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America's Choice
Children's Health or Corporate Profit
The American People's Dioxin Report
Technical Support Document
November 1999
Center for Health, Environment and Justice
Falls Church, VA

Dedication

The American People's Dioxin Report is dedicated to the memory of Dr. David P. Rall. David Rall was committed to understanding how the environment affects health. He helped establish the National Institute of Environmental Health Sciences as one of the world's leading research institutions on the health effects caused by low level chronic exposures to chemicals in the environment. His leadership will be sorely missed.

Acknowledgments

The Center For Health, Environment and Justice's Science Director, Stephen Lester, deserves special thanks and recognition for his tireless hours of collecting data and the most recent scientific reports on dioxin and human health which went into this report. He also coordinated and worked with the writers, researchers, and scientific peer review group.

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Peer Reviewers:

Dr. Linda Birnbaum, US Environmental Protection Agency
Dr. Mark Cohen, National Oceanographic and Atmospheric Administration
Dr. Barry Commoner, Queens College
Dr. Lynn Goldman, John Hopkins University School of Public Health
Dr. Philip Landrigan, Mt. Sinai School of Medicine
Dr. George Lucier, National Institute for Environmental Health Sciences
Dr. David Ozonoff, Boston University School of Public Health
Dr. David Rall, retired, former Director, National Toxicology Program.

Preface

This Technical Support Document (TSD) is the third and final section of America's Choice: Children's Health or Corporate Profit - The American People's Dioxin Report. The first section is a summary of the newest scientific research on the health effects of dioxin, detailing how American families are being silently poisoned by dioxin. The second section of the report provides a set of policy recommendations developed by a diverse group of more than 50 people, including grassroots activists who live near dioxin-contaminated sites or dioxin-producing facilities, activists who work on policy development, and scientific researchers. These policy recommendations provide clear workable solutions to eliminating dioxin sources without devastating our economy. The first two sections of the report can be found together in a separate document.

This Technical Support Document provides the scientific basis and support for the conclusions and recommendations made in the report. This document describes where dioxin comes from, how it moves through the environment and gets into our food, how it builds up in our bodies, and how it is affects our health and our children. Particular emphasis is given to how dioxin affects the immune, reproductive, and developmental systems of the human body and how it causes cancer. This Technical Support Document provides the reader with a scientific discussion and analysis of the latest research on dioxin, including extensive references and an overall characterization of the risk of dioxin to public health.

The American People's Dioxin Report is intended to accomplish three goals:

- Provide up-to-date scientific information and research on the effects of dioxin on human health;
- Provide the American people with suggested protective policies which they can ask their elected officials to enact to prevent this life-threatening chemical from harming our families; and
- Engage the American people in a national debate, community by community, on the nature of government in our society--how people became powerless as the corporations became powerful--and why our government protects the right to pollute more than it protects the American people's health.

The Center for Health, Environment and Justice is working with a diverse network of organizations and individuals to protect our health by reclaiming our right to safe food, safe water and safe breast milk by eliminating sources of dioxin. Over the next six to eight months public meetings and other gatherings will take place in cities across the country. These meetings will begin the public discussions about how to safeguard the health of the American people and launch efforts to establish protective policies.

This new effort to eliminate dioxin exposures is part of CHEJ's Stop Dioxin Exposure Campaign, which began in 1995. This report builds upon the still valid scientific information found in Dying From Dioxin. Dying From Dioxin contains organizing ideas and non-technical explanations of dioxin and its impacts. The information in the book is still very useful for non-scientific audiences. To obtain a copy contact CHEJ's offices.

--- Lois Marie Gibbs
Executive Director
Center For Health, Environment and Justice
November, 1999

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American People's Dioxin Report

Technical Support Document

Executive Summary

This Technical Support Document (TSD) to America's Choice: Children's Health or Corporate Profit - The American People's Dioxin Report discusses the latest scientific research on the toxic effects caused by or associated with exposure to dioxin. This document is intended to inform the public and their representatives in government so appropriate action can be taken to safeguard the health of the American people. It is clear that there is an extensive body of high quality scientific information describing the toxic effects of dioxin in people. This data indicates that dioxin is a potent chemical that produces a wide variety of toxic effects in animals and that some of these effects are

occurring in people.

The TSD's overall conclusion is that the American people are at serious risk from their daily intake of dioxin in food. This exposure appears to be affecting the growth and development of children, notably the development of the immune, reproductive and nervous systems, in particular, cognitive and learning abilities. While exposure of the general population occurs through ingestion of many common foods, children exposed in utero during critical periods of development appear to be the most sensitive and vulnerable to the toxic effects of dioxin.

The newest studies on dioxin's effects on human health lead to the following conclusions:

- All American children are born with dioxin in their bodies. The greatest impact of this exposure appears to be to the growth and development of children. Disrupted sexual development, birth defects and damage to the immune system may result.

- Dioxin exposure has been associated with IQ deficits, increased prevalence of withdrawn/depressed behavior, adverse effects on attentional processes, and an increase in hyperactive behavior in children. These effects have been documented in 42-month old Dutch children whose exposure to dioxins and PCBs came primarily before birth. The children's mothers were exposed to "background" levels of dioxins and PCBs as a result of the daily ingestion of dioxin in food.

- Dioxin exposure has been associated with alterations in immune function including increased susceptibility to infections and changes in T-cell lymphocyte populations. These effects have been reported in 42-month old Dutch children exposed to dioxins and PCBs primarily before birth. Altered immune function, reported at birth, 3, and 18 months of age, persists to 42 months of age in these children. Reported immune effects included an increase in middle ear infections and chicken pox, and a decrease in allergic reactions.

- There is evidence of both developmental and reproductive effects in children exposed to dioxin. These effects include defects in permanent teeth, adverse effects on thyroid hormones, altered sex ratio (more females than males), and increased respiratory infections.

- Hormonal effects associated with dioxin exposures in humans include a decrease in testosterone in dioxin-exposed workers and a decrease in thyroid hormones following prenatal exposure to background levels of dioxin in infants.

- Dioxin interferes with the hormone insulin and alters glucose tolerance which leads to diabetes. New studies of soldiers exposed to Agent Orange and residents of Seveso, Italy add to the existing evidence from studies of workers that

exposure to dioxin increases the risk of developing diabetes.

- The average daily intake of dioxin in food poses a substantial cancer risk to the general American population. The lifetime risk of getting cancer from exposure to dioxin is 1 in 10,000 for the general American population and 1 in 1,000 for highly exposed members of the population. These risks are 100 and 1,000 times higher, respectively, than the generally "acceptable" one-in-a-million cancer risk for carcinogens.

- Updates of ongoing studies of cancer rates in dioxin-exposed workers in the U.S. and Germany, and in residents of Seveso, Italy all indicate increasing cancer rates in the highest exposure groups. These studies provide strong support for the decision by the World Health Organization's International Agency for Research on Cancer (IARC) to define dioxin (TCDD) as a "known human carcinogen." This decision is further supported by evidence from animal studies and data on dioxin's mechanisms of action in the body.

- Nearly all Americans are exposed to dioxin through ingestion of common foods, especially meat and dairy products. Dairy cows and beef cattle absorb dioxin by eating dioxin contaminated feed crops. The crops become contaminated by airborne dioxins that settle onto soil and plants. Dioxins enter the air from thousands of sources including incinerators that burn medical, municipal, and hazardous waste, chemical processing facilities that use chlorine to make products such as pesticides and PVC plastic, and metal refining and smelting operations.

- The average daily intake of the American people is already well above two federal guidelines for "safe" exposure. The American average daily intake is more than 200 times higher than the Environmental Protection Agency's cancer risk guideline and over twice the Agency for Toxic Substances and Disease Registry's lowest adverse effect level.

- Some groups of people are at higher risk of exposure to dioxin. These groups include children, nursing infants, some workers, people who eat fish as a main staple of their diet, such as some indigenous peoples and fishermen, and people who live near dioxin release sites. These groups of people are likely exposed to at least 10 times as much dioxin as the general population.

- The average daily exposure of dioxin and dioxin-like chemicals in the U.S. is approximately 3-6 pg TEQ/kg body weight per day. Nursing infants ingest about 50 times this much each day.

- Dioxin accumulates in biological tissue. The average tissue or "body burden" level of Americans ranges from 36 to 58 ng TEQ/kg lipid (36-58 ppt). Approximately 10% of the population may have tissue levels as much as three times higher than this level.

- There is a small difference between the body burdens of dioxins that cause adverse non-cancer effects in animals and average levels in the general human population. Some people who have above average levels are already suffering from the adverse effects of exposure to dioxin.

- While TCDD is the most toxic form of dioxin, 90% of the total toxicity resulting from exposure to dioxins is due to dioxin-like compounds other than TCDD.

- There is an extensive body of high quality, published information on the toxicity of dioxin. This body of data indicates that dioxin is a potent toxin which produces a wide variety of adverse effects in animals and that some of these effects are likely already occurring in people.

Dioxin is an ubiquitous poison that is in our food and causes many toxic effects in people and animals. The neurodevelopmental and reproductive effects observed in children may be the most disturbing new evidence of dioxin's toxicity. These small shifts in cognitive ability or thyroid levels may be just the tip of the iceberg of our understanding of the impact of dioxin on the general American population.

We know that the daily intake of Americans is already too high, and exceeds two federal risk guidelines. We also know that some members of the general population are particularly sensitive and that others are exposed to dioxins at greater than the average daily levels. These are infants and children, people who live near contaminated sites, fishermen and indigenous people who rely on fish as a main staple of their diet, some workers, and others with high exposures. These groups have suffered a disproportionate share of dioxin exposure and many already suffer the adverse health effects caused by these exposures.

We agree with the World Health Organization who recommended that "every effort should be made to limit environmental releases of dioxin and related compounds to the extent feasible in order to reduce their presence in the food chains, thereby resulting in continued reductions in human body burdens..." (WHO, 1998). Americans have a choice: take action to protect public health by eliminating dioxin creation or continue to allow dioxin to be created and not burden industry with the short term transition costs of eliminating dioxin and related compounds.

Chapter 1

Background

Dioxin has been the subject of government study for more than twenty years. The history of its progress through the regulatory system is full of controversy, industry pressure, politics, and delays. This chapter outlines that history - from the time dioxin's toxicity was first discovered to the most recent delays in the United States Environmental Protection Agency's (EPA) release of a comprehensive assessment of dioxin's health effects.

Adverse health effects caused by exposure to dioxins were first reported in chemical workers at plants manufacturing chlorinated phenols in Michigan in the 1930s (Butler, 1937); in Nitro, West Virginia in the 1940s (Ashe, 1949; Suskind, 1984); and in western Germany in the 1950s (Goldman, 1972; Suskind, 1977). The most pronounced effect observed in these workers was chloracne, a severe skin disease. Effects on the liver, and the nervous, endocrine, cardiovascular, gastrointestinal, and immune systems were also reported (Moses, 1984). It was not until 1957 that a German chemist discovered that the compound responsible for these toxic effects was 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Kimming, 1957).

One of the earliest findings of dioxin's toxicity in animals was that it caused birth defects in mice at very low levels (Courtney, 1971). This finding led to dioxin being characterized as "one of the most potent teratogenic environmental agents" (Pratt, 1984). The first evidence that dioxin causes cancer came from several animal studies completed in the late 1970's (Van Miller, 1977; Kociba, 1978). The most important of these, published in 1978 by a team of scientists from Dow Chemical Company, led by Richard Kociba, found liver cancer in rats exposed to very low levels of dioxin (Kociba, 1978). This study helped establish dioxin as one of the most potent animal carcinogens ever tested and, together with the finding of birth defects in mice, led to the general statement that dioxin is the "most toxic synthetic chemical known to man."

In 1982, the National Toxicology Program (NTP), a program of the U.S. Department of Health and Human Services, completed a major study of the carcinogenocity of dioxin and found cancer in both mice and rats exposed to levels of dioxin similar to those in the Kociba study (NTP, 1982, 1982a). In 1985, the EPA published a scientific review of the health effects of dioxin. This review served as the scientific basis for the dioxin risk assessments used for all EPA programs and established an "acceptable daily dose" for dioxin of 0.006 picograms per kilogram of body weight per day (pg/kg/day) (USEPA, 1985). At the time, this risk estimate was the lowest of any risk estimate defined by other state and federal agencies as well as by other countries. Industry protested that this estimate was too low (Webster, 1994).

By 1987, the EPA's National Dioxin Study had found dioxin in the effluent of paper mills across the country (USEPA, 1987), and the paper industry was pressuring the EPA to

reconsider its risk estimate. Shortly afterwards, the agency set up an internal workgroup of EPA staff to reassess the 1985 document. Most of this reassessment dealt with the different models of how chemicals like dioxin might cause cancer. Since the EPA workgroup could not agree on the best model, they averaged the acceptable risk values predicted by the various models and obtained a new risk value that was 16 times higher than EPA's original estimate. The EPA's Science Advisory Board (SAB), a group of non-government scientists, chastised the EPA workgroup for its unusual approach. Several of the models used to calculate the average risk value contradicted each other, and it did not make sense to average them to find one value. Since the SAB found no scientific basis for revising EPA's original 1985 risk value for dioxin, it was not changed (Commoner, 1994).

In October, 1990, the EPA and the Chlorine Institute, which represents companies that use or manufacture chlorine, co-sponsored a scientific meeting at the Banbury Center on Long Island, New York (Roberts, 1991). The purpose of the meeting was to review the latest data related to how dioxin might cause cancer. Most of the meeting focused on the role the "Ah" receptor (see Chapter Five) plays in mediating dioxin's toxic effects. The participants generally agreed that most, if not all, of dioxin's effects were mediated by binding to the Ah receptor. A few industry participants felt that the effects of a chemical operating through a receptor must have threshold, a dose below which there would be no effect (Commoner, 1994).

In April 1991, EPA Administrator William Reilly announced that the EPA would undertake a second reassessment of dioxin. He was responding to internal pressure from staff scientists who attended the Banbury meeting and felt that the 1985 report needed to be updated. The Chlorine Institute also pressured the agency to reassess the health effects of dioxin because they felt that the findings from the Banbury meeting meant that dioxin was much less toxic than previously thought.

According to Reilly, the primary focus of the second reassessment was the idea of a threshold, or "safe" level of dioxin exposure, a concept advanced by industry (Roberts, 1991). News of the reassessment, including the notion that dioxin was much less toxic than previously thought, was widely reported in the lay press (Gorman, 1991; Schneider, 1991).

Barely five months into the EPA's second reassessment, new research by George Lucier and Chris Portier of the National Institute for Environmental Health Sciences suggested that there was no threshold for some of dioxin's effects (Roberts, 1991a). These findings, presented at the Eleventh International Symposium on Chlorinated Dioxins and Related Compounds in September 1991, seriously weakened the threshold theory. In addition, new research presented at this meeting suggested that dioxin acted like a hormone, disrupting many systems of the body.

Much of the research that came out of the 1991 Symposium on Dioxin supported the findings of EPA's 1995 health assessment on dioxin. It also provided the basis for EPA's third assessment of dioxin, which was released by the agency as a "public review draft" in September 1994 (USEPA, 1994). In this report, the EPA incorporated all of the latest scientific evidence on the health effects, methods of exposure, and fate after entry into the body of all dioxin and dioxin-like chemicals. The report also emphasized that: dioxin can cause health problems other than, and possibly more injurious than, cancer; dioxin accumulates in biological tissues; the average level of dioxin in Americans' bodies is at or just below levels that cause some adverse health effects; and humans are exposed to dioxin primarily by eating a wide variety of common foods which contain small amounts of dioxin (USEPA, 1994a).

In 1995, the EPA's SAB endorsed all of the exposure document and most of the chapters of the health assessment document. The SAB made it clear that the scientific basis of the draft report was sound. It made only minor comments on the exposure document and suggested some changes in Chapter 8, Dose-Response Modeling, and Chapter 9, Risk Characterization, of the health assessment document (USEPA, 1995). After the SAB's review in 1995, the EPA announced that it would release the final health and exposure documents in the fall of 1995. This was the first of numerous missed deadlines and broken promises by EPA about the release of the final assessment document. In March 1997, the agency did submit a revised draft of Chapter 8 to a peer review committee, and the draft was approved with minor revisions (USEPA, 1997). However, the final version of Chapter 8 has still not been released to the public. Similarly, in April 1998, the EPA released an "external review draft" of the dioxin sources inventory, which updated the 1994 draft exposure document (USEPA, 1998). Though this document was also peer reviewed (ERG, 1998), the final version has not been released to the public. As of the fall of 1999, four years after the date given to the SAB for the release of the reassessment, there is still no final version of EPA's reassessment of the health effects of dioxin.

Although the EPA has failed to finalize its 1994 reassessment of dioxin, it has made new rules for dioxin-producing industries such as municipal waste incinerators (GPO, 1995), medical incinerators (GPO, 1997), and pulp and paper mills (GPO, 1998). These new rules, however, are all technology based, and not health-risk based.

In the past 20 years, a great deal of information, based on sound scientific research, has been discovered about dioxin's health effects on humans. Much of this work was summarized very well by the EPA in their draft reassessment document (USEPA, 1994). Since that time, however, there have been many delays which have prevented EPA from finalizing their assessment and providing decision-makers at all levels of government with the scientific basis and understanding needed to make decisions to protect the American people from exposures to dioxin. Without this information, exposures to dioxin that could be avoided, reduced, or eliminated will continue. This report is intended to make the latest information on dioxin available so that an informed public and their

representatives in government can take action to safeguard the health of the American people.

Chapter 2

The Chemistry and Environmental Fate of Dioxin

Chemistry

Dioxins, furans, and polychlorinated biphenyls (PCBs) are a family of chemicals with related properties and toxicity. Dioxin and dioxin-like chemicals are composed of two benzene rings hooked together in one of three different ways (Figure 2-1). If they are hooked together by a six-member ring containing two oxygens, they belong to the family of dibenzo-p-dioxins. If they are hooked together by a five-member ring containing one oxygen, they belong to the family of dibenzofurans. If they are hooked together directly, they are called biphenyls. The dioxins and furans have three rings in their structure, but the biphenyls have only two.

Figure 2-1: Diagrams of Dioxin, Furan, and Biphenyl

Each of the hydrogen atoms in the benzene rings can be chemically replaced by chlorine atoms. To keep track of where the chorines are, the molecules are numbered as shown in Figure 2-1, and the name of the molecule gives both the number of chlorines (by the prefix tetra, penta, hexa, etc.) and the location of the chlorines. For example, 2,3,7,8-tetrachlorodibenzodioxin has 4 chlorines at positions 2, 3, 7 and 8. Table 2-1 shows the number of possible molecules that can be formed, depending on the number and arrangement of chlorines. For example, two chlorines can arrange on the dioxin molecule in 10 different ways, so there are ten different dichloro-p-dioxins. All together, there are 75 different dioxins, or polychlorinated dibenzodioxins (PCDDs), 135 different furans, or polychlorinated dibenzofurans (PCDFs), and 209 different polychlorinated biphenyls (PCBs). Bromine, an element closely related to chlorine, can also replace the hydrogens and form similar compounds. Chloro- and bromo- congeners can exist together. Both chlorinated and brominated chemicals are toxic, but the chlorinated ones are more common. Very little is known about the brominated congeners.

Not all of these chemicals have dioxin-like toxicity, and the toxic ones are not equally toxic. Only 7 of the 75 dioxins, 10 of the 135 furans, and 12 of the 209 PCBs have dioxin-like toxicity. These 29 different dioxins, furans, and PCBs all exhibit similar toxic effects caused by a common mechanism: binding to a complex molecule known as the aryl hydrocarbon or "Ah" receptor (see Chapter Five). It is believed that the tighter the binding to the Ah receptor, the more toxic the chemical. The most potent member of this family is 2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD, which also has the greatest

affinity for the Ah receptor. One key factor in toxicity is the number of chlorines in the molecule: those with three or fewer chlorines lack dioxin-like toxicity. Another key factor is where the chlorines are attached. In dioxins and furans, it is critical that chlorines be at the 2, 3, 7, and 8 positions. In PCBs, it is critical that the corresponding positions, which are 3, 3', 4, and 4', have chlorines. The chlorine number and position probably affects the toxicity of the molecules by changing their shapes, which in turn determines binding to the Ah receptor. For simplicity, the terms "dioxin" and "dioxins" are used in this report to refer to any of the dioxin family members that act as Ah receptor ligands and elicit dioxin like effects.

Toxic Equivalents (TEQs)

Because this family of related chemicals contains so many different members, assessing the risk
of an environmental sample contaminated with them is difficult. Initially, a contaminated sample's risk was measured according to the concentration of 2,3,7,8-TCDD, the most toxic of the chemical family members, present in the mixture. However, laboratory tests showed that this approach greatly underestimated the toxicity of the sample. The current approach uses Toxic Equivalency Factors or TEFs to estimate Toxic Equivalents, or TEQs. This approach was first used in the late 1970s (Eadon, 1986) when a transformer fire contaminated an office building with dioxins, furans and PCBs, and it was critical to determine when the building was safe for reoccupation. In this approach, each form of dioxin or dioxin-like chemical is assigned a toxicity factor, which is then multiplied by its concentration in a complex mixture to obtain its TEQ. The TEQs of each chemical are summed to give a total TEQ for the sample. The most toxic form of dioxin, 2,3,7,8-TCDD, is assigned a toxic equivalency factor (TEF) of 1. Each of the 17 toxic dioxins/furans and 12 PCBs is then assigned a "toxicity factor" that estimates its toxicity relative to TCDD. For example, a dioxin or dioxin-like substance that is half as toxic as TCDD is assigned a toxic equivalency factor of 0.5 and so on down to 0.00001. The toxicity factor for each dioxin and furan is shown in Table 2-1, and for PCBs in Table 2-2.

TABLE 2-1 Toxic Equivalency Factors (TEF) for Dioxins and Furans

Chemical	Toxicity Factor		
	Mammals	Fish	Birds
2,3,7,8-tetrachlorodibenzo	1	1	1

dioxin			
1,2,3,7,8-pentachlorodibenzo dioxin	1	1	1
1,2,3,4,7,8-hexachlorodibenzo dioxin	0.1	0.5	0.05
1,2,3,6,7,8-hexachlorodibenzo dioxin	0.1	0.01	0.01
1,2,3,7,8,9-hexachlorodibenzo dioxin	0.1	0.01	0.1
1,2,3,4,6,7,8-heptachlorodibenzo dioxin	0.01	0.001	<0.001
octachlorodibenzo dioxin	0.0001	<0.0001	0.0001
2,3,7,8-tetrachlorodibenzo furan	0.1	0.05	1
1,2,3,7,8-pentachlorodibenzo furan	0.05	0.05	0.1
2,3,4,7,8-pentachlorodibenzo furan	0.5	0.5	1
1,2,3,4,7,8-hexachlorodibenzo furan	0.1	0.1	0.1
1,2,3,6,7,8-hexachlorodibenzo furan	0.1	0.1	0.1
1,2,3,7,8,9-hexachlorodibenzo furan	0.1	0.1	0.1

2,3,4,6,7,8-hexachlorodibenzofuran	0.1	0.1	0.1
1,2,3,4,6,7,8-heptachlorodibenzofuran	0.01	0.01	0.01
1,2,3,4,7,8,9-heptachlorodibenzofuran	0.01	0.01	0.01
octachlorodibenzofuran	0.0001	<0.0001	0.0001

Source: Van den Berg, 1998

Table 2-2 Toxic Equivalency Factors (TEFs) for Dioxin-Like PCB's

Common Identifier	Chemical	Toxicity Factor		
		Mammals	**Fish**	**Birds**
77	3,3',4,4'-tetrachlorinated biphenyl	0.0001	0.0001	0.05
81	3,4,4',5-tetrachlorinated biphenyl	0.0001	0.0005	0.1
105	2,3,3',4,4'-pentachlorinated biphenyl	0.0001	<0.000005	0.0001
114	2,3,4,4',5-pentachlorinated biphenyl	0.0005	<0.000005	0.0001
118	2,3',4,4',5-pentachlorinated biphenyl	0.0001	<0.000005	0.00001
123	2',3,4,4',5-pentachlorinated biphenyl	0.0001	<0.000005	0.00001
126	3,3',4,4',5-pentachlorinated biphenyl	0.1	0.005	0.1
156	2,3,3',4,4',5-hexachlorinated biphenyl	0.0005	<0.000005	0.0001

157	2,3,3',4,4',5'-hexachlorinated biphenyl	0.0005	<0.000005	0.0001
167	2,3',4,4',5,5'-hexachlorinated biphenyl	0.00001	<0.000005	0.00001
169	3,3',4,4',5,5'-hexachlorinated biphenyl	0.01	0.00005	0.001
189	2,3,3',4,4',5,5'-heptachlorinated biphenyl	0.0001	<0.000005	0.00001

Source: Van den Berg, 1998

The use of TEQ to estimate the toxicity of a complex mixture of dioxins is based on several assumptions (Van den Berg, 1998). The first assumption is that chemicals with dioxin-like toxicity share a common mechanism of toxicity that begins by binding to the Ah receptor. Only those dioxins, furans and PCBs that bind to the Ah receptor are considered to have dioxin-like activity. Some non dioxin-like PCBs can produce toxic effects that act by another mechanism, and these toxicities are not accounted for by the TEQ. A second assumption is that the toxicity of chemicals is additive. Should chemicals interact in a synergistic or antagonistic manner, then the practice of adding the TEQs would not be valid. The third assumption or convention, adopted to harmonize many different studies, is the estimation of toxicity factors in orders of magnitude; i.e. 1, 0.1, 0.01, and 0.001 and so on.

The concept of TEQs has evolved considerably since the late 1970s when they were first used. At the first consensus meeting on TEQs held by the World Health Organization in 1993, it was agreed that TEQs were a useful, but interim, measure which should be re-evaluated every five years. At the second consensus meeting in 1998, the database of literature was updated and the scientific basis for each toxicity factor reviewed (Van den Berg, 1998). Consensus was reached on toxicity factors (TEFs) for PCBs. The second important result was the development of toxicity factors for fish and birds. Separate toxicity factors were developed because of an understanding that absorption, metabolism, and excretion for specific chemicals are different among species, and because of the possible differences in binding to the Ah receptor (see Tables 2-1 and 2-2). Third, a few of the toxicity factors from 1993 were revised: 1,2,3,7,8-pentachloro-dibenzodioxin was revised from 0.5 to 1.0 and octachlorodibenzo-p-dioxin and furan were both revised from 0.001 to 0.0001 (Van den Berg, 1998).

TEQs assume that the toxicity of chemicals in a complex mixture is additive, but that assumption is not always true. Examples of synergism, where the presence of one chemical enhances the toxicity of a second (Safe 1990; Van Birgelen, 1996, 1996a), and antagonism, where the presence of one chemical reduces the toxicity of a second, are

known (Safe, 1990; Morrisey, 1992; Smialowicz, 1997). Based on many studies, it is estimated that these antagonistic and synergistic interactions are not common and that the assumption of additivity is a reasonable approximation that is unlikely to lead to errors more than two-fold, especially at levels typically found in the environment.

Certain dioxin-like chemicals account for most of the family's toxicity. For example, PCBs account for approximately 50% and TCDD accounts for 10% of the toxicity of dioxin in cow and human milk (Ahlborg, 1994). Most of the toxicity of dioxin in human samples is attributed to TCDD, PCDD, 4-pentachlorodibenzofuran, and PCB 126.

Just how valid is the TEQ concept and how valid are the assumptions that underlie the setting of the toxicity factors? One way to determine if the concept is valid is to measure the toxicity of a mixture and to compare this to the calculated toxicity using the TEQ. This has been done for several mixtures and the use of TEQ was found to be reasonable. For example, when evaluated with TEQ, a contaminated sample of leachate from the Love Canal chemical waste site had an estimated 5-10 micrograms (ug) of TCDD, whereas measured toxicity was 3 ug TCDD (Silkworth, 1989). The TEQ of soot from a PCB transformer fire correlated well with the measured toxicity (Eadon, 1986). And, TEQ methods reasonably predict toxicity of mixtures in the laboratory (Viluksila, 1998, 1998a).

TEQs based on an analysis of soil or sediment are not very useful for estimating toxicity to wildlife or humans, because the various dioxin-like chemicals are absorbed, metabolized, and excreted differently by different animals. For dioxins, the 2,3,7,8 forms are preferentially enriched in mammals, birds, and fish. Thus, it is best to use an analysis of the animal's food source or the animal itself to estimate toxicity rather than an environmental sample such as sediment or soil.

Physical Properties and Environmental Fate

Dioxin-like chemicals share many physical properties that affect how they behave in the environment. They dissolve poorly in water, but very well in oils, fats, and organic solvents. They adhere strongly to organic components of soil and water and therefore do not wash out easily. They have a low vapor pressure which means they do not evaporate readily. Since they do not react with oxygen or water and are not broken down by bacteria, they persist in the environment for long periods of time. They can be broken down very slowly by sunlight, but only under certain conditions. The most stable members of the group have four or more chlorines (Zook, 1994; IARC, 1997; ATSDR, 1998).

Because PCBs are somewhat more reactive than dioxin, they are more easily broken down by sunlight, air, and bacteria. PCBs will volatilize and, depending on the congener and the air temperature, can be found in the vapor phase as much as 90% of the time (Murray, 1992; Cohen, 1997). They will gradually settle out (dry deposition) or be washed out by rainfall (wet deposition) and may slowly evaporate back into air (Baker, 1990). Furans, dioxins, and PCBs eventually settle on terrestrial plants where they enter the food chain. Dioxins fall out onto crops that are fed to dairy cows and beef cattle where they accumulate in the milk and meat of these animals. Dioxins are not well metabolized in the body and thus are not eliminated in urine or feces. Rather, they dissolve and accumulate in the body fat of these animals. People who consume the contaminated meat and dairy products ingest substantial amounts of dioxins. Generally, plants do not absorb dioxins through their roots, although there appear to be a few exceptions. Studies have shown that some plants (zucchini, pumpkin, and perhaps carrots) seem to absorb dioxin through their roots (Schroll, 1993; Hulster, 1994; Muller, 1994).

Dioxins and furans are not manufactured intentionally. Instead, they form as an unintended contaminant or byproduct during combustion or during the manufacture of certain chlorinated chemicals. Most dioxins and furans escape into the environment from incinerator stacks, pulp and paper industry discharges, and the manufacture of polyvinyl chloride (PVC) plastic, chlorinated solvents, and chlorinated pesticides (USEPA, 1998) (see Chapter Three). Some also escape into the environment from the burning of toxic chemicals in cement kilns and from metal smelting and refining (USEPA, 1998). PCBs were once produced intentionally in large quantities, and, though their manufacture is now banned, existing products containing PCBs are still used and discarded. Once released into the atmosphere, dioxins and furans stay suspended a long time and may travel all over the world before settling (IARC, 1997). In the air, dioxins are usually bound to particles such as incinerator ash and are shielded from photodegradation. Eventually, they settle to earth. On surface soil, it may take from 9 to 15 years to degrade half of the dioxin in the top 0.1 centimeters (cm) and 25 to 100 years to degrade half the dioxin in the subsurface soil below 0.1 cm (Paustenbach, 1992). In Times Beach, Missouri, it took 16 months before half of the dioxin in the top eighth of an inch of contaminated soil was photodegraded (Freeman, 1987). Dioxin below that level did not wash out with rain, nor did it evaporate. Whether it settles directly on water or ends up there due to soil erosion, dioxins end up in the bottom muds and sediments of rivers, lakes and ocean. These chemicals are taken up by aquatic organisms and are concentrated as they move up the food chain to fish and eventually to humans.

