On the other hand, they may be indicative of the fundamental level at which dioxin produces its biological impact and may represent a continuum of response expected from these fundamental changes. While all may not be adverse effects (some may be adaptive and of neutral consequence), several effects have been noted in human studies or in primates that deserve special mention.

9.7.6.1. *Circulating Reproductive Hormones*

Two cross-sectional epidemiologic studies have detected an association between levels of male reproductive hormones and exposure to TCDD. Decreased testosterone levels were detected in two of the three studies where testosterone was evaluated and luteinizing hormone (LH) was increased in one of the two studies evaluating that end point. The fact that the results are based on a single sample rather than on the currently preferred series of three samples adds to the uncertainty of these findings. Animal data are available to support the plausibility of these findings. The mechanism(s) responsible for this effect are largely unknown, but changes in receptor level or function and hormone metabolism and homeostasis need to be investigated. If these data continue to hold up in future observations, their clinical significance will need to be further evaluated. Follow-up studies are currently under way.

9.7.6.2. *Diabetes and Fasting Serum Glucose Levels*

Epidemiologic evidence has been presented to suggest an increased risk of diabetes and for an elevated prevalence of abnormal fasting serum glucose levels with dioxin exposure. Three studies found that individuals with elevated serum levels of TCDD had a slight but statistically significant or borderline significant increased risk for developing diabetes or having elevated fasting serum glucose. There are virtually no animal data to corroborate these findings although some data have indicated effects of TCDD on glucose metabolism and insulin function. While the findings of a greater prevalence of elevated fasting glucose may presage the development of diabetes, in the NIOSH study of chemical workers, the traditional risk factors for diabetes (age, body mass index or weight, and family history of diabetes) appear substantially more influential than TCDD exposure in the development of the disease.

9.7.6.3. *Enzyme Induction*

One of the best characterized effects of exposure to dioxin-like compounds is the induction of cytochrome P-450 1A1 (CYP1A1). CYP1A1 is one of a family of proteins involved in the activation and detoxification of both endogenous and exogenous chemicals.

Dioxin also increases the activity of a number of other enzymes involved in biotransformation reactions. Increased activity of these enzymes has been implicated mechanistically in the toxic responses seen in animals in response to dioxin-like compounds. For example, it has been hypothesized that increases in UDP-glucuronyltransferases leads to elimination of thyroxine and may lead indirectly to increased thyroid-stimulating hormone synthesis by the pituitary and subsequent hyperplastic and hypertrophic responses by the thyroid. There is speculation that such prolonged stimulation may lead to the thyroid tumors seen in both rats and mice exposed to TCDD. Therefore, while changes in enzyme activity in response to dioxin and related compounds may result in detoxification of certain chemicals, examples exist in experimental animals of changed metabolism leading directly or indirectly to adverse effects, some as severe as cancer. Data to confirm this effect of dioxin and related compounds in humans are not available.

9.7.6.4. *Gamma Glutamyl Transferase (GGT) Activity*

GGT is one of the many hepatic enzymes that are measured in human serum to evaluate liver toxicity. Of these, GGT is the only hepatic enzyme found in a number of human studies to be chronically elevated in adults exposed to high levels of TCDD. The consistency of the findings in a number of studies suggests that the finding may reflect a true effect of exposure but for which the clinical significance is unclear. Long term, pathologic 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. There are few animal data to support these findings.

9.7.6.5. *Endometriosis*

Endometriosis is a serious disorder of the female reproductive system that is of unknown etiology and a major cause of infertility in women. A recent study has determined that chronic exposure to TCDD increases the risk of endometriosis in rhesus monkeys (Reier et al., 1993). The incidence and severity of the disease were dose dependent. Additional studies are under way to further evaluate these observations in rhesus monkeys, and studies are planned to evaluate women exposed to TCDD after the accident at Seveso for any