Dioxins can easily re-enter the environment when muds and sediments are disturbed, such as in the dredging of a harbor. When buried in landfills or trapped in soil, this chemical family tends to stay in place unless carried away by oily substances or organic solvents such as benzene and toluene, which are often present in landfills. At Love Canal, New York, dioxin moved long distances from the canal into storm sewers and a creek in exactly this manner (DOH, 1981).

Dioxin Formation

Dioxins and furans form whenever chlorine-containing compounds are exposed to high heat or catalysts in the presence of organic material (Pennise, 1996). They form at temperatures as high as 1,000° Celsius (C) (Hunsinger, 1997) and as low as 20° C in the gas, liquid, or even solid phase (Klimm, 1998). In incinerators, dioxins form at temperatures from 200° C to 1,000° C (Hunsinger, 1997), with optimum formation temperatures varying from 300° to 600° C (Yasuhara, 1988; Huang, 1995). At 800° C, pure dioxins decompose almost completely, but dioxins bound to particles remain intact even at 1,150° C (Esposito, 1980). They form primarily when incinerator gases cool as they flow toward the stack. Trace metals, especially copper and zinc, may act as catalysts (Acharya, 1991).

Whatever the mechanism, chlorine is essential. Apparently any form of it, whether organic (such as found in PVC plastic, pentachlorophenol, or PCBs) or inorganic (such as potassium chloride, sodium chloride, cuprous or cupric chloride, or ferric chloride), can participate in the reactions (Addink, 1995; Danish EPA, 1997). A better understanding of mechanism is important to reducing dioxin emissions. If organic chlorinated precursors are important, then dioxin emissions can be prevented by removing PVC, polychlorinated benzenes, and polychlorinated phenols from wastes. Alternately, if even simple forms of chlorine lead to significant dioxin formation, then prevention will depend on following chlorine through its cycle of use in industrial processes, keeping track of every product and waste that are potential dioxin carriers, and perhaps even sharply curtailing chlorine use.

Chapter 3

Sources of Dioxin

"[I]t can be concluded that dioxins were largely anthropogenic and associated with events taking place around 1935-40. What were these events? The explanation is likely to be the introduction of chlorinated organic compounds (polyvinyl chloride and chlorinated pesticides are but two examples) in the 1935-40 time-frame...[T]he introduction of these chlorinated products into wastes that were combusted appears to be

the most likely cause of the increased dioxin deposition measured in sediments."

U.S. Environmental Protection Agency
Science Advisory Board, 1995.

Dioxins are found everywhere in the world - in water, air, soil, and sediment - even in places where dioxin or dioxin-containing products have never been used. This broad distribution is evidence that the sources are multiple and that dioxins can travel long distances. Dioxins get into the environment from industrial air emissions, wastewater discharges, disposal activities, and from burning material containing chlorine. Airborne dioxin eventually settles onto soil, plants, and water. It then moves up the food chains and may end up in people's bodies. In this chapter, we will examine how and where dioxins are released into the environment. Most of the estimates in this chapter are from the United States Environmental Protection Agency's (EPA) *The Inventory of Sources of Dioxin in the United States* (USEPA, 1998). The EPA acknowledges that there are many limitations with its estimates, and they are discussed later in this chapter. The most serious limitation is the lack of sufficient data to make accurate estimates of the amount of dioxin released into the environment by different sources.

Background

The EPA assembled one of the first listings of U.S. dioxin sources in 1980. The list identifies 25 commercial chemicals whose manufacture have a "high probability" of forming dioxin. Of these 25 chemicals, 15 are chlorinated compounds whose manufacture is expected to form polychlorinated dioxin and furan by-products. The remainder are brominated, fluorinated or iodinated compounds whose manufacture is expected to form polyhalogenated dioxins other than the chlorinated species.

The list also identifies 55 chemicals whose manufacture has the "possibility"of forming dioxin by-products. In addition, the list identifies 18 pesticides whose manufacture is "highly likely" to form dioxin by-products and another 20 whose manufacture has a "reasonable probability" of forming dioxin by-products. The EPA also identifies municipal waste incinerators, some industrial incinerators, and cigarettes as dioxin sources, and notes that combustion of naturally occurring compounds "in the presence of chlorine-containing compounds (e.g., DDT or polyvinyl chloride) can lead to the formation of chlorinated dioxins" (Esposito, 1980).

By 1985, the EPA concluded that the "primary sources of PCDD [dioxin] contamination in the environment result from the manufacture of chlorophenols and their derivatives and the subsequent disposal of wastes from these industries." Five categories of dioxin sources were identified: manufacturing processes, municipal incinerators, other combustion processes, chemical disposal sites, and photochemical processes (USEPA, 1985). In 1987, EPA released the results of a two-year nationwide study of contamination by the most potent of the dioxins, 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD). The study lists sites where the pesticide 2,4,5-trichlorophenol was produced or used and sites for the disposal of the associated wastes. In addition, combustion sources and production sites of other chemicals where 2,3,7,8-TCDD may have been produced are examined, as are levels of 2,3,7,8-TCDD in fish and in urban and rural soils (USEPA, 1987).

U.S. Inventories of Dioxin Sources and Emissions

In 1994, the EPA released a review draft of its second dioxin "reassessment." This report contains the first detailed inventory of U.S. dioxin sources and their estimated releases. It identified 31 source categories (facilities or activities that are known or suspected to release dioxins into air, water, land, and products) and estimated that the amount of dioxin released to air, water, land and products totaled 11,660 grams of toxic equivalents per year (gm TEQ/yr) (see Table 3-1) (USEPA, 1994b). One year later, the EPA's Science Advisory Board (SAB) reviewed the 1994 dioxin inventory and recommended revisions (USEPA, 1995). The EPA released a revised inventory in 1998 in the a report, *The Inventory of Sources of Dioxin in the United States*. Though it describes at least 70 source categories for dioxin, EPA only considers the 54 in Table 2-2 of their report to be part of its inventory. Estimated dioxin releases for some of these 54 sources, though in the report's text, are either not in that table, are summarized in another table, Table 2-5 (of the EPA report), or are missing. Nevertheless, the inventory estimates that dioxins released from these 54 source categories in reference year 1995 totaled 28,023 gm TEQ/yr (Table 3-1) (USEPA, 1998).

Table 3-1 Estimated Dioxin Releases to the Environment in the U.S.

	1994 Draft Inventory	**1998 Draft Inventory**
Dioxin Releases	Total, gm TEQ/yr*	Total, gm TEQ/yr*
To Air	9,300	2,745 **
To Water	110	20
To Land	2,100	208

To Products	150	25,050
Total	**11,660**	**28,023**

Source: USEPA, 1994b, 1998
* The values presented here are central estimates.
** USEPA considers the national dioxin inventory to consist only of those source categories and emissions presented in Table 2-2 of the inventory document. If preliminary estimates presented in the document, but not included on Table 2-2 are included, then dioxin releases to air are 4,925 gm TEQ/yr and total releases are 30,203 gm TEQ/yr.

As Table 3-1 shows, most of the dioxin is released into the air. However, because dioxins released into the water easily end up in fish people eat, dioxin emissions to water are also significant to human health. Dioxins disposed on land, mostly in landfills and as sewage sludge that is spread on farmland, can also pose significant health risks because of movement of dioxin out of these disposal sites.

Overview of EPA's 1998 Inventory of Sources of Dioxin

The EPA estimated how much dioxin was released into air, water, land, and commercial products in the U.S. in 1995 (Table 3-2). These estimates include only the 54 source categories for which EPA has sufficient confidence in the available data (USEPA, 1998). For example, estimates for air releases are made for only 20 of the 54 source categories. Preliminary estimates for a number of source categories are not included in EPA's inventory. Estimates are considered preliminary because they lack direct emissions measurements or because there are large uncertainties in the activity level or applicability of available data from facilities in other countries. Dioxins released from negligible sources, or dioxins already present in the environment in "reservoir" sources are not included or are listed as having "no emissions." No estimates are made for some source categories for which there is evidence of emissions. Detailed summaries of many of these specific sources are included in Appendix A. EPA estimates of these same sources using data from 1987 are also shown in Table 3-2.

Table 3-2 Inventory of Sources of Dioxin in the United States (gm TEQ/year)
Reference Year 1995 Reference Year 1987

Air: lower central upper lower central upper							
Municipal waste incineration	492	1,100	2,460		3,540	7915	17,698
Secondary copper smelting	171	541	1,710		96	304	960
Medical waste incineration	151	477	1,510		781	2,470	7,810
Forest, brush and straw fires	64.5	208	645		53.8	170	538
Cement kilns (hazardous waste burning)	48.4	153	484		37	117	370
Coal combustion	32.6	72.8	163		28	62.6	140
Wood combustion - residential	19.8	62.8	198		28.3	89.6	283
Wood combustion - industrial	13.0	29.1	65		12.3	27.5	61.5
Vehicle fuel combustion - diesel	10.6	33.5	106		8.3	26.3	83.2
Cement kilns (non hazardous waste burning)	5.6	17.8	56.3		4.3	13.7	43.3
Secondary aluminum smelting	5.4	17	53.8		3.0	9.5	30
Oil combustion - industrial/utility	2.9	9.3	29		4.9	15.5	49
Sewage sludge incineration	2.7	6	13.4		2.7	6	13.4
Hazardous waste incineration	2.6	5.7	12.8		2.2	5	11.2
Vehicle fuel combustion - unleaded	2	6.3	20		1.2	3.8	12
Kraft recovery boilers	1	2.3	5		0.9	2	4.5
Secondary lead smelters	0.73	1.63	3.65		0.55	1.22	2.73
Cigarette combustion	0.25	0.81	2.5		0.31	1	3.1
Boilers/industrial furnaces	0.12	0.38	1.2		0.24	0.77	2.4
Crematoria	0.07	0.24	0.75		0.05	0.16	0.51
Total	**1,026**	**2,745**	**7,541**		**4,616**	**11,274**	**28,220**
Products:							
Pentachlorophenol-treated wood	17,700	25,000	35,400		25,500	36,000	51,957

Bleached chemical wood pulp and paper mills	17.0	24.1	34.0		375	505	714
Dioxazine dyes and pigments	0.11	0.36	1.1		20	64	200
2,4-Dichlorophenoxy acetic acid	13.0	18.4	26.0		15.1	21.3	30.2
Non-incinerated municipal sludge	4.0	7	12.5		4	7	12.5
Total	**17,734**	**25,050**	**35,474**		**25,914**	**36,597**	**51,957**
Land:							
Non-incinerated municipal sludge	120	207	375		120	207	375
Bleached chemical wood pulp and paper mills	1	1.4	2		10	14.1	20
Total	**121**	**208**	**377**		**130**	**221**	**395**
Water:							
Bleached chemical wood pulp and paper mills	**13.8**	**19.5**	**27.6**		**252**	**356**	**504**

Note: It is not known what fraction, if any, of the estimated emissions from forest fires represents a "reservoir" source. Source: USEPA, 1994b, 1998

The estimates in Table 3-2 include an average or "best guess" estimate as the central value of a range of high and low estimates for each source (USEPA, 1998). Based on the quantity and quality of data available to EPA, the estimates are evaluated as follows: for high confidence estimates, the range between the high and low estimates is two fold; for medium confidence estimates, the range is about five fold; for low confidence estimates, the range is ten fold. For example, for municipal waste incineration, the difference between the lower and upper estimates is five fold. This means that EPA has medium confidence in the data used to make the estimates. Of the 20 source categories for which estimates for air emissions are made, EPA has low confidence in 13 of them and medium confidence in the remaining seven. EPA does not have high confidence in any of the air source categories. These confidence ratings are discussed in more detail below.

As seen in the Table 3-2, the major sources of dioxin can be found in two major categories: combustion and metal refineries. Combustion sources account for nearly 80% of the national inventory of dioxin released to air and includes burning of medical,

municipal, and hazardous waste, sewage sludge, hazardous waste in cement kilns, fuels such as coal, wood and petroleum products to generate power or energy, and uncontrolled burning, such as forest and brush fires (see Appendix A, Tables A-1, A-2, and A-3 for summaries). It is unclear what portion of the emissions during forest and brush fires is actually resuspension from residues deposited on leaves rather than newly formed dioxins (Clement, 1991; McLachlan, 1998).

The second significant group of sources is metal smelting, refining, and processing which includes primary and secondary metals operations, iron ore sintering, steel production, and scrap metal recovery. This category accounts for about 20% of the national inventory of dioxin released to air. A summary of the metal smelting and processing sources is presented in Appendix A.

In the 1998 EPA inventory, dioxins released to the air and products are the most thoroughly characterized (Table 3-3). For approximately 85% of source categories, releases to air are either estimated, given preliminary estimates or classified as having negligible or no releases.* Preliminary estimates of emissions to air are made for 12 source categories including open burning of household waste, landfill fires, iron ore sintering plants, and coal combustion from residential homes. None of these preliminary estimates are included in EPA's final inventory.

These preliminary estimates add up to an additional 2,180 gm TEQ/year and potentially increase the total dioxins released to air to 4,925 gm TEQ/year (see Table 3-3). Eleven source categories are considered negligible, and no estimates are made for eight source categories for which there is evidence of dioxin emissions. Dioxins released in products are similarly characterized for 91%

** EPA concluded that some source categories do not have any releases to air. In such cases, EPA used the term "not applicable."*

(49/54) of source categories. However, as EPA notes, only a few commercial chemicals are included in their inventory because only a limited number have been analyzed for dioxins (USEPA, 1998). Dioxins released to land and water are much less well characterized: only 37% (20/54) of the source categories for land and 44% (24/54) of the source categories for water for which evidence of dioxin releases exists are characterized.

Table 3-3 USEPA's Assessment of Dioxin Releases To Air, Water, Land, and Products in

1995					
	Number of Source Categories	**Dioxin Release gm TEQ/yr**		**Number of Source Categories**	**Dioxin Release gm TEQ/yr**
Releases into the Air Releases to Land					
Estimates	20	2,745	Estimates	2	208
Preliminary Estimates	12	2,180	Preliminary Estimates	0	
Negligible	11		Negligible	9	
No emissions	2		No emissions	9	
Evidence only	8		Evidence only	34	
Total	53 *	4,925	Total	54	208
Releases to Water Releases to Products					
Estimates	1	20	Estimates	5	25,050
Preliminary Estimates	0		Preliminary Estimates	0	
Negligible	9		Negligible	2	
No emissions	14		No emissions	42	
Evidence only	30		Evidence only	5	
Total	54	20	Total	54	25,050

*** One source category for which no estimate was made is included in an estimate for another source category. Source: USEPA, 1998**

Confidence Ratings of Dioxin Emissions Estimates

EPA estimates emissions by multiplying activity levels (amount of waste burned, quantity of chemical produced, etc.) and emission factors (quantity of dioxin released per unit of activity). The strength of each estimate is based on the confidence ratings of the activity levels and emission factors. EPA's rating system is shown in Table 3-4.

Table 3-4 Confidence Rating Scheme for U.S. Dioxin Emission Estimates

Confidence Rating	Activity Level Estimate	Emission Factor Estimate
High	Derived from comprehensive survey.	Derived from comprehensive survey.
Medium	Based on estimates of average plant activity level and number of plants.	Derived from testing at a limited but reasonable number of few or limited survey facilities believed to be representative of source category.
Low	Based on expert judgment or unpublished estimates.	Derived from testing at only a few, possibly non-representative facilities or from similar source categories of foreign surveys where differences in industry practices may be likely.

Source: USEPA, 1998

As shown in Table 3-5, EPA presented no confidence ratings for activity levels, emission factors or other characterizations for 61% of the source categories identified as having potential dioxin emissions to the air; 97% of source categories with potential releases to water; 95% of source categories with potential releases to land; and 58% of source categories with potential releases in products. In summary, EPA's confidence in dioxin releases to air, water, land, and products is relatively poor.

Table 3-5 Confidence Ratings of Activity Levels and Emission Factors

		Confidence Ratings

		Activity Levels				Emission Factors			
Potential Emissions	**Number of Source Categories Characterized**	**No Rating**	**Low**	**Medium**	**High**	**No Rating**	**Low**	**Medium**	**High**
To Air	54	33	2	3	16	33	13	8	0
To Water	40	39	0	0	1	39	0	0	1
To Land	45	43	0	0	2	43	0	0	2
To Products	12	7	1	0	4	7	0	1	4

Source: USEPA, 1998

Sources Not Included in the EPA's Inventory

The EPA only makes estimates of source categories for which they have sufficient confidence in the available data (USEPA, 1998). Preliminary estimates are made for some source categories (Table 3-6), but these estimates are not included in the national inventory. In addition, EPA makes judgements about some source categories that they feel contribute "negligible" emissions to the national inventory (Table 3-7). For other source categories, no estimates are made even though there is some evidence of emissions (Table 3-8). EPA notes that for most sources there are insufficient data to quantify the dioxins they release to air, water, or soil, yet there are apparently no efforts to collect such data. Some of these source categories, if included, would contribute substantially to the National Inventory.

Table 3-6 Preliminary estimates for source categories in air that were not included in the National Inventory (reference year 1995)

Potential Emissions Source		**Estimated Emissions (gm TEQ/yr)**
Backyard trash burning		1,000

Landfill fires		1,000
Iron ore sintering		100
Accidental vehicle fires		10
Asphalt mixing plants		10
Coke production		10
Combustion of landfill gas in flares		10
Electric arc furnaces		10
Ferrous metal foundries		10
Residential/commercial coal combustion		10
Residential/commercial oil combustion		10
Biogas Combustion		0.1

Source: USEPA 1998

Table 3-7 Source categories defined as contributing "negligible" air emissions to the National Inventory (they contribute less than 1 gm TEQ/year or non-existent)
Chlorinated phenol production
Emissions from pentachlorophenol manufacture
Chlorobenzene production
Dioxazine dyes/pigments
2,4-D production
PCB leaks/spills
Carbon reactivation
Drum and barrel reclamation
Combustion of leaded vehicle fuel

Tall oil-based liquid soaps

Source: USEPA, 1998

Table 3-8 Source categories for which no estimates were made even though there was some evidence of emissions
Accidental fires (structural)
PVC manufacture/production
Uncontrolled combustion of PCBs
Scrap electric wire recovery
Petroleum refinery catalyst regeneration
Biological formation - biotransformation of chlorophenols and dioxins/furans
Photochemical formation - phototransformation of chlorophenols and photolysis of dioxins/furans

Source: USEPA, 1998

For example, there are at least three U.S. magnesium production facilities in Texas, Utah and Washington, which have produced approximately 117,000 metric tons of magnesium in 1998 (USGS, 1999). These facilities use an electrolytic process that relies on chlorine-based technology that is known to generate dioxins (Oehme, 1989). The dioxin emission factor derived for the Magnola/Noranda magnesium production facility in Asbestos, Canada, is 38,000 nanograms (ng) TEQ per kg of magnesium produced. Dioxin emissions to air are 2 ng TEQ/kg of magnesium produced, from stacks; and 30-140 ng TEQ/kg of magnesium produced, volatilized from land. Estimated dioxin emissions to land are 300-1,400 ng TEQ/kg of magnesium produced; and 8,000-29,000 ng TEQ/kg of magnesium produced are transferred off-site (Bramley, 1998). These data suggest that magnesium production is a potentially large, but as-yet undetermined, dioxin source in the U.S.

Another potentially significant source category not included in the EPA inventory is the production and use of elemental chlorine, sodium hypochlorite, or metal chlorides. In each instance, the EPA cited the lack of data from U.S. facilities as the reason for not including these sources in the inventory. However, data is available from European facilities (USEPA, 1998). The production of PVC is identified as a source by EPA, but is not included in the inventory for the same reason - the lack of data on U.S. facilities. Greenpeace estimated that dioxin emissions from PVC production range from 500 to 1,000 gm TEQ per year (Thornton, 1994). This estimate would place PVC production among the largest sources of dioxin emissions in the U.S. Industry estimates are much lower (See appendix A).

The production of dioxin from accidental fires in homes and buildings that contain PVC wiring, carpeting, flooring, siding, molded furniture and other chlorinated plastics is also omitted from the inventory. There were over 500,000 structural fires in the U.S. in 1995 (USDOC, 1997), and several studies document that burning PVC plastic results in dioxin formation (Thiesen, 1989; Christmann, 1989). Several estimates of dioxin releases from accidental/structural fires have been made. Thomas (1995) estimated that 20 gm of TEQ are released annually from structural fires in the U.S. and Lorenz (1996) estimated that from between 78 to 212 gm TEQ are released annually from structural fires in Germany. Others believe that dioxin emissions from accidental/structural fires may be even greater, rivaling annual emissions from medical or municipal waste incinerators (Thornton, 1995).

Perhaps the largest known sources of dioxin omitted from EPA's National Inventory are the reservoir sources, which include dioxin in the bottom sediments of water bodies, dioxin in landfills, and dioxin in the hundreds of contaminated industrial and hazardous waste sites around the country.

Most of these sites are not cleaned up and are a major threat to their surrounding communities.
Dioxins at the bottom of oceans, lakes, and rivers are covered by new sediments each year, but if they are stirred up by dredging, storms, or floods, substantial amounts may re-enter the environment.

Another reservoir of dioxin is created by the fallout of airborne dioxin onto soil and vegetation (Weiss, 1998). Soil and vegetative surfaces are continually covered by fallout. The amount of dioxin on these surfaces will vary mostly depending on the proximity to different sources. However, construction, erosion, and other earth-moving activities such as dredging, can cause dioxin near the surface to re-enter the environment. For example, dioxin deposited on forest leaves by airborne fallout re-enters the environment during forest fires and brush fires. The EPA estimates that reservoir sources contain between 15 to 36 times the amount of dioxin typically generated in one year (USEPA, 1994b). Thus,

there is a great potential for future exposure from these sources even if all dioxin creation is stopped today.

For a number of the dioxin sources not included in the EPA inventory, estimates have been made for inventories of other countries or regions and/or identified in the scientific literature. These include estimates for:

- Waste oil disposal (Dyke, 1997)
- Run-off from roads (Dyke, 1997)
- Rubber vulcanization (Lexen, 1993)
- Electrolytic production of magnesium and aluminum (Danish EPA, 1997)
- Nickel refining (Danish EPA, 1997)
- Combustion of landfill gas (Fiedler, 1992; Koning, 1993; Bremmer, 1994;

Wevers, 1995; Douben, 1995; UKDE, 1995; Eduljee, 1996)

- Asphalt mixing plants (Koning, 1993; Bremmer, 1994; Douben, 1995)
- Accidental fires (Wevers, 1995)
- Carbon reactivation furnaces (Douben, 1995, Eduljee, 1996)
- Lime production (Wevers, 1995; Douben, 1995; UKDE, 1995; Eduljee, 1996)
- Ceramics and glass manufacture (Douben, 1995; Eduljee, 1996)
- Emissions from pentachlorophenol-treated wood (Douben, 1995; Eduljee, 1996)

Still other potential sources of dioxin identified by EPA or other sources (listed in parentheses) but omitted in the national inventory are:

- Textile manufacturing
- Dry cleaning operations
- Phthalocyanine dyes and pigments (Huntzinger, 1991)
- Printing inks (Santl, 1994)
- Drinking water treatment
- Small commercial and industrial furnaces and boilers
- High-rise apartment incinerators (Laber, 1994)

Differences Between 1998 and 1994 Estimates

EPA's estimated emissions of dioxin released into the air are lower in 1998 than in 1994. While this may appear to be good news, there are so many uncertainties in these estimates as well as significant sources not included in the 1998 inventory, that it is difficult to draw any meaningful conclusions about trends. There are several obvious differences between the two reference years. For one, the 1998 inventory adds the large release of 25,000 gm TEQ of pentachlorophenol. Also, since the 1994 report, dioxins released to the air have changed. Many municipal and medical waste incinerators, which

were both significant source categories of air releases reported in the 1994 inventory, are closed due in large part to the efforts of grassroots community based organizations. The resulting reduction in estimated air releases is offset to a great degree by the identification of two new large source categories in the 1998 report, open burning of household waste and landfill fires. Although both of these newly identified sources have preliminary estimated releases greater than 1,000 gm TEQ/yr, neither are included in EPA's National Inventory shown in Table 3-2.

Releases to Water

Because of the impact of point source discharges of dioxins to water on anglers and Native Americans, the SAB specifically asked EPA to further investigate these discharges in its review of the 1994 report (USEPA, 1995). Nonetheless, in both the 1994 and 1998 inventories, EPA estimates releases to water from only one source category, pulp and paper mills. Dioxins released to water from these sources are estimated to be 110 gm TEQ/yr in 1994 and 19.5 gm TEQ/yr in the 1998 report. This change is attributed to "process changes of a pollution prevention nature" and the availability of new, more accurate data (USEPA, 1998). Municipal waste water treatment systems, or publicly owned treatment works (POTWs), are listed as dioxin sources in the 1994 inventory, but not in the 1998 inventory, even though EPA provides a preliminary estimate of 163 gm TEQ/yr (USEPA, 1998).

In the 1998 report, EPA notes that there are insufficient data for most industrial and commercial facilities to quantify the dioxins they release to water, yet there are apparently no efforts to collect such data. According to EPA, there is one program in which chemical manufacturers report the amount of dioxin in their products, but not in their waste discharges. And since most dioxins formed during chemical and pesticide manufacture end up in wastes and waste treatment residues, this program can only identify a few dioxin sources among chemical and pesticide manufacturers.

Releases to Land

Another major difference between the 1994 and 1998 inventories is the treatment of dioxins released to landfills. In the 1994 inventory, dioxins released to landfills are included with those released to land and are estimated to total 2,100 gm TEQ/yr. This dioxin contaminated waste comes mostly from municipal incinerators (ash), POTWs (sludge), and cement kilns (dust) (USEPA, 1994b). These releases are not included in the 1998 inventory.

In the 1998 report, EPA establishes a new policy that seems to serve no purpose other

than to enable it to exclude dioxins released to landfills from the national dioxin inventory. It claims that "properly designed and operated landfills are considered to achieve long term isolation from the circulating environment" (USEPA, 1998). EPA's unsubstantiated assessment of landfill performance is contradicted by studies showing that dioxin-like chemicals evaporate from landfills and leach into groundwater from both existing and redundant landfills (USEPA, 1987; Bracewell, 1993; Hiraoka, 1993). A recent assessment of sustainable landfills concludes that "liner failure will occur ultimately, and that in the long term, the escape of waste materials and their products of degradation is inevitable" (Westlake, 1997).

EPA's recent decision to exclude dioxins that are taken to landfills from its inventory contradicts its earlier designation of incinerator ash disposal in landfills as a source category (USEPA, 1992). Moreover, this policy is highly inconsistent with those of other national inventories such as the European Union, the United Kingdom, and Denmark (Dyke, 1997; NRWSEA, 1997; Danish EPA, 1997). The U.K. inventory includes estimated dioxin releases to land/landfills from 23 source categories, with municipal incinerator ash being the largest contributor (Dyke, 1997). The European Union inventory estimates that dioxins released via residues and sent to landfills are of the same order of magnitude as those to air (NRWSEA, 1997). The U.K. inventory concludes: "Releases to land appear greater than those to air or water" (Dyke, 1997).

In its 1995 review, the SAB recommended that EPA evaluate more thoroughly the potential release of dioxins from reservoirs, including sediments, which SAB notes "might indeed be important" (USEPA, 1995). Nonetheless, EPA's new inventory lists only one source category, chlorophenol-treated wood, as a dioxin reservoir. The agency does, however, identify several
other reservoir sources - soils, sediments and vegetation - in its explanation of why these reservoirs are not included in its inventory (USEPA, 1998). EPA states that "no empirical evidence exists on the magnitude of reservoir emissions from soil to air." EPA presents no estimate of the quantity of dioxin-contaminated soils and sediments in the 1998 report. However, the U.S. Congress' Office of Technology Assessment estimated in 1991 that there were 500 million kilograms of dioxin-contaminated soil in the U.S. that require treatment (USOTA, 1991). If 1 part per billion (ppb) of 2,3,7,8-TCDD is the minimum level that requires treatment, this soil is a reservoir of at least 500 gm TEQ. It is also important to note that landfills, as described earlier, readily meet EPA's definition of a reservoir sources, as discussed above.

Other Inventories

The EPA's inventory of dioxin sources is one of only a few efforts to identify and estimate dioxin emissions from different sources. Other estimates exist in the U.S. (Travis, 1991; Thomas, 1995); Canada (Sheffield, 1985); Sweden (Rappe, 1991; Lexen,

1993); Germany (Fiedler, 1992); The Netherlands (Koning, 1993; Bremmer, 1994); Switzerland (Schatowitz, 1993); Austria (Riss, 1993); Belgium (Wevers, 1995); and Great Britain (Douben, 1995; UKDE, 1995). Also, the Center for the Biology of Natural Systems (CBNS) at Queens College in New York has collaborated with EPA to generate a separate estimate of dioxin emissions to air (Commoner, 1998). This study, headed by Dr. Barry Commoner, estimates total annual dioxin emissions for both the U.S. and Canada from 20 source categories to be 4,350 gm TEQ, of which 3,890 gm TEQ are generated in the U.S. and 460 gm TEQ are generated in Canada (Commoner, 1998). The survey found that 86% of the total dioxin emitted in the U.S. results from only five types of sources: municipal waste incinerators, medical waste incinerators, secondary copper smelters, cement kilns that burn hazardous waste, and iron sintering plants. Two of these, municipal and medical waste incinerators, account for 2,640 gm TEQ/year, nearly two-thirds of the total emissions in the U.S. This estimate agrees fairly well with EPA's total estimate of 2,745 gm TEQ/yr (4,925 gm TEQ/year if preliminary estimates are included) for these sources. CBNS comments that "given the uncertainties inherent in such estimate inventories ... the actual values may be several times greater or smaller than the mid-point" (Commoner, 1998).

Chapter 4

Exposure Levels in Americans

Simply by eating, Americans have accumulated harmful or almost harmful levels of dioxin in their bodies. Some segments of the population, such as nursing babies and people who eat a diet high in animal fat or foods contaminated because of their proximity to dioxin release sites, have been exposed to higher than average levels of dioxin. Others, such as Vietnam veterans and some chemical plant workers, have accumulated additional dioxins because of their exposure to Agent Orange or chemicals in the workplace. Previous chapters in this report have discussed the chemistry of dioxin and its sources. This chapter discusses how exposure to dioxin is measured, how much dioxin exposure the average American and certain highly exposed groups have experienced, attempts by several government agencies to establish "safe" levels of dioxin exposure, and whether such safe levels exist.

Daily Intake in the U.S.

One way of assessing health risks from exposure to dioxin is to measure the average

American's daily intake of dioxin. It is estimated that, simply by eating, the average American's daily dioxin intake ranges from 18 to 192 picograms (pg) TEQ per day. The EPA estimated that the average 150-pound adult ingests 120 pg of dioxin TEQ per day (Schaum, 1994). This is equivalent to a 75-kilogram adult ingesting 1.6 pg TEQ per kilogram of body weight (bw) per day. As discussed later, nursing infants ingest 35-118 pg/kg bw/day, far more than the average adult (Schecter, 1994a; Patandin, 1999).

In one of the few surveys of dioxin levels in food from U.S. supermarkets, Schecter (1994) estimated that the average American adult ingests 0.3 to 3 pg TEQ/kg bw daily. This is equivalent to a 65 kg (143 pound) adult ingesting 18 to 192 pg TEQ/day, a value that compares well with EPA's estimate of 120 pg TEQ/kg bw/day. The daily intake is less in strict vegetarians (Schecter, 1998). In a more recent survey of 100 food samples from supermarkets in Binghamton, New York; Chicago, Illinois; Louisville, Kentucky; Atlanta, Georgia; and San Diego, California, Schecter (1996) estimated that the average daily U.S. intake of dioxins for a 65 kg (143 pounds) adult ranges from 34 to 167 pg TEQ. This is equivalent to a daily adult intake of 0.52 to 2.57 pg TEQ/kg bw. If dioxin-like PCBs are also included, the daily adult intake increases to 1.16 to 3.57 pg TEQ/kg bw. These estimates agree with Schecter's original estimate and with EPA estimate.

Daily Intake in Other Countries

Studies conducted in other industrialized countries found estimates similar to U.S. values for the daily intake of dioxin. A survey of 100 food samples collected from commercial outlets in Canada reported a total daily dioxin intake of about 0.8 pg TEQ/kg body weight (Ryan, 1997). An earlier Canadian survey of different foods from the U.S. and Canada reported a total daily intake of 1.8 pg TEQ/kg body weight for a 60 kg adult (Birmingham, 1989). A German study of 22 samples of different foods estimated an average daily dioxin intake of 164 pg TEQ for a 70 kg (154 pounds) adult, equivalent to an average daily intake of 2.3 pg TEQ/kg body weight (Beck, 1989; Beck 1994). A second German study reported a daily intake of 85 pg TEQ (Furst, 1990). In the United Kingdom, it was estimated that the average daily intake of dioxin is 125 pg TEQ (Startin, 1994). The World Health Organization estimated that average daily intake of dioxin and dioxin-like PCBs in industrialized countries ranges from 3 to 6 pg/kg/day (WHO, 1998). Even people who live in remote places are affected by dioxin. The Inuit Eskimos of northern Canada have a high intake of dioxins and PCBs because their primary diet is fish, seal and whale, all animals high in the food chain and high in fat (Dewailly, 1994).

Dioxin Content in Foods

Approximately 90% (Schecter, 1994; Schaum, 1994; ATSDR, 1998), and perhaps as much as 98% (Hattemer-Frey, 1989), of the dioxin that Americans are exposed to comes from the foods they regularly eat. Though dioxin has been found in all organs of the body (Schecter, 1999), it accumulate mostly in the fats of meat, fish, and milk. Consequently, when people consume these foods, they also consume dioxins. As Table 4-1 indicates, ground beef has the highest dioxin content, 1.5 pg/gm (equivalent to 1.5 parts per trillion or ppt), of many common foods.

By multiplying the average dioxin content in a food by its average consumption rate (Schaum, 1994) (see Table 4-2), the total daily intake of dioxins from different food types by the average American (Figure 4-1) can be determined. As Figure 4-1 indicates, of the 119 pg/day taken in daily by the average American, about 97% (115.7 pg/day) comes from beef, chicken, pork, fish, and dairy products (cheese, milk, and eggs). However, the estimates in Figure 4-1 may not accurately reflect the dangers that consumption of some foods pose. For example, Figure 4-1 indicates that the average daily intake of dioxins through fish is low in the U.S., but that is because average fish consumption in the U.S. is low. If fish consumption were to increase, or if certain segments of the population who consume more fish than average were to be examined, measured daily intake of dioxins through fish would also increase. Also, as discussed below, certain segments of the population, either because of their diet or lifestyle, consume more dioxin in their food than the average American.

Table 4-1 Dioxin Levels in U.S. Foods

Food Type	**pg/gm TEQ (ppt)**
Ground beef	1.5
Soft blue cheese	0.7
Beef rib steak	0.65
Lamb sirloin	0.4
Heavy cream	0.4
Soft cream cheese	0.3
American cheese sticks	0.3
Pork chops	0.3
Bologna	0.12

Cottage cheese	0.04
Beef rib/sirloin tip	0.04
Chicken drumstick	0.03
Haddock	0.03
Cooked ham	0.03
Perch	0.023
Cod	0.023

Source: Schecter, 1994

Note: The amount of dioxin ingested when certain foods are eaten can be estimated by multiplying the average level of dioxin in that food (Table 4-1) by its average consumption rate (Table 4-2).

Source: USEPA, 1994b

Table 4-2 Consumption Rate for Various Food Groups			
Food Group	Consumption Rate (gm/day)	Low Range of Dioxin in Food (pg/gm) (ppt)	High Range of Dioxin in Food (pg/gm) (ppt)
Fruits and Vegetables	283	---	---
Milk	254	0.04	0.04
Beef	88	0.04	1.50
Other Dairy Products	55	0.04	0.70
Poultry	31	0.03	0.03
Pork	28	0.03	0.30
Fish	18	0.02	0.13

Source: Schecter, 1994

Body Burden Levels of Dioxin

In addition to measuring an organism's exposure to dioxin by its daily intake, its exposure can also be estimated by its "body burden." An organism's body burden not only estimates dioxins currently present in its body per kilogram of body weight, but it also reflects its total accumulation of dioxins. In contrast to daily intake, which is calculated in picograms (trillionths of a gram)/kg bw/day, body burdens are measured in nanograms (billionths of a gram)/kg bw. Two methods of estimating body burden may be used. The first one, the only option available until recently, estimates the body burden of dioxin in

an organism by analyzing a sample of its fat, which is difficult to obtain and expensive to analyze.

he second method, a result of the discovery that dioxins are carried in the lipid part of the blood and that these lipids reflect the concentration of dioxins in fat tissue (Papke, 1989; Schecter, 1990), makes it possible to calculate body burdens from blood samples. Whichever of the two methods is used to calculate body burdens, the following assumptions are made (DeVito, 1995): dioxins are almost equally distributed in the body lipids (fat), and all tissues have the same lipid-adjusted concentrations of TCDD; lipid-adjusted serum levels are equivalent to lipid-adjusted adipose tissue levels; and approximately 22% of the body weight of the average adult is lipids. Given these assumptions, body burden levels are calculated by multiplying lipid-adjusted serum or fat tissue concentrations (expressed as ng TCDD/kg or ng TEQ/kg) by 0.22, the fraction of fat in the body.

The most extensive survey of dioxin body burdens in humans, the National Human Adipose Tissue Survey (NHATS), conducted by the EPA in 1982 to monitor the level of selected chemicals in the general U.S. population (USEPA, 1991), used the fat sample method. In that survey, fat tissue samples from 865 individuals from different regions of the country indicated a national average dioxin tissue concentration of 28 ng TEQ per kilogram lipid or 28 ppt (USEPA, 1991; Orband, 1994). These levels show an increase with age, but there are no differences between races, sexes, or geographical regions of the U.S. (USEPA 1994b). In 1987, the survey was repeated, and the results suggest some decrease in average dioxin concentrations, but the decrease may be due to improved analytical methods or to other issues involving methods of study. For most congeners, including TCDD, the differences between 1982 and 1987 dioxin levels are not statistically significant (USEPA, 1994b).

Studies using the newer blood sampling technique are less extensive. In a study of 100 Americans, Schecter (1991) reported an average lipid-adjusted dioxin concentration of 41 ng/kg whole blood (combined PCDDs and PCDFs) and a range of 28 to 41 ng TEQ/kg lipid (Schecter, 1994a). In addition, average lipid-adjusted dioxin-like PCB concentrations range from 8 to 17 ng TEQ/kg tissue (Patterson, 1994). If the average PCBs levels are added to the average dioxin levels, the average total tissue concentrations range from 36 to 58 ng TEQ/kg lipid (DeVito, 1995).

Using the average tissue concentrations from the studies above, the estimated national average body burden of dioxins is 6 to 9 ng TEQ/kg body weight. If dioxin-like PCBs are included, the average body burden of dioxins ranges from 8 to 13 ng TEQ/kg body weight. In these estimates, TCDD contributes approximately 15% of the total TEQ (DeVito, 1995). These estimates represent average body burdens for a middle-aged person. Individuals vary, but, in general, younger people have lower body burdens than

older people.

Highly Exposed Groups

The reported levels of dioxin in meat, poultry, fish, and dairy products that most Americans are exposed to are averaged from food sources all over the U.S. However, people who eat a great deal of local food that is heavily contaminated with dioxin can accumulate higher than average dioxin concentrations. For example, though the uptake of dioxins by plant roots is very poor (Startin, 1994), if air emissions containing dioxins from large garbage incinerators, copper smelters, or similar dioxin sources settles on homegrown and farm crops, people who eat these plants may be exposed to higher than average levels of dioxins. Animals and animal products raised near a local dioxin source are more easily contaminated by dioxin than plants are. A subsistence farmer who lives near a large source of dioxin emissions and consumes the milk and beef produced on the farm risks a high exposure to dioxin.

People who fish for recreation and subsistence, or people who consume a great deal of freshwater, farm-grown or coastal fish or shellfish contaminated with dioxin also risk higher than average exposure to dioxin (USEPA, 1987; Hodson, 1992; USEPA 1992a). It has been demonstrated that levels of TCDD are higher in fish caught downstream from pulp and paper mills than fish caught where there are no paper mills (USEPA, 1987), and that concentrations of dioxin in sport fish and shellfish from dioxin-contaminated waters can be at least an order of magnitude higher than in commercial fish and shellfish purchased in a supermarket (ATSDR, 1998).

In EPA's 1987 National Dioxin Study, 38% of the fish caught downstream from paper mills have dioxin concentrations greater than 5 ppt, some as high as 85 ppt (Kuehl, 1989). These levels are higher than the average dioxin levels found in ground beef, which contains the highest levels of dioxin in food (see Table 4-1). Currently, 66 advisories in 21 states restrict consumption of dioxin-contaminated fish and shellfish. In addition, three states have statewide advisories for dioxins in their marine waters (USEPA 1998a). The level of concern is set in each individual state, but many states use the FDA tolerance level of greater than 25 ppt, but less than 50 ppt of dioxin in fish flesh to advise consumers to restrict consumption of dioxin contaminated fish and shellfish (ATSDR, 1998).

High Exposure from Accidents, the Workplace, and Agent Orange

People living in dioxin-contaminated communities such as Times Beach, Missouri; Jacksonville, Arkansas; and Pensacola, Florida have been, and in some cases still are, exposed to dioxins leaking from contaminated sites. These dioxins are in addition to those which people are already exposed to from food. Furthermore, dioxins have been identified in some form at 110 Superfund sites across the country (ATSDR, 1998), and people living near these sites may be exposed to higher than average levels of dioxins. Workers in industries that manufacture or produce products that are contaminated with dioxins have the highest exposures to dioxins. Among these workers are chemical industry workers, herbicide production and packaging workers, some herbicide and pesticide applicators, and some electrical workers. Workers and fire fighters responding to fires that burn polyvinyl chloride (PVC) or PCBs may also have especially high exposures. Some Vietnamese people and Vietnam veterans have higher than average dioxin body burdens because of their exposure to dioxin-contaminated Agent Orange. Residents of communities such as Seveso, Italy and Nitro, West Virginia, where industrial accidents released large amounts of dioxins into the community, are another group of highly exposed individuals (ATSDR, 1998). Discussions of each of these highly exposed groups can be found in other chapters of this report.

Dioxin in Breast Milk

Dioxin accumulates in breast milk because it readily dissolves in the milk's rich fat content. During nursing, it is transferred from mother to her baby (Schecter 1990a; McLachlan, 1993; Pluim, 1993; Abraham, 1994, 1996; Dahl, 1995) who may absorb as much as 95% of the dioxin in the milk (McLachlan, 1993; Pluim, 1993). Several studies reporting dioxin in human breast milk (ATSDR, 1998; Schecter, 1994b) indicate that levels range from 20 to 30 ng/kg TEQ in industrial countries and from 3 to 13 ng/kg TEQ in less industrialized countries (Table 4-3). The World Health Organization reports a worldwide mean of 20 ng/kg TEQ (fat), with values ranging from a low of 3.1 ng/kg TEQ (fat) to a high of 110 ng/kg TEQ (fat) (IARC, 1997).

The levels of dioxins in breast milk vary according to several circumstances. For example, because lactating mothers accelerate the elimination of dioxins from their bodies (Schecter, 1987), dioxin concentrations decrease as nursing continues. Concentrations are highest in the first weeks after delivery, and after a year of nursing, a baby ingests less dioxin from nursing than it does when it is two or three months old. The levels are measurably reduced by six weeks (Rogan, 1987).

Table 4-3 - Dioxin Levels in Pooled Breast Milk Samples

from Various Countries (ng/kg, lipid)

	PCDD TEQ		PCDF TEQ		TOTAL TEQ
Vietnam - Da Nang	18		16		34
Japan	12		15		27
Germany	13		14		27
Canada	18		8		26
USA	12		8		26
Vietnam - Ho Chi Minh City	13		6		19
South Africa - White	9		4		13
Pakistan	9		4		13
Russia	5		7		12
South Africa - Black	7		2		9
Vietnam - Hanoi	5		4		9
Thailand	1		2		3
Cambodia	2		1		3
PCDD = Polychlorinated dibenzodioxins PCDF = Polychlorinated dibenzofurans					

Source: Schecter, 1994b

Additionally, dioxin levels are 20-30% lower in mothers nursing their second child than in mothers nursing their first child (Rogan, 1986; Furst, 1989). Total dioxins in the serum lipids of nursing mothers decrease by approximately 50% after 2 years of nursing (Schecter, 1996a).

It may be possible for mothers to reduce dioxins in their fat stores by pumping their breasts between feedings and discarding the milk. They may further reduce their dioxin intake and eliminate more of it by changing to a low animal fat or vegetarian diet. Women who are long-term strict vegetarian or on a low fat diet have less dioxins in their breast milk than other women (Schecter, 1998). However, because dioxin is present in

long term fat stores, changing to a vegetarian diet at the time of the baby's birth is unlikely to change breast milk dioxin levels (Pluim, 1994).

Though women have about the same concentration of dioxins in fat stores regardless of weight, overweight women take much longer than women of normal weight to eliminate their store of dioxins. Also, older women nursing their first baby have higher levels of dioxins in milk simply because they have had a longer time to accumulate them (Bates, 1994). A number of studies show that mothers of experimental animals also transfer dioxins to their nursing infants, who may then accumulate higher levels of dioxins than their mothers have (Bowman, 1989; Abbott, 1996). During nursing, rhesus monkeys transfer 17 to 44% of their dioxins to their infants (Bowman, 1989), and concentrations in the fat of the infants increase to four times that in the fat of their mothers. The infants of breast-fed marmoset monkeys have higher concentrations of 2,3,7,8-TCDD in their livers than do their mothers (Hagenmaier, 1990).

The Daily Intake of Nursing Infants

Nursing infants ingest considerably more dioxins each day than adults. Because of this, they are considered a heavily exposed population. Table 4-4 shows the results of two studies, one in the U.S. and the other in the Netherlands, that estimated daily intake of dioxins according to the age of the infant. The U.S. study found that nursing infants typically consume between 35 and 53 pg TEQ/kg body weight per day from breast milk (Schecter, 1994). The more current Dutch study, however, found that nursing infants typically consume about 112-118 pg/TEQ/kg bw/day (Patandin, 1999). If the Dutch study is correct and infants consume dioxin at the rate of about 112-118 pg/TEQ/kg bw/day, and adults typically take in only about 2.2 pg TEQ/kg/ bw/day (Schecter, 1999), then nursing infants consume about 50 times more dioxin per day than adults do. Schecter (1996a) estimated that approximately 10-12% of total lifetime exposure can occur via nursing.

Table 4-4 **Dioxin Equivalents (TEQ) Consumed Each Day at Different Ages**

U.S. Study*		**Dutch Study ****	
Age	picograms TEQ/kilogram body weight/day	Age	picograms TEQ/kilogram body weight/day

Breast-fed infant	35 - 53		Birth to 6 months		112 - 118
Formula-fed infant	0.07 - 0.16				
1-4 years	1 - 32		1-5 years		6.3 - 6.5
5-9 years	1 - 27		6-10 years		3.5 - 3.9
10-14 years	0.7 - 16		10-15 years		2.7 - 3.0
15-19 years 0.4 - 11			16-20 years		2.1 - 2.5
adult >20 years	0.3 - 3		20-25 years		2.2 - 2.4

Source: * Schecter, 1994
**** Patandin, 1999**

Breast-fed babies accumulate far more dioxin than do formula-fed babies. Abraham (1996) found that the intake of CDDs and CDFs is up to 50 times greater in breast-fed infants than it is in formula-fed infants. He also found that TEQ concentrations in blood from 11 month old formula-fed infants are less than one fourth the concentration of the mother's blood and about 10 times less than the concentration in infants that are breast-fed for six to seven months. Chapter Eight discusses dioxins' effects on children who are breast-fed.

"Safe" or "Tolerable" Dioxin Levels

Three separate federal or international agencies have established a "safe" or tolerable daily dose for dioxin (see Table 4-5). The first agency to do this was the EPA. In 1985, it determined that a "virtually safe dose" of dioxin is 0.006 pg/kg bw/day or 0.42 pg/day for a 150 pound (70 kilogram) adult (USEPA, 1985). In 1994, EPA raised this limit by almost 100 times when it released for public comment a draft reassessment report on dioxin that defined an dose of 0.01 pg TEQ/ kg bw/day, equivalent to 0.7 pg/day for a 150 pound adult, as posing a cancer risk of one additional cancer in one million people exposed, (USEPA, 1994a). This "risk dose equivalent" is designed to protect adults and does not include any added protection for children.

Table 4-5 Daily Intake of Dioxin (TEQ) Compared to Established Guidelines		
	Guideline	**Equivalent Intake for 70 kg**
EPA Risk Specific Dose	**0.01**	**0.70**
ATSDR Minimal Risk Level	**1.0**	**70.0**
WHO Tolerable Daily Intake	**1-4**	**70-280**
Average Daily Intake of Dioxin	**1-3**	**70-210**
Average Daily Intake of Dioxin and Dioxin-Like PCBs	**3-6**	**210-420**

The second agency to attempt to establish a safe daily intake of dioxins was the World Health Organization (WHO). In 1990, WHO established a Minimum Risk Level (MRL) of 10 pg/kg bw/day. A minimum risk level estimates the daily human exposure to a hazardous substance that is unlikely to cause appreciable risk of non-cancer health effects over a specified duration of exposure (ATSDR, 1998). In 1998, WHO came up with a revised calculation, called tolerable daily intake (TDI). Its range of 1 to 4 pg/kg bw/day (WHO, 1998), for a daily ingestion of 70 to 280 pg in a 70 kg adult, was substantially higher than EPA's 0.7 pg/day dose for a 70 kg adult.

In 1998, the Agency for Toxic Substances and Disease Registry (ATSDR) set minimum risk levels (MRLs) for acute, subacute, and chronic exposures to dioxin (ATSDR, 1998). ATSDR and WHO used the same studies but different endpoints to calculate MRLs. ATSDR used altered group behavior (Schantz, 1992), and WHO used object learning (Schantz, 1989). ATSDR's chronic MRL for dioxins came out to be 1 pg/kg bw/day. The WHO working group used an average daily intake of dioxins that ranges from 3 to 6 pg TEQ/kg bw (WHO, 1998). This range, even at the lower end, substantially exceeds the EPA risk specific dose and is at least three times the ATSDR MRL. The WHO acknowledged that its TDI range of 1-4 pg/kg bw/day overlaps the typical daily intake of dioxins in food, and that some people may be harmed by dioxin simply because they eat.

Nevertheless, the WHO workgroup accepts its TDI range of 1-4 pg/kg bw/day as "tolerable on a provisional basis as these reported subtle effects were not considered overtly adverse." The workgroup was also concerned about the health effects of dioxin-like compounds, but it stressed that "the upper range of the TDI of 4 pg TEQ/kg bw should be considered a maximal tolerated intake on a provisional basis and that the ultimate goal is to reduce human intake levels below 1 pg/kg bw/day" (WHO, 1998).

As Table 4-5 shows, no matter which agency's calculations are used to establish safe daily intake levels of dioxins, the average daily intake of the average person, approximately 120 pg, exceeds or equals them all. The average daily intake of Americans, which is about 2 pg/kg bw (Schecter, 1999) is more than 200 times higher than the EPA dose, twice the ATSDR MRL, and in the middle of the WHO TDI range. If dioxin-like PCBs are included, then the daily intake of dioxin is that much higher than these standard guidelines. Table 4-5 shows that the average daily intake of dioxin in the U.S. is well above these federal and international guidelines.

"Safe" Body Burden Levels

Knowing the average half-life of dioxin in the human body (7.5 years), and assuming that they are eliminated at a constant rate, current body burdens can be used to estimate previous body burdens (or dose equivalents) after a specific exposure event (DeVito, 1995). Furthermore, because body burden measurements account for pharmacokinetic differences across species and between individuals (Birnbaum, 1999), they can be used to compare the doses needed to produce similar adverse effects in different species. Such a comparison was made by a World Health Organization (WHO) working group, convened to re-evaluate the Tolerable Daily Intake for dioxin. The results, which indicate that body burdens in Americans are already at harmful or near harmful levels, are as follows. First, the WHO working group listed the most sensitive endpoints associated with exposure to dioxin in animals other than humans. As Table 4-6 indicates, the reproductive and immune systems are particularly sensitive to the toxic effects of dioxin. Most importantly, the lowest observed adverse effect levels (LOAELs), ranging from 10 to 73 ng/kg, are all within an order of magnitude (factor of 10) of the average "background" body burden of 10 ng/kg in the U.S. population. The WHO working group's results assume that body burdens are a reasonable exposure measure, that extrapolations of body burdens from single doses are valid, and that the TEQ system provides reasonable estimates of human body burdens.

Table 4-6 Animal Body Burden Levels Associated with Sensitive Adverse Effects

Body burden (ng/kg)	Species	Health Effect
73	Rats	Genital malformations (females) (Gray, 1997a)
50	Rats	Immune suppression (Gehrs, 1997,

		1998)
42	**Monkeys**	**Endometriosis (Rier, 1993)**
42	**Monkeys**	**Object learning (Schantz, 1989)**
28	**Rats**	**Decrease in sperm count (Gray, 1997)**
10	**Mice**	**Adult immune suppression (Burleson, 1996)**

Current body burdens in the background population: approximately 10 ng/kg
Source: WHO, 1998

Second, the WHO working group converted the body burdens for the animals in Table 4-6 to Estimated Daily Intake (EDI) values which, on a chronic basis, can lead to similar body burdens in humans. Assuming steady state conditions, EDIs are calculated using the formula:

EDI (ng/kg/day) = Body Burden (ng/kg) x [ln(2)/half-life]/f

where "f" is the fraction of the dose absorbed (assumed to be 50% from food), and the estimated half-life of TCDD in humans is assumed to be 7.5 years. This formula was used because it accounts for the large differences in the half-lives of dioxin-like compounds in various species (WHO, 1998). The results are shown in Table 4-7.

Table 4-7 Animal Body Burdens and Associated Human Estimated Daily Intakes (EDI)

Response	**Body Burden (ng/kg)**	**Human EDI (pg/kg bw/day)**
Genital malformations	**73**	**37**
Immune suppression	**50**	**25**
Learning deficits	**42**	**21**
Endometriosis	**42**	**42**
Sperm counts	**28**	**14**

Current body burdens in the background population: approximately 10 ng/kg
Source: WHO, 1998

Following its calculations, the WHO commented that "the lower doses giving rise to statistically significant effects in the most sensitive endpoints following exposure have resulted in body burdens (e.g. 3 to 73 ng of TCDD/kg) in the exposed animals that overlap, at the lower end, the range of body burdens expressed as TEQ that are found in the general population in industrialized countries exposed to background levels of PCDD, PCDFs and PCBs" (WHO, 1998).

This comparison shows that there are small differences between the body burdens of dioxins that cause adverse non-cancer effects in animals and average body burden levels in the general American population. Some people who have above average body burden levels are already suffering from the adverse effects of exposure to dioxin.

Another way to compare the effects of dioxin exposure between animals and humans is to examine the ratio between the lowest observed effect level (LOEL) in animals and human exposure. This ratio is called the "margin of exposure." As Table 4-7 shows, the lowest observed adverse effect levels, ranging from 28 to 73 ng/kg, are all within a factor of 10 of the average body burden of 10 ng/kg in the human population. This indicates that there is little or no "margin of exposure" (Birnbaum, 1999) or "margin of safety," as it is sometimes called. This is a very significant and worrisome observation.

The EPA prepared a table similar to Table 4-7 that includes both human and animal body burdens (USEPA,1994a). Their data (Table 4-8) indicate that the lowest body burden that is harmful in humans is 14 ng/kg, a level slightly above the "average" American's body burden of 9 ng/kg. Many Americans have dioxin levels above that average, and some are more sensitive to dioxins than others. The comparisons in Table 4-8 are the basis of the statement made several times in the EPA reassessment document that "levels of dioxins are at or near the levels known to cause harm" (USEPA, 1994a).

Table 4-8 Levels of Dioxin Known to Cause Health Problems

Body Burden (ng/kg)	Species	Health Effect
83	**Human**	**Decreased testosterone**
64	**Rats**	**Decreased sperm count**
54	**Monkeys**	**Endometriosis**
19	**Monkeys**	**Learning disability**
14	**Human**	**Decreased testes size**
14	**Human**	**Altered glucose tolerance**

7	**Mice**	**Increased susceptibility to viruses**
7	**Monkeys**	**Altered immune response**

Source: USEPA, 1994a

The information in Tables 4-6, 4-7, and 4-8 indicates that dioxin harms people at body burden levels ranging from 14 to 83 ng/kg, levels comparable to those that harm other animals. If depression of the immune system occurs at 7 ng/kg (Table 4-8), and Americans have an average dioxin body burden of 10 ng/kg, then the immune system of some Americans may be compromised, and any general increase in dioxin exposure may be even more harmful to the general population.

Whether one uses daily intake rates or body burdens, the levels of dioxin that Americans have been exposed to are harmful or just short of being near harmful. Dioxin is an ubiquitous toxin that reaches people in a most fundamental way: through our food. Whether that food comes from supermarket shelves, fish in a river, or breast milk, it contains measurable and often harmful amounts of dioxin.

Chapter 5

How Dioxin Affects the Human Body

Other chapters in this report deal in depth with how dioxin causes cancer, disrupts the immune system, and interferes with or damages other processes in humans and other animals. This chapter presents a more general understanding of how dioxin is distributed and absorbed once it enters the body, how it targets and is retained in certain organs, how it is processed, and, in particular, how it affects the functioning of life's fundamental unit, the cell.

Absorption and Distribution

Though studies on the health effects of dioxin in animals use prescribed doses under controlled conditions, studies of its health effects on humans are more complicated

because people are accidentally exposed to usually unmeasured doses under uncontrolled conditions with all kinds of complicating factors. Nevertheless, scientists are using sophisticated techniques to track even small quantities of dioxin in a living organism and uncover its effects on humans and other animals. Once dioxin is in the body, the bloodstream readily distributes it to all its organs (Olson, 1994). Because dioxin does not dissolve well in the blood (blood is mostly water), it stays there for only a short time and tends to accumulate in fatty tissues where it does dissolve, and in the liver which is very fatty (Van den Berg, 1994). Its distribution to the various internal organs depends on blood flow to a given organ, relative organ size, and the exposure dose (Olson, 1994). At low doses, a major portion of it ends up in fatty tissue. At high doses, for reasons which will be explained below, a higher proportion ends up in the liver (Abraham, 1988). There does not appear to be major differences in the tissue distribution of dioxins among species or strains of mice.

Absorption of a particular dioxin (a "congener") depends on how it is administered and on its molecular size and solubility (IARC, 1997). As with distribution of dioxin, there appears to be only minor differences in gastrointestinal absorption among rodents (Van den Berg, 1994; IARC, 1997).When taken orally as part of the diet, 50% to 90% of dioxin is absorbed (Olson, 1994; IARC, 1997). Absorption through the lungs is similar to that observed following oral exposure (Nessel, 1990, 1992; Diliberto, 1996). Absorption through the skin is much more limited (Nessel, 1992; Diliberto, 1996). Absorption from dermal exposure is probably less than 1% for all congeners (IARC, 1997).

Metabolism

One would expect that, after exposure to high doses of dioxin, more of the toxin would accumulate in fatty tissues where it dissolves best. Instead, a greater proportion of it ends up in the liver (Abraham, 1988). Apparently, the liver, when faced with high concentrations of this poorly soluble compound, makes more of the protein cytochrome P-450 1A2 (CYP1A2) to which dioxin binds (Olson, 1994; Diliberto, 1997; IARC, 1997). This protein is quite abundant in the liver, and it becomes more so in the presence of dioxin and related compounds (Voorman, 1987, 1989; Poland, 1989; 1989a). It does not bind to dioxin as tightly as does the Ah receptor described below, but, because of its abundance, it is the major protein to which dioxin binds.

Elimination and Persistence

The body excretes dioxin by first metabolizing or converting it to more water soluble and less harmful compounds in the liver. However, in both people and laboratory animals, these changes happen very slowly, so even low doses build up over time. Consequently, once in the body, dioxin is there to stay. Though it is excreted slowly from all animal

species tested, the rate of excretion differs among individuals and species. Half-lives (the amount of time it takes for half the amount of a given substance to be eliminated from the body) range from 11 days in the hamster to 2,120 days in humans (Olson, 1994). The half-life in rats ranges from 17 to 31 days (IARC, 1997) and is less in mice (Gasiewicz, 1983; Birnbaum, 1986). The half-life in rhesus monkeys is about 391 days (Bowman, 1989). In humans, dioxin is eliminated much slower than in other animals (IARC, 1997). Its half-life ranges from 5.8 to 14.1 years (Wolfe, 1994; Michalek, 1996; Grassman, 1998). This means that following chronic exposures, humans retain considerably more dioxin than do rats. A half-life of 9.7 years was calculated in a 42-year old male volunteer who ingested 105 ng of 2,3,7,8-TCDD (Schlatter, 1991). This calculation was based on data collected for 5 years following ingestion. More than 87% of this dose was absorbed from the gastrointestinal tract.

People with high body fat appear to store more dioxin and eliminate it more slowly than people with low body fat. Operation Ranch Hand soldiers with a high percent body fat who sprayed Agent Orange during the Vietnam war show a significantly longer half-life of 2,3,7,8-TCDD than those with low body fat (Wolfe, 1994). Since breast milk is rich in fat, mothers exposed to dioxin may accumulate considerable quantities of it in breast milk, and lactation would increase the rate at which it is eliminated. Unfortunately, nursing a baby inadvertently exposes it to dioxin, but generally, the benefits of nursing are believed to outweigh the risks (see Chapter Eight).

How Dioxin Affects Cells

Basically, dioxin affects cells by interfering with the expression (the turning on and off) of genes, which are responsible for making specific proteins. Nearly every cell in the human body contains the same genetic information in its chromosomes. The chromosomes are strands of DNA in the nucleus of the cell, and the genes are discrete pieces of this DNA. The chromosomes in people contain more than 100,000 genes, and these genes contain the directions for making proteins. Proteins make the body work. Not only are they the actual structure of the body, but they are its enzymes, hormones, antibodies, and a host of other endogenous substances that make virtually every part of the body function the way it does. Since different cells have specific functions, they only use some of their full set of genes to perform these functions. In fact, each cell keeps most of its genes turned off. For example, when development is completed in an embryo, the genes that control growth must be turned off, and the genes that control particular cell functions must be turned on, or "expressed." If this carefully programmed sequence is disrupted, then birth defects or developmental toxicity could result.

Dioxin interferes with this process of "knowing" when to turn genes off and on. For some genes, we know how dioxin turns them on, but for others, we do not. Under normal circumstances, genes function quickly, triggering a series of actions that determine

normal cell function and then shut off. However, with dioxin in the receptor site, the message is different and the normal process that determines cell functions is altered. With dioxin, gene function can either be blocked, or kept turned on inappropriately, such as occurs with cancer.

Dioxin readily enters a cell and binds to a soluble protein called the aryl hydrocarbon or "Ah" receptor. The Ah receptor is a highly conserved basic helix-loop-helix protein (Schmidt, 1996). It is the only known member of this group of proteins that is ligand activated (Birnbaum, 1999). The Ah receptor is found in the cells of all vertebrates (Hahn, 1994, 1995, 1997), in most if not all parts of the body, including the liver, lung, lymphocytes, and placenta (Okey, 1994; Rowlands, 1997). It exists in a multi-protein complex associated with heat shock and other proteins. Upon binding to a ligand, a conformational change results in release of the other proteins and association of the ligand binding unit, Arnt, or aryl hydrocarbon nuclear translocator.

The Ah receptor acts as a signal transducer and activator of gene transcription (DeVito, 1994; NTP, 1997). A signal transducer is a receptor protein that responds to a signal in a cell and allows that signal to be transmitted to another protein in a chain of events or cascade (see Figure 5-1). Gene transcription is the process of synthesizing a messenger RNA (mRNA) molecule from a specific sequence of DNA known as a gene. Once the mRNA is transcribed or synthesized, the message is then translated into a protein.

When dioxin binds to the Ah receptor (AhR), a complex is formed, becomes "activated," and can interact with other proteins (see Figure 5-1). One such protein is "Arnt" which helps move the Ah receptor-dioxin complex into the nucleus of the cell. Once inside the nucleus, the complex binds to DNA leading to alterations in gene expression.

Many genes can be expressed (turned on or off) by this dioxin complex, some of which are critical determinants of normal cell growth. The most commonly studied gene is the cytochrome P450 1A1 or CYP1A1 gene (Whitlock, 1993, 1996, 1999; Safe, 1995). The CYP1A1 gene encodes for a protein whose family members help detoxify or activate either the body's own hormones and other chemicals, or foreign chemicals such as dioxin and PCBs. A second process that alters cell signaling that involves activation of protein tyrosine kinases has been proposed (Matsumura, 1994). This mechanism is also shown in Figure 5-1. The precise mechanism involving the Ah receptor is not known.

Source:

Birnbaum, 1995

Several proteins involved in binding can modify the dioxin-AhR-Arnt complex and in turn produce proteins that influence hormone metabolism and growth factors affecting many biochemical and physiological processes including reproduction and the immune system. The complex can also cause genetic changes that lead to cell proliferation, increased risk for mutations, or cancer (Okey, 1994; Rowlands, 1997).

Although poorly understood, the Ah receptor system probably has a very important biological role. Many researchers believe that the Ah receptor has one natural ligand, or perhaps a few natural ligands. Recent genetic studies have revealed that expression of the Ah receptor is a requirement for proper embryonic development which appears to be a common function shared by many other helix-loop-helix proteins (Rowlands, 1997). If the Ah receptor system behaves like many other biological-signaling systems, first the expression of the ligands are probably tightly controlled in space and time, and, second, the ligands are degraded after use, perhaps by the CYP1 family of phase I enzymes. Dioxin, being foreign to biological systems, may cause problems because it interferes with these two signaling features.

Additionally, there is a family of closely related Arnt proteins that, when complexed with dioxin and the Ah receptor, may turn on different genes. This may account, in part, for

the diversity of actions of dioxin in different tissues. Furthermore, the Arnt and other involved proteins are defined by genes that can vary from individual to individual. This may explain why sensitivity to dioxin seems to vary widely among individuals. In some people, dioxin may cause chloracne, in others a suppression of the immune system, in others a change in hormone levels, and in others all of these problems. Additional research is needed to better understand the role and function of the Ah receptor and related Arnt proteins.

Many compounds with the right size and shape bind to the Ah receptor. Dioxin, being similar to natural aromatic hydrocarbons from plant foods like broccoli or cauliflower that enter the body, is one of these. However, whereas the naturally occurring compounds are easily and rapidly metabolized by a wide range of enzymes in the liver and then eliminated from the body, dioxin is not. Rather, it persists, ties up the Ah receptor, and prevents normal use of the site to regulate cell function. Also, while dioxin is bound to the receptor, a constant signal is sent to the nucleus to make more RNA and protein, simulating a switch stuck in the "on" position. Dioxin's persistence may prove to be critical in understanding how it causes its many varied effects.

The Ah receptor is believed to mediate most, if not all, of the biological and toxic effects induced by dioxins (Safe, 1990; Birnbaum, 1994; Okey, 1994; Hankinson, 1995; Van den Berg, 1998). In both rodents and humans, TCDD has the highest affinity (degree of binding) of the chlorinated dioxins and furans for the Ah receptor. It induces a wide spectrum of biological effects, including induction of gene expression, altered metabolism, altered cell growth and differentiation, and disruption of steroid hormone and growth factor signal transduction pathways (IARC, 1997; NTP, 1997). Similar Ah receptor-mediated responses are observed in both rodents (Birnbaum, 1994) and humans (Harper, 1991), suggesting common mechanisms of action. These responses occur at similar tissue concentrations (DeVito, 1995).

There are good reasons to believe that binding to the Ah receptor is central to the biological responses to dioxin. First, the Ah receptor binds to dioxin at the concentrations at which dioxin has its biological effects. Second, the more toxic a member of the dioxin family is, the more tightly it binds to the Ah receptor: TCDD binds more strongly than other dioxins. Third, genetic variants of the Ah receptor correlate with different responses to dioxin. C57BL/6 mice, which have Ah receptors with high binding affinity for dioxin, are sensitive to its toxic effects (Poland, 1980). DBA/2 and other mouse strains, which have Ah receptors with low affinity for dioxin, are much less sensitive to its effects (Okey, 1989). Fourth, Ah deficient mice strains show reduced or no toxic effects when exposed to dioxin compared to normal mice (Fernandez- Salguero, 1995, 1996; Schmidt, 1996; Mimura, 1997). These "knock-out" mice show signs of premature aging (Fernandez-Salguero, 1996) and difficulties during pregnancy including maintaining conceptuses, surviving during pregnancy and lactation, and rearing pups to weaning (Abbott, 1999).These knock-out mice were produced by using genetic techniques to

"knock-out" the gene that makes the Ah receptor protein.

As in other animals, human populations vary widely in Ah receptor binding affinity (Micka, 1997; Grassman, 1998). A recent study reports more than a 20 fold difference in Ah receptor binding affinity in 86 human placenta samples (Okey, 1997). In general, the Ah receptor in human tissues has a similar but slightly lower affinity for TCDD than it does in many other species (Moore, 1979, 1991; Chahoud, 1989; Egeland, 1994; Rowlands, 1995). The binding affinity of TCDD to the Ah receptor is high for all species examined, and while its effects vary widely from species to species (Van den Berg, 1998), many species are affected at dose levels that are within one order of magnitude (DeVito, 1995). Dioxin's binding affinity does not predict its lethal or toxic effects among species (Kleeman, 1988), nor does it predict whether humans are more or less sensitive to its effects than other species are (DeVito, 1995; ATSDR, 1998).

We know that dioxin is a very powerful and persistent substance that travels quickly through the blood stream, accumulates in fatty tissues and the liver, and that it affects a cell's gene expression by interacting with the Ah receptor. While initial binding to the Ah receptor is necessary for dioxin's toxicity, it does not fully explain its wide variety of toxic effects (DeVito, 1995; Sewall, 1995). To fully understand dioxin's toxicity, we need to study the steps after binding and activation, which appear to be critical, and the variety of additional cytoplasmic and nuclear proteins capable of interacting with the Ah receptor.

Chapter 6

Dioxin and the Immune System

The immune system is a complex, interdependent network of cells that detects and eliminates foreign invaders such as bacteria, viruses, parasites, and possible cancer cells. It also recognizes and avoids reacting against the body's own cells and tissues. The growth, maturation, and activity of the immune system's many cells is under the control of hormones or hormone-like chemicals. This chapter examines how upsetting the immune system can lead to allergies, inflammation, autoimmune diseases, and increased susceptibility to infection and cancer.

If the immune system is suppressed, one may be more susceptible to infectious diseases and to cancer. An over-active immune system can led to allergies, inflammation, and autoimmune diseases. In allergy, the immune system correctly recognizes something as foreign, such as ragweed pollen, but incorrectly reacts against it as if it could grow to be harmful. In inflammation, sometimes the immune system over-reacts and damages normal tissues inadvertently, such as in septic or toxic shock. In autoimmune diseases such as lupus and scleroderma, the immune system mistakes the body's own normal cells and molecules as foreign and attacks them.

A vital part of the immune system is the thymus gland. Located behind the breast bone, it is relatively large at birth and during childhood, but it shrinks relative to body weight. Actually, the size doesn't decrease much. Instead, with age, the thymus becomes more filled with fat. In the thymus, the immune system's T cells develop from extrathymic stem cells and, especially in the fetus and early infancy, develop the ability to distinguish between normal cells or "self" and foreign invaders. This process goes on throughout life. It is just in fetal and early life that the thymus' rate of production as a percent of the total system is greater.

One of the first and most consistently observed toxic effects of dioxin is a dramatic shrinking of the thymus in young animals (McConnell, 1978; Poland, 1982; Kerkvliet, 1994). Animals exposed to dioxin and dioxin-like chemicals before birth are more sensitive to immune suppression than exposed adults (Vos, 1974; Faith, 1977; Luster, 1980; Gehrs, 1997, 1998, 1999).

Evidence from studies of animals indicates that dioxin can suppress activity of the immune system at much lower doses than those which visibly shrink the thymus. For example, dioxin decreases the resistance of mice and other animals to infections and cancers (Thigpen, 1975; Vos, 1978; Thomas, 1979; Hinsdill, 1980; Luster, 1980; Clark, 1983; Tucker, 1986; White, 1986; House, 1990). Mice infected with influenza die at a higher rate if they are first exposed to a single dose of as little as 10 ng of dioxin per kg of body weight, the smallest single dose of dioxin ever observed to have a clearly toxic effect (House, 1990; Burleson, 1996).

Recent mass deaths of marine mammals may be due to increased susceptibility to viruses after exposure to pollutants. Captive seals fed fish from the contaminated Baltic Sea have several times more dioxin-like compounds, especially PCBs, and a weaker immune response than seals fed fish from the relatively cleaner Atlantic Ocean (Ross, 1995). The seals in these studies, like people, are not exposed to a defined dose of laboratory TCDD but to all of the dioxins and dioxin-like chemicals that are typically found in the environment and whose levels are high enough to alter the immune system. This study adds further support to the finding that the levels of dioxins found in the environment could be high enough to suppress the immune system. It is possible that other Baltic Sea

pollutants may have contributed to this effect as well.

How Dioxin Affects the Immune System

Dioxin is thought to affect individual cells of the immune system by first reacting with the Ah receptor (see Chapter Five). How it further affects the interaction of all the cell types in the entire immune system is not known, but the toxic effects are often greater than predicted from tests on isolated immune cells in a test tube. These indirect effects on the immune system may be in part due to hormonal systems or by altering the behavior of cells that nurture and support the primary cells of the immune system. Inbred strains of mice differ in their sensitivity to dioxin toxicity due to different genes (alleles) of the Ah receptor (Poland, 1987, 1990).

Sensitivity to lethal effects of dioxin caused by Ah alleles is correlated with differences in sensitivity of the immune system to much lower doses of dioxin, providing further evidence that the immunotoxicity operates through the Ah receptor. Studies indicate that Ah receptor knockout mice, whose Ah receptor gene is absent in all body tissues, are resistant to many of dioxin's effects, including those on the immune system (Fernandez-Salguro, 1995, 1996; Schmidt, 1996), and that dioxin must directly affect the Ah receptor in immune system cells to show its effects (Kerkvliet, 1994; ATSDR, 1998). These studies suggest that dioxin damages the immune system by turning on genes in the immune system's cells (Staples, 1998; Thurmond, 1999).

It may be that dioxin causes the Ah receptor, which partners with other important proteins, to draw those proteins out of circulation so that they cannot do their normal job in the immune system. Further studies on the Ah receptor knock-out mice and with other genetically modified mice will tell us more about why dioxin is so immunotoxic.

Dioxin and Human Immune Function

The information on dioxin's immunotoxicity to humans is limited, and sometimes contradictory or inconsistent. Nevertheless, the Ah receptor in mice and humans is very similar, as is their response to some of the toxic effects caused by exposure to dioxin (Birnbaum, 1994), so mice provide a good model for predicting the effect of dioxin in people. To protect public health, we should assume that it is likely that at least some people are as susceptible to the immunotoxic effects of dioxin as are sensitive mice.

Generally, people who are accidentally or occupationally exposed to dioxins, furans, or PCBs have more skin and respiratory system infections (Bekesi, 1979; Lu, 1985;

Jennings, 1988; Webb, 1989; Zober, 1994), middle ear infections (Chao, 1997), and exhibit more immune system damage than people who are not exposed. An initial study of residents at the Quail Run Mobile Home Park, a notorious dioxin-contaminated site, in Times Beach, Missouri, indicated that these residents had depressed cell-mediated immunity (delayed hypersensitivity) (Hoffman, 1986), but a subsequent study did not confirm this result. However, the second study found that the residents had a shift in thymosin alpha-1 protein levels correlated with the dioxin levels in their fat tissues (Evans, 1988).

In Germany, workers exposed to high levels of dioxin had reduced T-cell activities (Tonn, 1996); higher levels of IgA, IgG, IgM and complement (Ott, 1994); and impaired immune responses (Ernst, 1998). In the Air Force Ranch Hand study of Vietnam veterans who sprayed Agent Orange, significant positive associations were found between IgA and serum 2,3,7,8-TCDD levels (Roegner, 1991). The authors suggested that this rise in IgA is consistent with a subclinical inflammatory response of unknown origin. In Taiwan, children exposed to dioxin in the Yu-Cheng rice oil poisoning incident had several alterations in immune system function (Hsu,1994). In Seveso, Italy, one study showed that children exposed to dioxin after an accidental explosion had higher levels of complement activity, a set of proteins that take part in the immune response, higher values for lymphocyte responses to pytohemagglutinin and pokeweed mitogen, and increased numbers of peripheral lymphocytes (Bertazzi, 1998). However, a second study did not find any differences in immune systems between children from the more contaminated zones compared to the less contaminated areas (Reggiani, 1978). Recent studies using more sensitive techniques suggest that there may be differences between the children from the more contaminated zones compared to the less contaminated areas (Mocarelli, 1999).

In the Netherlands, a study of 207 normal children from birth suggested that those with high levels of dioxin and dioxin-like compounds in their bodies had more immune system alterations (and neurodevelopmental problems) after 42 months than did those with low dioxin levels. These findings are especially disturbing because the dioxin levels in the mothers of these children are similar to the average levels found in most industrialized countries (Weisglas-Kuperus, 1998).

Our understanding of the human immune system's response to dioxin is limited for several reasons. First, there are not good tests for measuring how the various parts of the system interact. Second, very little is known about the range of susceptibilities to dioxin exposure among individuals. We can be sure that if mice and rats have specific inbred strains which are very sensitive very resistant, that humans too will have such differences. Since we cannot tell these differences in humans, it is hard to tell if we are being consistent when we look for health effects.

Third, there have been many problems in the design of studies to determine dioxin's impact on the immune system (for example, determining who was exposed and at what levels). The problem of trying to understand toxicity of the immune system is especially difficult because it is an interrelated network. If one were to take snapshots of any complicated machine with interacting moving parts, such as a car engine, one would see engines at all different stages of the cycle. Some snapshots would have pistons up and some would have pistons down, but the photographs would not necessarily help one understand how the parts interact with each other or how the engine works. Similarly, by only looking at "snapshots" of immune mechanisms at certain points in time, we miss the way the parts connect and relate to each other. Despite these limitations in our understanding of the immune system, the growing body of evidence is consistent with human immune effects caused by exposure to dioxin at levels currently found in the environment.

Chapter 7

Dioxin as a Carcinogen

TCDD was declared a probable human carcinogen by the International Agency for Research on Cancer (IARC) in 1997 (IARC, 1997). It is one of the strongest carcinogens known, causing many kinds of cancers in both sexes of several species, including humans. Dioxin does not mutate DNA directly like most other carcinogens, but rather causes cancer indirectly through various mechanisms. It may increase the chance that a mutation caused by another agent is expressed by stimulating cell division (a cell with a mutation must divide for the cancer to be expressed). It may weaken the immune system's ability to recognize and destroy cancer cells (Chapter Six). It increases the production of cytochromes P4501A1 and P4501A2 (Chapter Five) which activate certain chemicals that bind to DNA and cause mutations. It may act like a hormone or disrupt normal hormonal action and stimulate the growth of hormone-sensitive cancers.

Studies in Animals

Four long-term studies on the effects of dioxin on mice, rats, and hamsters indicate that TCDD causes cancer in the liver, lung, tongue, roof of the mouth, nose, thyroid gland, thymus gland, adrenal gland, skin of the face, and under the skin (Kociba, 1978; NTP,

1982; Della Porta, 1987; Rao, 1988). Dioxin also causes cancer in fish (Johnson, 1992). An extensive review of the carcinogenicity of dioxin in animal studies can be found in IARC (1997), NTP (1997), and ATSDR (1998). All of these studies have found TCDD to be positive for carcinogenicity, including tumor promotion studies and in transgenic animals.

Human Studies

Demonstrating an association between dioxin and cancer in humans is difficult. Humans are usually exposed accidentally, in settings where exposure measurements are either inaccurate, inconsistent, or non-existent, and additional chemicals are often involved. For many years, two crude measurements of exposure were used: the number of years working at a job where exposure to dioxin occurred, and the presence of chloracne, a severe skin condition caused by high exposure to dioxin and other chlorinated compounds. Epidemiological studies linking exposure to dioxin with cancer are complicated because dioxin may cause several kinds of cancer, not all exposed people develop cancer, the cancer may be caused by agents other than dioxin, and it takes time before exposure to dioxin shows its effects.

Generally, the studies discussed below use one of two approaches. In the first approach, researchers look for more disease among people exposed to dioxin than among people who are not exposed. If significantly more exposed people have the disease, dioxin is linked to the disease. In the second, case-control, approach, people with a disease and suitable controls without it are questioned about possible exposure to the chemicals being studied. If a higher proportion of exposed people compared to controls have the disease, the disease is considered linked to the exposure. When working with rare diseases, as most cancers are, both types of studies give just about the same results. Because the earliest studies on the health effects of dioxin suggested that dioxin exposure might lead to an increased risk of soft tissue sarcoma and non-Hodgkin's lymphoma (NHL), case-control studies have largely concentrated on these two types of cancer.

In many of these studies, it is conventional to correlate dioxin exposure with a particular cancer with the term "relative risk" which is almost always accompanied by a "95% confidence range." A relative risk of 1.0 means that an exposed person is no more likely to develop cancer than an unexposed person. The 95% confidence range is the result of a statistical test. A relative risk of 2.0 means that an exposed person is twice as likely to develop cancer as an unexposed person. A relative risk of 4.0 with a 95% confidence range of 3 to 5 is quite significant, and cannot be explained as a chance occurrence. However, the same relative risk of 4.0 with a 95% confidence range of 0.5 to 7.5 is not very convincing because the confidence range includes a risk of 1.0, which means there is no increased risk. Therefore, a relative risk with a confidence range that includes 1.0 is said to be non-significant: the result could have occurred by chance.

There are many possible reasons for a wide confidence range. One of the most common is that the number of people studied was small. The smaller the number of people in the study, the wider the range. Conversely, the larger the number of people in the study, the smaller the 95% confidence range and the more likely it is that any observed risk is real. A well designed study will have a small confidence range. The confidence range will also vary with measurement accuracy and other variables in the study.

Cancer and Dioxin Exposure in the Workplace:

Four Key Studies

Four large studies, one in the United States, two in Germany, and one in the Netherlands, have produced very good data and establish a strong correlation between exposure to TCDD and cancer in humans. Most other studies are not very useful because there was little or no information on dioxin exposure levels.

United States: In the first study (Fingerhut, 1991), the National Institute of Occupational Safety and Health (NIOSH) tracked, for over 20 years, 5,172 people who worked at 12 United States plants that produced chemicals contaminated with dioxin. Men exposed for over one year had a 50% increase in stomach cancer, lung cancer, non-Hodgkin's lymphomas, Hodgkin's disease, and cancer of the soft and connective tissues. The relative risk for these cancers averaged 1.46 with a 95% confidence range of 1.2 to 1.8. The largest relative risk was 9.2 (95% confidence range of 1.9 to 27.0) for connective and soft tissue cancers. The high lung cancer rates could not be explained by cigarette smoking alone since other smoking related deaths did not increase.

After monitoring the workers more closely for six more years, Steenland (1999) showed that exposed workers were more likely to die of all types of cancers combined than were unexposed workers, and that the risk correlated directly with the amount of exposure. In a separate analysis of 608 workers who had chloracne (evidence of heavy exposure), the relative risk of death due to soft tissue cancer was 11.32 with a 95% confidence range of 2.33 to 33.10. There were also suggestive increases for respiratory cancer and multiple myeloma, but not for non-Hodgkin's lymphoma.

Germany: The second study is significant because it firmly establishes a dose-response relationship between dioxin exposure levels and relative risk for cancer. In this study, 1,583 workers (1,184 men, 399 women) from a BASF plant that produced herbicides contaminated with dioxin had a higher risk for all cancer types, including lung cancer (Manz, 1991). The relative risk of all cancers to these workers correlated directly with

intensity and duration of exposure to dioxin and its levels in their fat tissues. Workers exposed for less than 20 years had a relative risk of 1.1 (95% confidence range of 0.8 to 1.4). Workers exposed for more than 20 years had a relative risk of 2.6 (95% confidence range of 1.2 to 4.9). Lung cancer was also elevated in these workers. No increases in soft or connective tissue cancer were reported, but, because of the small number of workers involved in the study, less than one case of such cancer was expected. Relatively few female workers were included in this study, and only 7% of them were highly exposed. There was an increased risk only for breast cancers in these women.

A follow-up study (Flesch-Janys, 1995) that was updated three years later (Flesch-Janys, 1998) included only male workers employed at the plant from 1952 through 1992. The 1998 study carefully characterized and correlated work history with TCDD exposure levels, which allowed the data to be used in a risk assessment (Becher, 1998). New methods of estimating dose rates of TCDD and related chemicals in various departments of the plant were used, and workers were grouped into TCDD quartiles.

Compared to the general German population, the workers had a significantly higher risk of all types of cancer including a higher risk for specific cancers such as rectal and respiratory cancer and lymphosarcoma (a type of non-Hodgkin's lymphoma) correlating directly with the amount of TCDD exposure. The study is weakened by the reliance on death certificates for specific cancer diagnoses, by the relatively small size of the cohort, and by possible confounding with smoking. Overall the study adds to the evidence of an association between TCDD exposure and cancer mortality.

In the 1995 study, detailed industrial hygiene data and serum levels were collected from a subset of 190 workers (Flesch-Janys, 1995). Results indicated that the risk of death from cancer rises as estimated TCDD exposure levels rise. Other causes of death were examined in this way; total toxic equivalencies were estimated and gave similar results. Although the 1995 study is updated by the 1998 study, it nevertheless supports a dose-response relationship between TCDD exposure and total cancer mortality.

The 1995 study was criticized for basing exposure predictions on blood samples from a subset of surviving workers, choosing an atypical referent group, and using a new method of statistical analysis not used in previous analyses of the same cohort (Swaen, 1997). The authors responded by stating that blood samples can only be collected from surviving workers, and that the statistical analyses used were the most appropriate method. They also noted that, as was appropriate in an earlier analysis, the cohort was compared to the German population. The exchange clarifies some methodological points and further strengthens the interpretation that the data from this cohort support a dose-response relationship between dioxin exposure and total cancer mortality.

Data from this same German cohort was used to estimate lifetime cancer risk for a specific dose of TCDD (Becher, 1998). Several models were used to relate dose to total cancer mortality. The best fit to the data was a dose-response curve that was "concave" at low dose, and the lifetime risk for ingesting 1 picogram of TCDD per kilogram body weight per day was in the range 1.2×10^{-3} to 7.7×10^{-3}. This range is equivalent to a risk range of approximately 1 to 8 in 1,000 people. Risks higher than one-in-a-million are generally considered unacceptable (NRC, 1994).

Additional estimates of lifetime risk of inhalation of dioxin were made, and an evaluation of the model output for evidence of a threshold was inconclusive. This assessment builds on the strengths of the exposure assessment for the cohort and provides additional evidence of a dose-response relationship between TCDD exposure and cancer mortality.

Germany: A third study examined 247 workers employed at a German chemical manufacturing company that produced 2,4,5-trichlorophenol contaminated with dioxin (Zober, 1990). Workers employed at the company over 20 years had twice as much chance of getting cancer, including lung cancer, as people who did not work there. The relative risk was 2.0 with a 95% confidence range of 1.2 to 3.2. As with the two studies previously mentioned, people with the highest dioxin exposure had the highest cancer risks. The relative risk for lung cancer was also elevated. An update on this study elaborated on and verified the original results (Zober, 1997).

Netherlands: The fourth study, part of a larger international study by IARC, examined a small cohort of Dutch workers exposed to various herbicides and contaminants while working at a chemical factory from 1955 through 1991 (Hooiveld, 1998). An accident also exposed some workers to higher levels of contaminants including PCDD. This study was particularly good because dioxin exposure levels were carefully estimated and validated with a sub-set of workers whose serum levels of PCDD and other contaminants were correlated with their work histories. The results showed that workers had higher risks for all cancer types, generally in proportion to serum TCDD levels, including total cancers, respiratory cancer, urinary tract cancer, prostate cancer, and non-Hodgkin's lymphoma. The limitations of the study are the reliance on death certificates to diagnose specific cancers and the small size of the exposed population. This study provides additional support for an association between dioxin exposure and total cancer mortality.

The IARC Study: Increased risk of cancer associated with TCDD exposure in the work place is substantiated by results from a large international cohort established in 1980 by the International Agency for Research on Cancer (IARC). In this effort, IARC examined 36 cohorts from 12 countries, consisting of a total of 21,863 male and female workers who produced or sprayed phenoxy herbicides or chlorophenols during the years 1939 to 1992. The initial report published in 1991 covered over 18,000 workers, including 1,500 women, from 10 European counties. Despite problems in the definitions of exposure and

in the length of follow-up, the study found an increased incidence of deaths from several forms of cancer, including lung cancer and soft tissue sarcoma, and a decrease in the incidence of breast cancer in women (Saracci, 1991).

This study has since been enlarged to include cohorts of herbicide workers in the United States (Fingerhut, 1991) and Germany (Manz, 1991; Flesch-Janys, 1995; Becher, 1996). It now includes practically all of the phenoxy herbicide production workers who have ever been studied (Kogevinas, 1997). Workers from 1939 to 1992 were studied, and exposures were reconstructed using job records, company exposure questionnaires, and serum and adipose tissue dioxin levels. Workers exposed to TCDD or higher chlorinated dioxins had higher, but not statistically significant, cancer mortality from soft tissue sarcoma compared to national rates (6 deaths, standard mortality ratio risk (SMR) = 2.03, 95% confidence interval [CI] of 0.75 to 4.43). Mortality from all cancers combined (SMR = 1.12, 95% CI = 1.04-1.21), lung cancer (SMR = 1.12, 95% CI = 0.98-1.28, and non-Hodgkin's lymphoma (SMR = 1.39, 95% CI= 0.89-2.06) were slightly elevated. Risks for all cancers combined, sarcoma, and lymphoma increased with time since first exposure to herbicides contaminated with dioxins. Kidney cancer was among the cancers showing an overall statistically significant increase in mortality (Kogevinas, 1997).

This study indicates that exposure to herbicides contaminated with dioxins is associated with a small increase in overall cancer risk and in risk for specific cancers. In response to criticism that including the low mortality cohorts from the United States and Germany may have biased the updated study (Mundt, 1998), IARC pointed out that the excess in all cancer mortality persists when the German and U.S. cohorts are removed, and that lifestyle factors like smoking do not appear to confound the results (Kogevinas, 1998). In particular, IARC noted the deficit of deaths from non-malignant respiratory disease in the combined data. The criticism by Mundt does not alter the evidence that dioxin exposure is associated with increased cancer mortality.

A study of a portion of the larger IARC cohort consisting of 13,831 workers exposed to herbicides and contaminants in twelve countries between 1939 and 1992 indicates that mortality from soft tissue sarcoma is twice as high, although not statistically significant, and that mortality from total cancer, non-Hodgkin's lymphoma and lung cancer were modestly higher for members of the cohort exposed to dioxin than for those not exposed (Boffetta, 1998).

Miscellaneous Studies in Workers

Three studies of pulp and paper-mill workers potentially exposed to high levels of dioxins indicate that they are not at a higher risk for non-Hodgkin's lymphoma, lung cancer, or stomach cancer (Robinson, 1986; Jappinen, 1987; Henneberger, 1989).

However, these studies were of short duration and had problems estimating exposures. Studies of the effects of dioxin exposure on worker groups from Denmark (Lynge, 1985, 1987, 1993), Britain (Coggon, 1986, 1991), the Netherlands (Bueno de Mesquita, 1993), and Sweden (Wiklund, 1986, 1988, 1989) lack good data, but there are isolated instances of excess risk for multiple myeloma and cervical cancer in women and malignant melanoma in men (Lynge, 1993). The longer the follow-up time, the greater the likelihood of observing cancer.

Agricultural workers in Sweden (Eriksson, 1981, 1990; Hardell, 1979, 1981, 1988) and New Zealand (Smith, 1982, 1983, 1984, 1986; Pearce, 1986, 1987) who used the herbicide 2,4,5-T contaminated with dioxin had a high risk of developing soft tissue sarcomas (2.3 in Sweden and 3.0 in New Zealand). Agricultural workers from Nebraska (Zahm, 1990), Kansas (Hoar, 1986), and Iowa and Minnesota (Cantor, 1992) exposed to dioxin-contaminated herbicides also had a high risk of developing non-Hodgkin's lymphoma, but the risks were lower than in the Swedish studies.

The earliest studies on dioxin suggested that increases due to dioxin exposure might be found in soft tissue sarcoma and non-Hodgkin's lymphoma, and so case-control studies have largely concentrated on these two types of cancer. A historical summary of studies associating herbicides and related compounds with non-Hodgkin's lymphoma and a discussion of various etiologic theories has been published (Hardell, 1998). The authors suggest that chemicals, including dioxin, may act as co-carcinogens along with viruses and other inducers of immunosuppression to cause non-Hodgkin's lymphoma.

This article provides no new data or additional evidence of an association between dioxin and cancer in humans although it suggests a mechanism by which one particular cancer may be caused. In several instances, poor exposure information renders studies conducted on pesticide applicators, railroad workers, and other workers who may have been exposed to dioxin or dioxin-like chemicals virtually useless because it's unclear what exposure might have caused any observed effects.

Another re-evaluation of existing data found a link between soft tissue sarcoma and exposures to dioxin (Hoppin, 1998). This study used data from the Selected Cancers Study, which was originally intended to examine the risk of Agent Orange exposure in Vietnam on soft tissue sarcoma risk. This subsequent analysis is focused on other risk factors, primarily occupational exposures to chlorophenol and other exposures for machinists, leather workers, woodworkers handling treated wood, and other occupations. There were 295 cases and 1,908 controls with data deemed adequate for inclusion in the analyses presented. No information on dioxin contamination of chlorophenols was available and no serum TCDD levels were analyzed in this study. Results indicated a significantly increased risk of soft tissue sarcoma with increasing years of exposure to chlorophenols. This was especially evident in those exposed to cutting fluids as

machinists.

This study included a large number of cases, virtually all of which were confirmed as soft tissue sarcomas by consulting pathologists. There was also a wealth of information on potential confounding exposures such as medical radiation, and the use of sophisticated statistical techniques. The weakness of the study for the purpose of assessing cancer risk associated with TCDD is that no information on TCDD levels in the workplace or in the study subjects was available. The first author considers the study silent on the issue of TCDD and its association with soft tissue sarcoma.

Non-Occupational Studies: Seveso, Italy

In 1976, over 30,000 people in Seveso, Italy, an industrial town north of Milan, were exposed to high levels of dioxin after an explosion in a chemical manufacturing plant. Thousands of people were affected, some were severely exposed, and many developed chloracne. The population has been studied for 15 years, which is not very long considering that most cancers take between 20 and 30 years to develop.

The most recent update (Bertazzi, 1997) on cancer mortality linked to the Seveso accident delineates three exposure zones: Zone A (highest exposures), Zone B (moderate exposures), and Zone R (lowest exposures). Rectal cancer and leukemia deaths in men and multiple myeloma deaths in women are significantly excessive in Zone B. Non-significant increases in cancer mortality also occurred: digestive cancer deaths in women in Zones A and B; Hodgkin's disease deaths in both men and women in Zone B; and soft tissue sarcoma deaths in males in Zone R.

There have been only a small number of deaths from specific types of cancer since the Seveso accident, but there is a suggestive association between the number of years since the accident or length of stay in the contaminated area and some cancers such as multiple myeloma in women (Bertazzi, 1993). Earlier studies found increased incidence of connective and soft tissue sarcomas among males, and of some relatively rare blood-related and liver cancers in both males and females (Bertazzi, 1993).

The strengths of the 1997 update are the large sample size and wealth of information collected on exposure and other health outcomes. However, it does potentially misclassify exposures in individuals who left the various Zones for periods of time after the accident, and relies on death certificates to diagnose cancer. For example, death certificates from males in Zone B that indicate cancer of the pleura (RR=5.3; 95% C.I.=1.1-15.5) do not specify whether this is mesothelioma or sarcoma. The 15-year mortality follow-up is still too short to describe the complete range of cancer that might

be expected in the exposed population. Overall, the Bertazzi 1997 update supports an association between dioxin exposure and cancer mortality in humans.

Twenty years after the Seveso accident, Landi (1998) carefully examined plasma TCDD levels of 62 people from Zones A and B and 59 controls from outside the contaminated areas. Though the sample size of exposed subjects was relatively small, the authors performed a multivariate analysis of the data that looked for associations between plasma TCDD levels, gender, and various other factors such as weight loss and consumption of locally grown vegetables or animals. TCDD levels were significantly higher in females and persisted for two decades after the accident in all areas examined.

The strength of this study is its careful examination of the plasma TCDD levels and its multivariate analysis of the factors influencing the observed gender difference. The weakness is the relatively small number of exposed subjects on which the analyses are based. This study introduces evidence that may add to an understanding of the mechanism of TCDD carcinogenesis in humans, but no conclusions can be reached based on this article alone.

Cancer and Dioxin in Contaminated Food

In 1968, 1,900 people in Yusho, Japan ate food cooked with rice-oil contaminated with PCBs and dibenzofurans. Though the exposures were massive, they lasted only a few months. In addition to many non-cancerous effects, liver cancers increased significantly (9 observed compared to 1.6 expected), and lung cancer increased significantly in males (Kuratsune, 1988). In 1979, 2,000 people in Yu-Cheng, Taiwan were exposed in a similar accident. There has not been enough time for cancer to develop among those who were exposed.

Cancer and Dioxin Exposure in Vietnam

Many studies have examined cancer incidence in Vietnam veterans due to their exposure to Agent Orange (a mixture of the herbicides 2,4,5-T and 2,4-D which are both contaminated with dioxin). Individually, most of these studies show no association between cancer incidence and exposure to Agent Orange in Vietnam veterans. However, a panel of scientists brought together by the National Academy of Sciences Institute of Medicine carefully examined all of the evidence from these studies and concluded that there is a positive association between exposure to herbicides and soft tissue sarcoma, non-Hodgkin's lymphoma, Hodgkin's disease, and chloracne.

They also found suggestive evidence linking exposure to herbicides with increased risk for respiratory cancers (lung, larynx, trachea), prostate cancer, multiple myeloma, acute and subacute peripheral neuropathy, spina bifida and porphyria cutanea tarda (a liver disorder) (IOM, 1996). With one exception, dioxin levels in fat and serum for small groups of male and female Vietnam veterans were indistinguishable from the general U.S. population (Wolfe, 1990). The exception was veterans of Operation Ranch Hand, a small group of 1,261 soldiers who suffered high exposure when they sprayed Agent Orange from aircraft and on the ground in Vietnam. Their dioxin levels were only three times higher than controls (12.4 ppt compared to 4.2 ppt), though a subgroup of "non-flying enlisted men" had very high dioxin levels (23.6 ppt).

In an on-going study that began in 1982 (Michalek, 1998) of mortality among the Air Force Ranch Hands, there have been 30 cancer deaths, 19 of which occurred in veterans at least twenty years after their service in Vietnam. The overall increase in risk of cancer death in this group is 1.1 (95% confidence range of 0.7 to 1.6). Nine Ranch Hands who died from cancer of the bronchus and lung also represent an increased risk of 1.3 (95% confidence range of 0.6 to 2.3). One Ranch Hand soldier died from soft-tissue sarcoma, one from multiple myeloma, and one from an unspecified type of lymphoma. These findings are consistent with an earlier report that shows an elevated risk of soft tissue sarcomas in Vietnam veterans who went into areas that were sprayed with Agent Orange (Michalek, 1990). However, in that study, the number of cases reported was too small (in part, because the soldiers were so young) to have any statistical significance.

The strength of the 1998 Ranch Hand mortality study is its focus on a particular cohort with opportunity for Agent Orange exposure, and its attempt to carefully document subsequent health outcomes. Its weaknesses are the small size of the cohort and therefore its limited power to detect increases in specific causes of disease such as cancer. The findings with respect to cancer mortality in this report are too limited to draw meaningful inferences.

To determine if exposure to Agent Orange during service in Vietnam was linked to increased risk of lung cancer, the Veterans Administration compared lung cancer cases diagnosed between 1983 and 1990 to two sets of controls from its medical centers (Mahan, 1997). Exposure was measured by branch of service, military occupation, and troop location in Vietnam. One set of controls had colon cancer, and the other was randomly selected from the patient treatment files. Different exposure categories were delineated based on branch of service, military occupation, and troop location. Results indicated an increased risk of lung cancer (odds ratio=1.39; 95%C.I.=1.01 to 1.92). The mean age of the cases was only 38.4 years, a very young age for development of lung cancer. The authors conclude that "there is no evidence of increased risk of lung cancer associated with service in Vietnam at this time."

The strengths of this study are its reliance on hospital records as the basis of diagnosis and its attempt to develop surrogate exposure measures of several types. The weaknesses are the short time since exposure, and therefore short latency, for development of lung cancer from any exposure in the Vietnam war, and the author's over-reliance on statistical criteria for drawing inferences about the risks of lung cancer. The study was done too early to determine the overall risk of lung cancer from exposures in Vietnam. It nonetheless provides early suggestive evidence of increased lung cancer risk which should be examined at a more appropriate time period.

Summary

All available evidence indicates that dioxin exposure causes cancer in humans in a dose-dependent fashion. Additional research will clarify how dioxin causes cancer in specific human organs and organ systems. The most important studies in the recent literature that bear on the carcinogenicity of dioxins in humans are the series of studies by Flesch-Janys and colleagues in Germany and by Bertazzi and colleagues in Italy. The studies of the German chemical plant workers attempt to quantify the dose-response relationship between estimated TCDD exposure and total cancer mortality. The Italian studies of mortality among those exposed to the Seveso plant accident also focus on cancer mortality in populations stratified by exposure level. Both research groups recognize limitations and uncertainties in estimation of exposure and definition of specific causes of death, among other typical limitations of epidemiologic studies. However, both series of recent studies strengthen the conclusion that dioxin exposure is related to cancer mortality in humans in a dose-dependent fashion. This was also found in the Dioxin Workers Registry update published by Steenland and colleagues in 1999. Additional clinical and biomarker research is expected to illuminate the mechanism of dioxin carcinogenicity. Likewise, additional examination of cancer incidence (in addition to mortality) in the Seveso follow-up studies to the year 2002 will add evidence about the carcinogenicity of dioxin in specific human organs and organ systems. This will only deepen our quantitative and mechanistic understanding of the association which is already established.

Chapter 8

Reproductive and Developmental Effects of Dioxin

Dioxin exposure, even in single doses and at very low concentrations, may seriously

disrupt normal reproduction in humans and other animals. It may lower fertility, increase prenatal mortality, cause birth defects, and increase the risk of endometriosis. Though the mechanism by which dioxin acts is unclear, it apparently interferes with the production and function of many different hormones, growth factors, and enzymes. Its effects can be long term, and its toxicity is much more severe and consistent across species in early stages of development than it is in adults.

Fertility and Prenatal Mortality in Animals

In female rats, dioxin exposure at high doses suppresses ovarian function and the estrous cycle, and interferes with ovulation (Kociba, 1976; Barsotti, 1979; Allen, 1979). Concentrations of 0.1 micrograms per kilogram per day (ug/kg/day) decrease fertility and litter size, and if these offspring reproduce, they are sensitive at even lower doses, a second generation effect (Murray, 1979). In adult male rodents, dioxin reduces testosterone levels, sperm counts, and fertility, as well as appetite and body weight, though at substantially larger doses than required to affect fetuses or infants (Kociba, 1976).

Dioxin exposure alters the ovarian cycle and decreases fertility in monkeys. Females on diets of 0, 5, and 25 parts per trillion (ppt) dioxin did not seem to be affected themselves, but only seven of eight females on the 5 ppt diet conceived, and only six had live births; and only one of eight females on the 25 ppt diet had a live birth. All controls had successful live births (Bowman, 1989). Cumulative doses of dioxin ranging from 1 to 500 ug/kg body weight cause prenatal mortality in monkeys, guinea pigs, rabbits, rats, hamsters, and mice (monkeys and guinea pigs are most sensitive, followed by rabbits, rats, hamsters, and mice). Timing of the exposure is critical. At day 14 of pregnancy, fetal guinea pigs die from a single dose of 1.5 ug/kg of body weight. This dose also killed the mother. Larger doses are needed later in pregnancy (Couture, 1990).

In mice, cleft palate can be induced by TCDD exposure when it is administered to pregnant dams on gestation days 6-12. After day 12, the ability of TCDD to cause cleft palate diminishes with time, so that there is no palatal clefting when TCDD is administered after day 13, even though birth normally occurs between days 19 and 21 in the mouse (Couture, 1990).

Dioxin is also lethal to fish and birds in the early stages of their development. Young fish may be especially sensitive (Kleeman, 1988; Walker, 1991). The LD_{50} (the concentration of dioxin necessary to kill 50% of treated eggs) for fertilized lake trout eggs is 65 picograms per kilogram (pg/kg) egg weight. Eggs are more sensitive than juveniles. The LD_{50} for rainbow trout juveniles is 10 ug/kg (ppb) body weight, whereas it is only 0.4 ug/kg egg weight for embryos (Greig, 1973). Similarly, bird eggs die from dioxin doses

100 to 200 times less than it takes to kill an adult bird (Greig, 1973; Allred, 1977).

Dioxin and Birth Defects in Animals

The degree to which dioxin causes birth defects varies from species to species. It causes heart defects in chicks (Cheung, 1981, 1981a), asymmetric brains in herons and cormorants hatched from eggs containing 60 ppt (Henshel, 1997), and cleft palates and hydronephrosis (a birth defect in which excessive growth on the lining of the ureter blocks the flow of urine and causes kidney enlargement and damage) in newborn rats (Khera, 1973) and mice (Courtney, 1971; Neubert, 1972). Cells from the developing palates of mice are particularly sensitive to dioxin exposure. Human cells are affected, but at higher concentrations than mouse cells.

Exposure to dioxin is detrimental to the development of the male reproductive system. Fetal male rats seem to be particularly sensitive to dioxin exposure. Among other effects, it decreases the size of or deforms their testes, penis, prostate, seminal vesicles, and sperm (ATSDR, 1998).

Pregnant rats exposed to a single dose of TCDD of 1 ug/kg body weight give birth to males with low sperm count and testosterone levels, delayed testicular descent, and altered sexual behavior (Mably, 1991, 1992, 1992a, 1992b; Peterson, 1993; Gray 1993, 1995). The male offspring from these exposed mothers take much longer to successfully mate with receptive females, mate at longer intervals, and assume female mating positions when in the presence of other males.

At this same dose level given on day 15 of pregnancy, sperm production drops (Gray, 1997) and sexual behavior changes permanently; smaller doses of as little as 0.064 ug/kg body weight have the same result, but it may not be permanent (Mably, 1992b). Similar effects occur to fetal hamsters exposed to dioxin at 2 ug/kg body weight on day 12 of pregnancy (Gray, 1995). Fetal mice are less sensitive than rats and hamsters (Theobold, 1997). At concentrations that have no effect on testosterone levels, dioxin permanently alters the distribution of androgen receptors and delays cellular differentiation in the prostate of male rats (Roman, 1998).

Although the actual mechanism by which dioxin produces its reproductive toxicity in males is unknown, many of dioxin's effects on males appear to occur either because it decreases testosterone production or because it disrupts the feedback communication mechanism among the hypothalamus, the pituitary gland, and the testes, which is called the hypothalamic-pituitary- gonadal (HPG) axis. This feedback mechanism controls normal testosterone levels in the body. Normally, when testosterone levels are low, the

pituitary gland secretes leutenizing hormone (LH), which sends signals to the testes to produce more testosterone. The testosterone surges in fetal and early life help set in place or "imprint" this feedback system in the HPG axis. Therefore, if dioxin interferes with this imprinting by reducing the testosterone surges in fetal and early life, it could explain why the testes of adults do not respond normally to LH, and testosterone levels remain low. Dioxin's effect on testosterone may alter sexual differentiation of the brain during these early developmental stages when male hormones imprint the brain for male-mating behavior, male-type response in the hypothalamus, and male-type biochemical response to hormones such as LH. Dioxin's precise effects on testosterone remain to be determined.

Dioxin also disrupts the development of the female reproductive system. Pregnant rats given a TCDD dose of 1 ug/kg of body weight on day 15 of pregnancy give birth to females with delayed puberty and abnormal genitals including a permanent thread of tissue across the vaginal opening (Gray, 1995a, 1997a; Flaws, 1997; Heimler, 1998). Female offspring also had fewer of certain sized follicles in their ovaries, indicating a direct effect of TCDD on ovarian development (Heimler, 1998). Dioxin may reduce estrogen activity either by decreasing its synthesis, decreasing its receptor concentrations, or increasing its metabolism. Evidence for all three mechanisms exist (Chaffin, 1996; Heimler, 1998a; Tian, 1998).

Endometriosis

Endometriosis is a disease in which endometrial cells from the lining of the uterus inappropriately grow outside of the uterus. They grow on the ovaries, on the outside of the uterus or fallopian tubes, or elsewhere in the abdominal cavity. These abnormal implants are thought to occur when menstrual flow is reversed, but they may also occur when normal physiological processes do not remove them. Like normal uterine cells, these cells respond to the hormones of the monthly menstrual cycle and proliferate, and bleeding ensues. Endometriosis causes pain during menstruation and intercourse and is a common cause of infertility. The symptoms grow worse as the endometriosis spreads.

More than five million women in the United States are affected by endometriosis, and the number appears to be increasing. This is partly due to better diagnosis and partly to a real increase in the disease. From 1965 to 1994, there has been a 250% increase in hysterectomies for women between the ages of 15 and 24, and a 186% increase for women between the ages of 25 and 34, who suffer from endometriosis (Berger, 1995).

Endometriosis likely results from the interaction of genetic and environmental factors (Zeyneloglu, 1997; Baranova, 1997; Kennedy, 1998). Dioxin is one such environmental factor, and it has been shown to cause a marked increase in the severity of endometriosis

in primates. From 1977 to 1982, three groups of rhesus monkeys were fed a diet with zero, 5 ppt, and 25 ppt dioxin. After the dietary exposure ended, the monkeys were kept with their colony. From 1989 to 1992, three of the female monkeys died due to blockage of the intestinal tract by severe endometriosis. Examination of the remaining monkeys showed moderate to severe endometriosis in 17% of the zero exposure group, 71% of the 5 ppt group, and 86% of the 25 ppt group (Rier, 1993).

How dioxin contributes to the development of endometriosis is not fully understood, but it more than likely disrupts the endocrine and immune systems. In rats, dioxin appears to interfere with the capacity of progesterone to suppress surgically induced endometrial implants (Cummings, 1995). Abnormal expression of cytokines, the signaling molecules of the immune system, may also play a role (Osteen, 1997). Dioxin can also promote the growth of surgically induced endometriosis in rats and mice (Cummings, 1995; Johnson, 1997).

These findings prompted a number of studies to determine if humans with endometriosis have higher than normal levels of dioxin. Few of these studies have been completed though one study in Israel reports higher concentrations of dioxin in the blood of women with endometriosis than in controls (Mayani, 1997).

Dioxin and its Effects in Humans

In humans, testosterone levels change dramatically in infancy, and there are no well controlled studies that correlate infant male hormone levels to fetal dioxin exposure. However, in the NIOSH study, workers with high levels of exposure to dioxin have higher levels of LH and lower levels of testosterone than controls (Egeland, 1994). Operation Ranch Hand veterans with the highest dioxin levels have the lowest testosterone levels and four times as many "unspecified testicular abnormalities" as do members of a control group (Roegner, 1991). These findings are consistent with results from studies in animals.

Several analyses draw different conclusions about trends in human sperm counts and birth defects involving the male reproductive tract. Some studies indicate that human sperm counts have substantially declined over the past 50 years and that the incidence of some birth defects involving the male reproductive tract has increased in industrialized countries (Carlsen, 1992; Irvine, 1994; Auger, 1995). In a review and analysis of 61 papers published between 1938 and 1991, Carlsen et al (1992) concluded that there had been a substantial decline in semen quality. Other studies have not found such a decline in human sperm counts (Paulsen, 1996), and the study design and statistical techniques used in the Carlsen meta-analysis have been criticized (Fisch, 1996). A re-analysis of the same data, using various statistical techniques, confirmed the original conclusions but

showed that the decline is in the United States and Europe but not in non-Western countries (Swan, 1997). One autopsy study designed to compare sperm quality data from similar populations concluded that the percentage of men with normal healthy sperm production declined from 56% to 27% from 1981 to 1991 (Pajarinen, 1997). The degree to which dioxin may contribute to these findings is unclear.

The incidence of cryptorchidism, a condition in which one or both testes fail to descend into the scrotum during fetal development, is increasing (Giwercman, 1993). Similarly, the incidence of hypospadias, a condition in which the opening is not at the tip but rather on the underside of the shaft of the penis, is increasing (Paulozzi, 1997). Though no evidence directly links these trends to dioxin exposure, the findings are consistent with the effects of fetal dioxin exposure on the reproductive systems of other animals, which are characterized by incomplete masculinization of the reproductive tract.

When rice oil accidentally contaminated with PCBs and furans was consumed in Yusho, Japan and in Yu-Cheng, Taiwan, effects on fetal development in humans were observed. There was high infant mortality, especially in babies with hyperpigmented (over colored) skin, another sign of dioxin toxicity. The mothers of almost all affected babies had chloracne, suggesting that the dose they were exposed to was quite high. Babies in both incidents had abnormal fingernails and toenails, conjunctivitis, teeth at birth, altered eruption of permanent teeth, and abnormally shaped tooth roots (Kuratsune, 1989; Chen, 1992). Accelerated tooth eruption is also seen in newborn mice exposed to TCDD (Madhukar, 1984). Similarly, several children born near the hazardous waste site at Love Canal, New York had tooth abnormalities such as double sets of teeth and teeth at birth (DOH, 1981).

In the Yusho and Yu-Cheng incidents, exposed children also had developmental delays, speech problems, behavioral difficulties, and impaired intellectual development (Rogan, 1988; Hsu, 1994; Masuda, 1994). The Yusho children had low birth weights and retarded growth (Masuda, 1994). The Yu-Cheng children later developed hearing loss and middle ear disease (Chao, 1997).

A study of 402 births from mothers exposed to the dioxin-contaminated oil spread on roadways to control dust in Times Beach, Missouri shows no apparent increase in fetal deaths or low birth weight babies. An observed two to three-fold increase in nervous system birth defects and undescended testicles is not statistically significant (the small sample size required a six-fold increase to achieve statistical significance) (Stockbauer, 1988).

In the seven years following the 1977 industrial accident that released large amounts of dioxin in Seveso, Italy, the most heavily exposed mothers gave birth to almost twice as

many girls as boys (Mocarelli, 1996). The mechanism by which dioxin may have caused this effect on sex ratios is unclear. This effect on sex ratio is not seen in the Yu-cheng children (Rogan, 1999).

Vietnam Veterans and Reproductive Effects

Both the Vietnamese people and American soldiers were exposed to variable amounts of the dioxin-contaminated herbicide Agent Orange, which was widely sprayed as a defoliant in South Vietnam during the Vietnam War. Hatch (1984) reports an average of 6 birth defects per 1,000 births from North Vietnamese veteran fathers who never served in South Vietnam compared to an average of 29 birth defects per 1,000 births from veteran fathers who did serve in South Vietnam. In a very large study of North Vietnamese veterans involving 121,000 pregnancies, spontaneous abortions are significantly higher and birth defects are slightly, but not significantly, higher in exposed compared to non-exposed cohorts (Hatch 1984; Constable, 1985).

A study by the Centers for Disease Control (CDC) reports a higher incidence of spina bifida, cleft lip and cleft palate, hydrocephalus, and childhood cancers in children of American Vietnam veterans than in controls (Erickson, 1984). An American Legion study shows a higher incidence of miscarriages of fetuses fathered by Vietnam veterans compared to controls (Stellman, 1988). The median blood dioxin levels in several small groups of veterans who handled Agent Orange
regularly is 25 pg/g of blood fat compared to controls with 3.9 pg/g (Kahn, 1988). Veterans in Operation Ranch Hand, considered the most highly exposed group of Vietnam veterans, had dioxin concentrations of 12.4 pg/g compared to a control group of non-exposed soldiers with 4.2 pg/g. Some of the men in this group had dioxin levels as high as 166 pg/g (Wolfe, 1992).

Ranch Hand veterans who fathered children during or after service in Southeast Asia did not have any increased risk for spontaneous abortions, still births, major birth defects or delays in development compared to a group of Air Force veterans not involved in Operation Ranch Hand (Wolfe, 1995). In some groups, miscarriages are higher than expected, but the increases do not match well with the blood level of dioxin. One study reports a decrease in sperm count in Ranch Hand veterans (CDC, 1988).

The data from the studies of Vietnam veterans are limited by the lack of data on exposure at the time of conception. Dioxin levels were measured up to 25 years after exposure and extrapolated back to an initial TCDD level at the time of conception (IARC, 1997). But these calculations may not be precise. The power of the study for detecting an increase in the rate of specific birth defects is also limited because of the small numbers in each exposure group.

Neurodevelopmental Effects of Dioxin in Humans

Though the neurodevelopmental effects of dioxin exposure have been studied in animals and humans, epidemiological research in humans is complicated by the occurrence of PCB exposure along with dioxin. Both molecules have many of the same properties, are persistent in the environment, and tend to accumulate in adipose tissue, including breast milk. Consequently, fetuses and infants exposed to dioxin are also exposed to PCBs. Some PCBs (so-called coplanar or non-ortho PCBs) have striking dioxin-like toxicity; others (ortho-PCBs) are shaped sufficiently different from TCDD and have other mechanisms of toxicity (see Chapter Two). For example, some PCB molecules may mimic thyroid hormone and interfere with its transport into the developing brain in ways that dioxin does not seem to (Porterfield, 1994). Some PCBs also interfere with neurotransmitter levels in the developing brain. Dioxin can also deter thyroid levels by increasing the rate of elimination.

Studies designed to identify toxic effects of PCBs and dioxin usually distinguish between those PCBs with dioxin-like effects and those that do not. Pregnant rats exposed to 2 ug/kg/day of dioxin-like PCBs during days 10 to 20 of pregnancy give birth to hyperactive offspring with impaired learning capacity (Holene, 1995). Monkeys exposed to dioxin during fetal development and through breast milk demonstrate specific learning disabilities, and the dioxin-exposed infants cling to the mothers much more than unexposed infants do. This behavior, also seen in lead- exposed infants, is interpreted to mean that the mothers must provide more care than normal to the infants (Schantz, 1986, 1989). Developmental effects on hearing ability have been reported in rates exposed to PCBs 125 and 126. Perinatal maternal PCB exposure caused low frequency hearing deficits (Goldey, 1995, 1998; Herr, 1996; Crofton, 1999).

The Dutch Studies

A research group in the Netherlands has examined effects in children whose mothers were exposed to "background" levels of dioxins and PCBs. These background levels result from the daily intake of dioxin in food by the mother. In this study, breast milk samples were analyzed for 17 dioxins and furans and 3 planar PCBs as a measure of prenatal exposure. Cord and maternal plasma PCB levels were used as measures for prenatal PCB exposure (Koopman-Esseboom, 1994). The study group consisted of 207 children who were breast-fed and 207 children who were formula fed (Lanting, 1998; Patandin, 1999a). These researchers found that background levels of dioxins/ PCBs were associated with reduced birth weight and reduced growth from birth through 3 months of

age (Patandin, 1998); delays in psychomotor development at 3 months (Koopman-Esseboom, 1996); neurodevelopmental delays at two weeks (Huisman, 1995) and 18 months (Huisman, 1995a); alterations in thyroid hormones at birth and at 3 months (Koopman-Esseboom, 1994a); and with alterations in immune status from birth to 42 months (Weisglas-Kuperus, 1995, 1999). All of these effects were seen in children exposed prenatally to background levels of dioxins/PCBs. These effects are summarized in Table 8-1.

The adverse neurological effects found at birth and at 18 months could not be detected at 42 months (Lanting, 1998a). However, a decrease in cognitive function as measured by a 4 point deficit in IQ was measured for the first time at 42 months (Patandin, 1999b). This difference may be explained by the different testing procedures. Cognitive ability was measured by the Kaufmann Assessment Battery for Children (K-ABC) which is a quantitative measure of a child's cognitive abilities, whereas the neurological examination is a qualitative measure of brain development (Patandin, 1999b). Other neurodevelopmental and behavioral effects found at 42 months to be associated with exposure to prenatal dioxins/PCBs, include a decrease in high play activity, an increase in non play activity, and an increase in the errors of omission in the beginning of a task (Patandin, 1999c), and an increased prevalence of being withdrawn and depressed (Patandin, 1999d). These effects are also included in Table 8-1.

In addition, delays in psychomotor development at 7 months (Koopman-Esseboom, 1996); alterations in thyroid hormones at 3 months (Koopman-Esseboom, 1994a); alterations in immune status as indicated by an increased prevalence of recurrent middle ear infections and decreased prevalence of allergic reactions to food, pollen, dust and pets at 42 months (Weisglas-Kuperus, 1999); an increase in mean reaction times, a decrease in sustained attention, and an increase in hyperactive behavior at 42 months (Patandin, 1999c) were associated with background dioxins/PCBs exposure postnatally. These effects are summarized in Table 8-2.

Developmental effects in humans have also been reported in a Finnish cohort exposed to background levels of dioxins. Evidence of effects on tooth development has been reported by Alaluusua and colleagues who have been following children exposed to dioxin-tainted breast milk (Alaluusua, 1996, 1996a, 1999). Some of these children have soft, discolored molars. While researchers in Japan (Kuratsune, 1989) and Taiwan (Rogan, 1988) have reported yellowish-brown tooth discolorations in children exposed to very high dioxin levels, Alaluusua found similar discolorations in children with moderate (background) dioxin exposure. Laboratory experiments also found that TCDD induced tooth mottling in adult rats (Alaluusua, 1993). The mechanism of action may be through effects on cellular receptors for epidermal growth factor (EGF) which contributes to tissue development (Partanen, 1998). The exposure window is in early infancy, when teeth are forming.

Table 8-1Associations Between Prenatal Exposure to PCBs and Dioxins, and Adverse Outcomes from Birth Until 42 Months of Age.		
Parameter	**Age**	**Outcome**
Endocrine effects (Koopman-Esseboom, 1994a)		
Thyroid hormone	pregnancy	Increase in TT3 and decrease in TT4 in mothers with PCB/dioxin TEQ
	10 days	Increase in TSH in child with PCB/dioxin TEQ
		Decrease in TT4 and FT4 with dioxin-TEQ
	3 m	Increase in TSH in child with PCB/dioxin TEQ
Growth effects (Patandin, 1998)		
Fetal and postnatal growth	birth	Decrease in birth weight with PCB cord and PCB maternal
	0-3 m	Decrease in growth rate for weight, length and head circumference, with PCB cord and PCB maternal
Neurodevelopmental and behavior effects (Huisman, 1995, 1995a; Koopman-Esseboom, 1996; Lanting, 1998a; Patandin, 1999b,		
Bayley Scales	3 m	Decrease in Psychomotor score withPCB maternal
Neurological examination	10 days	Hypotonicity with planar PCB-TEQ
		Decrease in NOS with PCB/dioxin TEQ
	18 m	Decrease in NOS with PCB cord and PCB maternal
Cognitive abilities (K-ABC)	42 m	Decrease in cognitive scale with PCB maternal
		Decrease in sequential processing with PCB maternal
		Decrease in simultaneous processing withPCB maternal and PCB cord
Free play observation	42 m	Decrease in high level play with PCB cord andPCB maternal
		Increase in non-play behavior with PCB cord

Vigilance task	42 m	Increase in errors of omission in the beginning of a task withPCB cord
Teacher CBCL	42 m	Increase in prevalence of being withdrawn/depressed with PCB cord PCB maternal and PCB/dioxin TEQ
Immunological effects (Weisglas-Kuperus, 1995, 1999)		
T-cell markers	birth	Increase in TcR+ T cells with PCB/dioxin TEQ
	3 m	Decrease in Monocyte and Granulocyte with PCB/dioxin TEQ
	18 m	Increase in CD8+, TcR+ and TcR+ T cells with PCB/dioxin TEQ
	42 m	Increase in total T-cells, CD8+, TcR+ and CD3+ HLA-DR+ T cells with PCB maternal
Health status	42 m	Increase in prevalence of chickenpox with PCB maternal
Humoral immunity	42 m	Decrease in antibody levels to measles with PCB cord
NOS: Neurological Optimality Score at birth using Prechtl and at 18 months using Hempel/Touwen. K-ABC: Dutch Kaufman Assessment Battery for Children. CBCL: Child Behavior Checklist. PCB = sum PCBs measured in either maternal or cord blood.		

Source: Patandin, 1999

Table 8-2Associations Between Postnatal Exposure to PCBs and Dioxins and Adverse Outcome from Birth Until 42 Months of Age

Parameter	**Age**	**Outcome**
Neurodevelopmental and behavior effects (Koopman-Esseboom, 1996; Patandin, 1999c)		
Bayley Scales	7 m	Increase in psychomotor development with lactational PCB/dioxin exposure
Vigilance task	42 m	Increase in mean reaction time with PCB

		42-month
		Decrease in sustained attention with PCB 42-month
GBO	42 m	Increase in activity with PCB 42-month
Immunological effects (Weisglas-Kuperus, 1995, 1999)		
T- cells	3 m	Decrease in monocytes and granulocytes with lactational PCB/dioxin TEQ exposure
Heath status	42 m	Increase in prevalence of recurrent middle ear infections Decrease in prevalence of allergic reaction to food, pollen, dust and pets with PCB 42-month
GBO: Groninger Behavior Observation Scale adapted for 42 month olds. Lactational exposure was defined as PCB/dioxin = the sum of measured PCBs and dioxins TEQ in breast milk multiplied by the number of breast-feeding weeks. Current PCB body burden assessed from PCB 42-months represents mainly lactational transfer of maternal PCBs (the breast-fed group) and in part during gestation (the formula-fed group).		

Source: Patandin, 1999

Dioxin and Thyroid Hormone in Humans

During fetal development, thyroid hormone is essential for normal brain development. It enters the fetal brain almost exclusively in the form of thyroxine (T4) attached to a carrier protein. Clinical studies demonstrate that children born with hypothyroidism are at risk of mental retardation (Refetoff, 1993; Rovet, 1993; Brucker-Davis, 1995; Hauser, 1998). Though there is considerable uncertainty about the impact of only slight decreases in thyroxine levels during fetal and infant development, subtle neurological effects may occur. In pre-term and low birth weight babies, low thyroid hormone in the first weeks of life is associated with higher than normal risk of neurological disorders, including the need for special education by age nine (Den Ouden, 1996).

Rats exposed to dioxin-like PCBs *in utero* and through breast milk have lower than normal levels of total and free thyroid hormone (T4) levels (Morse, 1993). Women with higher than normal levels of dioxin and PCBs in breast milk (expressed as TEQs) have infants with lower than normal levels of maternal thyroid hormone and higher than

normal levels of thyroid stimulating hormone (TSH) (Koopman-Esseboom, 1994). Thyroid hormone levels are depressed, though within normal limits, in the most highly exposed two-week old infants, but the deficiency is no longer detectable in three-month old infants. No persistent relationship exists between thyroid levels and neurological development, though it is possible that learning or behavioral effects only become apparent at a later age.

Effects of Exposure to Dioxin Through Breast Milk

Several research groups have evaluated the impact of feeding children breast milk contaminated with dioxins and PCBs (Gladen, 1988; Jacobson, 1990; Lanting, 1998; Patandin, 1999a). A series of studies on a group of Dutch infants found that most adverse associations were found with prenatal exposure and not with postnatal or current exposure (see Tables 8-1 and 8-2). In this study, dioxins and PCBs were measured in maternal and cord blood and in breast milk (see discussion of Dutch Studies above).

As shown in Table 8-1, significant associations between prenatal background PCB/dioxin exposure and adverse effects were found. Some effects were found during infancy (neonatal and 18 months) including neurological effects and reduced birth weight and growth rate. Some of these effects are still present at preschool age (immunological effects). Other effects are not revealed until preschool age including poorer cognitive functioning, less focused attention, and withdrawn/depressed behavior. It's uncertain whether these adverse effects are transient or whether they will persist with time.

These studies also show that breast-feeding is associated with improved psychomotor and mental development at 7 months of age compared to formula-fed children (Koopman-Esseboom, 1996). At 18 months of age, there is also an advantageous effect of breast feeding on the quality of movements in terms of fluency (Huisman, 1995a). This beneficial effect of breast-feeding on the fluency of movements continued at 42 months of age (Lanting, 1998b).

These studies have clearly concluded that most adverse effects are associated with *in utero* dioxin exposure, not with breast-feeding. The authors suggest that strategies should be directed toward reducing PCB and dioxin intake through the food chain at all ages, by lowering consumption of animal products and processed foods, not by discouraging breast-feeding (Patandin, 1999).

In addition to this evidence that breast-fed children perform better on a battery on neurodevelopmental tests, there are also many benefits to nursing (Jelliffee, 1988;

Lawrence, 1994). Numerous studies on infant nutrition show that breast feeding has a beneficial effect on growth, morbidity, and neurological and cognitive functioning later in life (Rogan, 1993; Lanting, 1994; Gordon, 1997). Children who are breast-fed repeatedly show small increases over formula-fed children in mean test scores on measures of intelligence and academic achievement (Fergusson, 1982). Children who were breast-fed had higher developmental scores at 18 months and higher intelligence quotients assessed at 7.5 years compared to those who were not (Lucas, 1992). Recently it was reported that breast-feeding is associated with small but detectable increases in child cognitive ability and educational achievement (Horwood, 1998).

Breast milk is also a perfect food for infants, containing all the nutrients in ideal proportions for optimal growth and development. Beneficial nutrients include long chain polyunsaturated fatty acids (PUFA), thyroid hormone, prolactin, gonadotropin hormone, adrenal gland hormones, nucleotides, and epidermal growth factors (EGF) (Koldovsky, 1987). Breast milk also contains the mother's natural immunities which aid the infant in fighting infections. The psychological benefits of nursing are invaluable as well (La Leche League, 1994).

Although infants are exposed to higher levels of dioxins and PCBs from breast-feeding than from formula diets, the Dutch studies provide strong evidence that most adverse effects are associated with *in utero* exposure and not postnatal exposure. These findings are consistent with the findings of Gladen and Rogan (1991) who found cognitive deficits through 2 years of age, but not at 3, 4, or 5 years of age in children who had prenatal exposures to PCBs. They also support the recommendations of the World Health Organization and the Agency for Toxic Substances and Disease Registry which both promote and support breast feeding (WHO, 1998; ATSDR, 1998).

Chapter 9

Other Health Effects of Dioxin

As seen in previous chapters, dioxin, in particular 2,3,7,8-TCDD, has a wide range of health effects that differ between species, within species, and at different stages of development within an individual (Schecter, 1994c; IARC, 1997; ATSDR, 1998). Its effects on the immune system, cancer, and reproduction have been discussed in detail in Chapters Six, Seven, and Eight. This chapter reviews and elaborates on a few of those

effects, but primarily it discusses in more detail some of the health effects that were not covered in those chapters: the harmful effects that dioxin has on the skin, the liver, the thyroid gland, glucose levels, the endocrine system, the heart, and the lungs.

Animal Studies

An overview of the non-cancer health effects observed in animals is shown in Table 9-1. At high doses, dioxin causes death, weight loss, oxidative stress, lymphoid atrophy (especially of the thymus where T-cells are produced), gonadal atrophy, and skin changes, including chloracne that is similar to that observed in humans. Relatively high doses also cause liver damage (including fatty infiltration), changes in the number and kinds of cells, and porphyrin accumulation (over-production of a protein related to red blood cell formation). Short-term exposure to dioxin increases thyroid weight and thyroid dysfunction. It also disrupts the receptor levels, concentrations, and transport of a wide variety of hormones: estrogens, androgens, glucocorticoids, thyroid hormones, insulin, gastrin and melatonin (Birnbaum, 1994, 1998). Chronic exposure to TCDD causes oxidative stress, heart problems, including damage to the myocardium, heart valves and arterial walls, and upsets cardiac and vascular integrity. Chronic exposure also causes bronchiolar and alveolar changes in the lung that are similar to chronic bronchitis in humans.

Exposure to dioxin upsets numerous physiological processes. It decreases the ability to store vitamin A, changes the amount of protein and cholesterol in the blood, alters expression of cytochrome P450 and mixed oxidase functions, alters enzyme levels in the liver, decreases serum albumin, disrupts bone marrow production, and affects growth and differentiation by down-regulating epidermal growth factor (EGF) receptors. Some biochemical effects are difficult to classify as toxic or adverse, but they clearly represent molecular and cellular responses to dioxin (Birnbaum, 1994). Not all effects occur in all species, and many are tissue specific.

Table 9-1 Non-Cancer Health Effects in Animals

Loss of body weight (wasting syndrome)	Brewster, 1984; Peterson, 1984; Kelling, 1985; Max, 1987; Lakshman, 1988, 1989, 1991
Atrophy (shrinking) of: • Lymphoid tissue (especially thymus)	• Luster, 1980, 1985; Chastain, 1985; Vos, 1991Kociba, 1976; Chahoud, 1989

• Gonads • Uterus	• Romkes, 1988; DeVito, 1992
Liver damage • Fatty infiltration/ Hyperplasia • Porphyria (blockage in heme biosynthesis which produces toxic by-products) • Reduced number of estrogen receptors • Altered enzyme levels in the liver (indicative of liver damage) • Increase in liver size	• McConnell, 1978; Moore, 1979; Turner, 1983; USEPA, 1984, 1985; WHO, 1989; • Goldstein, 1973, 1982; Elder, 1976, 1978; Jones, 1981; Cantoni, 1981; Smith, 1982; DeVerneuil, 1983; USEPA, 1984, 1985; WHO, 1989 • Goldstein, 1990; Lin, 1991, 1991a; Safe, 1991 • Zinkl, 1973; Elder, 1976, 1978, 1982; Kociba, 1976, 1978; Gasiewicz, 1980; Olson, 1980; Jones, 1980; Cantoni, 1984, 1984a; • Vos, 1974a; Allen, 1977; McConnell, 1978, 1978a; Kociba, 1978; Gasiewicz, 1980
Changes in skin (chloracne, changes in pigmentation)	Jones, 1962; Schwetz, 1973; Olson, 1980; Knutson, 1980, 1982; Gierthy, 1984, 1985; Osborne, 1985; Hudson, 1986; WHO, 1989; Hebert, 1990, 1990a; Enan, 1992
Increased thyroid weight & thyroid dysfunction	Neal, 1979; USEPA, 1984, 1985; Henry, 1987; WHO, 1989
Endocrine (hormonal) effects • Estrogens (decrease in estrogen & estrogen receptors) • Androgens (reduced testosterone)	• Barsotti, 1979; Umbreit, 1988; Romkes, 1988; Spink, 1990; DeVito, 1992 • Neal, 1979; Mittler, 1984; Keys, 1985; Moore, 1985, 1991; Kleeman, 1990

• Glucocorticoids, thyroid hormones, insulin and gastrin	• Sunahara, 1989; Goldstein, 1990; Lin, 1991, 1991a; Birnbaum, 1994
Oxidative stress	WHO, 1989; Wahba, 1989, 1989a, 1990, 1990a; Pohjanvirta, 1989; Alsharif, 1990; Stohs, 1990; Hassoun, 1998
Changes in bronchiolar & alveolar tissue (similar to chronic bronchitis in humans)	Allen, 1977; Van Miller, 1977; Kociba, 1979; NTP, 1982, 1982a
Effects on the Heart • Changes in heart muscle (damage to myocardium, heart valves, and arterial walls) • Effects on cardiac and vascular integrity	• Buu-Hoi, 1972; Kociba, 1978; Brewster, 1987; Kelling, 1987; Canga, 1988; Hermansky, 1988; Olson, 1980 • Allen, 1967, 1977; Norback, 1973
Decreased ability to store vitamin A	Aitio, 1979; Thunberg, 1979, 1983; Hakansson, 1989, 1989a, 1991; Puhvel, 1991
Biochemical changes • Damage to adrenal glands • Increased levels of certain proteins known as cytochrome P450 and mixed-function oxidases • Changes in lipid function (amount of cholesterol and lipoprotein in blood) • Decreased serum albumin & effects on bone marrow	• USEPA, 1984, 1985; WHO, 1989 • USEPA, 1984, 1985; Hahn, 1988; WHO, 1989; Rifkind, 1990 • Grieg, 1973; Zinkl, 1973; McConnell, 1978, 1978a, 1979; Gasiewiecz, 1979, 1980; Olson, 1980; Brewster, 1984; USEPA, 1984, 1985; Schiller, 1986; Lakshman, 1988, 1989, 1991; Pohjanvirta, 1989; Wahba, 1989, 1989a, 1990, 1990a; WHO, 1989; Alsharif, 1990; Stohs, 1990 • Luster, 1980, 1985; Chastian, 1985
Suppression of the immune system	Vos, 1973, 1978, 1989; Clark, 1981;

	Kerkvliet, 1984; Tucker, 1986; Sonawane, 1988; Benjamini, 1991; Holsapple, 1991, 1991a; Smialowicz, 1994
Reproductive effects Endometriosis	Rier, 1993; Cummings, 1996; Johnson, 1997
Developmental effects on animals prenatally exposed • Growth retardation • Lymphoid atrophy (shrinking) • Hemorrhage (bleeding) • Edema (fluid retention) • Fetal death (spontaneous abortion) • Birth defects (cleft palate) • Altered body set-point temperature Reproductive problems and anomalies in reproductive organs • Hearing deficits • Immunotoxicity and changes in the immune system • Learning deficits	• Birnbaum, 1994 • Birnbaum, 1998 • USEPA, 1984, 1985; WHO, 1989 • Firestone, 1973; Schwetz, 1973 • Bowman, 1989 • Couture, 1990 • Gordon, 1995, 1996 • Mably, 1992, 1992a; Gray, 1995, 1995a, 1997 • Goldey, 1996, 1998; Herr, 1996; Crofton, 1999 • Hong, 1989; Neubert, 1992, 1993; Narasimhan, 1994; Ross, 1996; Gehrs, 1997,1998, 1999 • Schantz, 1989

As discussed in Chapter Eight, dioxin is particularly toxic to early stages of growth and development. Dioxin adversely affects the developing immune, reproductive, nervous, and lymph systems. It causes cleft palate, hemorrhage and edema, impairs hearing, and retards growth. It also alters the set point for body temperature in rats and hamsters (Gordon, 1995, 1996). Alteration of the set point for body weight is the hypothesized mechanism for the wasting syndrome resulting from dioxin exposure. As studies in monkeys show, offspring prenatally exposed to dioxin may have more spontaneous abortions, learning deficits, and damaged immune systems. The lethal doses of dioxin are also much smaller in early stages of development for all species tested.

Evidence of Human Health Effects

In this chapter, two kinds of information are used to assess the effects of exposure to dioxin on human health: information obtained directly from studying humans exposed to dioxin, and information obtained from studying experimental animals exposed to dioxin.

For the many reasons mentioned in Chapter Seven, studies of dioxin's adverse effects in humans are fewer than studies of its effects in other animals. However, several epidemiological studies provide reliable data on dioxin's effects on people. These studies are the cancer mortality studies of German workers (Zober, 1990; Ott, 1994; Flesch-Janys, 1995, 1998); studies of the Seveso, Italy residents (Bertazzi, 1989, 1993, 1997, 1999); the NIOSH study of U.S. chemical workers (Sweeney, 1989, 1993; Fingerhut, 1991; Steenland, 1999); the study of Dutch workers (Hooiveld, 1998) and the large international cohort of workers being followed by IARC (Kogevinas, 1997). These studies are discussed in detail in Chapter Seven.

There are also many studies that have linked non-cancer effects in people to dioxin exposures. These studies include the Ranch Hand study of Vietnam war veterans (Michalek, 1990, 1998, 1999; Roegner, 1991; Egeland, 1994; Grubbs, 1995); studies of the residents of Seveso, Italy (Bertazzi, 1994, 1998; Mocarelli, 1996; Pesatori, 1998); studies of the residents of Times Beach, Missouri (Webb, 1989); studies of developmental effects in Dutch children (see Chapter Eight); and studies of people exposed to contaminated oil in Taiwan (Hsu, 1994) and Japan (Masuda, 1994).

Evidence of health effects in humans is also provided by a national Dioxin Registry that is being maintained by ATSDR as part of the National Exposure Registry. This registry tracks health effects in populations exposed to dioxin at four sites in Missouri. Thus far, the following health effects were significantly elevated for the dioxin-exposed populations: all cancers, skin rashes, stroke, urinary track disorders, and anemia (ATSDR, 1996).

Animals as Predictors of Effects in Humans

The evidence of dioxin's effects on people is based largely on exposures at relatively high levels. Much less is known about what happens to people who are exposed to lifelong low level environmental exposures, or to what is generally considered "background" exposures. This is what the general American population is exposed to through the daily ingestion of common foods. Better characterization of dose-response relationships and the factors associated with individual variations in susceptibility are needed to evaluate the risks to humans from these exposures (Grassman, 1998).

One way to increase the confidence that a particular adverse effect observed in an epidemiological study was associated with exposure to dioxin is to compare the dose of dioxin that is required to produce an effect in animals to the dose of dioxin in humans that is associated with that same or with a similar effect. These comparisons can be made by using "body burden levels" where the body burden is the total accumulation of dioxin in the body per kilogram of body weight (see Chapter Four). This body burden approach provides a "dose equivalent" or tissue concentration measure on a body weight basis that is associated with an adverse effect. Body burdens can be used to compare effects observed in experimental animals using a high dose with those observed in humans who, in general, are exposed to much lower doses.

For some toxic effects in animals, such as lethality and weight loss, it is clear that there are marked differences in susceptibility to dioxins (IARC, 1997). For other effects such as reproductive and developmental effects, most animal species respond at similar doses (DeVito, 1995; Grassman, 1998). Thus, the dose of dioxin that produces a particular effect in experimental animals might be expected to be similar to the dose of dioxin associated with that same effect in humans. Using this body burden approach, researchers at the EPA compared the body burden levels of dioxin that produced effects in experimental animals to body burden levels associated with effects in humans based on clinical findings from epidemiological studies (DeVito, 1995).

These researchers made the following observations:

- Humans and rats are equally sensitive to TCDD-induced biochemical changes when compared on a total body burden basis;
- Humans may be approximately 280 times more sensitive to the testosterone decreasing effects of dioxin compared to rats;
- Immune alterations including increased viral infections and altered lymphocyte subsets in marmoset monkeys occurred at body burden levels equivalent to human background exposures; and
- Endometriosis occurs in experimental animals at body burden levels less than 10 times the average background exposure to humans.

This study provides strong evidence that the body burden levels associated with adverse health effects are "similar between animals and humans." The authors go on to say that "some individuals may respond to dioxin exposures with cancer and non-cancer effects at body burden levels within two orders of magnitude [a factor of 100] of those in the

general population."

Researchers at the National Institute of Environmental Health Sciences (NIEHS) agree that animal models are good predictors of dioxin's effects in people. They provide the following reasons why this is so:

- The reproductive, developmental, immunologic, and carcinogenic responses to dioxin which can be seen in humans also occur in animal models;
- The preponderance of biochemical effects induced by dioxins in both animals and humans are mediated by the Ah receptor;
- Animal dosing regimes can be varied to examine the range of exposures encountered n human populations;
- Dose metrics based on internal dose (tissue dose and body burden) can be used to compare responses across species as these parameters take into account species differences in clearance rates; and
- Biochemical responses to dioxins in animal models show qualitative and quantitative similarity to those observed in humans (Grassman, 1998).

Chloracne and other Effects on the Skin

Chloracne is the most common symptom and the hallmark effect that follows high dose exposure to dioxin. A serious skin disorder that begins with an acne-like appearance two to four weeks after exposure, it progresses to pus-filled boils, pimples that may be colored more darkly than the rest of the face, blackheads, and cysts that sometimes persist for years (Reggiani, 1980). Chloracne may disappear after exposure to dioxin ceases, or it may remain for more than 10 years (Suskind, 1984), or even for an average of 26 years, after exposure (Moses, 1984). In Seveso, Italy, most (88%) of the reported chloracne cases were children (Bertazzi, 1998). Those who had chloracne also had frequent gastrointestinal symptoms and eye irritation, and abnormal levels of the liver enzymes GGT (gamma-glutamyltranspeptidase), AAT (alanine aminotransferase), and ALA-U (urinary aminolevulinic acid) (Bertazzi, 1994). These enzymes are indicators of liver damage (see discussion below), and also result from high dose exposures. In addition to chloracne, people exposed to dioxin at Seveso had several transient skin effects including conjunctivitis, sebaceous cysts and inflammation (Reggiani, 1980; Bertazzi, 1994). Inflammation of the eye lids or blepharitis (squamous metaplasia of meibomian glands) was also reported.

Although chloracne usually occurs after exposure to a high dose of dioxin, the amount of dioxin exposure that causes chloracne varies from individual to individual. Some people develop it at body burdens of approximately three times the background level (Beck, 1989). Though chloracne is a marker of dioxin exposure, adverse health effects from dioxin exposure can occur without it.

Chemical workers in New Jersey and West Virginia experienced hirsutism (abnormal distribution of facial hair) and hyperpigmentation (overcoloration of the skin) after exposure to dioxin (Ashe, 1950; Suskind, 1953; Bleiberg, 1964; Poland, 1971). The 1996 update of Veterans and Agent Orange study reported that there is "suggestive" evidence that Vietnam veterans exposed to dioxin may have above average susceptibility to porphyria cutanea tarda (PCT), a rare liver disease that can involve thinning and blistering of the skin in areas exposed to the sun (IOM, 1996). German workers (Pazderova-Vejlupkova, 1981), New Jersey chemical plant workers (Bleiberg, 1964), and Czechoslovakian workers (Jirasek, 1973, 1974) all had a higher than expected incidence of PCT after exposure to dioxin. In the New Jersey workers, PCT symptoms (but not chloracne) disappeared six years after exposure (Poland, 1971). PCT in these workers may have been associated more with worker exposure to hexachlorobenzene (HCB), a known porphyriagenic compound, than with TCDD exposure (Jones, 1986). PCT was not associated with TCDD exposure in Calvert's (1994) study of workers exposed to dioxin. Neither chloracne nor PCT were found in residents of Times Beach exposed to dioxin (Stehr, 1986). Porphyria may require a mixture of dioxins and PCBs. Both animal data (van Birgelen, 1996, 1996a) and human data (Hryhorczuk, 1998) indicate that there are multiple mechanisms involved.

Effects on the Liver

Exposure to dioxin consistently increases liver size in animals, and the same effect is seen in people, especially after accidental exposure. The liver usually reverts to its normal size with time (Ashe, 1950; Suskind, 1953; Reggiani, 1980; Bertazzi, 1998). One of the liver's functions is to metabolize and clear foreign substances from the body. When there are large doses of dioxin in the body, the liver sequesters the dioxin from the body. There is also evidence that the liver sequesters dioxin when the body burden rises because the body is exposed to small doses over a long period of time. In any case, an enlarged liver is a sign that the body is overloaded with toxic chemicals.

Gamma glutamyl transferase (GGT) is a liver enzyme that can spill over into the blood when the liver is damaged. Its presence is often used in standard clinical biochemistry screens as a biomarker of dioxin exposure, particularly at high doses. High GGT levels are found in animals exposed to dioxin, and they were found in the following humans exposed to dioxin: residents of Seveso, Italy (Mocarelli, 1986; Bertazzi, 1989); workers with chloracne in Nitro, West Virginia (Moses, 1984); and trichlorophenol (TCP)

production workers (May, 1982; Martin, 1984; Moses, 1984; Calvert, 1992). In his study of TCP production workers, Calvert (1992) found no evidence of liver disease, but he noted that the high levels of GGT persist for 15-36 years. Both the Vietnam Experience Study and the U.S. Air Force Ranch Hand study reported significantly higher than expected levels of GGT among veterans exposed to dioxin (CDC, 1988; Roegner, 1991). Pazderova-Vejlupkova (1981) observed mild liver lesions in one-third of a cohort of German workers, and Zober (1994) observed marginal increases in chronic liver disease among these same workers in the highest exposure group.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were higher than normal in the Seveso children (Caramaschi, 1981; Mocarelli, 1986) and in TCP production workers (May, 1973; Jirasek, 1974). Both effects may have been transient effects of acute exposure. D-Glucaric acid (DGA) excretion, another measure of liver damage, was higher than normal in adults who lived in Seveso at the time of the accident, but by 1981, levels were within a normal range (Ideo, 1985). Martin (1984) observed a similar effect in TCP workers exposed to dioxin. Workers exposed to dioxin at Dow Chemical in Midland, Michigan (Bond, 1987) and workers exposed to dioxin after an accident in Nitro, West Virginia (Suskind, 1984) had slightly more ulcers than normal. However, Ranch Hand veterans, workers in the NIOSH study, and workers in Fingerhut's study (1991) did not experience ulcers when exposed to increasing dioxin levels. More Australian soldiers exposed to Agent Orange in Vietnam died of digestive diseases than veterans who were not exposed (Fett, 1987). More Ranch Hand veterans, who sprayed Agent Orange, died from digestive disease, notably chronic liver disease and cirrhosis, than other veterans (Michalek, 1998). Masuda (1994) observed that more males and females exposed to contaminated rice oil at Yusho died from chronic liver disease and cirrhosis than individuals who were not exposed and that more of the exposed females died from gastric and duodenal ulcers than females who were not exposed to the contaminated rice oil. Masuda (1994) also reported that more females who were exposed died from kidney disease than females who were not exposed.

Effects on Thyroid Function

Thyroid hormone is very important to human brain development, and deficiencies of it (hypothyroidism) during fetal life or during early infancy can lead to mental retardation, hearing loss, and speech problems (Porterfield, 1994). Even if they have normal IQs, children with hypothyroidism may have language comprehension problems, impaired learning and memory, and hyperactive behavior - all problems similar to those experienced by the children exposed to PCBs and dioxins from contaminated rice oil in the Yusho and Yu-cheng incidents (Porterfield, 1994). Structurally similar to thyroid hormone, PCBs and dioxin bind to important proteins and cause effects that resemble hypothyroidism (Bastomsky, 1977; Potter, 1983, 1986; Pazdernik, 1985; Henry, 1987; Roth, 1988; Muzi, 1989).

Though some studies (Suskind, 1984; Ott, 1994), fail to show that exposure to dioxin has any effects on the thyroid gland in adult humans, Bahn et al. (1980) reported hypothyroidism in workers exposed to dioxin-like polybrominated biphenyls, and Zober et al. (1994) found that, over a 36 year period, workers exposed to dioxin in a chemical reactor accident had a higher than normal incidence of thyroid disease and appendicitis. Veterans in the 1987 Ranch Hand study exposed to dioxin had lower than normal, but not statistically significant, uptake of triidothryonine (T3) (Roenger, 1991), and those with higher than normal serum levels of 2,3,7,8-TCDD had slightly above normal, but not statistically significant, mean levels of thyroid stimulating hormone (TSH) in both the 1987 study and 1992 follow-up (Roenger, 1991; Grubbs, 1995). Low T3 uptake and high TSH levels are signs of thyroid imbalance.

The effects of dioxins on the thyroid are most severe in prenatal stages and in babies that nurse on contaminated breast milk. Nagayama (1998) reported that Japanese babies exposed to background levels of PCDDs, PCDFs and PCBs in breast milk had adverse thyroid function, including low serum thyroxin (T4) and high TSH levels. In contrast, Pluim (1992, 1993a) reported that breast-fed babies of mothers with the highest dioxin levels in their milk had higher than normal levels of T4 and concluded that dioxin modulates the hypothalmic pituitary thyroid regulatory system.

Similarly, Koopman-Esseboom (1994) found dioxin levels in mother's milk to be significantly correlated with infant plasma levels of TSH at the second week and third month, and inversely correlated with total T3 pre-delivery, and with mothers' total T3 post-delivery. The infants with higher dioxin levels showed a statistically significant increase for total T3 and free T4 measured during the second week of life. These two Dutch studies of nursing infants suggest that ingestion of breast milk with elevated levels of dioxins may alter thyroid function. Both studies are limited by a short observation period and a lack of controls for other factors which might affect thyroid status.

Diabetes

Dioxin interferes with the hormone insulin, alters glucose tolerance, and can lead to diabetes. In one study of 55 exposed workers evaluated 10 years after exposure, 50% of the workers are diabetic or have abnormal glucose tolerance, an early indicator of diabetes (Pazderova- Vejlupkova, 1981). Subsequent to this finding, several studies examined either fasting glucose levels or the number of diabetics in dioxin-exposed populations; although the results vary from study to study, the most reliable studies consistently show that dioxin increases the risk of diabetes. In the NIOSH study, the risk of diabetes increases 12% for every 100 picogram dioxin/gram (pg/g) of lipid in the blood (Sweeney, 1992). In the Ranch Hand study, Roegner (1991) found that veterans with blood dioxin greater than 33.3 pg/g have a relative risk of 2.5 for diabetes. In a

follow-up study of the Ranch Hand veterans, Henriksen et al (1997) found that veterans exposed to dioxin have a relative risk of 1.4 for glucose abnormalities, 1.5 for diabetes, and 2.3 for the use of oral medications to control diabetes. These authors also found that Ranch
Hand veterans exposed to dioxin develop diabetes earlier than other veterans, and that non-diabetic Ranch Hand veterans exposed to dioxin have a relative risk of 3.4 for serum insulin abnormalities. Wolfe (1992a) reported that Ranch Hand personnel exposed to dioxin and who satisfy the case definition of diabetes develop diabetes earlier than other veterans. The ongoing follow-up study of the Seveso residents reported a significant increase in mortality from diabetes in females in the second highest exposure group (Zone B) and a slightly elevated increase in males (Pesatori, 1998). Diabetes deaths in the highest exposure zone showed a suggestive but not statistically significant increase, because the number of deaths were too few to draw any conclusions.

Although exposure to dioxin alters glucose homeostasis in humans, rats, and guinea pigs (Zinkl, 1973; Enan, 1992), it is puzzling that dioxin does not raise plasma glucose levels in animal studies as it does in humans (USEPA, 1994d). Perhaps the difference in dietary fat intake between humans and other animals is responsible. Human diets are between 30% and 50% fat, and a high proportion of it is saturated. Other animals' diets are usually between 8% and 15% fat, and the fats are unsaturated. If animals were fed a diet comparable to humans and then tested for plasma glucose and diabetes, the discrepancy between the effects of dioxin exposure on plasma glucose levels in humans and other animals might disappear.

Other Hormonal Effects

As noted in Table 9-1, exposure to dioxin has a variety of effects on hormone function in animals. Several studies show that exposure to dioxin has similar effects in humans. In the NIOSH cohort, dioxin-exposed workers have lower than normal testosterone levels and higher than normal follicle-stimulating and luteinizing hormone levels, both of which reduce sperm counts (Egeland, 1994). In Vietnam Ranch Hand veterans, who were exposed to dioxin at much lower levels than the NIOSH cohort, lower testosterone levels were also found, but these levels did not differ significantly from controls (Longnecker, 1997). Moses (1984) reported that workers with chloracne who were exposed to dioxin in Nitro, West Virginia have higher than expected sexual dysfunction and lower than expected libido. Boys prenatally exposed to rice oil contaminated with PCBs and PCDFs in Taiwan have small penises at puberty (Guo, 1993).

Effects on the Nervous System

As it does in other animals, dioxin damages the nervous system in humans (Table 9-2). Russian workers exposed to the herbicide 2,4,5-T reported the following symptoms: headache, sleepiness, insomnia, irritability, fatigue, weakness, pain near the heart, reduced memory and reduced potency. Confirmed symptoms were still present 18 years later (Basharova, 1997). In Seveso, mild transient peripheral neuropathy was associated with chloracne and higher than normal level liver enzymes (Filippini, 1981; Bertazzi, 1998;). Moses (1984) reported that one group of TCP production workers exposed to dioxin has significant neuropathy, but other groups of exposed workers do not (Suskind 1984; Hoffman, 1986; CDC, 1988, 1988a; Assennato, 1989; Sweeney, 1993). Acute and subacute periperphal neuropathy is documented in Vietnam veterans exposed to dioxin (IOM, 1996). Although some neurological effects from exposure to dioxin are transient, they persist in some adults for 10 or 18 years (Pazderova-Vejlupkova, 1981; Basharova, 1997). BASF workers exposed to dioxin also report persistent peripheral nervous system and sense organ disorders (Zober, 1994), as well as increased upper respiratory tract infections and skin disorders.

Webb (1989) reported that a group of 41 Missouri residents with measured 2,3,7,8-TCDD serum lipid levels have abnormal neurological symptoms, including abdominal pain sensation in lower extremities, abnormal vibratory sensation, and abnormal reflexes; these effects are not dose-related. Singer (1982) reported that 55 workers in Jacksonville, Arkansas exposed to 2,4-D and 2,4,5-T have significantly lower median motor nerve and sural nerve conduction velocities than a control group not exposed to these phenoxy herbicides.

Table 9-2 Neurological Symptoms Reported After Dioxin Exposure

headaches	**muscle weakness**	**reduced sex drive**
dizziness	**nervousness**	**difficulties with erection**
irritability	**anxiety**	**decreased mental efficiency**
depression	**crying spells**	**depression**
insomnia	**apathy, fatigue**	**loss of feeling in extremities**
tremor	**slowed thinking**	**tingling in toes and fingers**
numbness	**social withdrawal**	**slowed nerve conduction velocity**
anorexia	**trouble concentrating**	

Source: Ashe, 1950; Baader, 1951; Suskind, 1953; Bauer, 1961; Goldman, 1972; Jirasek, 1974; Oliver, 1975; Pazderova-Vejlipkova, 1981

Dioxin may be particularly toxic to the developing nervous system, and its effects may be permanent. Pregnant women who ate contaminated rice oil in Yu-Cheng delivered babies that were developmentally delayed and had speech problems. When 8 to 14 years old, these children still had low IQ test results, more behavior problems than normal, and tended to be hyperactive (Guo, 1992, 1993). Although the rice oil did not contain TCDD, it contained PCBs, PCDFs and polychlorinated quaterphenyls (PCQs), which affect the human body in the same way that TCDD does.

Cardiovascular Effects

As noted in Table 9-1, at high doses, dioxin damages heart muscle and valves in animals other than humans. Most studies in humans, however, indicate no correlation between exposure to dioxin and death from all diseases of the circulatory system (Bond, 1987; Bertazzi, 1989, 1992; Zober, 1990; Coggon, 1991; Fingerhut, 1991; Collins, 1993; Bueno de Mesquita, 1993; Flesch-Janys, 1995). Critics of these results suspect that the studies are biased by the "healthy worker" effect, a bias introduced into a study when workers are compared to non-workers: since workers are generally healthier and more physically active than non-workers, it is spurious to compare the health of the two groups since the risk for heart disease decreases with physical activity. It makes more sense to compare workers who are exposed to dioxin and workers who are not exposed to it. In such a study at a herbicide-producing plant in Germany, Flesch-Janys (1995) reported a strong dose-dependent relationship between deaths from cancer or ischemic heart disease and exposure to PCDD/Fs. Workers with the highest exposure to PCDD/Fs had a relative risk of 2.48 for ischemic heart disease.

Other studies on the effects of dioxin exposure on heart disease also control for the healthy worker effect. Vos (1977) reported that members of an accident clean-up crew exposed to dioxin are more likely to die from myocardial infarction than were unexposed members of the clean-up crew. In contrast, workers exposed to 2,4,5-T following an accident at a Nitro, West Virginia plant show no more signs of cardiovascular disease than unexposed workers thirty years after the accident (Suskind, 1984). Bueno de Mesquita (1993) reported that trichlorophenol workers in Holland have a slightly higher mortality rate from stroke than non-exposed workers, and Bond (1987) found that workers with chloracne in Michigan are twice as likely to die from a stroke than unexposed workers are.

In another study which controlled for the healthy worker effect, Ranch Hand veterans and Australian conscripts who served in Vietnam have a higher risk for diseases of the

circulatory system than veterans not exposed (Fett, 1987; Michalek, 1990, 1997, 1998; Wolfe, 1994); few differences in deaths from heart disease are observed between the two groups. Ranch Hand Veterans with the highest dioxin levels have higher blood pressure, more arrhythmias, and more abnormal pulses than other veterans (Roegner, 1991). In Seveso, more males exposed to dioxin die from cardiovascular disease, particularly from chronic ischemic heart disease, than unexposed males, and more females exposed to dioxin die from chronic rheumatic heart disease and hypertension than do females not exposed to dioxin (Bertazzi, 1998).

It is unclear if dioxins increase the level of cholesterol and triglycerides in the blood. In the majority of studies, the levels of these two substances in workers exposed to dioxin are normal (Moses, 1984; Suskind, 1984; Mocarelli, 1986; Hoffman, 1986; Assennato, 1989; Webb, 1989; Ott, 1994; Calvert, 1996), but in several studies, workers exposed to dioxin have higher than normal levels of total cholesterol (Suskind, 1984; Martin, 1984) and triglycerides (Martin 1984; Calvert, 1996). Ranch Hand veterans tested in 1987 have higher than normal levels of both cholesterol and triglycerides (Roenger, 1991). However, when tested again in 1992, their cholesterol levels are normal, but their triglyceride levels are still high (Grubbs, 1995).

Pulmonary Effects

Long-term exposure to dioxin in rats, mice, and monkeys causes changes in the lung that are consistent with chronic bronchitis in humans (Table 9-1). In humans, acute exposure to dioxin causes respiratory problems (Goldman, 1972; Zack, 1980). In the initial 1987 study, Ranch Hand veterans with the highest serum dioxin levels score lower on lung function tests than other veterans (Roegner, 1991), but all veterans score equally well in the 1992 follow-up (Grubbs, 1995). Suskind (1984) reported that chemical workers exposed to dioxin have lower pulmonary function than workers who were not exposed, but the results have been questioned because the average age of the exposed group is 10 years older than the controls. Bertazzi (1998) observed that more residents of Seveso exposed to dioxin die from chronic obstructive pulmonary disease than expected.

Summary

The adverse effects of dioxin exposure to human health are varied and numerous. A concise summary is almost impossible. The EPA examined the evidence for each of the health effects of dioxin, taking into account the number of scientific studies, the quality of the studies, the time of exposure and appearance of an illness, the degree of exposure, the consistency among studies, and whether the diseases observed fit with the known

biochemical actions of dioxin. It then determined which effects are known to be caused by dioxin, which effects are probably caused by dioxin, and which effects are likely to be caused by dioxin. This characterization of dioxin's effects on human health is shown in Table 9-3. Finally, a summary of many of the adverse health effects associated with dioxin and dioxin-like chemicals in humans is provided in Table 9-4.

Table 9-4 - Overall Health Effects Associated with Dioxin and Dioxin-Like Chemicals

Cancer - lung, stomach, soft tissue, liver
Skin disorders - chloracne, hyperpigmentation, hirsutism
Male reproductive toxicity - reduced sperm count, testicular atrophy, abnormal testis structure, reduced size of genital organs, lower male hormone levels, feminization of hormonal, and behavioral responses
Female reproductive toxicity - hormonal changes, decreased fertility, inability to maintain pregnancy, ovarian dysfunction, endometriosis
On unborn fetus - birth defects, alterations in reproductive system, decreased sperm count, altered mating behavior, structural abnormalities in female genitalia, reduced fertility, delayed puberty, neurological problems, developmental problems
Hormonal Changes including alterations in sex, thyroid, digestive and other hormones)
Immune suppression and increased susceptibility to infectious diseases
Metabolic changes including altered glucose response, decreased insulin levels, altered fat metabolism, weight loss, wasting syndrome, fetal death
Neurological damage to the central and peripheral nervous systems including impaired neurological development and subsequent cognitive deficits
Damage to liver, thymus, spleen, and bone marrow
Damage to heart leading to arrhythmias
Diabetes
Lung Problems

Table 9-4 Overall Health Effects Associated with Dioxin and Dioxin-Like Chemicals

Cancer

- lung
- stomach
- soft tissue
- liver

Male Reproductive Toxicity

- reduced sperm count
- testicular atrophy
- abnormal testis structure
- reduced testis size
- lower male hormone levels

Hormonal Changes

- altered sex hormones
- altered thyroid hormones
- altered digestive hormones

Metabolic Changes

- altered glucose response
- decreased insulin levels
- altered fat metabolism
- weight loss
- wasting syndrome

- feminization of hormonal and behavioral responses

Female Reproductive Toxicity

- hormonal changes
- decreased fertility
- inability to maintain pregnancy
- ovarian dysfunction
- endometriosis

Developmental Toxicity

- birth defects
- alterations in reproductive system
- decreased sperm count
- altered mating behavior
- structural abnormalities in female genitalia
- reduced fertility
- delayed puberty
- neurological problems

Immune Suppression

- increased susceptibility to infectious disease

- fetal death
- diabetes

Damage to the central and peripheral nervous systems

- impaired neurological development
- subsequent cognitive deficits

Organ Damage

- liver
- thymus
- spleen
- bone marrow
- lungs
- heart - leading to arrhythmias

Skin Disorders

- chloracne

- hyperpigmentation
  hirsutism

Source: DeVito, 1994; Schecter, 1994c; Birnbaum, 1994; USEPA, 1994; IARC, 1997; ATSDR, 1998; Birnbaum, 1998; WHO, 1998.

Chapter 10

Risk Characterization

According to EPA policy, assessing risks has four main components: 1) hazard identification; 2) dose-response assessment; 3) exposure analysis; and 4) risk characterization (USEPA, 1995a). In order, these can roughly be translated as: 1) What

effects can a toxic compound cause? 2) At what doses do these effects occur? 3) To what levels of the toxin are people exposed? 4) Based on the answers to first three questions, are people likely to be at risk?

Risk characterization is the summarizing step of risk assessment. It integrates information from the first three components of the risk assessment into an overall conclusion about risks that is "complete, informative and useful to decision-makers" (USEPA, 1995b). The goal of risk characterization is to estimate whether exposure might cause harm, not to say what to do about it. Though risk assessment is important, it does not by itself determine policies on how to reduce exposure. In fact, some people argue that risk assessment should be a fairly minor determinant of policy and that the availability of alternative processes which reduce or eliminate the pollutant need to be considered. Policy making is complex and, at its core, political.

The theoretical separation between risk assessment and decision-making does not mean that risk assessment is devoid of policy implications. Although dioxin is one of the most intensely studied of all toxic compounds, our knowledge of it is still quite incomplete. Numerous assumptions with varying degrees of technical and policy content are therefore used to fill the gaps in our knowledge.

Scope

This chapter draws on information from other chapters and summarizes the scientific evidence used to assess the risks of chronic, low level exposures to dioxin and dioxin-like compounds to the general population. This exposure is often referred to as "background" exposure. This term is misleading since we know that dioxin contamination of food is the result of primarily airborne releases from thousands of anthropogenic sources. To use the term "background" implies that nothing can be done about these exposures when, in fact, many of these sources can be eliminated.

Other dioxin risk assessments have been recently produced. The EPA produced its draft document in 1994, but it has not been finalized (USEPA, 1994a). The Agency for Toxic Substances and Disease Registry (ATSDR) recently completed a toxicological profile that sets minimum risk levels for dioxins (ATSDR, 1998). The World Health Organization (WHO) revised its tolerable daily intake for dioxins in 1998 (WHO, 1998)[(1)] . Efforts by individuals include those by Birnbaum (1999) and Webster (1994). Though these risk assessments are good ones, none integrates all the known information about dioxin's effects on human health, and none has the same sense of urgency to enact public policy on dioxin as this report.

Dioxin and Dioxin-like Compounds

The term "dioxin" is often imprecisely used. Some people restrict its use to only 2,3,7,8-TCDD (TCDD), the best studied and most potent "dioxin." Some extend its use to a whole class of compounds thought to have effects qualitatively similar to TCDD, and whose effects are mediated by the Ah receptor (AhR). In this report, the terms "dioxin" and "dioxins" are used to refer to any of the dioxin family members that act as AhR ligands and elicit dioxin-like effects. These compounds meet the following four criteria which were used by the World Health Organization committee to assign toxic equivalency factors (Van den Berg, 1998).

First, they have a structural relationship to the polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Second, their toxicity takes effect only after they bind to the aryl hydrocarbon receptor (AhR), though this binding alone is not sufficient for toxic effects to occur. The growing evidence for this criterion comes from studies of genetics, molecular biology, structural activity, and other disciplines that study the toxicity of these compounds.

Though still poorly understood, the Ah receptor (AhR) system probably has a very important biological role. Many researchers believe that the AhR has one natural ligand, or perhaps a few natural ligands. If the AhR system behaves like many other biological-signaling systems, the expression of the ligands is probably tightly controlled in space and time, and the ligands are degraded after use, perhaps by the CYP1 family of phase I enzymes. Dioxin-like compounds, being foreign to biological systems, may cause problems because they violate these two signaling system features.

Third, they must cause dioxin-like biochemical and toxic effects. This criterion eliminates compounds that may bind to the AhR but do not lead to the full complement of dioxin-like effects. Fourth, they must be persistent and accumulate in living tissue. This important criterion eliminates compounds which cannot be absorbed or are not persistent, especially compounds with very short half-lives. However, it may prove incorrect in some cases. For example, the biological effects of dioxin probably depend less on exposure than on the concentrations present in a target organ over a critical length of time. In large doses, some toxic compounds that are not persistent may have effects similar to the cumulative effects of the more persistent dioxins.

In other instances, nonhalogenated AhR agonists found in certain cooked foods, though they may not have the full range of dioxin-like effects (Van den Berg, 1998; Pohjanvirta, 1998), may account for a significant amount of the dioxin-like activity in a person's body burden (Safe, 1995a). These nonhalogenated AhR agonists, derived from food or from certain polycyclic aromatic hydrocarbons, require additional research to determine their

relative contribution to body burden levels of dioxins. All things considered, when persistence is taken into account, the PCDDs, PCDFs, and PCBs generally appear to contribute most of the toxicity in a person's body burden (DeVito, 1996).

The four criteria used to define dioxin-like compounds are not perfect. For example, dioxin-like compounds conventionally include subsets of the PCDDs, PCDFs, and PCBs, but some PCBs which are not considered dioxin-like have their own spectrum of toxic effects. Also, there are a large number of polychlorinated and polyhalogenated compounds that meet the four criteria outlined above but have not yet been considered in the TEF approach (Van den Berg, 1998).

For example, though the World Health Organization has not yet evaluated whether hexachlorobenzene is dioxin-like, van Birgelen et al. (1998) argue that it should be. Though some assays measure it as much less potent than 2,3,7,8-TCDD, it is persistent and present in the environment in large quantities. Finley et al. (1998) argue differently. Inclusion of hexachlorobenzene may depend on the question being asked (Birnbaum, 1999a). If we want to know the complete amount of dioxin-like activity in a mixture, the answer may be yes.

Toxic Equivalents

Though the dioxin-like compounds are all thought to act in the same way, they are not all equally toxic. Their different toxicities may be due to unique pharmacokinetics and/or strengths of binding to the AhR. Therefore, the risk of each of these unique compounds is assessed by rating their toxicities relative to 2,3,7,8-TCDD, which is assigned a value of "1." The resulting order of magnitude estimates are called toxic equivalency factors (TEFs). These factors were recently updated by the World Health Organization (Van den Berg, 1998).

Although the TEQ system is widely used, there is some dissatisfaction with it (Van den Berg, 1998; Birnbaum, 1999a). First, it assumes that the total dioxin-like activity of a mixture of these compounds can be estimated by multiplying each compound's concentration by its toxic equivalency factors and then adding all of them together to obtain the toxic equivalency (TEQ) of the mixture. However, some of these dioxin-like compounds antagonize each other's effects, and some act in a synergistic manner. For example, Smialowicz et al. (1997) reported that a non dioxin-like PCB antagonizes an immunosuppressive effect of dioxin, though admittedly only when the PCB is present in very high doses that are unlikely to be seen in the environment. Synergism also occurs, though only if one or more compounds in a mixture acts by a non-dioxin mechanism. It also occurs when low doses of dioxin and nondioxin-like PCBs affect thyroid hormone

levels (van Birgelen, 1997).

Second, the World Health Organization has established toxic equivalency factors only for PCDDs, PCDFs and certain PCBs. It acknowledges that many other compounds meet the basic four criteria, but that there is insufficient data to estimate their toxic equivalency factors. Consequently, these other compounds are effectively assigned an interim equivalency factor of zero, leading to an underestimate of exposure to dioxin-like compounds. This underestimate may be significant. For example, if hexachlorobenzene were included, van Birgelen (1998) suggested that, in most countries, it could add 10-60% to total TEQ in human milk, and, in some countries, up to a six-fold increase. It may be possible to correct for underestimates of toxicity that result from non-inclusion of some dioxin-like compounds by comparing TEQ estimates with the toxicity estimates from bioassays. The bioassay results are larger for some environmental samples (Tillitt, 1996, 1998), perhaps because the bioassay measures dioxin-like compounds that are not included using the TEQ approach. By virtue of the criteria we have adopted to define dioxins and dioxin-like compounds, effects not mediated by the AhR are not accounted for with the TEQ approach.

Overall, the TEQ system is imperfect, but it is a basically reasonable way of estimating the toxicity of a mixture of dioxin-like compounds; there is good experimental support for the assumptions that underlie it (Van den Berg, 1998; Birnbaum, 1999a). It permits extrapolation from 2,3,7,8-TCDD, a compound about which a great deal is known, to other compounds about which much less is known.

Sources

In the U.S., dioxins are unwanted byproducts of various kinds of combustion and chemical reactions involving chlorine. PCBs can also be formed during combustion of chlorine-containing compounds. Though still in use, PCBs are no longer intentionally produced in the U.S. The EPA's latest inventory of U.S. emissions of dioxins, and to a lesser degree dioxin-like PCBs, was released in 1998 (USEPA, 1998). In the document, emissions are characterized according to whether they are released into the air, water, or soil. Since most dioxins are emitted into the air, air emissions are the major focus of EPA's document. Of known air emissions, municipal solid waste incineration is ranked first, secondary copper smelting second, and medical waste incineration third. Less is known about releases to water, products, or soil.

Although much has been done to characterize dioxin emissions, a great deal of information is still needed. For example, known U.S. emissions of dioxins are dwarfed by the amount unintentionally produced during the manufacture of the wood preservative

pentachlorophenol (USEPA, 1998). Since most of these dioxins end up in treated wood, and their fates are unknown, EPA does not list them in its emissions inventory. Another concern is incinerator ash which is also not included in the dioxin inventory. EPA defines incinerator ash as not being released to the environment because it must be disposed of in permitted landfills. However, rules defining ash toxicity are not based on levels of dioxins and allow this ash to be used as daily cover at landfills. There have also been proposals to use incinerator ash as a road-building material, although the fate of the dioxin in the ash is unknown in such a situation.

Even municipal waste incinerators, one of the most studied sources of dioxin emissions (Webster, 1998), need further study. The conditions leading to *de novo* synthesis of dioxins in the cooler parts of incinerators are still not completely understood. The relationship between emissions and chlorine content of the fuel is controversial. Another major unknown is the fate of accumulated dioxins in soil, sediment, and organic matter such as leaves that are disturbed and redistributed back into the environment. Also, many sources of dioxins, including building fires, backyard burning, and cigarette smoking, are very poorly characterized. There are even suggestions that dioxin is formed by living organisms.

Environmental Fate

Depending on ambient temperature and each congener's vapor pressure, dioxins released into the air are emitted as particulates or vapor. The more chlorinated compounds tend to adsorb to particulates and therefore are more protected from chemical and UV degradation. This protection may account for the larger relative abundance of the higher chlorinated compounds in the environment. Airborne dioxins can be carried large distances downwind from their sources and contribute significantly to local deposition (Cohen, 1995; Commoner, 1998). An EPA Science Advisory Board committee stated "...any national model framework to guide exposure assessments for large combustor operations must address both local (i.e., within 50 km) impacts on human health and the environment, and also the contribution of the operations of any single combustor to the more distant (>50 km) regional impacts" (USEPA, 1994c). Local risk assessments should therefore be comprehensive and include the cumulative impact of all sources, not just impacts from a single point source.

Eventually, airborne dioxins settle as particulates or vapor to earth, where they become a major source of contamination of the terrestrial food chain. Their deposition on plants consumed by farm animals is of particular concern because dioxins accumulate in the meat and milk of these animals and then end up in humans. Because dioxins are not very water soluble, when they settle onto waterways, they tend to become buried in sediments or remain suspended in the water as particulates for long periods of time. They move up

the food chain into fish and then eventually into humans.

Though progress has been made in determining how airborne dioxins contaminate food (Cohen, 1995; Commoner, 1998), determining if other pathways of dioxin exposure contaminate foods is generally difficult. Contamination may be direct, as when dioxin-containing sludge are applied to crops. Or, as recently happened in Belgium, it may be indirect. There, PCBs (dioxin-like PCBs accounted for 80% of the total TEQ content) somehow found their way into a batch of chicken feed and caused hatching problems and sickness in chickens. When the news became public, bans on many Belgian products were instituted around the world. Had the toxin levels been lower and gone undetected, a large number of people may have been exposed to dioxin. Food in the U.S. is not exempt from dioxin contamination. Though it is not routinely monitored for dioxin contamination, some surveys have revealed unexpected sources of dioxin in food.

Exposure

Although some people are exposed to dioxin-like compounds on the job or as a result of chemical accidents, this report is principally concerned with how people are exposed continuously to low levels of dioxins, the so-called "background" exposure. But these levels are not natural, but rather are a product of the chlorinated chemical industry. It is inaccurate and misleading to refer to these exposures as "background."

Exposure to dioxins can be measured as a function of daily intake or as a function of body burden. For most Americans, about 90% of dioxin exposure comes from common foods, especially meat, fish and dairy products. There are two reasons for this. One is that dioxin accumulates in fats increasing in concentration at each step in the food chain. The other is that fat-containing foods are prominent in Western diets. According to the World Health Organization, the average adult in Western countries consumes about 1-3 picograms TEQ per kilogram body weight (pg TEQ/kg bw) daily (WHO, 1998). Daily intake increases to 3 to 6 pg TEQ/kg bw if dioxin-like PCBs are included. The average daily intake of dioxins for Americans has been estimated at 2.2 pg TEQ/kg bw (Schecter, 1999). These exposure estimates are based on chemical analysis of foods, food consumption rates, estimates of exposure by other means such as inhalation and skin, and pharmacokinetic extrapolations based on the levels found in people.

The daily intake of dioxin by infants and nursing children is significantly higher than adults. This occurs for several reasons. First, dioxin crosses from the mother into the placenta. Then, after the baby is born, large doses of dioxin-like compounds are transmitted from the mother's milk to her nursing baby. Babies fed with breast milk are exposed on a daily basis to about 50 times more dioxin than adults per kilogram of body

weight. On a daily basis, toddlers are exposed to about three times more dioxin than adults on a body weight basis (Patandin, 1999).

In addition to early developmental stages and infants, other highly exposed groups include subsistence farmers, people who fish for a living and for sport, and indigenous people. Indigenous people who eat fish and sea mammals from the Arctic regions are exposed to dioxin at higher than average levels because dioxin and PCB levels are particularly high in these foods (Papke, 1998). On the other hand, vegetarians have below-average body burden levels of dioxin (Schecter, 1998). People who live or obtain food near sources of dioxin emissions may have higher than average exposure to dioxins. In one instance, milk from cows living near an incinerator in the Netherlands that emitted large amounts of dioxins was declared hazardous waste (MPH, 1989). Animals foraging on soil contaminated with dioxin concentrations in the low part per trillions have been found to accumulate significant amounts of dioxins (Stephens, 1995).

Another common way of measuring exposure to dioxin is to measure body burden levels. Body burden, the total amount of dioxins in an organism's body per kilogram of body weight, reflects the total accumulation of dioxins over an organism's lifetime. Because dioxins are persistent and accumulate in fat, body burdens can be estimated by sampling fat tissues or blood lipids. The dioxin concentrations in fat tissue and in blood lipids are in equilibrium (Papke, 1998). In humans, body burdens are obtained by multiplying concentrations of dioxins in lipids by 22%, the average fraction of the body that is fat (DeVito, 1995). In animals, they are measured experimentally or estimated by using pharmacokinetic models. Keeping in mind that the levels of dioxin in adipose tissue in a human being increase with age, increase after weight loss, and decrease in a breastfeeding mother, the concentration of dioxin in the general population is about 10-30 ppt TEQ. These concentrations are higher if dioxin-like PCBs are included. The average American adult's body burden of dioxin is roughly 10 ng TEQ/kg bw (DeVito, 1995; Birnbaum 1999). Though the range of concentrations for body burdens found in the U.S. population is not well known, there is some evidence for log-normal distribution of concentrations of 2,3,7,8-TCDD. This means that about 5% of the population would be expected to have a body burden of dioxin of about 20 ng TEQ/kg and about 1% would be expected to have a body burden of about 30 ng TEQ/kg.

Trends

Overall, the emissions of dioxin into the environment seem to be decreasing. In lake sediment cores, concentrations of dioxins are low until about the 1930s, the period of rapid growth of the chlorinated chemical industry.[(2)] From that point on, concentrations rise until about the 1960s and 1970s and fall steadily afterwards. Although the scope of the dioxin problem between the 1930s and the 1960s was not understood at the time, it is

likely that environmental measures aimed at other pollutants coincidentally reduced emissions of dioxins.

Before the 1980s, estimates of dioxin emissions into the environment are poor. However, between 1987 and 1995, EPA notes a substantial drop in estimated dioxin emissions into the air, much of this decrease being due to the closing or retrofitting of a number of incinerators emitting very large quantities of dioxins (Webster, 1998). These incinerators were equipped with so-called "hot-sided" electrostatic precipitators (ESPs), which provide good conditions for *de novo* dioxin formation (see Appendix A).

Other changes in regulations and technology responsible for decreased emissions of dioxins into the environment include the ban on leaded gasoline in the U.S. for on-road vehicles; the ban on 2,4,5-T (2,4-D and pentachlorophenol are still allowed); and, in the last decade, the reduced emissions of 2,3,7,8-TCDD and 2,3,7,8-TCDF, the main congeners of dioxin produced during chlorine bleaching in pulp and paper mills. This reduction in dioxin emissions from the pulp and paper industry resulted mostly from the conversion from using chlorine gas to using chlorine dioxide for bleaching paper. This process still produces dioxin, though in lesser quantities.

The recent reductions in dioxin emissions into the environment are partially reflected, though not unequivocally so, in the body burden levels of dioxins in humans. For example, over the last decade, TEQ levels in breast milk and blood have fallen in Western Europe (Papke, 1998). In the U.S., data on body burden levels for the same time period are very sparse, and trends in body burdens are less clear than they are in Europe (Schecter, 1996b). Levels of dioxins in North American lake sediments have decreased, however. It is unclear if the levels of dioxins released into the environment will continue to decline, will plateau or will increase. Furthermore, the TEQ trend data apply to the more conventional dioxin-like compounds; it is possible that other dioxin-like compounds may have different trends.[(3)]

Pharmacokinetics, Body Burden and its Implications

The biological effects of a toxin depend on the concentrations of that substance in a target organ over a critical time period. These concentrations in turn depend on three important pharmacokinetic factors: the absorption, distribution and persistence of the toxin throughout the body. For example, with regard to absorption, 2,3,7,8-TCDD is much more easily absorbed than OCDD resulting in more TCDD than OCDD remaining in the body. PCDDs, PCDFs and PCBs dissolve and accumulate in fat, or they may accumulate in the liver, bound to an inducible protein.

With regard to persistence, PCDDs, PCDFs and PCBs, depending on their chlorine substitution, are quite persistent and slowly eliminated from the body. For example, 2,3,7,8-TCDD has a half-life of five to ten years in humans. In contrast, benzo(a)pyrene, an AhR-binding polynuclear aromatic hydrocarbon (PAH), has a half-life of only about 4.4 hours in humans (DeVito, 1996). Given the same chronic dose, levels of TCDD remaining in the body would be much higher than benzo(a)pyrene. Persistence also varies between humans and other animals: whereas in humans the half-life of 2,3,7,8-TCDD is five to ten years, in rats it is less than a month. Given the same chronic dose, TCDD accumulates to much higher levels in humans than it does in rats in the same amount of time.

The pharmacokinetics of dioxins, which vary in different species and at different stages of development within a species, must be taken into account when extrapolating from one species to another. Traditionally, to account for these differences, NOAEL (no observed adverse effect level)[(4)]or LOAEL (lowest observed adverse effect level) doses in animals are divided by a safety factor. However, these safety factors are often not large enough to account for pharmacokinetic differences, especially given the much longer persistence of dioxin in humans than rodents. Therefore, assessing human health risks from experimental animal health risks is best accomplished by comparing the body burdens of dioxins in the different species. Whereas humans and other animals may respond differently to the same daily intake or dose of dioxin, they are likely to respond similarly when affected by the same body burden (DeVito, 1995).

Another way of comparing the effects of dioxin exposure between humans and other animals is to examine the ratio between the animal LOAEL or NOAEL and human exposure. This ratio is called the "margin of exposure" or MOE. This ratio has been called the margin of safety in the past (Hallenbeck, 1986), but the word "safety" carries an explicit value judgement. While for certain sensitive effects, margins of exposure based on dose are typically about a factor of several hundred, MOEs based on body burdens can be less than a factor of ten (DeVito, 1995). This is a very significant and worrisome observation. Still, body burden comparisons may only be suitable for certain effects, such as reversible effects and those on reproduction and development (Hurst, 1998; Kim, 1998). Also, it may not be appropriate to compare body burdens based on a single ingested dose with body burdens due to chronic doses.

Sensitive Non-Cancer Effects

Studies of dioxin's effects in experimental animals indicate that in addition to cancer, it causes a host of toxic effects. It has adverse effects on the liver and skin, on development, and on the reproductive, immune and nervous systems (see Chapters Six, Eight, and Nine). A number of biochemical and cellular effects in animals occur at body burdens of

about 10 ng/kg or less, levels comparable to those found in the average person (Birnbaum 1998; WHO 1998). Some of the more sensitive endpoints in animals are induction of CYP1A1 and CYP1A2 enzymes, downregulation of the EGF receptor, increased expression of the cytokine IL-1, oxidative damage, and alterations in lymphocyte subsets. These observations suggest that dioxin causes biological effects at levels comparable to those found in the average person. It is not yet known if these effects are adverse, or if they are part of a mechanism leading to toxicity.

Table 10-1 lists some sensitive adverse effects of dioxin and the body burdens estimated to cause those effects in animals. The body burdens are incremental, and even control animals (rats and mice) have body burdens of dioxin-like compounds of about 4 ng/kg (WHO 1998), presumably due to the presence of these compounds in animal feed. Due to differences in the assumptions used to estimate body burdens, the values listed in Table 10-1 differ somewhat from earlier estimates (USEPA 1994a; Webster 1994, 1994a; DeVito, 1995), but not in ways that affect our overall conclusions.

The effects are divided into two groups based on whether dioxin exposure is chronic or single dose. As discussed earlier, chronic doses more closely represent dioxin exposure to the average person. Acute doses are still of interest, but comparisons with human body burdens are difficult to interpret because of the way in which acute doses of dioxin are initially redistributed
throughout the body. The salient features and some supporting data from the studies listed in Table 10-1 are discussed below.

Table 10-1 Some Sensitive Endpoints of Dioxin Exposure

			Incremental Body Burden	
Species	**Effect**	**Dose**	**(ng/kg)**	**Reference**
Rhesus	Object learning	~ 160 pg/kg/d	42*	Schantz, 1989
Rhesus	Endometriosis	~ 160 pg/kg/d	42**	Rier, 1993
Rat	Genital malformation	200 ng/kg #	73*	Gray, 1997a
Rat	Immune suppression	100 ng/kg #	50*	Gehrs, 1998, 1997
Rat	Decreased sperm count	64 ng/kg #	28*	Gray, 1997

Mouse	Immune suppression (viral susceptibility)	10 ng/kg #	10	Burleson, 1996
Current Average Body Burden Levels in People ("Background")			~10	

Rodent background body burdens are about 4 ng/kg
 # Single dose on specific day of pregnancy
*** Estimated maternal body burden above background.**
**** Estimated body burdens above background.**
Not used by WHO to set the TDI (see text).

Source: WHO, 1998; Birnbaum, 1999.

Developmental neurotoxicity: Subtle deficits in object learning are observed in the offspring of rhesus monkeys chronically exposed to dioxin *in utero* and to breast milk (Schantz, 1989). Seo et al (1999) observed long-lasting learning and memory effects in rats exposed to similar dioxin levels. Rats of both sexes have deficits in nonspatial learning, but males have enhanced spatial learning. The latter result may represent a response strategy rather than improvement in learning or memory.

Endometriosis: The incidence and severity of endometriosis in rhesus monkeys chronically exposed to dioxin rises as the dose of exposure increases (Rier, 1993). This study has been criticized because the clinical history of the monkeys was not reported (ATSDR, 1998) and abdominal surgery may be a risk factor for endometriosis in monkeys. However, the exposed and control monkeys were treated identically, and underwent only fat biopsies prior to laparoscopy. Surgically-induced endometriosis is enhanced by exposure to dioxin in cynomolgus monkeys (Yang, 1998). As endometriosis does not occur naturally in rodents, it is not present in early classic reproductive toxicology studies of rats (Murray, 1979). However, endometriosis-like lesions can be surgically-induced in rodents, and dioxin promotes these lesions in rats and mice (Cummings, 1996; Johnson, 1997). In human endometrial tissue, AhR is expressed, suggesting that it is involved during the reproductive phase of this tissue (Kuchenhoff, 1999).

Effects on the Developing Reproductive System: Pregnant rats exposed to a single dose of dioxin during organogenesis give birth to both male and female offspring with permanent damage to their reproductive systems (Gray, 1997, 1997a).

Immunotoxicity: Offspring of pregnant female rats exposed to dioxin have "suppressed delayed type hypersensitivity"which renders an animal more susceptible to immune infections (Gehrs, 1997, 1998). Captive harbor seals fed Baltic fish with 210 ng TEQ/kg lipid in their blubber develop delayed type hypersensitivity relative to controls fed cleaner Atlantic fish with only 62 ng TEQ/kg lipid in their blubber (Ross, 1995). Dioxin may suppress immune responses in virally infected individuals. Eight week old mice treated with 10 ng/kg of dioxin die more frequently than controls when exposed to influenza virus one week later (Burleson, 1996). The World Health Organization (1998) did not use the results of this study to derive a tolerable daily intake of dioxin because there was no dose-response effect in the study, and because "children from Seveso with chloracne, who had been exposed acutely to high doses of TCDD, exhibited only minor transient alterations in various non-specific immune system parameters."[(5)]

On the other hand, the Agency for Toxic Substances and Disease Registry (ATSDR, 1998) used the study as the basis for its Minimum Risk Level (MRL) for acute exposure to dioxin. This experiment reveals the most sensitive adverse effect of dioxin exposure yet identified. Additional research on viral susceptibility after exposure to dioxin is needed.

It seems evident from Table 10-1 that in experimental animals, the reproductive, immune, and nervous systems are particularly sensitive to dioxin's toxic effects. It also seems evident that the body burdens in experimental animals at which these toxic effects occur are within an order of magnitude (factor of 10) of those that occur in the average American. This conclusion depends on several important assumptions: i) body burden is a reasonable exposure measure; ii) extrapolation from single doses to body burdens is acceptable; and iii) the TEQ system provides a reasonable estimate of human body burden.

However, confirmation that dioxin's toxic effects in experimental animals also occur in humans is not an easy task. As mentioned elsewhere in this report, extrapolating health risks of dioxins from animals to humans is not always possible. Furthermore, epidemiological studies of the effects of dioxins in humans cannot be done under the same controlled situations as studies of dioxin's effects in experimental animals. In spite of these difficulties, the following similarities between humans and experimental animals allow reasonable extrapolations of dioxin's effects from one species to the other. Experimental animals and humans both have the AhR and associated factors; they both share a number of similar biochemical responses; and, on a body burden basis, many human responses to dioxins are reasonably comparable to the responses in experimental animals (DeVito, 1995).

Also, epidemiological data from high exposure situations, and studies of dioxin's adverse effects in highly exposed groups provide evidence that some of its effects in experimental

animals also occur in humans. Children of women exposed *in utero* to a complex mixture of PCDFs, PCBs and other compounds in the Taiwan rice oil poisoning incident (Yu-cheng) suffer a number of effects including damage to the nervous and respiratory system (Hsu, 1994); higher than normal incidence of middle ear infections (Chao, 1997); and reduced penis size at adolescence (Guo, 1993). Children from Seveso with chloracne experienced transient changes in immune parameters, but no adverse immunological effects were reported, although the immune effects seen in animals were not examined. Also, the sex ratio (48 girls to 26 boys) of children born in Seveso was not normal for several years following dioxin exposure, but the same effect is not seen after PCB/PCDF exposure in the Yu-cheng children (Mocarelli, 1996; Rogan, 1999). Though a major study of women exposed to dioxin at Seveso is underway, the existing epidemiological evidence showing the effect of dioxin exposure on endometriosis is limited and mixed. One study in Israel found higher levels of dioxin in the blood of women with endometriosis than in controls (Mayani, 1997). Workers with chloracne who worked at the Nitro, West Virginia trichlorophenol plant reported higher than expected sexual dysfunction and lower than normal libido (Moses, 1984). Additional human information on the effects of dioxin is available from studies of less highly exposed populations.

Effects Observed in the General Population

The Dutch Cohort

Some of the most important evidence that dioxin exposure adversely affects the general public comes from a study of a cohort of children from the general population of the Netherlands (Lanting 1998; Patandin 1999). In that study, children exposed to dioxins and PCBs demonstrate neurodevelopmental, immune system, and thyroid hormone effects. Most effects were more associated with *in utero* exposure to dioxin/PCBs than to exposure through breast milk. Many effects were subtle, and some diminished over time. However, some effects, including a 4 point IQ deficit (Patandin, 1999b), high incidence of chickenpox, and a low number of antibodies to measles (Weisglas-Kuperus, 1999), persist at 42 months of age. In addition, altered but not abnormal levels of lymphocyte subsets and thyroid-related hormones in children up to three months old are associated with exposure to PCBs and dioxins (Weisglas-Kuperus, 1995). These and other effects found in this study are discussed in detail in Chapter Eight. It is often difficult to distinguish if effects are due to dioxins or to PCBs because the compounds typically occur together. Many of the effects observed in this study appear to be associated more with PCBs than with dioxins, though certain early endpoints are related to dioxins.

The Finnish Cohort

Relatively new evidence of harmful effects of dioxin in the general population comes from studies of teeth. A study of breast-fed Finnish children reports an association between dioxin exposure and hypo-mineralization defects of permanent teeth (Alaluusua, 1996, 1996a, 1999). Because permanent teeth in humans are mineralized during the first two years of life, a child's exposure to dioxin in this study was estimated by multiplying the TEQ in the breast milk of mothers by the length of breast feeding. The authors found that as the concentration of dioxin in breast milk went up, the more frequent and more severe the tooth defects. These findings contrast with the results of the Dutch cohort where effects were observed primarily in children exposed *in utero*. Incorporation of dioxin-like PCBs into the exposure measure does not alter the results. Teeth defects are also observed in the rice oil poisonings in Taiwan (Rogan, 1992). It would be very useful to repeat the Finnish study in other groups of children exposed to dioxin.

There are some toxicological data to support effects of dioxin on tooth development. Dioxin causes defects of dental hard tissues in rats (Alaluusua, 1993),[(6)] perhaps by altering the action of epidermal growth factor receptor (Partanen, 1998). McNulty (1985) observed dental defects and changes in ameloblasts (enamel-forming cells) in rhesus monkeys exposed to PCBs.

Miscellaneous Studies: U.S., Germany, and Japan

In two American studies that do not measure dioxins (Rogan, 1991; Jacobson, 1996), and in an ongoing German study (Winneke, 1998), neurodevelopmental effects are associated with low-level PCB exposure; some of the results differ among the studies. In a study of children from the general Japanese population, exposure to dioxin-like compounds are associated with adverse effects on thyroid hormones and the immune system (Nagayama, 1998, 1998a).

In summary, some evidence indicates that dioxin exposure causes developmental effects in children from the general population. However, as exposure to "background" levels of dioxin-like and non-dioxin-like compounds typically take place together, it is often difficult to sort out their respective effects. Whereas the effects on the development of the nervous system are more associated with *in utero* exposure, the dental effects are more strongly associated with dioxin exposure from breast milk, a finding consistent with the timing of tooth mineralization in humans.

Derivations of "Tolerable" Daily Intake Based on Non-Cancer Effects

Based on data similar to that in Table 10-1, a number of national and international agencies have discussed or proposed "tolerable" daily intakes (TDIs) of dioxin-like

compounds for non-cancer risks. The approaches of three such agencies, the EPA, the Agency for Toxic Substances and Disease Registry (ATSDR), and the World Health Organization (WHO), are summarized below.

EPA Does Not Set a Reference Dose

The EPA assesses the non-cancer risks from exposure to a chemical by setting a Reference Dose (RfD), a chronic dose below which an appreciable risk of adverse non-cancer effects is unlikely to occur. Reference doses are calculated by dividing a sensitive "no observed adverse affect level" (NOAEL) or "lowest observed adverse effect level" (LOAEL) observed in animal studies by an uncertainty ("safety") factor, a factor that attempts to correct for uncertainties such as differences in pharmacokinetics when extrapolating between species. As early as 1992 or before, when dioxin's toxic effects were being reassessed, the EPA was one of the first government agencies to raise public concern about non-cancer risks posed by "background," or body burden levels of dioxin. By 1994, the EPA concluded that members of the general population with a higher than average exposure to dioxin may be at risk for a number of dioxin's adverse effects (USEPA 1994a).

However, EPA realized that setting a reference dose for dioxin posed an unusual circumstance. If the agency applied the standard uncertainty factors to account for species differences and sensitive populations to the lowest effect observed in studies of animals exposed to dioxin, the resulting reference dose levels for dioxin would be 10 to 100 times below the current estimates of daily intake in the general population (USEPA, 1994a). As a result, EPA decided to ignore its own standard methods for determining reference doses and declined to set a RfD at the time. The situation has not changed since. The EPA noted that "MOEs (margins of exposure) in the range of 100-1,000 are generally considered adequate to rule out the likelihood of significant effects occurring in humans based on sensitive animal responses. The average levels of intake of dioxin-like compounds in terms of TEQs in humans described above would be well within a factor of 100 of levels representing lowest observed adverse effect levels in laboratory animals exposed to TCDD or TCDD equivalents. For several of the effects noted in animals, a MOE of less than a factor of 10, based on intake levels or body burdens, is likely to exist" (USEPA, 1994a).

ATSDR Sets Minimum Risk Levels

The ATSDR assessed the non-cancer risks from dioxin exposure by setting minimal risk levels (MRLs). Defined similarly to the EPA's RfDs, MRLs are set for acute, sub-chronic, and chronic exposures to dioxins. The chronic MRL was based on dioxin's developmental neurotoxicity in rhesus monkeys. In choosing this study, ATSDR ignored

the more sensitive effects in Table 10-1 that result from single doses of dioxin. ATSDR considers the endometriosis results less convincing than the developmental neurotoxicity results, but indicates that the MRLs calculated from using either endpoint are about the same.[7]

Although the World Health Organization chose to use the effects measured on object learning (Schantz, 1989) as an endpoint in their calculation of a Tolerable Daily Intake (TDI), ATSDR chose to examine a different endpoint, altered group behavior (Schantz, 1992) in the same study of rhesus monkeys. ATSDR then applied an uncertainty factor of 90 (3 for a minimal LOAEL, 3 for interspecies extrapolation, and 10 for human variability)[8]to the LOAEL dose of 120 pg/kg/day to yield a chronic minimal risk level of 1 pg/kg bw/day.

World Health Organization Sets a Daily Tolerable Intake

The World Health Organization assessed the non-cancer risks from dioxin exposure by setting a Tolerable Daily Intake (TDI). In deriving the TDI, the World Health Organization estimated that the chronic daily intake of dioxin for the body burdens associated with the toxic effects in Table 10-1 (omitting viral susceptibility) was 14-37 pg/kg bw/day. WHO used this wide range of dioxin exposures because of the uncertainties involved in extrapolating body burden levels of dioxin from the experiments using in most cases a single ingested dose of dioxin. WHO then divided this value by a safety factor of 10 and obtained a TDI of 1-4 pg/kg bw/day.

In arriving at its uncertainty factor of 10, the World Health Organization reasoned as follows. First, though it uses LOAELs instead of NOAELs, it considers LOAELs to be within a two to three-fold difference of NOAELs. Second, it argued that, though there are potential differences in susceptibility to dioxin exposure between other animals and humans, some of the endpoints are about equally sensitive. WHO avoided the need for a safety factor to account for interspecies differences in dioxin's pharmacokinetics by using body burdens to scale across species and by arguing that the differences in half-lives between dioxin congeners are not very great.

In speaking about its TDI, the World Health Organization commented that it "recognized that certain subtle effects may be occurring in some sections of the general population of industrialized countries at current intake levels (2-6 TEQ pg/kg bw/day) and body burdens (4-12 TEQ ng/kg bw), but found it tolerable on a provisional basis as these reported subtle effects were not considered overtly adverse and there were questions as to the contributions of non-dioxin-like compounds to the observed effects. The consultation therefore stressed that the upper range of the TDI of 4 pg TEQ/kg bw should be considered a maximal tolerated intake on a provisional basis and that the ultimate goal is

to reduce human intake levels below 1 pg/kg bw/day" (WHO, 1998).

The World Health Organization also states that the effects of dioxin found in infants are associated with *in utero* exposure rather than with exposure from breast feeding. The executive summary of the WHO report - all that is in the public domain (see footnote 1 of this chapter) - did not discuss the Finnish epidemiological studies on teeth because the studies were not available at the time of their review. Since breast feeding has beneficial effects, World Health Organization reiterates its position that "the current evidence does not support an alteration of WHO recommendations which promote and support breast feeding" (WHO, 1998).

With the release of the evaluations by the WHO and ATSDR in 1998, the case for non-cancer risks to the general American population from low level dioxin exposures was, if anything, stronger than that faced by EPA in 1994. Nevertheless, though all three agencies use some of the same key data about dioxin's effects on human health, differences in judgment, assumptions, and policy lead them to different conclusions about tolerable daily intakes or minimum risk levels. Had EPA followed its own guidelines and set a RfD for dioxin, it would have been below the current dioxin intake. EPA argues that in this instance, when low level exposures (referred to by the agency as "background intake") exceed the calculated RfD, it is inappropriate to use a RfD for evaluating incremental exposure of dioxin (USEPA, 1994a). However, when assessing risks of exposure, the assessment should be based on total exposure to a toxin, not just on incremental exposure. Addressing only incremental exposure ignores the dioxin already accumulated in the body.

Although ATSDR and WHO both use the data from rhesus monkey studies to calculate their respective chronic MRL and TDI, their results differ significantly. By using body burdens to extrapolate across species, the WHO accounts for the 7.5 year half-life of TCDD in humans compared to its 1.3 year half-life in rhesus monkeys. However, ATSDR's interspecies uncertainty factor of 3, though it may reasonably account for dioxin's different pharmacokinetics among different species, does not adequately account for pharmacokinetic differences of other dioxin congeners. In its favor, however, ATSDR explicitly uses an uncertainty factor of 10 for human variability and 3 for a LOAEL.

The WHO's decision to use body burden levels to avoid using an uncertainty factor to account for the pharmacokinetic differences among dioxin congeners is unconventional. WHO's justification for using an overall uncertainty factor of 10 to account for the combined effects of LOAELs, interspecies sensitivity, and intraspecies sensitivity is weak and insufficiently protective. Factors of ten have traditionally been applied for each of these individually, but not for all three collectively. Indeed, a factor of 10 might be used just for susceptibility within humans. Though such factors are essentially arbitrary, they have considerable policy implications. Nevertheless, to its credit, WHO reduced its initial

tolerable daily intake of 2,3,7,8-TCDD from 10 pg/kg bw to 1-4 pg/kg bw of TEQ (with a goal of below 1). This represents a major shift in WHO's perspective.

Cancer

An increased incidence of cancer is observed in animals and humans at body burdens higher than those listed in Table 10-1. From this perspective, cancer is a less sensitive endpoint. This does not mean that dioxins cannot cause cancer at lower body burdens, only that it has not been detected at lower doses. In fact, because of the confounding effect of other carcinogens, the carcinogenicity of dioxins is probably undetectable at lower doses. Yet, because the ranking of dioxin's toxic effects in Table 10-1 is empirical and can depend on the statistical methods used to do the ranking, and because dioxins can potentially cause cancer at low doses, cancer risk characterizations from dioxin exposure are often given special attention.

Qualitative Carcinogenicity

The qualitative aspects of dioxin's carcinogenicity are reviewed by the International Agency for Research on Cancer (IARC, 1997) and by the U.S. National Toxicology Program (NTP, 1997).

IARC considers 2,3,7,8-TCDD to be a "group 1: carcinogenic to humans" substance. In making this judgement, IARC acknowledges the importance of the Ah receptor (AhR) in mediating dioxin's toxic effects in both humans and experimental animals. IARC did not classify other PCDDs and PCDFs because experimental and epidemiological data was scarce. NTP ranks dioxin as "reasonably anticipated to be a human carcinogen."[9]

Both agencies agree that 2,3,7,8-TCDD causes cancer in experimental animals, though the evidence for its carcinogenicity in humans is not as convincing on its own. However, the recent update of the NIOSH cohort of workers (Steenland, 1999), which finds that dioxin's carcinogenicity is directly proportional to exposure, and the most recent update from Seveso (Bertazzi, 1999) provide further confirmation of dioxin's carcinogenicity for humans.

Quantitative Carcinogenicity

Quantitative estimates of a chemical's carcinogenicity are often as important or more important than qualitative ones. To quantitatively estimate dioxin's carcinogenicity, EPA

calculates a "unit risk" value. A unit risk is the additional cancer risk produced by a lifetime average daily dose of 1 pg/kg/day. In its 1994 reassessment draft, EPA estimated that the lifetime risk for the American people of getting cancer from exposure to dioxin is 1 in 10,000 and that the risk attributable to dioxin for highly exposed members of the population is 1 in 1,000 (USEPA, 1994a). These risk estimates are based on ingesting a "risk specific dose" of 0.01 pg TEQ/kg bw/day over a 70-year lifetime. At this dose, there will be one additional cancer for every one million exposed people. One cancer per million is often considered an "acceptable risk" value (NRC, 1994). The risk specific dose of 0.01 pg TEQ/kg bw/day over a 70-year lifetime corresponds to a unit risk of 1 x 10^{-4} pg TEQ/kg/day. This estimate is virtually the same as its 1985 estimate, when 2,3,7,8-TCDD was described as the most potent animal carcinogen ever evaluated by the agency (USEPA, 1985).

These risk estimates, which are based on studies in animals, are within an order of magnitude of that derived from epidemiological studies of workers highly exposed to dioxin. A recent re-evaluation of one cohort using sophisticated statistical methods estimates an even higher unit risk of 10^{-3} (1 in 1,000) to 10^{-2} (1 in 100) (Becher, 1998). As the authors point out, questions about thresholds or the correct dose metric are often difficult, if not impossible, to answer with epidemiologic data.

If these estimates are correct, the low level exposure of the American people to dioxin-like compounds poses an uncertain but potentially substantial risk to the average person, a point made at least a decade ago (Commoner, 1985). Since the average daily intake of dioxin ranges from 1 to 3 pg/kg bw/day (3-6 pg/kg bw/day if dioxin-like PCBs are included), everyday the general American public is exposed to an estimated additional cancer risk of 1 cancer per 10,000 persons. The risk attributable to dioxin for highly exposed member of the population is 1 cancer per 1,000 persons. These risk estimates are 100 to 1,000 times higher than the one-in-a-million "acceptable" cancer risk.

EPA's procedure for calculating a unit risk has generated enormous controversy. This is primarily the result of EPA deciding that, unless there is compelling evidence to the contrary, at low doses, the dose-response curve for carcinogens has no threshold. This assumption is usually reserved for a carcinogen which is genotoxic by causing mutations, although when evidence is unclear EPA tends to try to err on the side of caution. Since dioxin has little or no direct mutagenic action and may work through a "promotional" mechanism,[(10)] many argue that the no threshold model does not apply to dioxin's carcinogenicity. When the Peer Review/Risk Characterization committee began reassessing dioxin's toxicity in 1994, it was argued that mediation by the AhR necessarily implies a threshold. Although this idea was discounted then (Melnick, 1996), the controversy continues.

Whichever estimate of cancer risk is used, they are uncertain for a number of reasons.

First, and perhaps most importantly, the no threshold assumption may be invalid and the shape of the dose-response curve at low doses is unknown. The body burdens of dioxin at which exposed workers and experimental animals have higher numbers of cancers are similar (Grassman, 1998; Steenland, 1999), and both are substantially higher than the body burden levels of dioxin in the general human population. If the dose response curve for dioxin's carcinogenicity is sub-linear or if a threshold exists, the EPA's unit risks may overestimate cancer risk to the general population from dioxin exposure.

Second, the EPA's unit risk calculations are based on a person's lifetime average daily dose. However, dioxin is particularly harmful in early stages of development (see the discussion below on the relationship of dioxin exposure and the incidence of breast cancer). Also, the EPA's unit risks are based on current exposures to dioxin; if exposure is decreasing, unit risks may be overestimated for some people and underestimated for others.

Third, some critics of EPA's unit risks suggest that dioxin actually reduces the incidence of some types of cancers, and that this effect of dioxin is not taken into account by the EPA. For example, the lowest dose of dioxin tested reduced the incidence of mammary tumors in rats, an effect that was not observed at higher doses (USEPA, 1994d). While the incidence of some types of cancer increased in the first ten years after the Seveso accident, female breast cancer decreased in zone A, closest to the chemical plant explosion. The relative risk (RR) in zone A was 0.5 (95% confidence intervals of 0.1-3.3). In zone B, the relative risk was 0.7 (0.4-1.4). In zone R, a decrease was seen only in those living longest in the area (Bertazzi, 1993). None of these increases were statistically significant. In contrast, breast cancer increased in a group of women occupationally exposed to dioxin at a German chemical plant: RR=2.2 (1.0-4.1) (Manz, 1991). The reasons for the apparently different relationships noted above between exposure to dioxin and the incidence of breast cancer are unclear. Because dioxin can be anti-estrogenic in some tissues, it is biologically possible for dioxin exposure to decrease the incidence of breast and certain other types of cancer. On the other hand, estrogen appears necessary for dioxin to cause liver cancer in female rats (USEPA, 1994d).

Perhaps more pertinent, the risk of breast cancer may depend on when exposure to dioxin occurs. Recent experiments indicate that female rats exposed to dioxin *in utero* have mammary glands that are incompletely developed and have an above normal susceptibility to carcinogens when 50 days old. Rats exposed to 2,3,7,8-TCDD *in utero* and then exposed to a mammary carcinogen, dimethylbenz(a)anthracene (DMBA), 50 days after birth have a dramatically higher incidence of mammary tumors than animals exposed to DMBA without having first been exposed *in utero* to 2,3,7,8-TCDD (Brown, 1998). These results suggest that the effect on breast cancer may be more complicated than previously thought. More importantly, it suggests that the timing of exposure may be very important. This finding is not surprising given other studies on the developmental effects of dioxin.

Fourth, cancer risk estimates are made without taking into account co-exposure to other agents. Yet, as the results of Brown et al. (1998) and others suggest, dioxin's toxicity may not only depend on the presence and influence of other chemicals, but may also depend on the presence and influence of viruses. For instance, in addition to their independent effect, PCBs may interact synergistically with the Epstein-Barr virus in causing non-Hodgkin's lymphoma (Rothman, 1997).

Conclusion: The American People are at Serious Risk from their

Daily Intake of Dioxin in Food

The weight of the evidence on the effects of dioxin on human health supports the following conclusions:

- All American children are born with dioxin in their bodies. The greatest impact of this exposure appears to be to the growth and development of children. Disrupted sexual development, birth defects and damage to the immune system may result.

- Dioxin exposure has been associated with IQ deficits, increased prevalence of withdrawn/depressed behavior, adverse effects on attentional processes, and an increase in hyperactive behavior in children. These effects have been reported in 42-month old Dutch children exposed primarily prenatally to dioxins and PCBs. The children's mothers were exposed to "background" levels of dioxins and PCBs as a result of the daily ingestion of dioxin in food.

- Dioxin exposure has been associated with alterations in immune function including increased susceptibility to infections and changes in T-cell lymphocyte populations. These effects have been reported in 42-month old Dutch children exposed primarily prenatally to dioxins and PCBs. Altered immune function, which was reported at birth, 3, and 18 months of age, persists to 42 months of age in these children. Reported immune effects included an increase in middle ear infections, chicken pox, and a decrease in allergic reactions.

- There is evidence of both developmental and reproductive effects in children exposed to dioxin. These effects include defects in permanent teeth, adverse effects on thyroid hormones, altered sex ratio (more females than males), and increased respiratory infections.

- Hormonal effects associated with dioxin exposures in humans include a decrease in testosterone in dioxin-exposed workers and a decrease in thyroid hormones

following prenatal exposure to background levels of dioxin in infants.

- Dioxin interferes with the hormone insulin and alters glucose tolerance which leads to diabetes. New studies of dioxin in soldiers exposed to Agent Orange and residents of Seveso, Italy add to the existing evidence from studies of workers that exposure to dioxin increases the risk of developing diabetes.

- The average daily intake of dioxin in food poses a substantial cancer risk to the general American population. The lifetime risk of getting cancer from exposure to dioxin is 1 in 10,000 for the general American population and 1 in 1,000 for highly exposed members of the population. These risks are 100 and 1,000 times, respectfully, higher than the one-in-a-million "acceptable" cancer risk for carcinogens.

- Updates of ongoing studies of cancer rates in dioxin-exposed workers in the U.S. and Germany, and in residents of Seveso, Italy all indicate increasing cancer rates in the highest exposure groups. These studies provide strong support for the decision by the World Health Organization's International Agency for Research on Cancer (IARC) to define dioxin (TCDD) as a "known human carcinogen." This decision is further supported by evidence from animal studies and data on dioxin's mechanisms of action in the body.

- Nearly all Americans are exposed to dioxin through ingestion of common food, mostly meat and dairy products. Dairy cows and beef cattle absorb dioxin by eating dioxin contaminated feed crops. The crops become contaminated by airborne dioxins that settle onto soil and plants. Dioxins enter the air from thousands of sources including incinerators that burn medical, municipal, and hazardous waste, chemical processing facilities that use chlorine to make products such as pesticides and PVC plastic, and metal refining and smelting operations.

- The average daily intake of the American people is already well above two federal guidelines for safe exposure. The average daily intake of the American people is more than 200 times higher than EPA's risk cancer guideline, over twice ATSDR's lowest adverse effect level.

- At higher risk of exposure to dioxin are children, nursing infants, some workers, people who eat fish as a main staple of their diet, such as some indigenous people and fishermen, and people who live near dioxin release sites. These groups of people are likely exposed to at least 10 times as much dioxin as the general population.

- The average daily exposure of dioxin and dioxin-like chemicals in the U.S. is approximately 3-6 pg TEQ/kg body weight per day. Nursing infants ingest about 50 times this much each day.

- Dioxin accumulates in biological tissue. The average tissue or "body burden"

level of Americans ranges from 36 to 58 ng TEQ/kg lipid (36-58 ppt). Approximately 10% of the population may have tissue levels as much as three times higher than this level.

- There is a small difference between the body burdens of dioxins that cause adverse non-cancer effects in animals and average levels in the general human population. Some people who have above average levels are already suffering from the adverse effects of exposure to dioxin.

- While TCDD is the most toxic form of dioxin, 90% of the total toxicity resulting from exposure to dioxins is due to dioxin-like compounds other than TCDD.

- There is an extensive body of high quality, published information on the toxicity of dioxin. This body of data indicates that dioxin is a potent toxin which produces a wide variety of adverse effects in animals and that some of these effects are likely already occurring in people.

Dioxin is an ubiquitous poison that is in our food and causes many toxic effects in people and animals. The neurodevelopmental and reproductive effects observed in children may be the most disturbing new evidence of dioxin's toxicity. The small shifts in cognitive ability or thyroid levels may be just the tip of the iceberg of our understanding of the impact of dioxin on the general American population.

We know that the daily intake of Americans is already too high, and exceeds several federal risk guidelines. We also know that some members of the general population are particularly sensitive and that others are exposed to dioxins at greater than the average daily levels. These are infants and children, people who live near contaminated sites, fishermen and indigenous people who rely on fish as a main staple of their diet, some workers, and others with high exposures. These groups have suffered a disproportionate share of dioxin exposure and many already suffer the adverse health effects caused by these exposures.

We agree with the World Health Organization who recommended that "every effort should be made to limit environmental releases of dioxin and related compounds to the extent feasible in order to reduce their presence in the food chains, thereby resulting in continued reductions in human body burdens..." (WHO, 1998). Americans have a choice: take action to protect public health by eliminating dioxin creation or continue to allow dioxin to be created and not burden industry with the short term costs of eliminating dioxin and related compounds.

1. The full WHO report is not yet available, although a press release and a summary have appeared (Van Leeuwen, 1998). In this report, we refer to the executive summary of December 1998. While this report states that it is not yet formal and "should not be

quoted or cited and is personal use only," it is now in the public domain (Montague, 1999).

2. This pattern is not observed for PCDD in some marine sediment cores (Hashimoto, 1990, 1995), but there is some concern about the validity of the results (Alcock, 1996). The increase in PCDD/PCDF sediment concentrations in a Scottish lake begins in the mid-1800s (Rose, 1997). This pattern may be connected to the extensive industry in the UK last century (Alcock, 1998), including the forerunner of the modern chlorine industry (Webster, 1994).

3. A recent analysis of polybrominated diphenyl ethers (PBDEs) in Swedish breast milk show dramatic increases over the last two decades, doubling every 5 years (Noren, 1998). PBDEs are used as fire retardants; their toxicity is reviewed by Pijnenburg et al. (1995). If they are dioxin-like--and there is not much evidence to support it--their TEFs are very small (Hornung, 1996; Murk, 1996).

4. NOAELs and LOAELs depend not only on biology but also on the study design that includes such factors as statistical power of an experiment to detect differences from controls.

5. Burleson's 10 ng/kg result has not been repeated yet. Burleson et al. (1996) confirmed earlier work by House et al. (1990) at 100 ng/kg and above. No one has examined the immunology of children from Seveso who were exposed in utero.

6. Unfortunately, only one very high dose of dioxin, 1,000 ug (plus controls), was examined in this study of Hans Wistar rats (resistant to lethality but not other effects). Repetition at lower doses would be useful.

7. ATSDR didn't use endometriosis but suggested potential uncertainty factors: 10 for LOAEL, 10 for human variability and 1 for interspecies extrapolation from animals to humans. ATSDR argued that since prevalence of endometriosis was higher in rhesus monkeys than in humans, humans are less susceptible. But these prevalence estimates are crude at best and, more importantly, may say little about susceptibility to dioxin. It also underestimates the difference in pharmacokinetics.

8. The experiment reported dose as ppt in feed. In extrapolating to pg/kg/day, different assumptions can lead to an estimated dose different from Table 10-1.

9. NTP had made an initial decision to rank dioxin as a known human carcinogen (NTP, 1997). However, critics challenged this decision arguing that NTP criteria require that there is "sufficient evidence" from human studies alone to classify a substance as a human carcinogen. The IARC process takes into account all evidence including mechanistic and animal data. According to NTP criteria, this additional data can only be used to lower rankings (NTP, 1998). NTP has not made a final decision whether to upgrade dioxin's status to a human carcinogen or not.

10. A good discussion of the meaning of the terminology is found in Chapter 6 of USEPA,1994d.